Table IV—Methaqualone Concentrations in Blood [a] at Various Times after Administration of a Single Oral Dose with and without Diphenhydramine

Time[b], hr	Treatment: Methaqualone HCl Capsule	Methaqualone HCl plus Diphenhydramine Elixir	Methaqualone–Diphenhydramine Capsule
0.5	0.38 ± 0.44	0.24 ± 0.25	0.60 ± 0.50
1	1.39 ± 0.68	1.34 ± 0.53	1.54 ± 0.53
2	1.38 ± 0.35	1.26 ± 0.31	1.56 ± 0.48
4	0.79 ± 0.23	0.81 ± 0.23	0.92 ± 0.35
8	0.49 ± 0.15	0.51 ± 0.20	0.54 ± 0.17
24	0.24 ± 0.07	0.25 ± 0.08	0.30 ± 0.10
32	0.21 ± 0.08	0.21 ± 0.08	0.26 ± 0.09
48	0.16 ± 0.07	0.16 ± 0.07	0.18 ± 0.06

[a] Values (μg/ml) are the mean ± *SD* for 12 subjects. [b] Means were calculated using the approximate time of blood collection; exact collection times were recorded and used for *AUC* and $t_{1/2}$ determinations.

used in therapeutic combination products (25 mg) did not affect blood levels of methaqualone or its major metabolite.

An important consideration, in comparing the present study conducted in healthy humans to previous studies conducted in the rat (3), may be the relative doses of diphenhydramine and methaqualone hydrochloride administered. Although the ratio of methaqualone–diphenhydramine doses were similar (8:1 for rats and 10:1 for humans), the doses administered per kilogram of body weight were higher in animal studies (40 mg/kg for methaqualone HCl; 5 mg/kg for diphenhydramine HCl) than in human studies (3.7 mg/kg for methaqualone HCl and 0.4 mg/kg for diphenhydramine HCl). For the one subject who received a fourth dose of methaqualone in combination with a larger dose of diphenhydramine (50 mg), the $t_{1/2}$ for methaqualone increased and the urinary metabolite excretion decreased with increasing diphenhydramine dosage. While the AUC_0^∞ had increased, after correction for the increase in $t_{1/2}$, the $AUC_0^\infty\beta$ had decreased with the larger diphenhydramine dosage. Thus, larger doses of diphenhydramine, as might be favored by drug abusers, may elicit a pharmacokinetic interaction. The possibility of a dose-dependent interaction was not specifically investigated and would require further study.

This study was concerned only with investigating a pharmacokinetic interaction, and therefore does not preclude the possibility of a pharmacological interaction between methaqualone and diphenhydramine.

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ACKNOWLEDGMENTS

The assistance of the Medical Research Council in the form of an operating grant (MA-6599) and studentship (CBS) is greatly appreciated.

Photolytic Decomposition of Hydrochlorothiazide

SWASONO R. TAMAT and DOUGLAS E. MOORE ˣ

Received January 25, 1982, from the *Department of Pharmacy, The University of Sydney, Sydney 2006, Australia.* Accepted for publication April 9, 1982.

Abstract □ Hydrochlorothiazide decomposes upon irradiation with near-UV light ($\lambda > 310$ nm) both in methanol and aqueous solutions. In the photolysis the chlorine substituent is removed to be replaced by either —H or —OR from the solvent ROH. Hydrolysis of the thiadiazine ring is superimposed upon the dechlorination. The presence of oxygen inhibits the decomposition. The mechanism of the photolysis is suggested to involve cation radical formation which facilitates the hydrolysis step. 5-Chloro-2,4-disulphonamido-aniline, the normal hydrolysis product from hydrochlorothiazide, is also susceptible to photolytic dechlorination by a similar mechanism.

Keyphrases □ Hydrochlorothiazide—photolytic decomposition, irradiation, dechlorination, hydrolysis □ Decomposition, photolytic—hydrochlorothiazide, irradiation, dechlorination, hydrolysis □ Hydrolysis—photolytic decomposition of hydrochlorothiazide, dechlorination, irradiation

Hydrochlorothiazide [6-chloro-3,4-dihydro-1,2,4-benzothiadiazine-7-sulphonamido-1,1-dioxide] (I) is a widely used diuretic effective in small doses. Within a few years of its introduction there were reports of its implication in skin photosensitization (1). From oxygen uptake measurements and free radical polymerization (2), I and some

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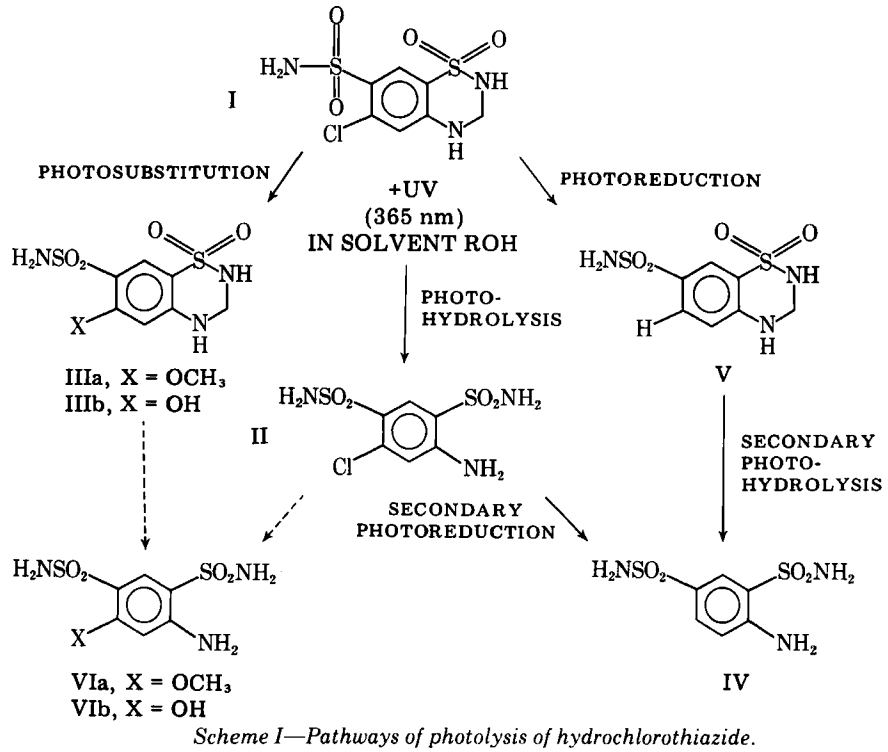

Scheme I—Pathways of photolysis of hydrochlorothiazide.

thiazide derivatives were found to be capable of acting as photosensitizers by both free radical and excited singlet molecular oxygen mechanisms. Subsequently, it was reported (3) that the chlorine substituent in I is photolabile.

In a study to determine the mutagenic activity of a number of drugs, I proved to be weakly mutagenic (4). Mutagenicity appeared to depend on the presence of an aromatic chlorine substituent and required activation by light. This study suggests that the drugs undergo photodehalogenation, yielding a reactive form, which can damage DNA. The nature of the photolysis products of I has now been examined and found to involve ring opening (hydrolysis) as well as dechlorination.

The chlorine substituent in 5-chloro-2,4-disulphonamidoaniline II, the normal hydrolysis product of I, has also been found to be photolabile.

EXPERIMENTAL

Materials—Hydrochlorothiazide (I)[1] and 5-chloro-2,4-disulphonamidoaniline (II)[2] were indicated to be >99% pure by high-performance liquid chromatography (HPLC).

2,4-Disulphonamidoaniline (IV) and 2,4-disulphonamido-5-methoxyaniline (VIa) were prepared by the action of chlorosulphonic acid on aniline and *m*-anisidine, respectively, followed by hydrolysis with ammonium hydroxide, by analogy to a previous method (5). Colorless crystals, mp 233–235° for IV and 252–253° for VIa, were obtained after recrystallization from water.

Cyclization of VIa with paraformaldehyde to 6-methoxy-3,4-dihydro-1,2,4-benzothiadiazine-7-sulphonamido-1,1-dioxide (referred to as methoxyhydrothiazide) (IIIa) was based on the method for I (6). Colorless crystals, mp 273–275°, were obtained after recrystallization from water. All compounds synthesized gave the expected NMR and mass spectra.

Trimethylanilinium hydroxide, a methylating agent for GC, was obtained as 2 *M* solution in methanol[3]. Acrylamide[4] was twice recrystallized from redistilled chloroform. All other chemicals and solvents were of analytical grade[5]. Doubly distilled water was used in aqueous systems.

Methods—A solution of I was irradiated at a concentration of 5×10^{-4} *M* in either methanol or water containing 5% methanol (to assist in dissolution). A medium pressure mercury lamp and a glass reaction vessel were used as previously described (3), so that the solution was exposed to UV light of >310-nm wavelength. The solution was presaturated with nitrogen or oxygen as required by bubbling for 90 min, and the gas flow was maintained to stir the solution during the irradiation. The product mixture was sampled at various times and analyzed by:

1. HPLC[6] with a fixed-wavelength UV detector at 254 nm and a reverse-phase 10-μm column (0.4 × 25 cm)[7] (mobile phase: 10% methanol in water);
2. GC[8] with hydrogen flame-ionization detector, nitrogen carrier gas, and phenyl methyl silicone stationary phase[9]. Following a previous method (7) the irradiated solution of I was concentrated 10-fold, and to 100 μl was added 2.5 μl of 2 *M* trimethylanilinium hydroxide. Methylation of I occurred in the heated injection port at 290°. The column and detector temperatures were 260° and 310°, respectively;
3. GC–chemical-ionization mass spectrometry[10] using the column as above and methane carrier gas.

Photopolymerization of acrylamide (0.125 *M*) initiated by I, II, IIIa, or chlorpromazine (8×10^{-5} *M*) was performed using the apparatus and procedures described previously (2).

RESULTS AND DISCUSSION

Photolysis of Hydrochlorothiazide in Methanol Solution—The photolysis of I dissolved in methanol saturated with nitrogen leads to dechlorination and hydrolysis of the thiadiazine ring in approximately equal proportions as shown in Scheme I. The quantum yield for Cl^- production was 0.18 ± 0.05, as previously reported (3). Under the conditions of irradiation used, the dechlorination was complete in ~6 hr, as determined by potentiometric titration. Other product formation is shown in Fig. 1 as a function of time from HPLC analysis of aliquots withdrawn at various times. For the major products the relative peak areas have been corrected by subsequent calibration with authentic samples of the compound. Confirmation of the identity of the major

[1] Ciba-Geigy, Sydney, Australia.
[2] A gift from Dr. J. A. Mollica, Ciba-Geigy Research and Development, Suffern, N.Y.
[3] Pierce Chemical Co., Rockford, Ill.
[4] BDH Chemicals, Poole, England.
[5] Ajax Chemicals, Sydney, Australia.
[6] Altex model 330.
[7] Hewlett-Packard Model 5720A.
[8] Brownlee RP-8.
[9] 100–120 mesh Gas Chrom Q, coated with 3% OV-1 packed in a 3 mm × 1-m silanized glass column.
[10] Finnigan 6110-9500 system.

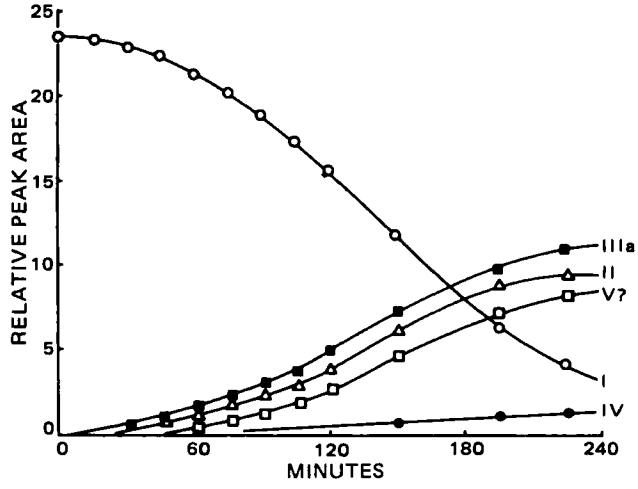

Figure 1—*Photodegradation of hydrochlorothiazide (0.5 mM) in oxygen-free methanol solution at 30° from HPLC analysis of aliquots withdrawn at various times.*

products was achieved by comparison of the chromatographic behavior of authentic samples of the hydrolysis product, 5-chloro-2,4-disulphonamidoaniline (II) and the reduced and substituted derivatives of I and II, namely, methoxyhydrothiazide (IIIa) and 2,4-disulphonamidoaniline (IV). On the basis of reported photoreactions of chloroaromatic compounds in methanol (8), coupled with the hydrolysis of I, other expected products were hydrothiazide (V) and 5-methoxy-2,4-disulphonamidoaniline (VIa). The latter compound was synthesized but its HPLC retention did not correspond to any of the photolysis products. Compound V, however, proved difficult to synthesize.

GC–MS analysis of trimethylanilinium hydroxide-treated samples of the photolysis mixture was complicated by the existence of such a large number of active protons in I and the photolysis products. Nevertheless, molecular ions corresponding to permethylated derivatives of II, IIIa, IV, and V were detected. For each one the GC peak height correlated approximately with the amounts detected by HPLC.

Figure 1 shows that dechlorination and hydrolysis occur simultaneously upon irradiation, where the concentrations of both II and IIIa build up to a maximum level after ~4 hr. Thereafter, II diminished as dechlorination proceeded as a secondary reaction. There was no hydrolysis detected in an unirradiated methanol solution of I after 6 hr at 30°, although complete hydrolysis can be achieved in ~1 hr by refluxing in 1 *M* NaOH, as reported previously (9).

Direct photolysis of II was also examined under the same conditions with the observation of dechlorination occurrence, leading predominantly to IV (~85%). A minor product was found, but its HPLC retention did not correspond to that of VIa. This correlated with the fact that VIa was not observed as a product from photolysis of I. In the GC–MS analysis of irradiated II a minor peak with *m/z* 319 was observed. This value

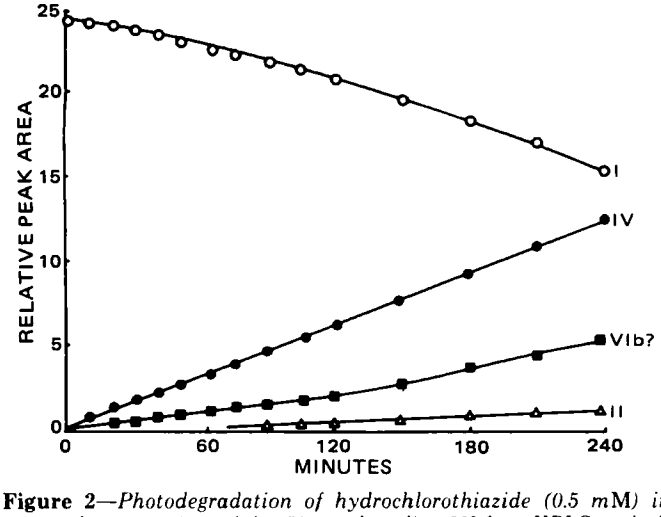

Figure 2—*Photodegradation of hydrochlorothiazide (0.5 mM) in oxygen-free water (containing 5% methanol) at 30° from HPLC analysis of aliquots withdrawn at various times.*

Table I—Rates of Photosensitized Polymerization of Acrylamide at 30°

Sensitizer, 8.3×10^{-5} *M*	Rate of Polymerization[a], mmoles liter^{-1} min^{-1}	
	pH 7.0 Buffer	Methanol
Hydrochlorothiazide (I)	1.58	0.39
5-Chloro-2,4-disulphonamido-aniline (II)	1.29	0.32
Methoxyhydrothiazide (IIIa)	3.47	0.16
Chlorpromazine	5.70	0.40

[a] Error in rate data ±5%.

corresponded to tetramethyl-V, suggesting that ring closure to form the thiadiazine ring had occurred to a small extent under irradiation. This assignment was confirmed by TLC separation, followed by solid-probe MS of the separated compounds without derivatization. That ring closure is a possibility comes from the observation that the hydrolysis of I is reversible (9).

When the methanol solution of I was saturated with oxygen before irradiation, both dechlorination and hydrolysis reactions were markedly inhibited. Loss of I and formation of II and IIIa occur at ~one-tenth the rate seen in the absence of oxygen. This is in agreement with the chloride yields already reported (3). Similarly, when II was irradiated in an oxygen-saturated solution, the dechlorination was inhibited, and only product IV was detected.

Photolysis of Hydrochlorothiazide in an Aqueous System—The solubility of I in pure water is relatively low, and the addition of 5% methanol was necessary to achieve the concentration of 0.5 m*M* used for the irradiation. Under these conditions and in the absence of oxygen, photodecomposition of I occurred at ~50% the rate in oxygen-free methanol, as shown in Fig. 2. It should be noted that the chloride ion appeared at the same rate in both solvents (3). The product distribution is seen to be different, with the hydrolyzed and dechlorinated IV as the dominant product. A relatively minor amount of the intermediate II was found, suggesting that dechlorination of I was occurring first followed by rapid hydrolysis. The other significant product was suspected of being VIb on the basis of its chromatographic retention, but samples collected were insufficient for direct MS confirmation. Molecular ions corresponding to permethylated compounds II, IV, and VIb were found by GC–MS analysis.

When the system was saturated with oxygen before irradiation, IV was the only product detected following a much slower reaction.

When the direct photolysis of II was examined in the aqueous system in the absence of oxygen, IV and the compound suspected of being VIb were found in the approximate ratio of 3:1.

Mechanism of the Photodegradation—The dechlorination step in the photolysis of both I and II leads to either reduction (Aryl-H) or substitution (Aryl-OR) involving the solvent ROH, as observed for other chloroaromatic compounds (8, 10). Additionally, photohydrolysis of the thiadiazine ring of I occurs, as has been observed for some other drugs susceptible to alkaline hydrolysis such as pentobarbitone (11) and indapamide (12).

It was proposed (8, 13) that photoreduction and photosubstitution of simple chloroaromatic compounds in methanol involves the formation of a pair of radical ions from the triplet state. The precursor of the reduction product (Aryl-H) is suggested to be a radical anion (Aryl-Cl^{-}), while a radical cation (Aryl-Cl^{+}) is postulated as the precursor of the substitution product (Aryl-OR). Alternatively, flash photolysis experiments with chlorpromazine in 2-propanol (10) led to the suggestion that a direct homolysis of the C–Cl bond occurs from the triplet state. We have observed (3) that chlorpromazine is moderately active as a triplet state photosensitizer in methanol solution, while in aqueous solution the predominance of photoionization as the primary photochemical event with phenothiazines (14) results in a significantly lower rate for this type of reaction.

Photoionization is reported for other heteroaromatic compounds not containing chlorine substituents *e.g.*, 4-hydroxybenzothiazole (15). The cation radical of chlorpromazine was postulated as the main initiating species of the photopolymerization of acrylamide following studies in cationic and anionic surfactants (16).

In view of the information available on the photochemistry of chlorpromazine, it was used as a standard against which the reactivity of other drugs might be compared. Hydrochlorothiazide was found to react in the same manner as chlorpromazine in triplet state photosensitization (3). The rates of photopolymerization of acrylamide in aqueous buffer and methanol solutions, sensitized by I, II, IIIa, and chlorpromazine were

measured, as shown in Table I. Since these four compounds have similar UV absorption characteristics, the rates of polymerization should provide an approximate indication of relative radical yields and reactivity. In aqueous solution the values of the rates suggest that cation radical production does not depend upon loss of chloride, as evidenced by the comparatively high rate sensitized by IIIa. However, in methanol solution the three compounds that lose chlorine show about the same rate of polymerization, a higher value than that for IIIa.

On the basis of the above results, the primary photochemical processes occurring on irradiation of hydrochlorothiazide appear similar to those found for chlorpromazine, with photoionization predominating in aqueous solution and triplet state formation mainly in methanol. However, it is possible that the polymerization technique is not an adequate indicator of radical ion formation in methanol where ion recombination or reaction with solvent may occur more readily than in water. A phenomenon that is indicative of excited state electron transfer is the quenching of fluorescence by an electron donor (17). We have observed that the fluorescence of I (50 μM in methanol) is quenched to 50% by 6 mM triethylamine. In contrast, the fluorescence of chlorpromazine is unaffected (18).

The fact that hydrolysis of I is stimulated by irradiation implies the formation of a cation radical in the thiadiazine ring, thereby rendering it more susceptible to attack by nucleophiles. This is most evident in the aqueous system where the expected intermediate IIIb was not detected. The dechlorination is presumably effected by the solvated electron formed in the photoionization.

In methanol the formation of cation radicals is indicated by the presence of significant amounts of the hydrolyzed compound II. However it is not clear if the cation radicals result from photoionization or radical-ion-pair formation from the triplet state. Flash photolysis experiments are needed to clarify the primary photochemical events. However, as pointed out elsewhere (10, 16), it is believed that the formation of free radicals following absorption of UV radiation is the significant factor in the initiation of a photobiological effect.

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ACKNOWLEDGMENTS

This work was made possible by the award of a Colombo Plan Scholarship to Swasono R. Tamat, and the generous assistance of Ciba-Geigy, Sydney.

NOTES

Evaluation of Various *N*-Substituted Azaspiranedione Derivatives as Potential Antimicrobial Agents

K. R. SCOTT *x, POLEDI G. KENNEDY *, MURRAY KEMP *, VASANT G. TELANG *, and H. W. MATTHEWS ‡

Received September 28, 1981, from the **College of Pharmacy and Pharmacal Sciences, Department of Biomedicinal Chemistry, Howard University, Washington, DC 20059*, and the ‡*Southern School of Pharmacy, Department of Biomedical Sciences, Mercer University, Atlanta, GA 30312.* Accepted for publication March 3, 1982.

Abstract □ A series of *N*-substituted hydrazines were condensed with various spiro[4.5] and [5.5]anhydrides and the resultant *N*-substituted azaspiranediones were evaluated for antimicrobial activity. None displayed any significant activity in a variety of organisms tested.

Keyphrases □ Antimicrobial agents—potential, evaluation of various *N*-substituted azaspiranedione derivatives □ Azaspiranedione—evaluation of various derivatives as potential antimicrobial agents

Previous works (1, 2) have shown a wide variety of biological effects for the azaspiro nucleus. It was of interest to extend this work to various 2-substituted-2-azaspiro-[4.5]decane-1,3-diones (I) and 3-substituted-3-azaspiro-[5.5]undecane-2,4-diones (II) and to evaluate these hydrazinoimides as potential antimicrobial agents.

It has been demonstrated (3–6) that in the fused 4-azacholestane ring various 4-alkyl-substituted derivatives

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