

Photolysis ($\lambda = 254$ nm) of phenylalanine in aqueous solution

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Abstract

Phenylalanine (Phe) was photolysed ($\lambda = 254$ nm) in deoxygenated and air-saturated aqueous solutions. The quantum yields of degradation ($\Phi(-\text{Phe})$) were found to be 5×10^{-2} and 5.4×10^{-2} respectively. In the absence of O_2 , eight isomeric (3-alanyl)-2-*exo*-bicyclo[3,1,0]hex-3-en-2-ols (AHBs) were formed. They were isolated (four as two binary mixtures only) and their structures determined by nuclear magnetic resonance (NMR) spectroscopy. Their yield remained almost constant in the presence of O_2 ($\Phi(\text{total AHBs}) = 4.2 \times 10^{-2}$) where the formation of three tyrosines ($\Phi(\text{total tyrosines}) = 4 \times 10^{-3}$) and phenylacetaldehyde ($\Phi = 5 \times 10^{-5}$) was also observed. In addition to these products, a short-lived ($t_{1/2} = 2.1$ h) product was formed which absorbed strongly at 305 nm. A good material balance between $\Phi(\text{products})$ and $\Phi(-\text{Phe})$ was obtained, suggesting that the unstable product must have a low quantum yield. In N_2O -containing solutions, N_2 was formed.

It was concluded that the eight AHBs arise from the excited singlet state via unstable benzvalene intermediates. The N_2O experiments suggest that photoionization also occurs at this wavelength. In the presence of O_2 , this may lead to the formation of tyrosines and phenylacetaldehyde.

Keywords: Photolysis; Phenylalanine

1. Introduction

There is a long-standing interest in the photochemistry of proteins and their subunits, the amino acids [1]. Surface waters contain low concentrations of glycoproteins which result from the excretion and biodegradation of algae (extracellular organic matter, EOM) [2]. On UV disinfection ($\lambda = 254$ nm) of these waters in drinking water processing, this material may undergo photolytic changes [3]. Thus in the context of the assessment of potential byproduct formation [3–5], the photolysis of aromatic amino acids has been investigated as a model system for the UV degradation of EOM.

In this paper, we report the 254 nm photolysis of phenylalanine (Phe) in aqueous solution. The photolyses of the other aromatic amino acids, tyrosine and tryptophan, have also been studied. These results will be published elsewhere.

The photochemistry of Phe has been studied previously [6–16]. The overall photodegradation quantum yield at 254 nm has been reported to be $\Phi(-\text{Phe}) = 0.019$ [13]. Photoionization occurs at 193 nm as a monophotonic process with a quantum yield of $\Phi(e_{\text{aq}}^-) = 6.6 \times 10^{-2}$ [16]. Laser excitation at 265 nm [7,8] and 248 nm [16] also causes photoionization, but at the lower energy of these quanta photoionization is

largely a biphotonic process. Due to the scatter of the data, extrapolation to zero laser intensity does not reveal whether there is also a significant monophotonic contribution at this wavelength [16]. The triplet state of Phe has been characterized by laser flash photolysis and its lifetime determined to be 3 μs [10]. Using the spin-trapping technique, a Phe-derived radical was identified that had lost the CO_2H group of Phe [11].

The photochemistry of Phe is much more poorly understood than the photophysics. It has been reported that tyrosines are formed in air-saturated solutions [6,14]. It has also been reported that new amino acids are formed, but their identification has not been discussed [6,9,17]. It is shown in this paper that they are of the alanyl-bicyclo[3,1,0]hexenol type and are the major products in the photolysis of Phe, both in the absence and presence of oxygen.

2. Experimental details

Phe (Aldrich) was used as received. Solutions were made up in Milli-Q-filtered (Millipore) water (5×10^{-3} mol dm^{-3} for preparative product studies; 10^{-3} mol dm^{-3} for kinetic studies unless stated otherwise). For the photolyses, a low-pressure Hg-arc (NK 30/89, Her-

aeus Noblelight) emitting essentially 254 nm radiation was used. The irradiation apparatus and actinometry have been described previously [18]. The fluence rate was typically 20 W m^{-2} .

The products were separated by high performance liquid chromatography (HPLC). A chromatogram is shown in Fig. 1. The same conditions were used to determine the consumption of Phe.

By cutting and rechromatographing, AHB-3b and (AHB-2a + AHB-4a) were also well separated. (AHB-2a + AHB-4a) remained unseparated (ratio, approximately 2:1) as did AHB-1a,b (ratio, approximately 3:2). The product with a retention time of 8 min was unstable, and it was not possible to isolate it by preparative HPLC for nuclear magnetic resonance (NMR) spectroscopy and gas chromatography/mass spectrometry (GC/MS) analysis as with the other products.

The eight major products (Fig. 1) are of the (3-alanyl)-2-*exo*-bicyclo[3,1,0]hex-3-en-2-ol type (AHB-1a,b–AHB-4a,b). Their NMR data were obtained on a Bruker AM-400 instrument and are compiled in Tables 1–7. Their structures are shown in Scheme 1 (see Section 3.2). For each of the four structures, AHB-1 to AHB-4, two diastereoisomers designated a and b were observed, whose NMR data were very similar and which were due to RR + SS and RS + SR combinations of the chiral moieties alanyl and bicyclohexene. No attempt was made to assign a and b to the individual diastereoisomeric structures.

The assignments given in Tables 1–7 were derived with the help of ^{13}C distortionless enhancement by polarization transfer (DEPT), H,C correlation spectroscopy (COSY) H,H spin decoupling and H,H nuclear

Overhauser effect (NOE) measurements. Two olefinic protons ($J_{\text{H-H}}^3 = 5.6 \text{ Hz}$) were observed in the ^1H NMR spectra of the bicyclo[3,1,0]hexenol derivatives AHB-1 and AHB-2 near 5.5 and 6.2 ppm respectively. In AHB-3 and AHB-4 one of these protons was replaced by an alkyl group. The atoms C_1 , C_5 and C_6 showed a chemical shift of about $\delta_{13\text{C}} = 30 \text{ ppm}$ as in normal aliphatic carbon, but much larger coupling constants of $J_{\text{H-}^{13}\text{C}} \approx 160\text{--}170 \text{ Hz}$. This indicates that C_1 , C_5 and C_6 form a cyclopropane ring, with near- sp^2 hybridization. The assignment of the ^{13}C signal of C_6 according to H,C COSY and DEPT indicates a CH_2 group in all of the hydroxy[3,1,0]bicyclohexene derivatives except AHB-1. In the diastereomeric pair AHB-1, C_6 is substituted with an alanyl group. The geminal ^1H – ^1H coupling of the high-field protons with coupling constants of typically 4 Hz provides further proof of a cyclopropane structure for these compounds. Protons 6a and 6b show signals at $\delta_{6\text{a}} = -0.04\text{--}0.2 \text{ ppm}$ and $\delta_{6\text{b}} = 0.91\text{--}0.97 \text{ ppm}$. The carbon atom linked to the hydroxyl group shows a typical ^{13}C shift of about 79 ppm. For compounds AHB-2b, AHB-3b and AHB-4b, ^1H – ^1H NOE measurements were carried out and in each case established the proximity of the two protons giving rise to the signals around $\delta = 4.2\text{--}4.4 \text{ ppm}$ and $\delta \approx 0 \text{ ppm}$. This demonstrates the endo configurations of both protons and hence the exo configuration of the hydroxyl group. The observation of a ${}_{\text{HH}}J_{1,2}^3$ value of about 0 Hz for these three compounds is in line with this conclusion since, according to Dreiding models, H_1 and $\text{H}_{2\text{-endo}}$ show a dihedral angle of about 90° . The same ${}_{\text{HH}}J_{1,2}^3$ value of about 0 Hz was also observed for all other isomers, which indicates that all possess an exo hydroxyl group. This conclusion is borne out by the ^1H NMR data reported for *exo*- and *endo*-2-methoxybicyclo[3,1,0]hex-3-ene; these compounds show ${}_{\text{HH}}J_{1,2}^3$ values of about 0 Hz and 6.3 Hz respectively, and $\delta_{6\text{a}}$ values of -0.11 and 0.52 ppm respectively [19]. The ^1H – ^1H NOE data also confirm the assignments of the NMR signals to positions 1–5.

For further confirmation, UV-irradiated material was brought to dryness and trimethylsilylated. The trimethylsilyl ethers were separated by gas chromatography and analysed by mass spectrometry (Hewlett Packard 5971A mass selective detector; column, 15 m PS-343.5; injection temperature, 200°C ; temperature programme, $50\text{--}270^\circ\text{C}$ at 6°C min^{-1}).

The ethers of the alanyl-trimethylsilyl-2-*exo*-bicyclo[3,1,0]hex-3-en-2-ols (molecular weight (MW), 399 Da) eluted at about 20 min. Their mass spectra were similar and only varied in the relative abundance of the ions. AHB-1 may serve as an example. Significant peaks were obtained at m/z (per cent in parentheses): 399 (2), 384 (3), 356 (2), 310 (2), 282 (52), 266 (4), 218 (100), 192 (74), 181 (6), 147 (23), 100 (29), 73 (100). The retention times and mass spectra of the

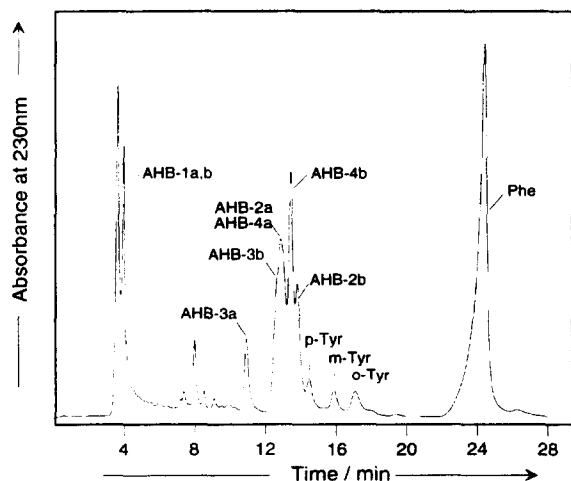


Fig. 1. Photolysis ($\lambda = 254 \text{ nm}$) of phenylalanine in air-saturated aqueous solution. Separation of the products by HPLC (pump: Gilson Model 303; detector: Spectra-Physics Model 770, fraction collector: Gilson Model 201; column (Macherey-Nagel): $250 \times 20 \text{ mm}$ Nucleosil-7- C_{18} ; eluent: water-methanol 97:3 (v/v), 1.0 ml min^{-1} , detection at 230 nm).

Table 1
 ^1H - and ^{13}C [^1H]-NMR spectra of 6-alanyl-2-*exo*-bicyclo[3,1,0]hex-3-en-2-ol AHB-1a,b in CD_3OD (^1H) and D_2O (^{13}C), respectively

Position	Chemical shift (δ)	^1H - ^1H coupling constants (Hz)			
1	1.64, 1.61	$J_{1,6}^3 = 3$	$J_{1,5}^3 = 6$		
2	4.43	$J_{2,3}^3 = 2$			
3	5.51	$J_{3,2}^3 = 2$	$J_{3,4}^3 = 5.5$	$J_{3,5}^3 = 2$	
4	6.185, 6.180	$J_{4,3}^3 = 5.5$	$J_{4,5}^3 = 2$		
5	1.92, 1.89	$J_{5,1}^3 = 6$	$J_{5,6}^3 = 3$	$J_{5,4}^3 = 2$	$J_{5,3}^3 = 2$
6	0.35	$J_{6,1}^3 = 3$	$J_{6,5}^3 = 3$	$J_{6,7a}^3 = 7$	$J_{6,7b}^3 = 7$
7a	1.83	$J_{7a,6}^3 = 7$	$J_{7a,8}^3 = 4.7, 5.3$	$J_{7a,7b}^2 = 14.5$	
7b	1.81, 1.77	$J_{7b,6}^3 = 7$	$J_{7b,8}^3 = 7.3, 6.7$	$J_{7b,7a}^2 = 14.5$	
8	3.57, 3.56	$J_{8,7a}^3 = 4.7, 5.3$	$J_{8,7b}^3 = 7.3, 6.7$		

Position	DEPT	Chemical shift	Position	DEPT	Chemical shift
1	CH	33.8, 33.4	6	CH	32.1, 31.8
2	CH	78.7	7	CH_2	35.53, 35.50
3	CH	132.4	8	CH	57.52, 57.49
4	CH	141.9, 141.8	9	C	177.3
5	CH	32.8, 32.7			

Table 2
 ^1H - and ^{13}C [^1H]-NMR spectra of 5-alanyl-2-*exo*-bicyclo[3,1,0]hex-3-en-2-ol AHB-2a in D_2O

Position	Chemical shift (δ)	^1H - ^1H coupling constants (Hz)			
1	1.53	$J_{1,6a}^3 = 4$	$J_{1,6b}^3 = 8.4$		
2	4.32	$J_{2,3}^2 = 2$			
3	5.44	$J_{3,4}^3 = 5.6$	$J_{3,2}^3 = 2$		
4	6.11	$J_{4,3}^3 = 5.6$			
6a	0.20	$J_{6a,1}^2 = 4$	$J_{6a,6b}^2 = 4$		
6b	0.93	$J_{6b,1}^3 = 8.4$	$J_{6b,6a}^2 = 4$		
7a	1.97	$J_{7a,8}^3 = 8$	$J_{7a,7b}^2 = 15$		
7b	2.07	$J_{7b,8}^3 = 5.8$	$J_{7b,7a}^2 = 15$		
8	3.73	$J_{8,7a}^3 = 8$	$J_{8,7b}^3 = 5.8$		

Position	DEPT	Chemical shift	$J_{1\text{H}-^{13}\text{C}}$	Position	DEPT	Chemical shift	$J_{1\text{H}-^{13}\text{C}}$
1	CH	32.6	170.3	6	CH_2	29.6	2×159.5
2	CH	79.1	144.2	7	CH_2	36.0	2×130.6
3	CH	132.4	167.4	8	CH	57.6	145.4
4	CH	142.5	164.8	9	C	177.4	
5	C	33.9					

trimethylsilylated tyrosines agreed with those of authentic material (Janssen).

Phenylacetaldehyde was determined by GC/MS after extraction of the irradiated solution with diethylether. Authentic material was available (Aldrich) for comparison. For quantification, this product was converted into its *O*-(2,3,4,5,6-pentafluoro-benzyl) oxime derivative with *O*-(2,3,4,5,6-pentafluoro-benzyl)hydroxylamine hydrochloride (Fluka) [20]. It was measured using the corresponding cyclopentanone derivative as internal standard to account for potential losses in the extraction process.

The material isolated by preparative HPLC (20 mg or more) was used for calibration in the determination of the quantum yields by HPLC. Quantitation of the tyrosines was performed by HPLC using a fluorescence detector (Merck/Hitachi). Yield vs. irradiation time plots were linear [21]. Quantum yields were calculated on the basis of the absorbance of Phe and the fluence rate was determined (for its determination see above).

Nitrogen yields from the photolysis of N_2O -containing aqueous solutions were determined by GC [22]. For different N_2O concentrations, the solutions were sat-

Table 3

 ^1H - and $^{13}\text{C}\{^1\text{H}\}$ -NMR spectra of 5-alanyl-2-*exo*-bicyclo[3,1,0]hex-3-en-2-ol AHB-2b in D_2O

Position	Chemical shift (δ)	^1H - ^1H coupling constants (Hz)		
1	1.55	$J_{1,6a}^3 = 4.2$	$J_{1,6b}^3 = 8.4$	$J_{1,3}^4 = 1.7$
2	4.33	$J_{2,3}^3 = 2$		
3	5.45	$J_{3,2}^3 = 2$	$J_{3,1}^4 = 1.7$	$J_{3,4}^3 = 5.6$
4	6.11	$J_{4,3}^3 = 5.6$		
6a	0.17	$J_{6a,1}^3 = 4.2$	$J_{6a,6b}^2 = 4.1$	
6b	0.90	$J_{6b,1}^3 = 8.4$	$J_{6b,6a}^2 = 4.1$	
7a	1.95	$J_{7a,8}^3 = 7.2$	$J_{7a,7b}^2 = 15.3$	
7b	2.16	$J_{7b,8}^3 = 5.4$	$J_{7b,7a}^2 = 15.3$	
8	3.72	$J_{8,7a}^3 = 7.2$	$J_{8,7b}^3 = 5.4$	

Position	DEPT	Chemical shift	$J_{\text{H-}^{13}\text{C}}$	Position	DEPT	Chemical shift	$J_{\text{H-}^{13}\text{C}}$
1	CH	32.9	166.6	6	CH_2	29.2	2×161.5
2	CH	78.9	150	7	CH_2	35.7	2×131.4
3	CH	132.7	168	8	CH	57.5	145.3
4	CH	142.8	164	9	C	177.5	
5	C	33.7					

Table 4

 ^1H - and $^{13}\text{C}\{^1\text{H}\}$ -NMR spectra of 4-alanyl-2-*exo*-bicyclo[3,1,0]hex-3-en-2-ol AHB-3a in D_2O

Position	Chemical shift (δ)	^1H - ^1H coupling constants (Hz)			
1	1.68	$J_{1,5}^3 = 5.5$	$J_{1,2}^3 = 1.5$	$J_{1,6a}^3 = 3.2$	$J_{1,6b}^3 = 7.6$
2	4.36	$J_{2,1}^3 = 1.5$	$J_{2,3}^3 = 2$		
3	5.24	$J_{3,2}^3 = 2$			
5	1.77	$J_{5,1}^3 = 5.5$	$J_{5,6b}^3 = 7.6$	$J_{5,6a}^3 = 3.2$	
6a	-0.06	$J_{6a,5}^3 = 3.2$	$J_{6a,1}^3 = 3.2$	$J_{6a,6b}^2 = 4.2$	
6b	0.93	$J_{6b,5}^3 = 7.6$	$J_{6b,1}^3 = 7.6$	$J_{6b,6a}^2 = 4.2$	
7a	2.69	$J_{7a,8}^3 = 7$	$J_{7a,7b}^2 = 15.2$		
7b	2.75	$J_{7b,8}^3 = 4.5$	$J_{7b,7a}^2 = 15.2$		
8	3.83	$J_{8,7a}^3 = 7$	$J_{8,7b}^3 = 4.5$		

Position	DEPT	Chemical shift	Position	DEPT	Chemical shift
1	CH	27.9	6	CH_2	23.0
2	CH	79.0	7	CH_2	34.7
3	CH	129.4	8	CH	56.4
4	C	151.8	9	C	176.9
5	CH	26.4			

urated with appropriate $\text{N}_2\text{O-Ar}$ mixtures prepared with the help of a Brooks gas mixer.

3. Results and discussion

3.1. Overall photodegradation

The overall photolytic degradation of Phe was followed under the conditions of "vanishing absorption" [23]. The Phe concentration was chosen as 10^{-5} mol dm^{-3} . Under this condition, the absorbance of 254 nm radiation is only 0.0014, i.e. the radiation is only attenuated by 1% or less over the 1 cm path length of

the photolysis cell used in these experiments. This condition allows the photolytic decay of Phe to be approximated by a first-order reaction even if, to a small extent, products absorbing more strongly at the excitation wavelength are formed. The analysis of unreacted Phe was performed by HPLC at 230 nm where Phe absorbs much more strongly than at 254 nm. HPLC conditions were chosen such that the photolytic products did not interfere with the quantification of Phe (Fig. 1). Fig. 2 shows a plot of $\log([\text{Phe}]/[\text{Phe}]_0)$ vs. the fluence ($[\text{Phe}]_0$ denotes the Phe concentration $[\text{Phe}]$ of an unirradiated sample). In Fig. 2 only the data obtained from an air-saturated Phe solution are plotted. In the absence of O_2 , the slope is slightly lower, but

Table 5

¹H- and ¹³C[¹H]-NMR spectra of 4-alanyl-2-*exo*-bicyclo[3,1,0]hex-3-en-2-ol AHB-3b in D₂O

Position	Chemical shift (δ)	¹ H– ¹ H coupling constants (Hz)			
1	1.67	J _{1,5} ³ = 5.5	J _{1,2} ³ = 1.5	J _{1,6a} ³ = 3.4	J _{1,6b} ³ = 7.7
2	4.37	J _{2,1} ³ = 1.5	J _{2,3} ³ = 2		
3	5.24	J _{3,2} ³ = 2			
5	1.78	J _{5,1} ³ = 5.5	J _{5,6b} ³ = 7.7	J _{5,6a} ³ = 3.4	
6a	–0.06	J _{6a,5} ³ = 3.4	J _{6a,1} ³ = 3.4	J _{6a,6b} ² = 4.2	
6b	0.91	J _{6b,5} ³ = 7.7	J _{6b,1} ³ = 7.7	J _{6b,6a} ² = 4.2	
7a	2.66	J _{7a,8} ³ = 7.5	J _{7a,7b} ² = 15		
7b	2.76	J _{7b,8} ³ = 4.5	J _{7b,7a} ² = 15		
8	3.81	J _{8,7a} ³ = 7.5	J _{8,7b} ³ = 4.5		

Position	DEPT	Chemical shift	Position	DEPT	Chemical shift
1	CH	27.9	6	CH ₂	23.2
2	CH	79.0	7	CH ₂	34.7
3	CH	129.4	8	CH	56.0
4	C	151.9	9	C	177
5	CH	26.4			

Table 6

¹H- and ¹³C[¹H]-NMR spectra of 3-alanyl-2-*exo*-bicyclo[3,1,0]-hex-3-en-2-ol AHB-4a in D₂O

Position	Chemical shift (δ)	¹ H– ¹ H coupling constants (Hz)		
1	1.63	J _{1,6a} ³ = 3.6	J _{5,6b} ³ = 7.5	J _{5,1} ³ = 5.5
2	4.26	–		
4	6.03	–		
5	1.81	J _{5,6a} ³ = 3.6	J _{1,6b} ³ = 7.5	J _{1,5} ³ = 5.5
6a	–0.08	J _{6a,1} ³ = 3.6	J _{6a,5} ³ = 3.6	J _{6a,6b} ² = 4
6b	0.87	J _{6b,1} ³ = 7.5	J _{6b,5} ³ = 7.5	J _{6b,6a} ² = 4
7a	2.65	J _{7a,8} ³ = 4.2	J _{7a,7b} ² = 15.5	
7b	2.38	J _{7b,8} ³ = 9.4	J _{7b,7a} ² = 15.5	
8	3.59	J _{8,7a} ³ = 4.2	J _{8,7b} ³ = 9.4	

Position	DEPT	Chemical shift	J _{1H-13C}	Position	DEPT	Chemical shift	J _{1H-13C}
1	CH	26.9	171.4	6	CH ₂	23.2	2 × 160
2	CH	80.2	142	7	CH ₂	32.2	2 × 129
3	C	139.3		8	CH	57.2	144
4	CH	141.1	166	9	C	177	
5	CH	24.6	170				

so close to that of air-saturated solution that its inclusion would have confused the graph. The good linearity of these plots shows that the first-order decay conditions are met and hence the quantum yields of Phe consumption can be calculated from the slopes. The values are $\Phi(-\text{Phe}) = 5 \times 10^{-2}$ in the absence of O₂ and $\Phi(-\text{Phe}) = 5.4 \times 10^{-2}$ in air-saturated solution. Our value is considerably higher than that (1.9×10^{-2}) reported in Ref. [13].

The very minor effect of oxygen is in contrast with the photolytic behaviour of the other aromatic amino acids, tyrosine and tryptophan. There, singlet oxygen production from the excited triplet state and the rapid reaction of singlet oxygen with these amino acids en-

hances (in addition to other reactions) the photodegradation in the presence of O₂ [21]. No attempts have been made in the present study to determine whether or not singlet oxygen is formed on Phe photolysis in the presence of oxygen. Similar to benzene and its alkyl derivatives, Phe is not expected to react noticeably with singlet oxygen. Thus, if this reactive intermediate were formed by the reaction of triplet Phe with oxygen, products from the reaction of singlet oxygen with Phe would not have been noticed, because at the low Phe concentrations used in these experiments singlet oxygen would stand little chance of reacting with Phe due to its short lifetime in H₂O ($k = 5 \times 10^5 \text{ s}^{-1}$) [24].

Table 7

¹H- and ¹³C[¹H]-NMR spectra of 3-alanyl-2-*exo*-bicyclo[3,1,0]-hex-3-en-2-ol AHB-4b in D₂O

Position	Chemical shift (δ)	¹ H- ¹ H coupling constants (Hz)		
1	1.60	J _{1,6a} ³ = 3.6	J _{1,6b} ³ = 7.6	J _{1,5} ³ = 6
2	4.22	—	—	—
4	6	—	—	—
5	1.79	J _{5,6a} ³ = 3.6	J _{5,6b} ³ = 7.6	J _{5,1} ³ = 6
6a	-0.11	J _{6a,1} ³ = 3.6	J _{6a,5} ³ = 3.6	J _{6a,6b} ² = 4
6b	0.86	J _{6b,1} ³ = 7.6	J _{6b,5} ³ = 7.6	J _{6b,6a} ² = 4
7a	2.63	J _{7a,8} ³ = 7.5	J _{7a,7b} ² = 15	—
7b	2.44	J _{7b,8} ³ = 4	J _{7b,7a} ² = 15	—
8	3.74	J _{8,7a} ³ = 7.5	J _{8,7b} ³ = 4	—

Position	DEPT	Chemical shift	J _{1H-13C}	Position	DEPT	Chemical shift	J _{1H-13C}
1	CH	26.7	177.6	6	CH ₂	23.1	164.4, 159.2
2	CH	80.6	148.0	7	CH ₂	32.1	2 × 130
3	C	138.2	—	8	CH	56.6	145.8
4	CH	142.0	164.7	9	C	176.8	—
5	CH	24.5	171.2				

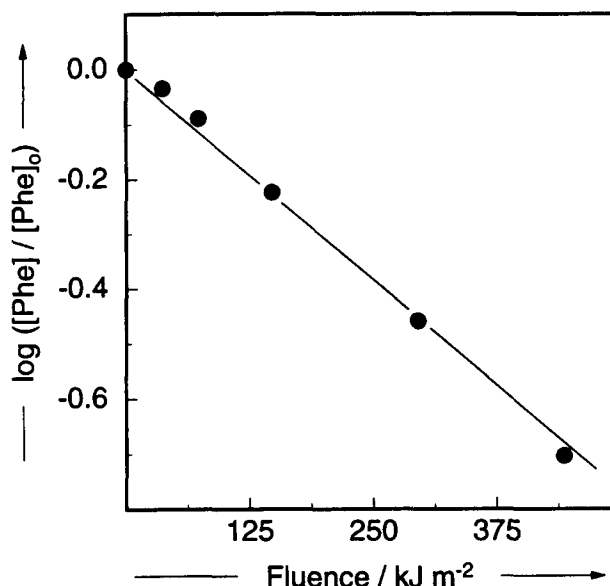


Fig. 2. Photolysis ($\lambda=254$ nm) of phenylalanine in air-saturated aqueous solutions. Kinetics of phenylalanine degradation (for details see text).

3.2. Photolysis in the absence of oxygen

In the absence of oxygen, only the alanyl-bicyclo[3,1,0]hexenols AHB-1a,b–AHB-4a,b were observed as products (Fig. 1). Their quantum yields are practically the same as in air-saturated solutions (Table 8, see Section 3.3). The tyrosines, well-documented products in the presence of O₂ (see below), are not formed under these conditions ($\Phi(\text{tyrosines}) \leq 10^{-6}$).

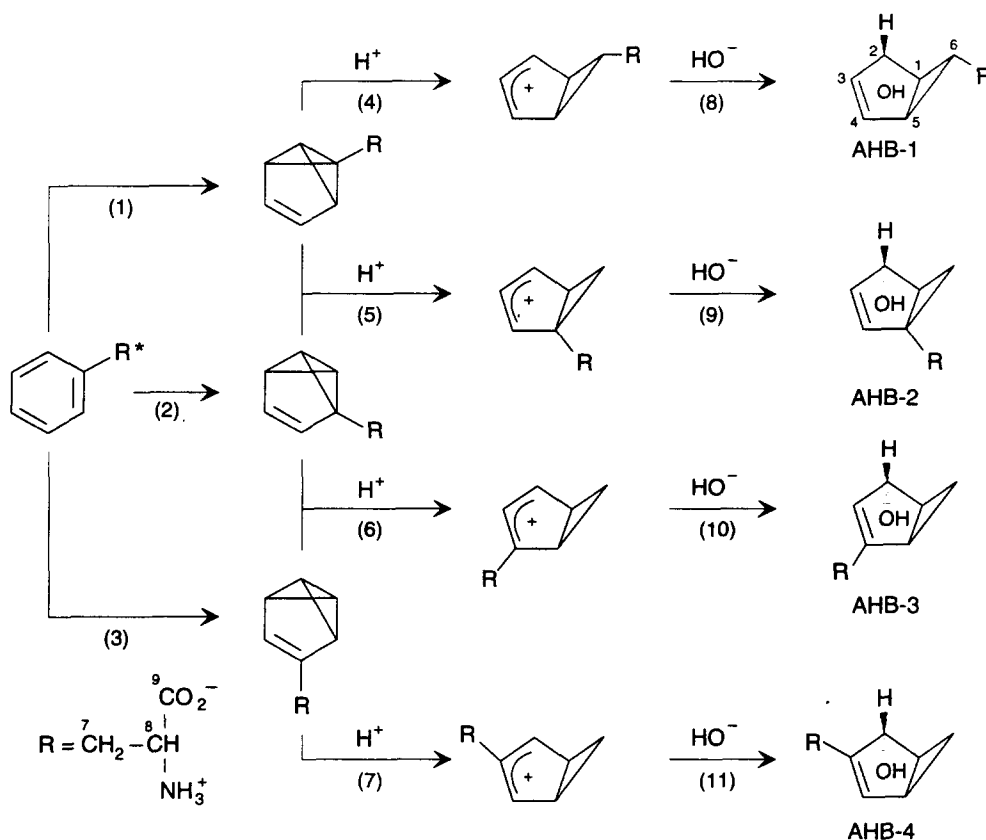
These eight products (AHB-1a,b–AHB-4a,b) are stereoisomers of the alanyl-bicyclo[3,1,0]hexenol type. Their structures are shown in Scheme 1. In all of them, the

amino acid function is retained, but the benzene ring has been broken up and fused into a two-ring structure consisting of a five-membered ring and a cyclopropane ring. One molecule of water is incorporated.

This type of reaction is not without precedent. It is well known that benzene and its alkyl derivatives give rise to (short-lived) benzvalenes on photoexcitation [25,26]. In protic solvents, the benzvalenes incorporate one solvent molecule [27]. Scheme 1 shows the various routes from benzvalene intermediates to the observed products.

As has been mentioned above, the quantum yields of AHB-1–AHB-4 are not reduced significantly when O₂ is allowed to take part in the photolytic reactions, and we conclude that these products must arise from the excited singlet state. The lifetime of the triplet state is 3 μ s and thus can be readily quenched by O₂ [10]. No other products have been identified in the absence of O₂, neither by HPLC nor GC/MS after trimethylsilylation.

However, there is evidence for the formation of an additional unstable product by UV spectroscopy (Fig. 3). This product absorbs strongly at 305 nm. Its yield increases linearly with increasing photolysis time. It must therefore be a primary photoproduct. Due to its short lifetime ($\tau_{1,2} = 2.1$ h, inset in Fig. 3), it was not possible to isolate and characterize this product. In the absence of O₂, $\Phi \times \epsilon = 3.9$ was found. In the presence of O₂, its yield is about three times higher. In the material balance (see Table 8, Section 3.3) there is not much room for a product with a high quantum yield. Hence we conclude that its absorption coefficient at the maximum must exceed 10 000 dm³ mol⁻¹ cm⁻¹.



Scheme 1.

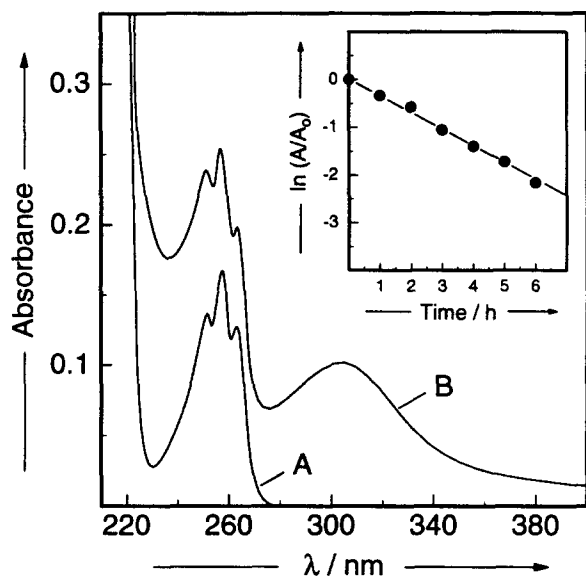


Fig. 3. Photolysis ($\lambda = 254 \text{ nm}$) of phenylalanine in air-saturated aqueous solutions ($10^{-3} \text{ mol dm}^{-3}$). Build-up of the labile photoproduct (fluence: 25 kJ m^{-2}). Inset: First-order decay of labile photoproduct measured at 310 nm.

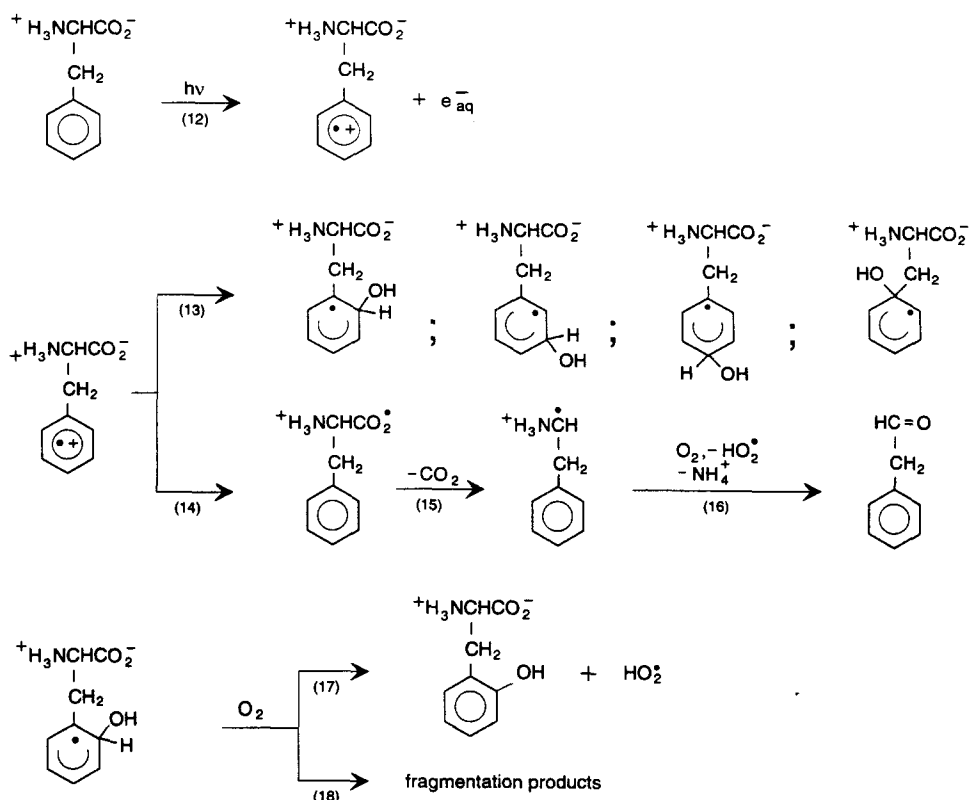
3.3. Photolysis in the presence of O_2

As has been mentioned above, $\Phi(-\text{Phe})$ is only slightly higher in the presence of oxygen than in its

absence. In the presence of O_2 , there are additional products (tyrosines) (Table 8) and the yield of the unstable product that absorbs strongly at 305 nm (Fig. 3) is enhanced.

The formation of the three tyrosines has been reported previously [6,14]. Hydroxylation in the presence of oxygen was also observed for tyrosine (formation of 3,4-dihydroxy-phenylalanine (dopa) and 2,4-dihydroxy-phenylalanine) [28]. Although, in the case of tyrosine, the mechanism leading to these products is not fully understood, it is clear that the precursor of these products is the tyrosine triplet state and photoionization does not lead to their formation [21]. In the present system, photoionization as a precursor of the tyrosines cannot be ruled out since it has been shown recently that Phe radical cations (produced by $\text{SO}_4^{\cdot-}$ radicals) react with water, and that the resulting substituted hydroxycyclohexadienyl radicals react with O_2 yielding the corresponding tyrosines (Scheme 2, reactions (12), (13) and (17)) [29].

A competing reaction of the Phe radical cation is electron transfer from the carboxylate group to the radical cation site (reaction (14)); this reaction is followed by subsequent decarboxylation (reaction (15)) [29]. The amino-substituted radical thus formed is expected to be oxidized by O_2 to yield the corresponding aldehyde (reaction (16)) [30]. In fact, phenylacetal-



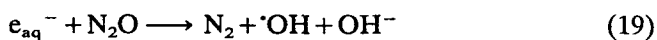
Scheme 2.

Table 8
Photolysis (254 nm) of phenylalanine in air-saturated aqueous solutions. Products and their quantum yields

Product	Quantum yield
Sum of alanyl-2- <i>exo</i> -bicyclo[3,1,0]hex-3-en-2-ols (AHB-1 -6 AHB-4)	4.2×10^{-2}
<i>o</i> -Tyrosine	1.5×10^{-3}
<i>m</i> -Tyrosine	1.3×10^{-3}
<i>p</i> -Tyrosine	1.2×10^{-3}
Phenylacetaldehyde	5.0×10^{-5}
Phenylalanine consumption	5.4×10^{-2}

dehyde has been found among the products, albeit with a low yield (Table 8).

The results of laser flash experiments indicate that photoionization may occur at 254 nm as a monophotonic process [16]. In order to obtain further information, we measured the N_2 yield formed during the photolysis of Phe in the presence of N_2O . N_2O scavenges solvated electrons with high efficiency ($k_{19} = 9.8 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$) [31]



As can be seen from Fig. 4, $\Phi(\text{N}_2)$ increases with increasing N_2O concentration. This is typical for this type of reaction [32], and indicates that only a fraction of the solvated electrons formed can escape the parent

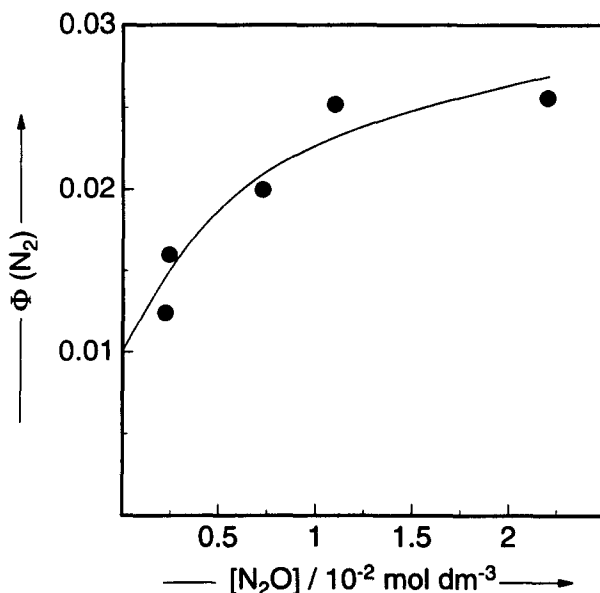


Fig. 4. Photolysis ($\lambda = 254 \text{ nm}$) of phenylalanine ($10^{-3} \text{ mol dm}^{-3}$) in N_2O -containing aqueous solutions. Quantum yield of N_2 -formation as a function of the N_2O concentration.

hole. Thus it is not unreasonable that, although $\Phi(\text{N}_2)$ at N_2O saturation ($2.2 \times 10^{-2} \text{ mol dm}^{-3}$) is as high as 2.6×10^{-2} , the total tyrosine yield is only $\Phi(\text{tyrosines}) = 4 \times 10^{-3}$ in air-saturated solution due to the lower scavenging capacity of the latter system

($[O_2] = 2.5 \times 10^{-4} \text{ mol dm}^{-3}$; $k(O_2 + e_{aq}^-) = 2 \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$) [31]. In addition, O_2 converts the Phe-derived hydroxycyclohexadienyl radicals into phenols with a yield of only approximately 50% [29] (for potential competing reactions, reaction (18), see Ref. [33]). The ortho to meta to para ratio of the tyrosines in the present study is not identical with that observed when the OH adducts formed in the $SO_4^{\cdot-}$ -induced reaction (via the Phe radical cation) are oxidized with $Fe(CN)_6^{3-}$ [29]. However, the oxidation of hydroxycyclohexadienyl radicals by oxygen is a rather complex reaction [33,34], and different ortho to meta to para ratios of the tyrosines were obtained from genuine OH adduct radicals when either $Fe(CN)_6^{3-}$ or O_2 was used as oxidant [29].

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