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Photochemical stability of biologically active compounds: V. Photochemical degradation of primaquine in an aqueous medium

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Summary

Quinoline antimalarials are photochemically unstable. Several of these drugs cause toxic reactions which may be ascribed to the photochemical degradation of the substances. The effect of light of wavelengths 320–600 nm on the aminoquinoline primaquine in aqueous solution was investigated. The main photochemical degradation products were isolated by means of preparative TLC. The samples were identified by mass spectrometry (EI, CI and high-resolution), ^{1}H-NMR spectroscopy and GC/MS.

Introduction

Primaquine is the only 8-aminoquinoline which is widely employed as an antimalarial drug at present. It was developed during World War II in an attempt to make a compound more potent and less toxic than the existing 8-aminoquinoline pamaquine (Goodman and Gilman, 1990). Primaquine which acts as a tissue schizontocide, is effective in eliminating the latent liver forms of *Plasmodium ovale* and *P. vivax* which may persist after suppressive treatment with chloroquine. Primaquine has no effect on the erythrocytic stages of plasmodia unless toxic doses are used. Hence primaquine is normally used in conjunction with a blood schizontocide such as chloroquine (Gustafsson et al., 1987; WHO Drug Information, 1988; Goodman and Gilman, 1990). Primaquine is not suitable for general prophylaxis due to severe adverse effects; methaemoglobinaemia and haemolysis are common side effects. These side effects are manifested particularly in patients with glucose-6-phosphate dehydrogenase deficiency (Warhurst, 1987; WHO Drug Information, 1988). In spite of this, prophylaxis with primaquine in addition to chloroquine might have to be consid-

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ered in areas where *P. ovale* and *P. vivax* are endemic (Reynolds, 1989).

Many of the antimalarials are quinoline derivatives and hence chemically related. The quinoline antimalarials include the 4-aminoquinolines chloroquine, hydroxychloroquine and amodiaquine, the 8-aminoquinoline primaquine, the 4-quinolinemethanol compound mefloquine and the cinchona alkaloid quinine. The quinoline antimalarials absorb light in the UVA (320–400 nm) and UVB (290–320 nm) regions, are photochemically unstable and should be protected from light (Florey, 1976, 1983, 1985; Reynolds, 1989). There are cutaneous and ocular toxic side effects associated with the use of several quinoline antimalarials which may be light induced (Moore and Hemmens, 1982).

Characterization of the photochemical properties of drugs and drug formulations is of interest both regarding in vitro stability and in vivo effects. Drugs are exposed to light during production, storage and use. Light exposure can influence the stability of drug formulations, and toxic photodecomposition products may be formed. There seems to be a correlation between photoinstability of a drug in vitro and adverse biological reactions of the compound in vivo (Epstein and Wintroub, 1985).

It has previously been shown that irradiation of hydroxychloroquine, mefloquine and chloroquine with UV/visible light in aqueous oxygen containing media causes structural changes of the molecular side chains, leaving the quinoline ring structures intact (Tønnesen et al., 1988; Tønnesen and Grislingaas, 1990; Nord et al., 1991). Brossi et al. (1987) have demonstrated that irradiation of primaquine in methylene chloride causes oxidation of the quinoline ring, but no stability data are available for primaquine in an aqueous medium. It is important to obtain knowledge of the photochemical stability in aqueous media since some of the severe side effects observed for the antimalarials may be light induced. The aim of this study was to investigate the photochemical degradation of primaquine in a medium simulating in vivo conditions and to compare the results with those previously reported for other quinoline derivatives under similar conditions.

Materials and Methods

Materials

Primaquine diphosphate (> 99% pure) was provided by Aldrich, U.S.A. All the other chemicals used were of p.a. grade.

Preparation of the samples

A 4×10^{-5} M solution of primaquine diphosphate in 0.05 M phosphate buffer pH 7.4 was exposed to continuous light of wavelengths 320–600 nm, 120 W (Hereaus immersion lamp system, high pressure mercury arc) for 1 h 15 min. To obtain light above 320 nm, a glass filter was placed between the sample and the light source. Subsequently the sample was adjusted to pH 11 with 1 M NaOH, extracted with chloroform and evaporated to dryness under vacuum. The residue was dissolved in dichloromethane prior to further analyses.

The photochemical degradation of primaquine was followed by semi-quantitative and qualitative TLC and UV spectroscopy.

The photochemical degradation products were isolated by means of preparative TLC. The samples were extracted from the silica gel with chloroform. The fractions were centrifuged for 5 min at $1100 \times g$ and evaporated to dryness under vacuum. The purity of the isolated fractions was checked by qualitative TLC. The fractions were stored at −20°C until further identification was carried out by use of mass spectrometry (EI, CI and high-resolution), NMR (^{1}H) and GC/MS.

TLC

The stationary phase was silica gel 60 F_{254} (Merck). The mobile phases were: *n*-hexane/acetone/diethylamine (5:3:2) (system I); ethanol/water/ammonia (18:1.65:0.35) (system II).

For qualitative evaluation of primaquine and degradation products both TLC systems were used. For preparative and semi-quantitative purposes TLC system I was chosen. A Shimadzu CS 9000 Dual-Wavelength Flying-Spot scanner was used for semi-quantitative studies of primaquine and degradation products. Detection wavelengths were: (UV) 265 nm; (fluorescence) excitation 366 nm, emission > 400 nm.

UV spectroscopy

The UV/visible spectra were recorded on a Shimadzu UV-260 spectrophotometer.

Mass spectrometry

Electron impact (EI) mass spectra and chemical ionization (CI) mass spectra (ionization gas: methane) were obtained with a VG Micromass 7070 F mass spectrometer via direct inlet. The ion source temperature was 220°C and the ionization potential was 70 eV.

High-resolution mass spectra were recorded on a VG Autospec via direct inlet and electron-impact. Peak match analyses were carried out. The reference substance was perfluorokerosene (PFK) and the resolution was 10000.

NMR

The ^{1}H-NMR spectra were recorded on a Bruker AM 500. The solvent was $CDCl_3$.

GC/MS

Each individual fraction was analysed by means of GC/MS. The GC/MS system used was a Hewlett Packard 5890 with a Hewlett Packard model MSD 5970 MS-detector; detector temperature 300°C. As stationary phase a Hewlett Packard Cross-Linked Methyl Silicone column was used. The length was 12 m, the inner diameter 0.20 mm and the thickness of the film coating 0.33 μm. A temperature programme was used, covering the range from 100 to 250°C with a temperature rise of 10°C per min. The temperature was then kept constant at 250°C for 10 min, giving a total run time of 25 min.

Results and Discussion

Solutions of primaquine (4×10^{-5} M) in phosphate buffer at pH 7.4 were irradiated for the isolation of photodegradation products. At this concentration primaquine formed seven major and several minor decomposition products. The main compounds could be isolated by means of preparative TLC. At concentrations above 4×10^{-5} M secondary reactions seemed to occur, leading to the formation of a very high number of degradation products. Phosphate buffer at pH 7.4 was used as the reaction medium to simulate physiological conditions. In this medium primaquine absorbs light below 500 nm with absorption maxima at 207, 260 and 350 nm. The samples were irradiated with light above 320 nm. Light in the UVA (320–400 nm) and visible (400–700 nm) range of the spectrum is likely to penetrate tissues and was therefore preferred in these studies. The optimal irradiation time was found to be 1 h 15 min. Further irradiation produced extensive formation of secondary degradation products.

A TLC chromatogram of primaquine irradiated in phosphate buffer for 1 h 15 min is shown in Fig. 1. The postulated degradation pattern of primaquine in phosphate buffer after exposure to light (320–600 nm) for 1 h 15 min is shown in Fig. 2. The MS fragments and NMR shifts of primaquine are given in Table 1. High-resolution MS data of the photochemical degradation products are given in Table 2, and the EI-MS fragmentations of the photochemical degradation products are illustrated in Fig. 3.

The structures of compounds 4, 5 and 8 (Fig. 2) were confirmed by ^{1}H-NMR, MS (EI, CI) and

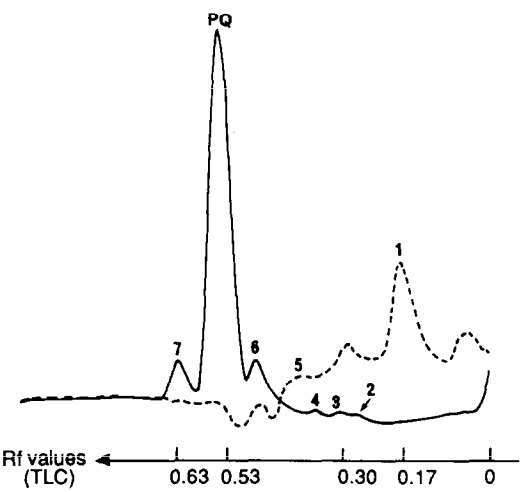


Fig. 1. Scan of a TLC chromatogram of primaquine in 0.05 M phosphate buffer pH 7.4 after exposure to light (320–600 nm) for 1 h 15 min. (- - - - - -) Fluorescence detection (excitation 366 nm; emission > 400 nm). (———) UV absorption detection (265 nm). Peak numbers 1–7 refer to the isolated fractions. PQ, primaquine. Fraction 4 contains compounds 4 and 5. Fractions 5–7 correspond to compounds 6–8, respectively.

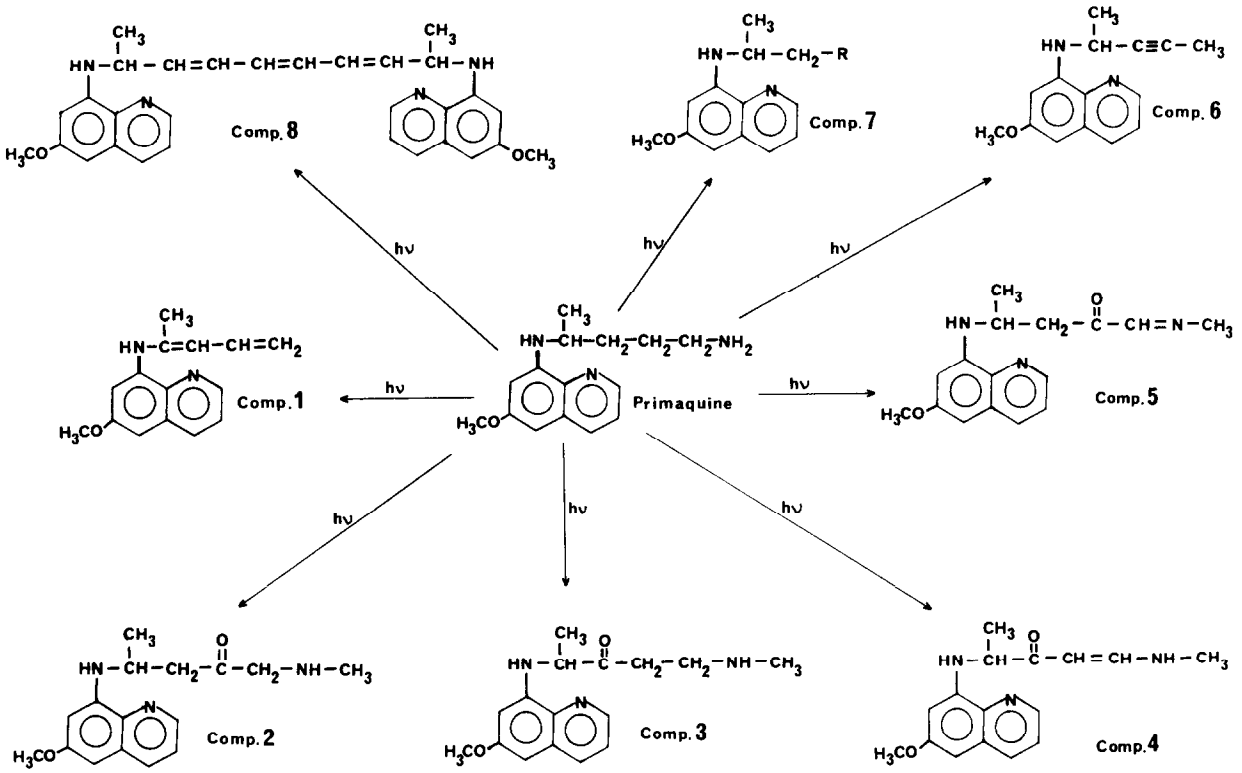


Fig. 2. Postulated degradation pattern of primaquine in 0.05 M phosphate buffer pH 7.4 after exposure to light (320–600 nm). (R in compound 7 is $C_9H_{12}NO_4$).

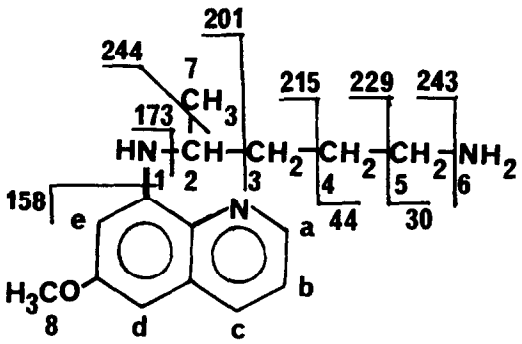
high-resolution MS. The identification of compounds 1–3 and 6 (Fig. 2) was based on MS (EI, CI) and high-resolution MS only. Compound 7 (Fig. 2) could not be fully identified. GC/MS analyses supported the identification of compounds 2, 4 and 5. Due to the low concentrations of the original primaquine solutions, it was very difficult to isolate sufficient of each sample to allow good NMR spectra to be obtained. The resulting NMR spectra were therefore used only for the identification of the quinoline ring systems. The MS and GC/MS spectra were used to deduce side chain structures.

Compounds 7 and 8 (Fig. 2) were the main degradation products. Both compounds have higher molecular weights than primaquine. The structure of compound 7 was not fully resolved, but ^{1}H-NMR, MS (EI, CI) and high-resolution MS indicated the structure of a highly oxygenated compound with molecular weight 413 (Table 2). The partial structure shown follows from the high-resolution MS of fragment m/z 201 (Fig. 3 and Table 2). Compound 8 clearly derives from two molecules of primaquine with loss of ammonia and hydrogen. The formation of such products is further indicated by an increase in the absorbance in the visible range of the primaquine spectrum. These products are not likely to form in vivo due to extensive dilution of the compounds by the body fluids and the probable reaction of intermediate radicals with endogenous substances. However, compounds 7 and 8 might be formed in vitro as a result of light exposure of the drug formulations.

The formation of compounds 2–5 (Fig. 2) shows that competitive reactions take place. The

TABLE 1

MS, GC / MS and NMR data of primaquine



High-resolution MS

Mean of measured values	Standard deviation ($n = 8$)	Deviation from theoretical value	Element composition
259.16948	0.00036	+1.0 mDa	$C_{15}H_{21}N_3O$

High-resolution MS of main fragment (magnet scan)

Measured value	Deviation from theoretical value	Element composition
201.1165	+13.7 mDa	$C_{12}H_{13}N_2O$

CI-MS

Fragments (molecular ion)	*m / z*	%
$M + C_2H_5^+$	288	2.3
$M(^{13}C) + H^+$	261	16.4
$M + H^+/M(^{13}C)^{+\cdot}$	260	100.0
$M^{+\cdot}$	259	78.4

EI-MS			GC/MS (retention time: 14.5 min)	
Main fragments	*m / z*	%	*m / z*	%
$M(^{13}C)^{+\cdot}$	260	3.3	260	3
$M^{+\cdot}$	259	18.1	259	19
$M(^{13}C) - NH_3/M - NH_2$	243	1.6	243	3
$M - NH_3$	242	8.7	242	18
	241	3.4	241	7
$C_9H_5N(OCH_3)(NHCH(CH_3)CH_2)$	215	2.9	215	2
$C_9H_5N(OCH_3)(NHCHCH_3)(^{13}C)$	202	14.6	202	13
$C_9H_5N(OCH_3)(NHCHCH_3)$	201	100.0	201	100
	186	12.2	186	18
	187	7.0	187	7
	176	15.6	176	25
	175	24.2	175	22
$C_9H_5N(OCH_3)(NH_2)$	174	12.7	174	8
$C_9H_5N(OCH_3)(NH)$	173	1.6	173	2
$C_9H_6N(OCH_3)$	159	10.5	159	11
$C_9H_5N(OCH_3)$	158	9.7	158	9
	84	10.7	84	6
	70	14.4	70	7
C_2H_6N	44	1.6	44	2
CH_2NH_2	30	20.1	30	14

(Continued overleaf)

TABLE 1 (continued)

2D ^{1}H-NMR (COSY)

Atom (group)	Chemical shift (ppm)	Area	Coupling ^{1}H
C_a	8.45 (–)	18.4	C_b, C_c
C_c	7.80 (d)	20.1	C_a, C_b
C_b	7.15 (q)	20.3	C_a, C_c
C_d, C_e	6.25 (–)	45.3	–
NH	6.00 (d)	20.4	C_2
OCH_3	3.75 (s)	65.4	–
C_2	3.50 (m)	24.6	NH, C_7
C_5	2.55 (m)	43.4	C_4
NH_2	1.80 (s)	39.2	–
C_3	1.60/1.40 (2 × m)	78.7	C_4
C_4	1.50 (m)		C_3, C_5
C_7	1.20 (d)	52.8	C_2

relative amounts formed showed large variation from one experiment to another while the other degradation products were formed in constant relative amounts. This was demonstrated by the use of semi-quantitative TLC, assuming equimolar absorbance of all the substances (Fig. 1).

The isolated degradation products emphasize that the quinoline ring remained intact after exposure of primaquine to light. This is in agreement with previous experience with other quinoline antimalarials (Tønnesen et al., 1988; Tønnesen and Grislingaas, 1990; Nord et al., 1991). The main EI-MS fragment from primaquine (m/z 201), characteristic for the quinoline ring and its substituents (Table 1), makes an important contribution to the EI-MS spectra of all the isolated fractions, except the fraction containing compound 1 (Fig. 3). The ^{1}H-NMR spectra of compounds 4, 5, 7 and 8 (Fig. 2) also demonstrate that the quinoline rings are intact, giving chemical shifts and couplings similar to primaquine in the high-shift area.

Compounds 2–5 (Fig. 2) are photooxidation products of primaquine, as determined by high-

TABLE 2

High-resolution MS data of the photochemical degradation products

Compound number	Ion	Mean of measured values	Standard deviation	Deviation from theoretical value	Element composition
1	molecular ion	240.12761	0.00052 (n = 8)	+1.4 mDa	$C_{15}H_{16}N_2O$
2	molecular ion	287.16451	0.00051 (n = 6)	+1.1 mDa	$C_{16}H_{21}N_3O_2$
	main fragment	201.10305	(magnet scan)	+0.3 mDa	$C_{12}H_{13}N_2O$
3	molecular ion	287.16564	0.00055 (n = 6)	+2.3 mDa	$C_{16}H_{21}N_3O_2$
	main fragment	201.102	(magnet scan)	−0.8 mDa	$C_{12}H_{13}N_2O$
4&5	molecular ion	285.14867	0.00060 (n = 7)	+0.9 mDa	$C_{16}H_{19}N_3O_2$
	main fragment	201.1024	(voltage scan)	−0.4 mDa	$C_{12}H_{13}N_2O$
6	molecular ion	240.12784	0.00055 (n = 8)	+1.6 mDa	$C_{15}H_{16}N_2O$
	important fragment	201.108	(magnet scan)	+5.2 mDa	$C_{12}H_{13}N_2O$
7	molecular ion	413.18735	0.00028 (n = 3)	−7.7 mDa	$C_{22}H_{27}N_3O_5$
	main fragment	318.11469	0.00076 (n = 8)	−6.9 mDa	$C_{16}H_{18}N_2O_5$
	important fragment	201.107	(magnet scan)	+4.2 mDa	$C_{12}H_{13}N_2O$
8	molecular ion	480.25324	0.00069 (n = 4)	+0.7 mDa	$C_{30}H_{32}N_4O_2$

resolution MS (Table 2). Oxidation can occur either in the quinoline ring leading to the formation of a dione as reported by Brossi et al. (1987), or in the side chain. In this case high-resolution MS of the m/z 201 fragments showed that the oxidation had not changed the quinoline ring and must be in the side chain (Table 2). This is contradictory to that reported for photodecomposition of primaquine in methylene chloride (Brossi et al., 1987), but in agreement with the previous investigations of other quinoline antimalarials in water (Tønnesen et al., 1988; Tønnesen and Grislingaas, 1990; Nord et al., 1991). The ^{1}H-NMR spectrum of compounds 4 and 5 (Fig. 2) also showed the presence of the methoxy group, identified by the signals at 3.85 and 3.90 ppm.

The m/z 215 fragment gives a strong peak in the EI-MS spectrum of compound 2, but the EI-MS spectrum of compound 3 lacks this fragment (Fig. 3). This suggests the positions of the carbonyl groups in the two compounds. For compound 2 an EI-MS McLaffertey rearrangement product of m/z 200 confirms the position of the carbonyl group. The EI-MS fragment of m/z 229 from compound 3 further supports the position of the carbonyl group in this compound.

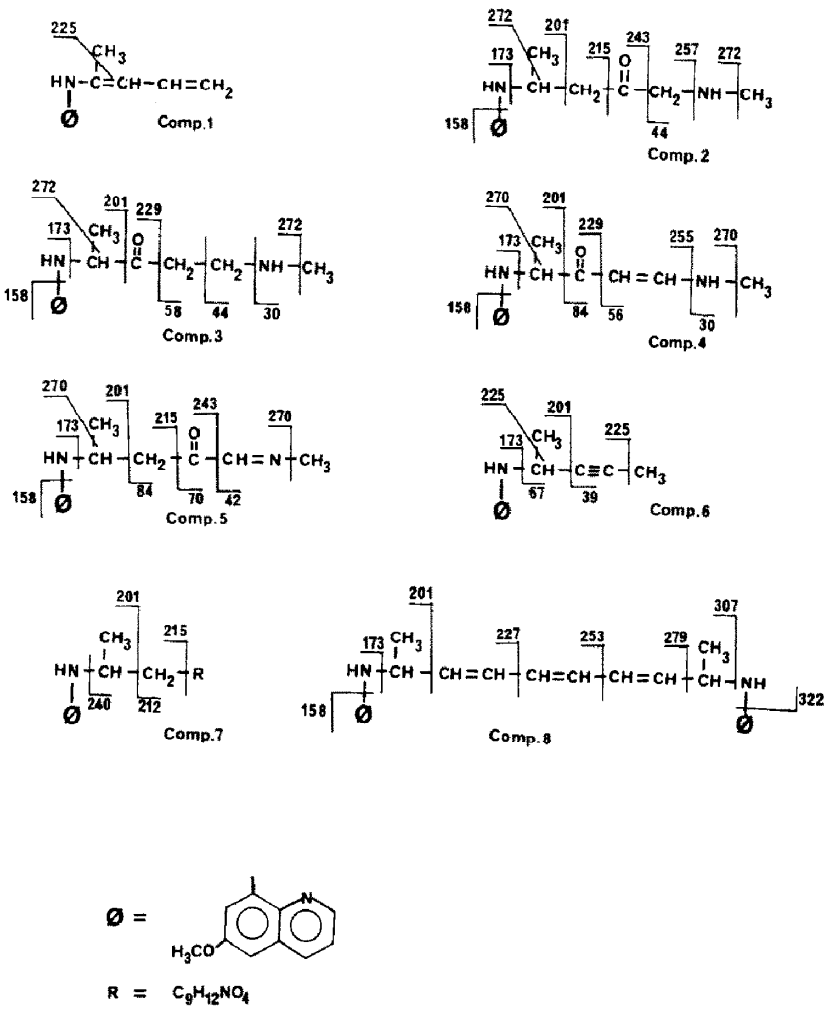


Fig. 3. EI-MS fragmentations of the photochemical degradation products.

The m/z 44 fragment is an important contributor in the spectra of compounds 2 and 3 (Fig. 3). It is a reasonable α-cleavage product of compound 2 carbonyl and amino groups and a α-cleavage product in respect of the amino group of compound 3.

GC/MS analyses showed that fraction 4 (Fig. 1) contained two compounds (4 and 5, Fig. 2) of m/z 285. The positions of the carbonyl groups in these compounds are deduced from the presence of the m/z 215 fragment in the GC/MS spectrum of compound 5 (retention time 16.2 min), and its absence in the GC/MS spectrum of compound 4 (retention time 16.4 min) (Fig. 3).

The EI-MS spectrum of compound 1 (Fig. 3) lacks the m/z 201 fragment, supporting the presence of an olefinic carbon atom. In contrast to this, the fragment of m/z 201 is an important contributor to the EI-MS spectrum of compound 6 (Fig. 3).

There are large differences in the charge density distribution in the aminoquinolines (Singer and Purcell, 1967). This is reflected in the different pK_a values of the quinoline nitrogens. The pK_a values are reported to be 3.2 and 8.4 for primaquine and chloroquine, respectively (Moore and Hemmens, 1982; Hufford and McChesney, 1983). In addition, the side chains of primaquine and chloroquine/hydroxychloroquine are attatched at different positions of the quinoline rings. These differences may lead to divergent photochemical reaction patterns.

The photodegradation products identified from primaquine are different from the reported metabolites of this drug (Baty et al., 1975; Moffat et al., 1986). For example, the important metabolite 6-methoxy-8-aminoquinoline could not be detected among the photodegradation products. This result is in agreement with the results of the photodecomposition of hydroxychloroquine (Tønnesen et al., 1988). For chloroquine however, 4-amino-7-chloroquinoline, which is the analog of 6-methoxy-8-aminoquinoline, formed the main degradation product after irradiation (Nord et al., 1991). Compounds 1, 6 and 8 formed from primaquine (Fig. 2) are analogous to photochemical decomposition products from hydroxychloroquine, although differing in the degree of unsaturation (Tønnesen et al., 1988). This suggests that no cyclization occurs in the side chain during the irradiation of primaquine.

Irradiation of primaquine, hydroxychloroquine and chloroquine in oxygen containing media causes cleavages of the side chains without cyclization. The quinoline structures remain intact, giving rise to photochemically active degradation products.

Conclusion

Irradiation of primaquine in an aqueous medium causes various cleavages of the side chain, leaving the quinoline structure unchanged. Hence, the degradation products are not photochemically inert, but may participate in secondary reactions. The main photodecomposition products (compounds 7 and 8, Fig. 2) are not likely to be formed in vivo, but may be formed in vitro after light exposure of drug formulations. It is obvious that different photochemical degradation products are formed dependent upon the experimental conditions. Further investigations will be carried out to evaluate the phototoxic behaviour of primaquine, the in vivo metabolites and the photochemical degradation products of this drug.

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