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Photochemical stability of biologically active compounds. IV. Photochemical degradation of chloroquine

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Summary

Chloroquine has been reported to give toxic reactions which may be ascribed to the photochemical degradation of the substance. The effects of light at wavelengths of 240–600 nm on solutions of chloroquine in isopropanol, and of light at wavelengths of 320–600 nm on solutions of chloroquine in buffer solutions were investigated. Seven of the photochemical decomposition products of chloroquine were isolated and identified by mass-spectrometry (EI, CI and high-resolution MS) and nuclear magnetic resonance spectroscopy (^{1}H-NMR).

Introduction

Drug-induced photosensitization causing adverse cutaneous reactions or toxic ocular responses in human represents severe side effects in the administration of several biologically active compounds.

Chloroquine is found to have a high affinity for the melanin-containing tissues of the skin and the eye (Salako, 1985). The drug has been reported to cause changes in skin pigmentation and bleaching of the hair (Isaacson et al., 1982; Dupre et al., 1985).

With long-term usage, chloroquine is likely to affect the cornea and the retina of the eye, leading to retinopathy (Tanenbaum and Tuffanelli, 1980; Ehrenfeld et al., 1986). The development of chloroquine retinopathy has been related to both the daily dose and the cumulative dose of the drug. Chloroquine-induced retinopathy rarely occurs for cumulative doses less than 300 g (Ehrenfeld et al., 1986), but it develops in almost all cases at daily doses exceeding 0.5 g (Isaacson et al., 1982).

The cutaneous and ocular side effects associated with the use of chloroquine are possibly phototoxic reactions (Moore and Hemmens, 1982). The mammalian retina is the only tissue in the body where light is focused continuously on a group of cells in a highly oxygenated environment (Andley, 1987). The present paper describes a study of the photochemical degradation of chloroquine in oxygen containing media. Identification of the main degradation products is of importance

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to evaluate further the possible phototoxic properties of chloroquine.

Materials and Methods

Materials

Pure chloroquine phosphate was provided by Rhone-Poulenc, France. All chemicals used were of p.a. grade (Merck).

Irradiation in buffer

0.013 g of chloroquine phosphate were dissolved in 250 ml of 0.05 M phosphate buffer, pH 7.4. After exposure to light, wavelength 320–600 nm, the sample was adjusted to pH 11 by addition of 1 M sodium hydroxide. 100 ml of the sample was extracted with 4×50 ml chloroform, and evaporated to dryness under vacuum. The photochemical degradation of chloroquine was followed by quantitative and qualitative TLC.

Irradiation in isopropanol

5.9 g of chloroquine phosphate were dissolved in 120 ml of distilled water. The sample was adjusted to pH 11 by addition of 1 M sodium hydroxide and extracted with 1×100 and 3×50 ml of chloroform. The combined extracts were washed with 50 ml water and dried by addition of sodium sulphate before evaporation to dryness under vacuum. 1.6 g of the isolated chloroquine base were dissolved in 500 ml isopropanol. After exposure to light, wavelength 240–600 nm, the sample was evaporated under vacuum. The photochemical degradation of chloroquine was followed by quantitative and qualitative TLC.

Isolation of photolysis products from chloroquine

1.6 g of the isolated chloroquine base were dissolved in 500 ml isopropanol. The sample was exposed to light of wavelength 240–600 nm for 3.5 h (Heraeus immersion lamp system). After exposure to light, portions of 62.5 ml of the sample (corresponding to 200 mg chloroquine base) were evaporated to dryness under vacuum. The residues were dissolved in 1 ml isopropanol. The degradation products were isolated by means of preparative TLC. The samples were extracted from the silica gel with 2×20 ml chloroform. After centrifugation for 5 min at 2300 rpm, the samples were evaporated to dryness under vacuum and stored at $-20\,^{\circ}C$ until further analyses were carried out.

The purity of the isolated fractions was controlled by qualitative TLC before further identification was carried out by MS (EI, CI and high-resolution MS) and (^{1}H-NMR) analysis.

Preparative TLC

Stationary phase was silica gel 60 F_{254} (Merck); mobile phase was chloroform/cyclohexane/diethylamine (5 : 4 : 1).

Quantitative TLC

TLC system: stationary phase was silica gel 60 F_{254} (Merck); mobile phase was chloroform/cyclohexane/diethylamine (5 : 4 : 1). For quantitation of chloroquine and degradation products a Shimadzu Dual-Wavelength Flying-Spot Scanner CS 9000 was used. Detection: UV, 337 nm.

Qualitative TLC

Stationary phase was silica gel 60 F_{254} (Merck). System I: mobile phase was chloroform/cyclohexane/diethylamine (5 : 4 : 1). System II: mobile phase was methanol/ammonia (100 : 3).

Irradiation

The samples were exposed to an immersion lamp with emission wavelengths of 240–600 nm, 120 W (Heraeus immersion lamp system). To obtain light above 320 nm, a glass filter was placed between the sample and the light source.

Mass spectrometry

Electron-impact (EI) mass spectra and chemical ionization (CI) mass spectra (ionization gas: ammonia) were obtained with a VG Micromass 7070 F mass spectrometer via direct inlet. The probe temperature was $220\,^{\circ}C$ and the ion potential was 70 eV. High resolution mass spectra were recorded on an AEI MS 902.

NMR

The NMR spectra were recorded on a VXR 300 MHz. The solvent was $CDCl_3$. The reference signal was $CDCl_3$.

Results and Discussion

The reaction rate of the photochemical degradation of chloroquine in aqueous media is low compared to the reaction rates measured in organic solvents (e.g. isopropanol). Isopropanol was therefore used as reaction medium for the isolation of the degradation products. Chloroquine phosphate is freely soluble in water, but poorly soluble in isopropanol. To obtain a sufficient concentration of chloroquine in isopropanol, chloroquine base was used. The base was isolated from an aqueous solution at pH 11 by extraction with chloroform.

Chloroquine in isopropanol (3.2 mg/ml) was irradiated with continuous light in the wavelength range 240–600 nm. The observed half-life of chloroquine under these conditions was 3.5 h. To obtain light above 320 nm, the light source was immersed in a glass jacket. This, however, resulted in a decrease in the photodecomposition rate of chloroquine. The main degradation products were shown to be identical after irradiation of the samples with unfiltered light or light above 320 nm. The samples were therefore exposed to continuous light (240–600 nm) due to a faster reaction rate.

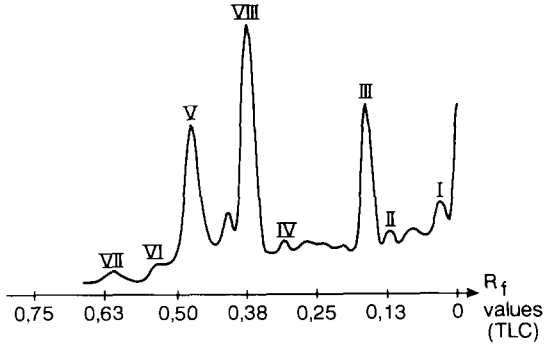


Fig. 2. TLC chromatogram of chloroquine in isopropanol after exposure to light (240–600 nm) for 4 h. Peak numbers I–VII refer to compounds **I–VII** (Fig. 1). Peak number VIII is chloroquine. The other peaks are not identified.

The proposed degradation pattern of chloroquine in isopropanol after exposure to light (240–600 nm) for 3.5 h is shown in Fig. 1. A TLC chromatogram of the sample is given in Fig. 2. The irradiation of chloroquine in isopropanol resulted in at least 15 degradation products. Seven of these products were isolated and identified. The structures of compounds **II, IV, V, VI** and **VII** were confirmed by NMR, MS (EI, CI) and high-

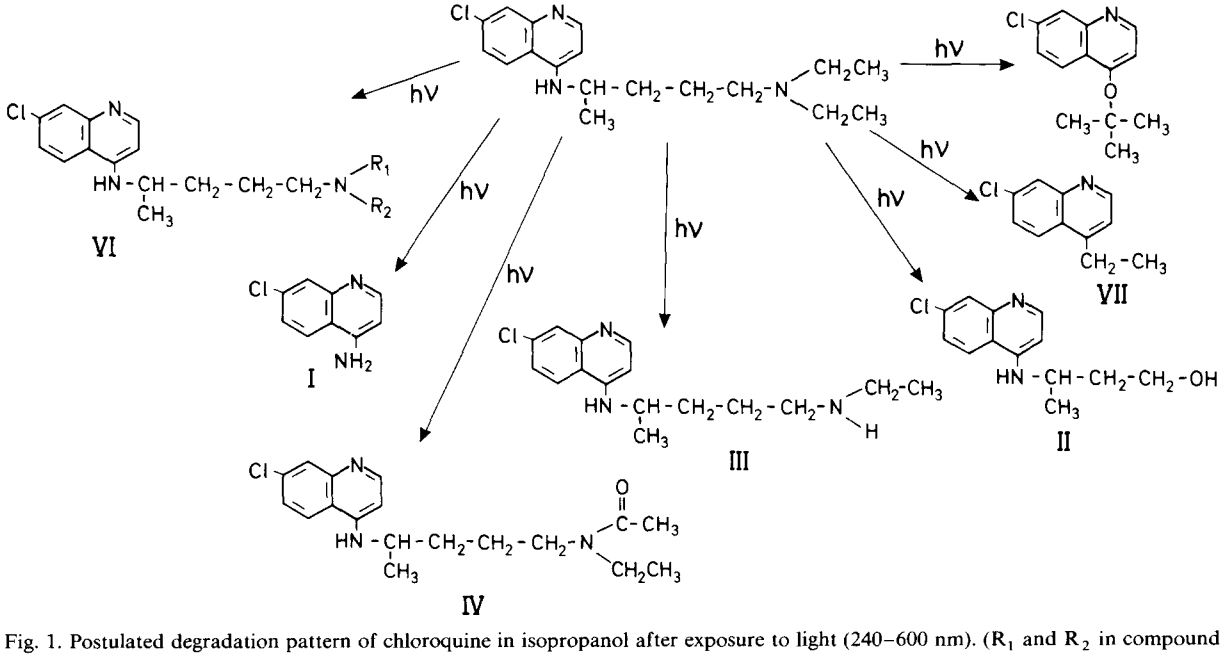
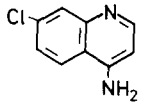


Fig. 1. Postulated degradation pattern of chloroquine in isopropanol after exposure to light (240–600 nm). (R_1 and R_2 in compound **VI** are not identified.)

TABLE 1

MS and NMR data of compound ***I****, identified as 4-amino-7-chloroquine*

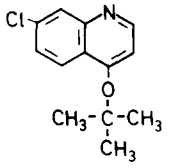


I

MS Fragment	*m/e*	%
M^+	178	100
$M^+ + 2$	180	32.2
$M-NH_2$	163	1.8
M–Cl	143	12.0
^{1}H-NMR	H-position	*J* (Hz)
C_{12}	8.46 (d)	5.13
C_{11}	6.53 (d)	5.13
C_{17}	7.64 (d)	8.79
C_{16}	7.34 (dd)	9.03
		1.71
C_{14}	7.92 (d)	2.20
N<u>H</u>	4.72 (s)	

TABLE 3

MS and NMR data of compound ***V****, identified as 7-chloro-4-(t-butoxy)quinoline*



V

MS Fragment	*m/e*	%	^{1}H-NMR	H position	*J* (Hz)
M^+	234	22.4	C_{12}	8.44 (d)	5.13
$M^+ + 2$	236	6.4	C_{11}	6.35 (d)	5.13
$M-CH_3$	220	18.9	C_{17}	7.60 (d)	9.03
$M-C_2H_6$	205	99.2	C_{16}	7.29 (dd)	9.03
$M-C_3H_9$	190	15.2			2.20
$M-C_4H_9$	178	28.7	C_{14}	7.89 (d)	1.95
$M-OC_4H_9$	162	18.4	$\underline{C}H_3$		
High-resolution MS			$\underline{C}H_3-\underline{C}-O$	1.2 (m)	
234: $C_{13}H_{13}ONCl$			$\underline{C}H_3$		

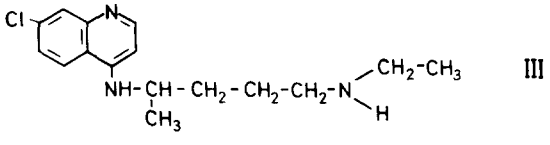
resolution MS. The identification of compounds **I** and **III** was based on NMR and MS (EI, CI) only.

4-Amino-7-chloroquinoline (compound **I**) and desethylchloroquine (compound **III**) were the main degradation products formed after irradiation of chloroquine in isopropanol (Tables 1 and 2). Desethylhydroxychloroquine was also isolated from the photochemical degradation of hydroxychloroquine (Tønnesen et al., 1988).

TLC analysis (system I and II) of a solution of

TABLE 2

MS and NMR data of compound ***III****, identified as desethylchloroquinoline*

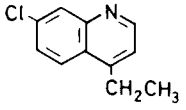


III

MS Fragment	*m/e*	%	^{1}H-NMR	H position	*J* (Hz)
M^+	291	15.8	C_{12}	8.42 (d)	5.70
$M^+ + 2$	293	5.5	C_{11}	6.31 (d)	5.70
$M-C_2H_5$	262	4.5	C_{17}	7.68 (d)	9.00
$M-NC_2H_6$	247	4.2	C_{16}	7.25 (dd)	9.00
$M-CH_2NC_2H_6$	233	7.5			2.10
$M-C_2H_4NC_2H_6$	219	32.6	C_{14}	7.85 (d)	1.80
$M-C_3H_6NC_2H_6$	205	53.1	N<u>H</u>	5.60 (d)	
$M-C_4H_9NC_2H_6$	190	5.8	$CH_3-C\underline{H}$	3.64 (m)	
$M-C_5H_{10}NC_2H_6$	179	100	$CH_2-N<^{CH_2}_{CH_2}$	2.50 (m)	
$M-NC_5H_{11}NC_2H_6$	162	9.6			
$C_4H_{10}N$	71	46.6			
C_3H_8N	58	96.1	$CH-C\underline{H}_2-C\underline{H}_2-CH_2$	1.66 (m)	
C_2H_6N	44	24.1	$C\underline{H}_3-CH$	1.28 (m)	
			$C\underline{H}_3-CH_2$	1.13 (m)	

TABLE 4

MS and NMR data of compound ***VII****, identified as 7-chloro-4-ethylchloroquine*



VII

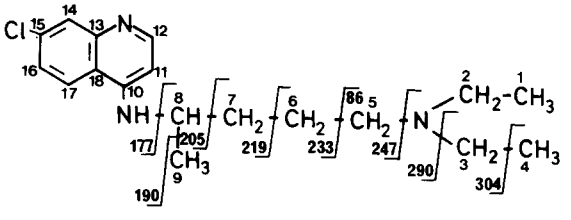
MS Fragment	*m/e*	%	^{1}H-NMR	H position	*J* (Hz)
M^+	191	100	C_{12}	8.80 (d)	4.40
$M^+ + 2$	193	33.5	C_{11}	6.59 (d)	4.40
$M-CH_3$	176	47.9	C_{17}	7.97 (d)	9.04
$M-C_2H_5$	162	3.6	C_{16}	7.50 (dd)	9.03
M–Cl	156	38.7	C_{14}	8.09 (d)	2.20
					2.20
High-resolution MS			$C\underline{H}_3-CH_2$	1.4 (m)	
191: $C_{11}H_{10}NCl$			$C\underline{H}_2-CH_3$	3.1 (m)	

chloroquine in isopropanol (3.2 mg/ml) irradiated for 3.5 h (240–600 nm) and a solution of chloroquine in buffer pH 7.4 (0.05 mg/ml) irradiated for 3 h (320–600 nm) indicated that the same main degradation products were formed in the two solvents (based on R_f values). Isopropanol forms several photodecomposition products of low molecular weight (Pacakova et al., 1985). Compounds **V** and **VII** were found to be condensation products formed from fragments of chloroquine and the reaction medium. Compounds **V** and **VII** are identified as 7-chloro-4-(*t*-butoxy)quinoline and 7-chloro-4-ethylquinoline respectively (Tables 3 and 4). The formation of these compounds can be explained by the cleavage of chloroquine in the C10 position followed by reactions with *t*-butanol and ethene, respectively (possible photodegradation products from isopropanol) (Pacakova et al., 1985).

Cleavage of the C5-C6 bond in chloroquine (Table 5) leads to the formation of compound **II**. Compound **II** is identified as 7-chloro-4-(1-methylpropanolamino)quinoline (Table 6). Compound **IV** is identified as 7-chloro-4-(1-methyl-4-(*N*-acetyl-*N*-ethylamino)butylamino)quinoline (Table 7). Compound **VI** is found to have the same molecular weight and elementary composition as

TABLE 5

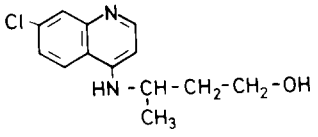
MS and NMR data of chloroquine



MS Fragment	*m/e*	%	^{1}H-NMR	H position	*J* (Hz)
M^+	319	7.5	C_{12}	8.51 (d)	5.70
$M^+ + 2$	321	2.5	C_{11}	6.42 (d)	5.70
$M-C_2H_5$	290	3.9	C_{17}	7.67 (d)	9.00
$M-NC_4H_{10}$	247	–	C_{16}	7.33 (dd)	9.00
$M-CH_2NC_4H_{10}$	233	0.7			2.10
$M-C_2H_4NC_4H_{10}$	219	2.7	C_{14}	7.94 (d)	2.10
$M-C_3H_6NC_4H_{10}$	205	3.0	N$\underline{H}$	5.30 (d)	7.20
$M-C_4H_8NC_4H_{10}$	191	1.6	$CH_3-C\underline{H}$	3.71 (m)	
$M-C_5H_8NC_4H_{10}$	179	3.2	$C\underline{H}_2-N<^{C\underline{H}_2}_{C\underline{H}_2}$	2.48 (m)	
$M-NC_5H_{11}NC_4H_{10}$	162	1.5			
$C_2H_4NC_4H_{10}$	99	6.1			
$CH_2NC_4H_{10}$	86	100	$CH-C\underline{H}_2-C\underline{H}_2-CH_2$	1.66 (m)	
NC_4H_{10}	73	6.6	$C\underline{H}_3-CH$	1.31 (d)	
C_4H_{10}	58	11.2	$C\underline{H}_3-CH_2$	1.01 (t)	

TABLE 6

MS and NMR data of compound ***II****, identified as 7-chloro-4-(1′-methylpropanolamino)quinoline*



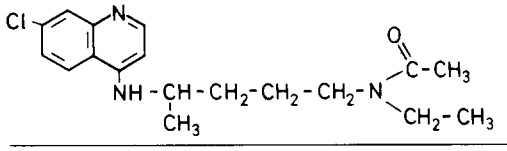
II

MS Fragment	*m/e*	%	^{1}H-NMR	H-position	*J* (Hz)
M^+	250	35.0	C_{12}	8.29 (d)	5.86
$M^+ + 2$	252	11.1	C_{11}	6.37 (d)	5.62
$M-CH_3$	235	11.3	C_{17}	7.66 (d)	9.28
$M-OH$	233	4.9	C_{16}	7.3 (dd)	8.8
$M-CH_2OH$	219	8.1			1.7
$M-Cl$	215	2.2	C_{14}	7.94 (d)	2.20
$M-CH_2CH_2OH$	205	100	$CH_3-C\underline{H}$	3.7 (m)	
$M-C_3H_8O$	191	5.7	$C\underline{H}_3-CH$	1.37 (d)	
$M-C_4H_9O$	178	82.0	$CH_2-C\underline{H}_2-OH$	3.0 (m)	
$M-NC_4H_{10}O$	162	8.7	$CH-C\underline{H}_2-CH_2$	–	

High-resolution MS:
250: $C_{13}H_{15}ON_2Cl$

TABLE 7

MS and NMR data of compound ***IV****, identified as 7-chloro-4-(1′-methyl-4′-(N-acetyl-N-ethylamino)butylamino)quinoline*



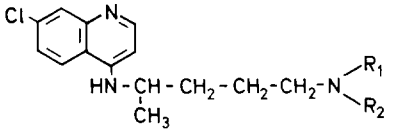
IV

MS Fragment	*m/e*	%	^{1}H-NMR	H position	*J* (Hz)
M^+	333	17.8	C_{12}	8.36 (d)	4.64
$M^+ + 2$	335	6.0	C_{11}	6.37 (d)	4.62
$M-C_2H_3O$	290	6.2	C_{17}	7.91 (d)	9.04
$M-C_2H_5$	304	1.6	C_{16}	7.33 (dd)	8.91
$M-NC_4H_8O$	247	6.2			1.96
$M-CH_2NC_4H_8O$	233	6.4	C_{14}	8.00 (d)	2.20
$M-C_2H_4NC_4H_8O$	219	69.8	$CH_3-C\underline{H}$	3.7 (m)	
$M-C_3H_6NC_4H_8O$	205	100	$C\underline{H}_2-N\langle^{C}_{CH_2}$	2.7 (m)	
$M-C_4H_9NC_4H_8O$	190	8.4			
$M-C_5H_{10}NC_4H_8O$	179	20.5			
$M-C_5H_{11}NC_4H_8O$	162	8.3	$CH-C\underline{H}_2-C\underline{H}_2-CH_2$	1.6 (m)	
C_2H_3O	43	20.2	$C\underline{H}_3-CH$	1.24 (m)	
NC_4H_8O	86	26.3	$C\underline{H}_3-CH_2$	1.15 (m)	
$CH_2NC_4H_8O$	100	16.1			

High-resolution MS:
333: $C_{18}H_{24}ON_3Cl$

TABLE 8

MS and NMR data of compound **VI** *(R_1 and R_2 are not identified)*



VI

MS Fragment	*m/e*	%	^{1}H-NMR	H position	*J* (Hz)
M^+	319	36.4	C_{12}	8.5 (d)	
$M^+ + 2$	321	12.0	C_{11}	6.3 (d)	5.1
$M-CH_3$	304	1.8	C_{17}	7.9 (d)	9.00
$M-C_2H_5$	290	24.9	C_{16}		
$M-NC_4H_{10}$	247	89.3	C_{14}	8.1 (d)	2.20
$M-CH_2NC_4H_{10}$	233	15.1	$CH_3-C\underline{H}$	3.7 (m)	
$M-C_2H_4NC_4H_{10}$	219	16.3	$C\underline{H}_2-N\begin{matrix}R_1\\R_2\end{matrix}$	2.7 (m)	
$M-C_3H_6NC_4H_{10}$	205	23.6			
$M-C_4H_9NC_4H_{10}$	191	18.8			
$M-C_5H_{10}NC_4H_{10}$	179	34.6	$CH-C\underline{H}_2-C\underline{H}_2-CH_2$	1.6 (m)	
$M-NC_5H_{11}NC_4H_{10}$	162	9.9	$C\underline{H}_3-CH$	1.2 (m)	
$C_3H_6NC_4H_{10}$	113	28.0			
$C_2H_4NC_4H_{10}$	99	46.3			
$CH_2NC_4H_{10}$	86	100			
High-resolution MS 319: $C_{18}H_{26}N_3Cl$					

chloroquine when analysed by high-resolution MS. The EI mass spectra of compound **VI** and chloroquine were identical with respect to the molecular peak at *m/e* 319 and the base peak at *m/e* 86. However, a difference in fragmentation pattern of the two compounds is demonstrated by the presence of the peak at *m/e* 247 (89%) in the EI mass spectrum of compound **VI** (Table 8). This peak cannot be identified in the MS spectrum of chloroquine (Table 5).

The R_f values (TLC system I) for the two substances were not identical (Fig. 2), indicating a structural difference between chloroquine and the product isolated. Based on the NMR and MS data obtained, this difference seems to be located in the side chain of the molecule.

Compound **VI** was formed as a minor product in the experiment and the lack of a signal corresponding to the C16 proton (^{1}H-NMR, compound **VI**, Table 8) might be ascribed to the small amount of sample available.

Based on the TLC analysis of samples of chloroquine after irradiation, compounds **I, III** and **V** could be identified initially in the photolysis of chloroquine (after 15 min irradiation). Compounds **II** and **IV** could be identified after 30 min irradiation, and compounds **VI** and **VII** after 45 min irradiation.

The signal with R_f corresponding to compound **I** increases steadily as a function of irradiation time. A large increase in signal intensity is observed after 3.5 h irradiation. This is probably due to the additional formation of compound **I** from the degradation of compounds **II** and **IV**.

The TLC analysis further indicates that compound **VII** is formed to a lesser extent than compound **V**.

Conclusion

Irradiation of chloroquine in oxygen-containing media causes cleavage of the side chain of the molecule, leaving the quinoline structure un-

changed. The chlorine atom remains intact in the C15 position of the molecule in all the degradation products isolated. This is in agreement with the results obtained previously on hydroxychloroquine (Tønnesen et al., 1988). The main photodecomposition products (compounds **I, III**) are also likely to be formed by normal metabolism of chloroquine in vivo (Salako, 1985). The reported phototoxicity of chloroquine might be ascribed to the drug itself or to the metabolites formed. Further investigations will therefore be carried out on the metabolites/degradation products of this drug.

Acknowledgements

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