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Photochemical stability of biologically active compounds. II. Photochemical decomposition of mefloquine in water

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Summary

Long-term treatment with antimalarials often causes side effects which are postulated to be phototoxic reactions. The photochemical degradation of mefloquine in water was carried out to evaluate the possible phototoxic properties of this drug. Six decomposition products were isolated by means of preparative TLC. The samples were identified by mass-spectrometry (EI, CI and high-resolution MS) and ^{1}H-NMR spectroscopy. The action spectrum for the photolysis of mefloquine was recorded.

Introduction

Human malaria is caused by four species of plasmodial parasites of which *Plasmodium falciparum* results in the most severe infections and is responsible for nearly all malaria-related deaths (WHO Drug Information, 1988). The resistance of *P. falciparum* to currently used antimalarials like chloroquine is growing. Efforts to find possible replacement drugs to which resistance has not yet been developed are therefore increasing. The long-recognized antimalarial activity of quinine has led to the development of structural analogs of which mefloquine seems to be a good alternative.

Mefloquine is still restricted in use for treatment of malaria, and should only be the drug of choice in areas with a high prevalence of multiple-drug resistance (Salako, 1985). Mefloquine is rapidly absorbed after an oral dose. The compound is strongly bound to plasma proteins (WHO Drug Information, 1988). Activity against multi-resistant falciparum malaria is obtained after a single dose of 500–1000 mg mefloquine base (Salako, 1985). After a single dose, an effective serum concentration may persist for more than 30 days. The mean whole blood half-life is reported to be 13.9 days (Florey, 1985).

Mefloquine seems to cause fewer side effects than the more widely used antimalarial drugs (e.g., chloroquine, hydroxychloroquine and quinine) (Salako, 1985, Gustafsson et al., 1987; WHO Drug Information, 1988). Changes in skin pigmentation and in the cornea and retina of the eye are side effects often associated with the long-term usage of antimalarials. These effects are possibly phototoxic reactions (Moore and Hemmens, 1982).

So far, no reports have appeared on the possible phototoxicity of mefloquine in humans. Long-term studies in rats given mefloquine in doses

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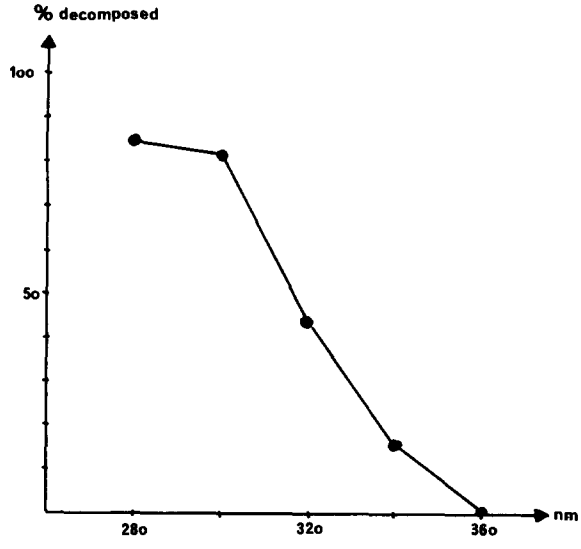


Fig. 1. Action spectrum of the photolysis of mefloquine hydrochloride in water.

equivalent to 2–3-times the usual human treatment dose showed the occurrence of ocular lesions including retinal degeneration (Florey, 1985).

The apparent low phototoxicity reported for mefloquine might be ascribable to the lack of information on long-term treatment with this compound in humans. For other antimalarials like hydroxychloroquine the retinopathy is dose-related and rarely occurs for cumulative doses below 200 g (Mills et al., 1981). If this is also the case with mefloquine, effects on the eye will only be detectable after several years of continuous treatment, a procedure on which no data are available yet.

The low phototoxicity observed with mefloquine can also be due to the chemical properties of the compound itself. Showing structural similarities to quinine, mefloquine is likely to be photolabile, which has also proved to be the case (Yoon and

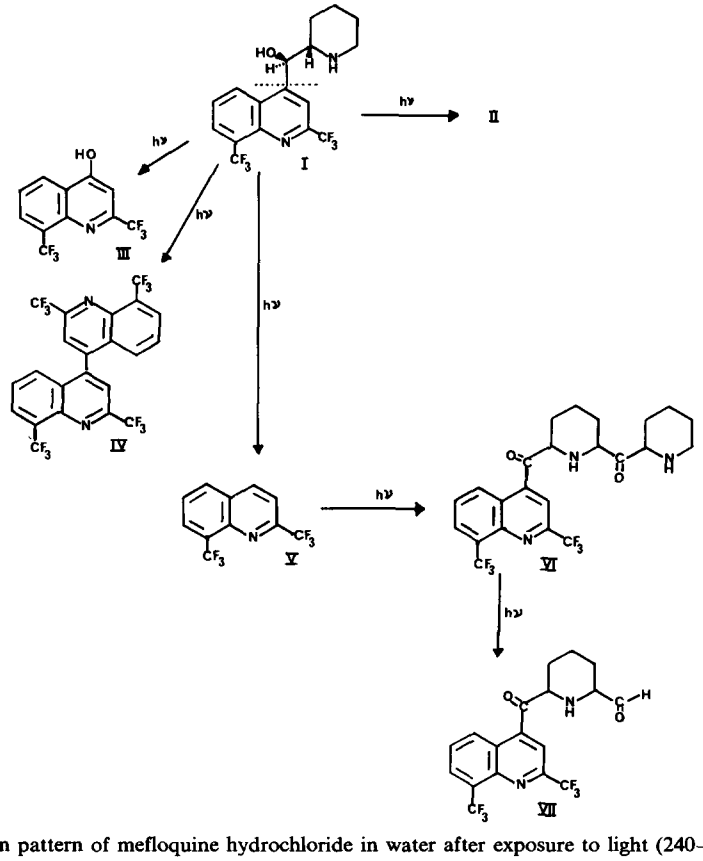


Fig. 2. Postulated degradation pattern of mefloquine hydrochloride in water after exposure to light (240–600 nm). Compound II is tentatively identified as the *threo* form of mefloquine.

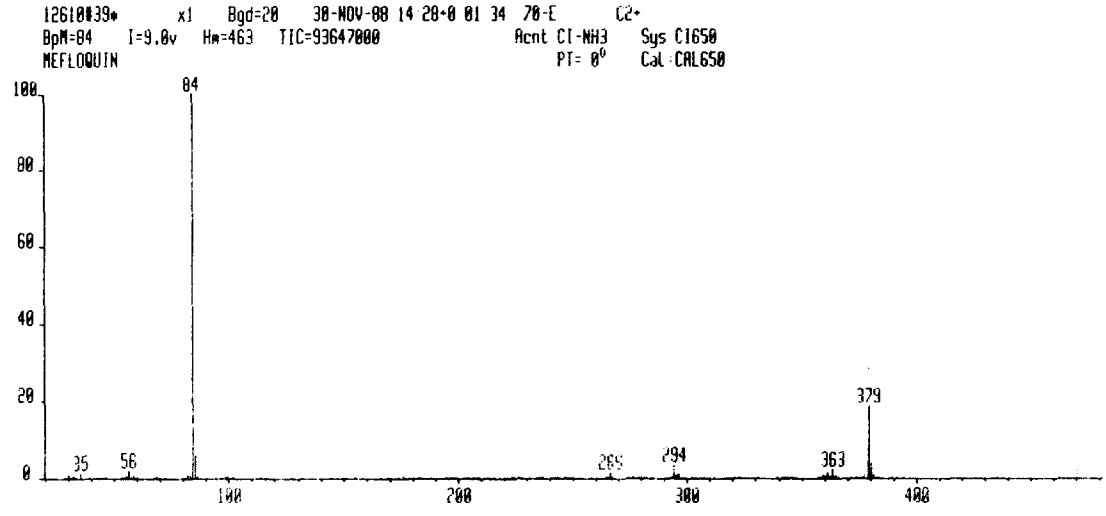


Fig. 3. CI-mass spectrum of mefloquine hydrochloride.

Epling, 1980; Epling and Yoon, 1982; Roche, internal report, 1986). Two decomposition products were identified after irradiation of mefloquine in methanol (deaerated system) with light of wavelength above 300 nm (Epling and Yoon, 1982). In vivo, however, the conditions should differ, since oxygen is not excluded from the tissues. It is therefore of interest to study the photochemical degradation of mefloquine in oxygen-containing media to evaluate the possible phototoxic properties of this drug after long-term treatment.

Materials and Methods

Materials

The *erythro* racemate of mefloquine hydrochloride was provided by Hoffmann-La Roche, Basle. All chemicals used were of p.a. grade.

Preparation of the samples

Mefloquine hydrochloride (50 mg) was dissolved in 500 ml water. After exposure to light, the sample was adjusted to pH 2 with 1 M HCl or to pH 10 with 1 M NaOH. The sample was then

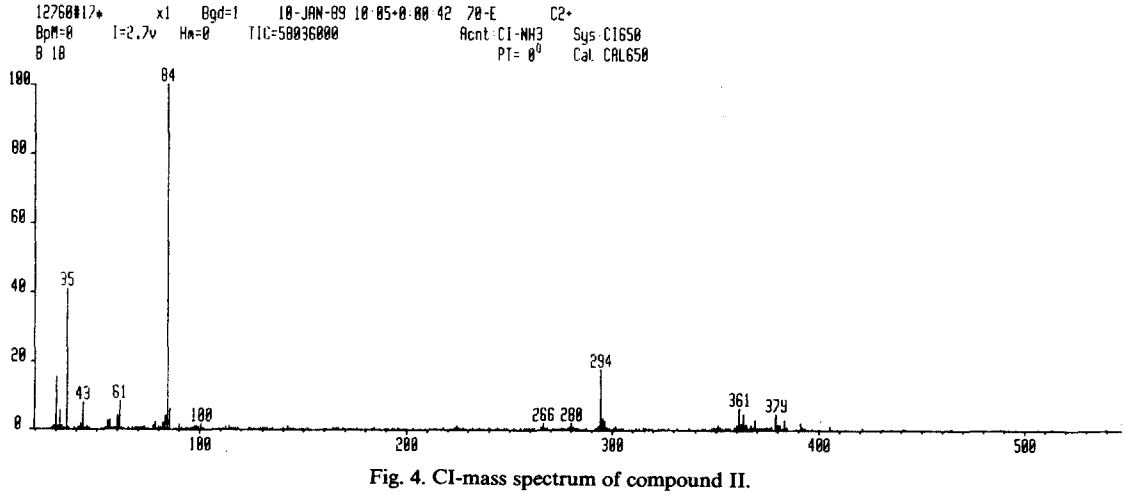


Fig. 4. CI-mass spectrum of compound II.

extracted with 3 × 100-ml volumes of dichloromethane. The combined extracts were evaporated to dryness under vacuum. The residue was dissolved in methanol for further analysis.

The photochemical degradation of mefloquine was followed by quantitative TLC, and the action spectrum for the decomposition was recorded.

After irradiation, photochemical degradation products were isolated by means of preparative TLC. Samples were extracted from silica gels with methanol/dichloromethane (1 : 1). The fractions were centrifuged for 10 min at 2300 rpm. The purity of the isolated fractions was monitored by TLC before carrying out further identification by mass spectrometry (EI, CI and high-resolution MS) and NMR (^{1}H).

Preparative and quantitative TLC

The stationary phase was silica gel (Merck), the mobile phase being dichloromethane/methanol/glacial acetic acid (8 : 1 : 1).

Irradiation

For the isolation of degradation products, samples were exposed to an immersion lamp with emission wavelengths of 240–600 nm, and power 120 W (Hereaus immersion lamp system). To provide light above 300 nm the immersion lamp was placed in a glass tube.

To obtain the action spectrum for the decomposition of mefloquine, a 900 W Photo-Irradiator (Applied Photophysics) was used. A bandwidth of 10 nm was used at all wavelengths. The energy output was quantified by a thermopile voltmeter (200 mV) (Applied Photophysics). The irradiation energy was kept constant at 80 W/cm^2 and the period of irradiation was 4 h.

Quantitative TLC

For quantitation of mefloquine and degradation products a Shimadzu CS 9000 dual-wavelength flying-spot scanner was used. Detection wavelengths: (UV) 223, 254 and 283 nm; (fluorescence) excitation, 366 nm; emission, > 400 nm.

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 °C and the ion potential was 70 eV. High-resolution mass spectra were recorded on an AEI MS 902.

NMR

The ^{1}H NMR spectra were recorded on a Varian XL 300.

Results and Discussion

Mefloquine hydrochloride is poorly soluble in water. For the isolation of degradation products a concentration of 0.1 mg/ml in water was chosen.

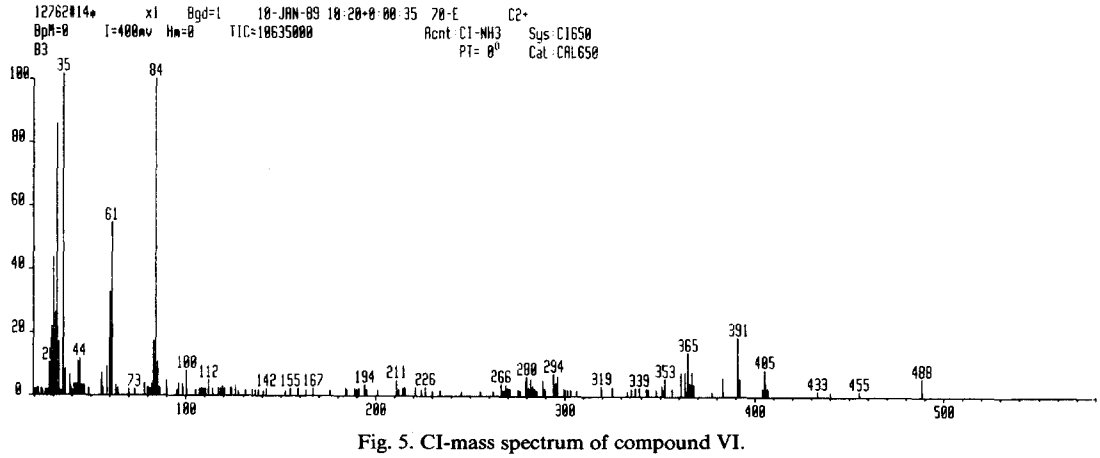


Fig. 5. CI-mass spectrum of compound VI.

After irradiation for 5.5 h a change in pH of the solution was observed from pH 6.8 to 3.7. The addition of buffer salts (phosphate) to the solution caused precipitation of the sample after 30 min irradiation under the given experimental conditions. Pure water was therefore chosen as the reaction medium in these experiments.

The action spectrum of the photolytic degradation of mefloquine hydrochloride in water at a fixed irradiation energy is given in Fig. 1. When irradiated with light in the region above 360 nm, no photochemical decomposition of mefloquine could be observed. Severe degradation occurred when the samples were exposed to light below 320 nm. This is in agreement with the UV/visible absorption spectrum of mefloquine in water (λ_{max} 282, 302 and 315 nm). The action spectrum shows that the influence of lower wavelengths in the UV region (290–400 nm), e.g., sunlight (cut-off at 290 nm), should not be neglected in assessing the photochemical stability of mefloquine.

For isolation and identification of the photochemical degradation products of mefloquine, the samples were irradiated with continuous light in the wavelength range 240–600 nm. The half-life of mefloquine under these conditions was 3 h. If the light source was immersed in a glass jacket to give light only above 320 nm, less than 20% mefloquine was decomposed after 4 h irradiation. The main degradation products were shown to be identical independent of the light source used. Mefloquine was therefore irradiated with unfiltered light in these experiments due to a faster reaction rate.

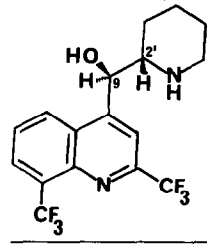
The postulated degradation pattern of mefloquine in water after exposure to light (240–600 nm) for 2 h is shown in Fig. 2. The irradiation of mefloquine in water (240–600 nm) resulted in four main degradation products (II–V). With the exception of the dimerization product (IV), the same degradation products were identified after irradiation of mefloquine with filtered light (above 320 nm).

Compound II was isolated after extraction of the samples at pH 10. The mass spectra of this compound were identical to the reference spectra of mefloquine (Figs 3 and 4). The NMR spectra (^{1}H) of the two compounds, however, showed a slight difference in location of the proton at the C-9 carbon. The C-9 proton in compound II showed a higher field (5.934 ppm) and a larger coupling constant (5.4 Hz) compared to the reference spectrum of mefloquine (Table 1). This indicates that compound II is the *threo* form of mefloquine (Florey, 1985).

Compounds III–VII were isolated after extraction of the samples at pH 2. The CI mass spectra give $M^+ = 282$ (base peak $m/e = 282$), $M^+ = 531$ (base peak $m/e = 266$) and $M^+ = 266$ (base peak $m/e = 266$) for compounds III–V, respectively. The CI mass spectra of compounds VI and VII

TABLE 1

^{1}H-NMR shifts of the erythro racemate of mefloquine hydrochloride in DMSO-d_6



Proton(s) at carbon	Chemical shift (ppm)	Resonance pattern	Coupling constant (Hz)
C-3	8.108	(s)	
C-5	8.400	(d)	7.5
C-6	7.981	(t)	7.8
C-7	9.021	(d)	7.8
C-9	6.127	(dd)	3.6 (2.7)
C-9 (OH)	6.809	(d)	4.2
C-2′	2.95	(m)	unresolved
C-3′	1.64	(m)	unresolved
C-4′	1.26	(m)	unresolved
C-5′	1.62	(m)	unresolved
C-6′	3.44	(m)	unresolved

TABLE 2

Mass fragments of photodecomposition products of mefloquine measured by high-resolution mass spectrometry

Compound	Mass fragment	Empirical formula
III	265	$C_{11}H_5F_6N$
IV	265	$C_{11}H_5F_6N$
V	265	$C_{11}H_5F_6N$
VI	404	$C_{18}H_{14}F_6N_2O_2$
VII	404	$C_{18}H_{14}F_6N_2O_2$

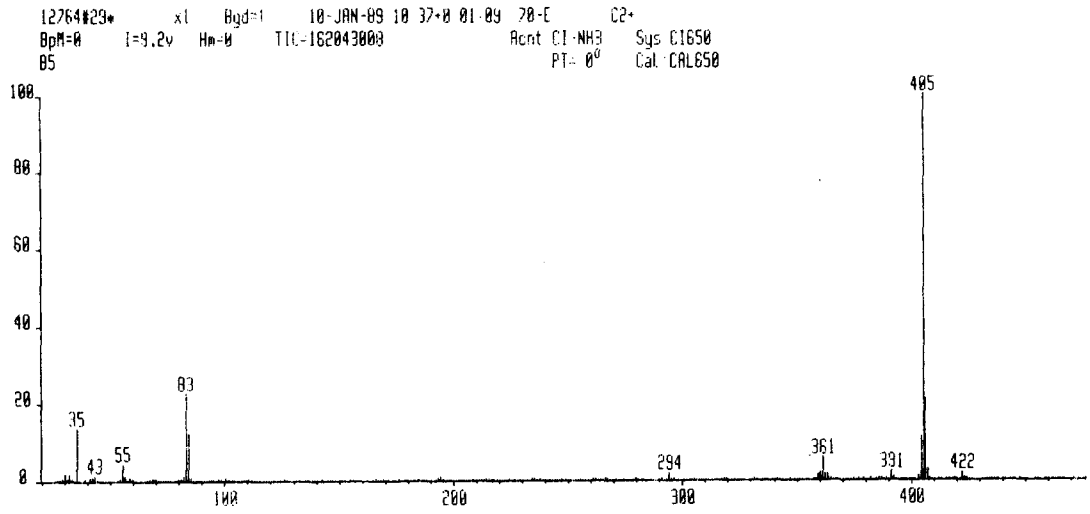


Fig. 6. CI-mass spectrum of compound VII ($m/z = 422$ is $M^+ + NH_3$).

are shown in Figs 5 and 6. High-resolution values of mass fragments from the isolated compounds are listed in Table 2.

Compounds VI and VII were formed in minor quantities. Based on the mass spectra of these compounds, they were postulated to be condensation products of radicals formed initially in the photolysis of mefloquine. The structures suggested (Fig. 2) were further underlined by ^{1}H-NMR studies. The C-9 protons were not present in the NMR spectra of compounds VI and VII. Irradiation of mefloquine in oxygen-containing media causes cleavage of the molecule which differs from what is observed after irradiation in deaerated systems (Yoon and Epling, 1980; Epling and Yoon, 1982). The main degradation products formed under aerobic conditions are strongly fluorescent compared to mefloquine. Moore and Hemmens (1982) have observed an apparent relation between fluorescence yield in vitro and photosensitizing ability in vivo of several antimalarials. To evaluate the possible phototoxicity of mefloquine further investigations will therefore be made on the photochemical properties of the main degradation products and on the mechanisms involved in the photolysis.

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