Photolytic Degradation of α-[(Dibutylamino)methyl]-6,8-dichloro-2-(3′,4′-dichlorophenyl)-4-quinoline Methanol: An Experimental Antimalarial

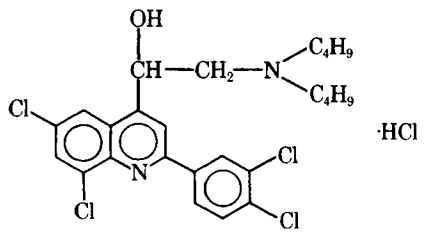
H. OKADA *§, V. STELLA *x, J. HASLAM ‡, and N. YATA *¶

Abstract □ A study of the effects of various storage conditions on the rate and products of degradation of the quinoline methanol antimalarial agent, α-[(dibutylamino)methyl]-6,8-dichloro-2-(3′,4′-dichlorophenyl)-4-quinoline methanol, was undertaken. The degradation was followed by high-pressure liquid chromatography and TLC in oxygenated and deoxygenated methanol, ethanol, chloroform, and chloroform–heptane mixtures under UV and laboratory fluorescent lighting irradiation, as well as in the absence of light. The kinetics of degradation confirmed the major catalyzing factor to be UV irradiation. The compound was stable in the absence of light and reasonably stable under fluorescent lighting both in the presence and absence of oxygen. The degradation resulted in a major product, 6,8-dichloro-2-(3′,4′-dichlorophenyl)-4-quinolinecarboxaldehyde, whose structure was confirmed by elemental analysis and IR, NMR, and mass spectral data.

Keyphrases □ α-[(Dibutylamino)methyl]-6,8-dichloro-2-(3′,4′-dichlorophenyl)-4-quinoline methanol—photolytic degradation □ Photolysis—degradation of α-[(dibutylamino)methyl]-6,8-dichloro-2-(3′,4′-dichlorophenyl)-4-quinoline methanol

The antimalarial agent α-[(dibutylamino)methyl]-6,8-dichloro-2-(3′,4′-dichlorophenyl)-4-quinoline methanol monohydrochloride[1] (I) has been shown to be useful in the treatment of resistant strains of *Plasmodium vivax* and *Plasmodium falciparum*. However, it has been shown to cause photosensitivity in some experimental animals and humans, although this effect is minor when compared to a number of other quinoline methanol antimalarials.

In the process of accumulating data on the physical and chemical properties of I and the free base form (II) in various organic solvents, it was noted that chemical degradation was occurring, particularly in standard solutions of II dissolved in chloroform and blood extractant solutions of II that were allowed to stand in the presence of laboratory lighting. Therefore, a study of the effects of various solvents and storage conditions on the rate and products of degradation of II was undertaken. The products of such degradation were of interest from a mechanistic viewpoint as well as from a toxicity viewpoint. That is, could the products of photolysis of II be the agents causing the phototoxicity reaction in animals and humans.



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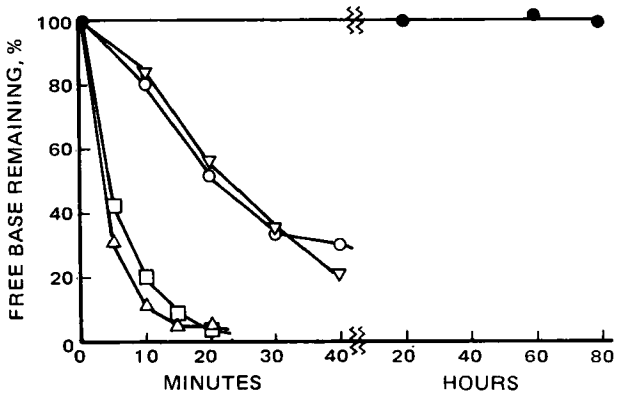


Figure 1—*Plot of percent of II remaining in various organic solvents as a function of time. Open points represent solutions exposed to direct sunlight; closed points represent a solution protected from direct irradiation or exposed only to laboratory fluorescent lighting. Key:* □, *methanol (0.001% II);* △, *20% methanol–ethanol (0.001% II);* ○, *chloroform (0.005% II); and* ▽, *20% chloroform–heptane (0.005% II).*

Most of the work was done on II and not I because preliminary data indicate that II is the predominant form of I at physiological pH[2].

RESULTS AND DISCUSSION

Degradation of II, which was followed quantitatively by high-pressure liquid chromatography (HPLC) and qualitatively by TLC, was found to be strongly catalyzed by UV irradiation, but solutions of II in various solvents were stable when protected from light. Exposure to normal laboratory fluorescent lighting catalyzed the breakdown of II but only after 4–5 days of exposure. Typical plots of the loss of II from UV-irradiated solutions in various solvents, concentrations, and UV energy sources, as a function of time, are shown in Figs. 1 and 2.

Solutions of II in chloroform exposed to UV irradiation, with and without oxygen-free nitrogen bubbled through the solution, showed no quantitative or qualitative differences in their degradation rates or products.

Factors other than the presence of UV irradiation affected the rate of photolytic decomposition of II. These factors included:

1. The concentration of II dissolved in the organic solvent. The higher the concentration of II in the solvent, the smaller was the percent loss of II per unit time. This result is consistent with the degradation rate passing from a rate-determining loss of II to an apparent pseudo-zero-order degradation dependent on the amount of incident UV energy (1).

[1] This compound, abbreviated WR-30,090, is currently under testing by the Walter Reed Research Institute. WR-30,090 itself is the hydrochloride salt. Most work in organic solvent and extraction systems requires the use of the free base because of the poor solubility of the hydrochloride salt in organic solvents.

[2] The pKa of I in aqueous solvents is suspected to be unpredictably low. The loss of I and II from aqueous solution and their unusual physical and chemical properties in aqueous solutions will be discussed later.

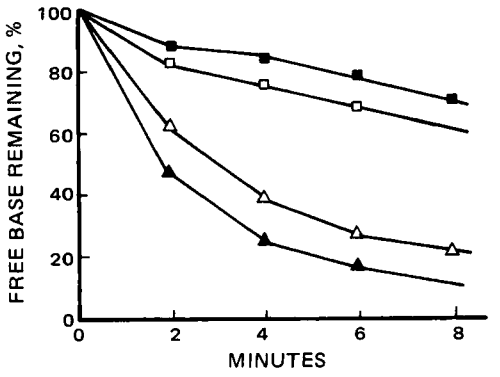


Figure 2—*Plot of percent of II remaining in various organic solvents containing various concentrations of II exposed to UV irradiation in a photochemical reactor at 3000 Å as a function of time. Key: ■, chloroform (0.005% II); □, chloroform (0.001% II); △, methanol (0.001% II); and ▲, methanol (0.0002% II).*

2. At the same concentration, the degradation of II was solvent dependent. UV-irradiated solutions of II degraded faster in methanol and methanol–ethanol than in chloroform and chloroform–heptane solvents.

3. Compound I, *i.e.*, the hydrochloride salt, was stable to short-term exposure to UV irradiation. Long-term exposure (>1 day) to irradiation at 2537 and 3000 Å in a photochemical reactor produced degradation, but the degradation products were numerous and not the same as the degradation products of II.

4. The degradation of II was quenched by the removal of the UV irradiation source; *i.e.*, the reaction was UV irradiation catalyzed and not just UV irradiation initiated. This result is adequately demonstrated in Fig. 3.

5. The UV-catalyzed degradation of I or II in aqueous and aqueous methanol solutions could not be studied because of apparent adsorption of II onto glass and its very poor aqueous solubility.

There did not appear to be any quantitative differences in degradation rates between solutions irradiated at 2537 or 3000 Å. However, corrections for light intensities, *etc.*, were not made.

Isolation of the major degradation product using column chromatography resulted in a yellow product, mp 195.5–197°, which gave methanol or ethanol solvates when recrystallized from methanol or ethanol solutions. The solvent-free compound gave a characteristic IR aldehyde carbonyl band at 1700 cm^{-1} and an aldehydic C—H stretch at 2860 cm^{-1}. Mass spectral data showed a peak at 368 and peaks at 370, 372, 374, and 376, consistent with P − 1, P + 1, P + 3, P + 5, and P + 7 peaks, respectively, of 6,8-dichloro-2-(3′,4′-dichloro)phenyl-4-quinolinecarboxaldehyde (III). A molecular weight of 35 was assumed as the base for chlorine. This structure was confirmed by NMR spectroscopy, and elemental analysis confirmed an empirical formula of $C_{16}H_7Cl_4NO$, the empirical formula for III (see *Experimental* for details).

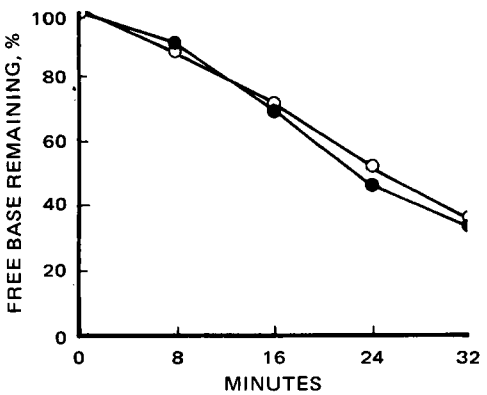
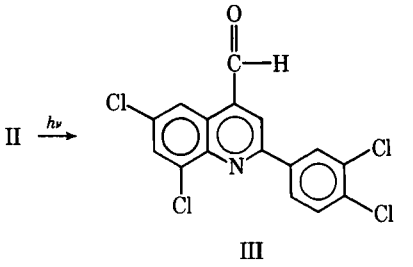


Figure 3—*Plot showing the effect of sunlight on the stability of II, 0.005% in chloroform. Key: ○, samples analyzed immediately upon sampling of the reaction mixture; and ●, samples taken at the same time but analyzed after 24 hr (these samples were protected from light during this period).*



III

Scheme I

A minor degradation product (as yet unidentified), mp 214–215.5°, was also isolated and is currently under investigation. By TLC, the reaction products did not appear to be solvent dependent. Isolation of the major degradation product from chloroform and methanol was accomplished. The characteristically yellow product, III (Scheme I), appeared to be the major degradation product in each case.

Davidson and Orton (2) recently showed that the fragmentation of 2-aminoethanols occurs in the presence of UV irradiation and suitable sensitizer molecules. Their results suggest that the mechanism illustrated in Scheme II may be involved in the fragmentation of 2-aminoethanols, where Y* is a sensitizer molecule such as anthraquinone, perylene, 2,3-diphenylquinoxaline, or acenaphtho[1,2-*b*]quinoxaline.

In the present study, it was initially felt that this sensitizer may have been the photoinduced free radicals in chloroform such as $Cl_3C\cdot$. Because the reactions proceeded just as fast if not faster in methanol and methanol–ethanol, it was felt that II, being a heterocyclic polychlorinated molecule, may be quite capable of acting as a sensitizer, catalyzing its own degradation *via* an autosensitizing photolytic decomposition.

The results of the present study concerning the decomposition of II to III in various organic solvents in the presence of UV irradiation appear consistent with the results of Davidson and Orton (2).

Because of the possible photoinduced fragmentation of other 2-amino alcohol antimalarial agents by UV irradiation to reactive aldehydes, a study of the susceptibility of these agents to photolysis and a correlation to their photosensitization properties might be desirable.

EXPERIMENTAL

Kinetic Studies—Solutions of II at various concentrations in a number of oxygenated and deoxygenated organic solvents were prepared. Methanol, methanol–ethanol, ethanol, chloroform, and chloroform–heptane were the various solvents[3] studied. Concentrations of II in the various solvents from 0.001 to 0.005% were studied. These solutions were exposed to sunlight, exposed to laboratory fluorescent lighting, protected from light by wrapping containers in aluminum foil, or photolyzed in a photochemical reactor[4] at 2537 or 3000 Å.

The degradation of II in the presence and absence of UV irradiation at ambient room temperature was followed by withdrawal of 5-μl samples from the quartz sample tubes at various predetermined times. Analysis of II was accomplished by HPLC[5]. The eluting solvent of choice was determined to be degassed and dried heptane–dioxane–methanol (80:20:5). An external standard of II dissolved in 20% chloroform–heptane protected from light was used. That this solution remained undegraded was confirmed by TLC. After isolation of one minor and one major degradation product, subsequent injections of these products on the HPLC column showed that neither interfered with the assay of II under the conditions described.

TLC using silica gel plates[6] with an eluting solvent of acetic

[3] All solvents were reagent grade quality from various sources.

[4] Rayonet Reactor, Southern New England Ultraviolet Co., Middletown, Conn. Two RPR 2537 Å lamps were used for the 2537-Å study, and two RPR 3000-Å lamps were used for the 3000-Å study. Ten-centimeter long quartz sample tubes, 1 cm in diameter, containing 5 or 15 ml of reaction solution provided the reaction vessel.

[5] Varian model 4000 liquid chromatograph equipped with a 280-nm detector and a Corasil II column (Waters Associates).

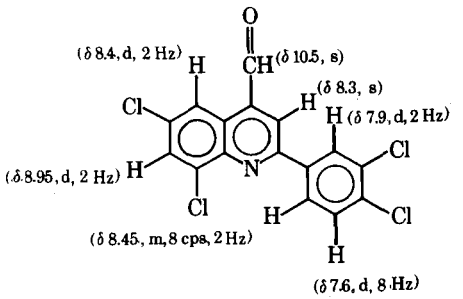
[6] Baker-Flex prepared silica gel plates.

acid–methanol–water (3:40:5) separated II satisfactorily from its apparent degradation products.

Preparation, Isolation, and Identification of Major Degradation Product—Eighty-seven milligrams of II dissolved in 50 ml of chloroform (or methanol) was exposed to direct UV irradiation in a photochemical reactor at 2537 Å for 2.5 hr. The photolyzed sample was evaporated to dryness, reconstituted in a minimum amount of chloroform, and added to a packed column[7]. The eluting solvent was chloroform. Fractionation resulted in the isolation of a small amount of unreacted II and approximately 50 mg of a yellow crystalline material, III, mp 195.5–197° (II, mp 129.5–130°; I, mp 215–218°).

Compound III was recrystallized from methanol or ethanol, either of which formed solvates with III, and dried overnight in a vacuum oven. TLC of III, using the previously described systems, showed a single spot with an R_f value of 0.70 (II, R_f 0.91), confirming it to be the major degradation material.

The IR spectrum[8] (KBr dispersion) of III gave a characteristic >C=O stretch at 1700 cm^{-1} and a C—H aldehyde stretch at 2860 cm^{-1}. NMR[9] of III, dissolved in $CDCl_3$ with tetramethylsilane as an internal standard, gave a spectrum consistent with the postulated aldehyde, III. The following assignments were tentatively made:

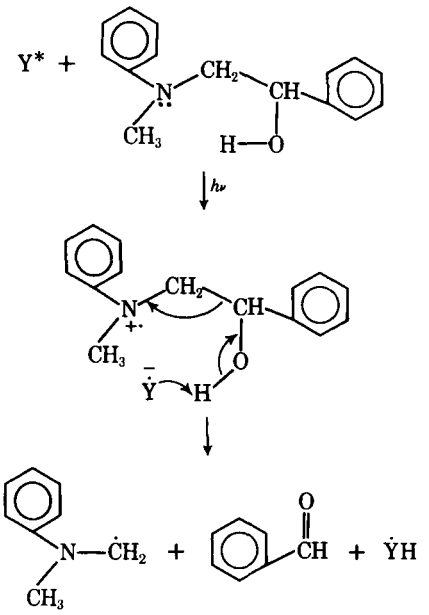


In the parentheses the first numbers refer to chemical shifts, in parts per million, from an internal standard of tetramethylsilane; s = singlet, d = doublet, and m = multiplet (also partially buried); and the last numbers are approximate spin-coupling constants. Integration was consistent with this structure when the single aldehydic proton was used as an internal standard.

If the molecular weight for the chlorine atom is assumed to be 35, the molecular weight of III is 369. A mass spectrum[10] of III showed a peak at 368 (P − 1), corresponding to $R—C{\equiv}O^+$, and a smaller P peak. The P + 1, P + 3, P + 5, and P + 7 peaks at 370, 372, 374, and 376 (as well as smaller P, P + 2, P + 4, P + 6, and P + 8 peaks), respectively, of magnitude relative to the P − 1 peak, 1.28:0.66:0.125:~0.01, confirm the presence of the four chlorine atoms in III. These relative peaks sizes are very close to the expected ratio of 1.31:0.64:0.14:0.01 (3). A peak corresponding to 369 − R′, where R′ = mass of —CHO at 340, is also consistent with the postulated structure of III.

Elemental analysis of III gave: C, 51.97; H, 2.08; N, 3.57. Calculated values for III, assuming the postulated structure, were: C, 51.75; H, 1.89; N, 3.77.

[7] Column containing 50 ml of Florisil, 100–200 mesh, packing material (Fisher Scientific Co.).
[8] Beckman IR-33 IR spectrophotometer.
[9] Varian T60 NMR spectrometer.
[10] Varian CH-5 mass spectrometer.



Scheme II

CONCLUSION

The degradation of the 2-aminoethanol (II), the free base form of I, in various organic solvents was found to be photoinduced, with the major degradation product being III. Compound II can be successfully extracted from blood or stored as standard solutions in various organic solvents as long as direct sunlight and long-term exposure to fluorescent lighting are excluded.

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§ Present address: Takeda Chemical Industries, Osaka, Japan.

¶ Present address: Faculty of Pharmaceutical Sciences, Osaka University, Osaka, Japan.

ˣ To whom inquiries should be directed.