Photosensitization by Drugs

DOUGLAS E. MOORE

Abstract □ UV irradiation (365 nm) of air-saturated methanol solutions of 20 drugs absorbing in the 300–400-nm region gave rise to oxygen uptake, as determined with a polarographic oxygen electrode. The drugs were tested for photosensitizing capability by either a Type I (free radical) or a Type II (singlet molecular oxygen) mechanism. This testing was done by the inclusion of either acrylamide or 2,5-dimethylfuran in the irradiated drug solution, with observation of the subsequent polymerization or oxidation, respectively. Phenothiazine and thiazide derivatives appear capable of photosensitization by both mechanisms; promethazine, trifluoperazine, and furosemide show relatively high reactivity. Diazepam (weak), hexachlorophene, aminacrine, pyrilamine, tetracycline, demeclocycline, quinine, and anthracene (strong) react only by a Type II mechanism, with a photosensitizing efficiency increasing in the order given. A correlation appears to exist with reports of *in vivo* photosensitivity.

Keyphrases □ Photosensitization—various drugs irradiated, polymerization of acrylamide determined by dilatometry or oxidation of 2,5-dimethylfuran determined using polarographic oxygen electrode □ UV irradiation—effect on various drugs, polymerization of acrylamide determined by dilatometry or oxidation of 2,5-dimethylfuran determined by polarographic oxygen electrode □ Polarography—oxygen electrode used to determine oxidation of 2,5-dimethylfuran by various drugs after UV irradiation □ Oxygen uptake—various drugs after UV irradiation, oxidation of 2,5-dimethylfuran determined using polarographic oxygen electrode

Certain drugs and chemicals increase the sensitivity of the skin to solar radiation (1, 2). While it is possible to avoid such side effects with the appropriate topical sunscreens or even by systemic treatment (3), a photochemical basis is needed from which to classify a drug as a potential photosensitizer.

The mechanism of the adverse reaction is believed to be initiated by the photosensitized oxidation of skin components, with the drug functioning as the photosensitizing agent. Implicated in this process are drugs with significant light absorption in that portion of the solar spectrum reaching the earth's surface (280–800 nm). Window glass, opaque to radiation below about 310 nm, is said to afford protection from the photosensitizing effects of several drugs (2).

The mechanisms by which photooxidation may occur were classified as follows (4). Type I is a free radical chain process generally termed autoxidation. Type II involves excited singlet molecular oxygen and is termed oxygenation. Recently, the tetracyclines, with absorption maxima around 370 nm, were shown (5) to act as photosensitizers by a Type II mechanism. Chlorpromazine, however, on irradiation (6), produced free radicals as well as singlet molecular oxygen, thereby implying both Type I and II mechanisms of photooxidation. The Type I and II mechanisms may relate to the photoallergic and phototoxic effects of a photosensitizing drug, respectively (6).

The present investigation examined the effects of UV irradiation on compounds with light absorption in the 300–400-nm region. Included were some phenothiazine and thiazide derivatives reported to have photosensitizing effects in humans. All irradiations were performed using a glass filter to restrict the incident light to wavelengths above 310 nm. Two substrates were employed to enable classification of the mechanism of a drug's participation, if any, in photooxidations. The substrates were 2,5-dimethylfuran, an efficient acceptor for singlet oxygen (7), and acrylamide, an effective free radical scavenger thereby undergoing polymerization (8).

EXPERIMENTAL

Apparatus—The experimental design was based on the polarographic oxygen electrode and irradiation apparatus used successfully to measure oxygen uptake in both the Type I photooxidation of benzaldehyde (9) and the Type II photooxidation of the tetracyclines (5). The cylindrical reaction vessel (30-ml volume) was constructed[1] of spectroscopic grade silica with plane parallel faces 38 mm in diameter and 20 mm apart. Two standard taper joints carried the oxygen electrode[2] and a belt-driven stirrer for oxygen uptake measurements.

For free radical polymerization measurements, the reaction vessel was converted into a dilatometer by fitting a glass stopper and a 100-mm length of 0.4-mm bore capillary glass tubing to the standard taper joints.

A glass filter[3] with 28% transmittance at 365 nm and an overall transmission range of 310–420 nm and a screen with a 35-mm diameter aperture were positioned between the reaction vessel and the irradiation source[4]. The whole apparatus was immersed in a thermostat at 30.0 ± 0.05°.

Materials—2,5-Dimethylfuran[5] was purified immediately before solution preparation by twice distilling at room temperature at a pressure of 10^{-4} mm Hg on an all-glass high vacuum line. Acrylamide[6] was twice recrystallized from distilled chloroform and dried under vacuum (mp 85°). Only when these purification procedures were adopted did the control irradiations of solutions not containing potential sensitizers show negligible reaction.

The drugs tested for photosensitizing ability (Tables I–III) were the purest grades available commercially. All irradiations were performed in solution in analytical reagent grade methanol[7]. In this solvent, the photochemistry is simpler than in aqueous solution.

Procedures—Three situations were studied for each drug.

Irradiation of Drug Alone—In this procedure, 1 ml of freshly prepared drug solution (2–8 m*M* in methanol) was added to 50 ml of air-saturated methanol at 30°. The mixture was quickly transferred to the reaction vessel, and the oxygen uptake was measured after irradiation as previously described (9). The absorption spectrum of the reaction mixture was recorded[8] both before and after irradiation.

Irradiation of Drug plus 2,5-Dimethylfuran—This procedure was identical with that for drug alone except that 50 ml of 2,5-dimethylfuran solution (0.0936 *M*) in methanol was used.

Irradiation of Drug plus Acrylamide—A weighed quantity (10–20 mg) of drug was dissolved in 50 ml of methanol in a 250-ml round-bottom flask, modified by addition of a sidearm to the neck. Solid acrylamide (0.300 g) was weighed into the dry reaction vessel, which was then connected to the sidearm. The flask was then connected to a high-vacuum line, and the contents were deaerated by three freeze–pump–thaw cycles. After deaeration, the flask was tilted so that the drug solution flowed into the reaction vessel, dissolving the acrylamide. The reaction vessel was removed from the sidearm, and the dilatometer capillary was quickly inserted. The reaction vessel was then placed in its irradiation position in the thermostat, and the movement of the liquid level in the capillary was observed with a cathetometer.

[1] Thermal Syndicate Ltd., Wallsend, England.
[2] Type E5046, Radiometer, Copenhagen, Denmark.
[3] Corning CS 7-37.
[4] Engelhard Hanovia 125-w medium pressure mercury lamp.
[5] Fluka AG, Switzerland.
[6] Eastman Kodak, Rochester, N.Y.
[7] Ajax Chemicals, Sydney, Australia.
[8] Varian Techtron model 635 UV–visible spectrophotometer.

Table I—Photooxidation of Phenothiazine Derivatives

Compound	λ_{max}, nm	Absorbance at 365 nm	Rate of Oxygen Uptake, μmoles/liter/min: Compound Alone (1.28×10^{-4} M)	Rate of Oxygen Uptake, μmoles/liter/min: Compound (1.28×10^{-4} M) plus Dimethylfuran (0.0936 M)
Promethazine hydrochloride	303	0.008	2.7	16.2
Promazine hydrochloride	304	0.010	2.4	13.4
Chlorpromazine hydrochloride	308	0.015	3.4	13.7
Prochlorperazine maleate	312	0.030	3.0	25.1
Trifluoperazine hydrochloride	313	0.072	5.9	44.7
Thioridazine hydrochloride	315	0.018	4.3	16.9
Chlorpromazine sulfoxide hydrochloride	342	0.012	2.5	8.4

RESULTS AND DISCUSSION

Photooxidation—The oxygen uptake rates measured for the UV-irradiated drug solutions in the absence and presence of dimethylfuran are recorded in Table I for phenothiazine derivatives, in Table II for thiazides and related compounds, and in Table III for miscellaneous compounds with absorption in the 300–400-nm region. Each value is the mean of at least three determinations with a maximum standard deviation of 5%. There was no dark reaction, and the oxygen uptake was linear with time, *i.e.*, zero order, indicating that the absorption of light was the rate-limiting factor in all cases except furosemide.

To assess relative photosensitizing ability, the drug concentration was selected to give an absorbance of approximately 0.5 at its wavelength of maximum absorption, λ_{max}, in the 300–400-nm region. For most drugs, the λ_{max} corresponds to the least intense absorption and in some cases is only a shoulder (sh) on the principal absorption peak. Comparisons are perhaps more realistically based on the absorbance of the drug solution at 365 nm, corresponding to the major irradiating wavelength.

The output of the medium pressure mercury lamp is discontinuous, the relevant lines being at 303, 313, 334, 365, and 405 nm with relative intensities of 23.9, 50, 9.3, 100, and 42, respectively (10). The presence of the glass filter means that the relative intensities incident on the reaction vessel are 0, 4.8, 5.3, 100, and 10.2, respectively. Therefore, most drugs listed in Tables I–III experience only a relatively small amount of light of a wavelength near to their λ_{max}. However, for most, the absorption band is broad, meaning that the 313- and 334-nm light is absorbed and that there is some residual absorbance at 365 nm (Tables I–III).

Phenothiazines—The phenothiazine derivatives react approximately in accordance with their absorbance at 365 nm (Table I). The UV absorption characteristics of these compounds are determined principally by the phenothiazine nucleus, with only minor effects due to the substituents (11). Therefore, the mechanism of energy transfer through the excited singlet and triplet states is expected to be similar within the phenothiazine series. Close similarities were found in the transient species formed on flash photolysis of chlorpromazine and promazine (6).

Chlorpromazine sulfoxide was identified as an oxidation product of chlorpromazine (12), yet it appears to be oxidized in proportion to its absorbance at 365 nm. In aqueous solution, chlorpromazine and its sulfoxide degrade in the presence of oxygen and UV light below 365 nm to give apparently similar products (12). Furthermore, with extended periods of irradiation of aqueous chlorpromazine solutions, numerous products are found (13). While some of these may be secondary in nature, the sulfoxide clearly is an intermediate rather than the final product.

Changes in the absorption spectrum of the irradiated phenothiazine were most marked with promethazine and prochlorperazine. Promethazine was the most reactive compound studied since the oxygen uptake rate (Table I) exceeded what might be expected from the absorbance at 365 nm. After 1 hr of irradiation of promethazine alone, the absorbance increased by 20% at the λ_{max} and from 0.01 to 0.18 at 342 nm, which could be indicative of sulfoxide formation. Furthermore, the solution developed a pink color, with a broad absorption centered on 506 nm, a phenomenon not seen with the other phenothiazines.

Prochlorperazine, on irradiation, showed the development of a broad shoulder at 330 nm and the narrowing of the original peak at 312 nm. Pending more study, including the analysis of transient species, no conclusions can be made. Spectral changes occurred for promazine, chlorpromazine, thioridazine, and trifluoperazine but were minor in comparison. The reaction of chlorpromazine sulfoxide could be followed by the decrease in absorbance at 342 nm.

Thiazides—Chlorothiazide and hydrochlorothiazide are reported to be potent photosensitizers, yet in the system studied here they showed less reactivity than the phenothiazines. This result may have been due in part to lower absorbances at 365 nm, although the results for cyclopenthiazide do not support that suggestion.

Hydrochlorothiazide and cyclopenthiazide had clearly defined absorption peaks at 318 nm. Upon irradiation, the peak structure gradually submerged to become a shoulder on the major absorption centered on about 270 nm, thereby resembling the chlorothiazide spectrum.

Chlorthalidone and furosemide, while not thiazides, were included because of similarities in their properties. Chlorthalidone had essentially zero absorbance above 300 nm, yet a very slight reactivity was observed. This substance was strongly susceptible to the shorter wavelength UV light, so this result may represent a very small amount transmitted through the filter.

Furosemide had a relatively high absorbance at 365 nm and a corresponding high photosensitizing capability. One unusual feature of the irradiation of furosemide alone was an initial rapid oxygen uptake, gradually decreasing to the steady value recorded in Table II. The initial reaction may have been due to formation of free radicals which were rapidly scavenged by oxygen molecules. The steady rate may, in fact, correspond to a secondary reaction. The overall reaction also could be

Table II—Photooxidation of Thiazides and Related Compounds

Compound	λ_{max}, nm	Concentration $\times 10^4$ M	Absorbance at 365 nm	Rate of Oxygen Uptake, μmoles/liter/min: Compound Alone	Rate of Oxygen Uptake, μmoles/liter/min: Compound plus Dimethylfuran (0.0936 M)
Chlorthalidone	284	1.5	<0.002	0.4	1.4
Hydrochlorothiazide	318	1.32	0.006	0.9	5.0
Chlorothiazide	280, 320 (sh)	1.30	0.006	1.4	3.4
Cyclopenthiazide	318	1.44	0.002	1.4	10.5
Furosemide	340	0.95	0.185	5.5	81.2

Table III—Photooxidation of Selected Compounds with UV Absorption in the 300–400-nm Region

Compound	λ_{max}, nm	Concentration $\times 10^4$ M	Absorbance at 365 nm	Rate of Oxygen Uptake, μmoles/liter/min: Compound Alone	Rate of Oxygen Uptake, μmoles/liter/min: Compound plus Dimethylfuran (0.0936 M)
Diazepam	316	1.93	0.005	0	3.9
Chlordiazepoxide	350 (sh)	1.90	0.335	1.8	2.0
Pyrilamine maleate	308	1.03	<0.002	1.1	4.8
Hexachlorophene	300, 317 (sh)	1.30	0.006	0.6	2.7
Quinine hydrochloride	335	0.84	0.003	7.8	38.8
Tetracycline hydrochloride	362	0.40	0.528	0.5	10.9
Demeclocycline hydrochloride	368	0.40	0.510	0.6	12.5
Anthracene	340, 357, 377	0.43	0.18	1.4	112.0
Aminacrine hydrochloride	382	0.68	0.210	0.8	4.1

Table IV—Photoinitiated Polymerization of Acrylamide Solutions Containing Phenothiazine and Thiazide Derivatives

Drug	Dilatometer Contraction Rate, mm/hr
Promethazine hydrochloride	60
Promazine hydrochloride	40
Chlorpromazine hydrochloride	26
Prochlorperazine maleate	38
Trifluoperazine hydrochloride	48
Thioridazine hydrochloride	19
Hydrochlorothiazide	10
Chlorothiazide	9
Cyclopenthiazide	11
Furosemide	28

followed by the decrease in absorbance of the peak at 340 nm.

Other Compounds—Of the substances listed in Table III, only quinine and anthracene showed a high photosensitizing ability. These compounds fluoresced strongly and also were able to transfer a considerable amount of energy to molecular oxygen. Aminacrine, also a strong fluorescer, was relatively inefficient as a photosensitizer.

Diazepam was a very weak sensitizer in neutral methanol solution, while chlordiazepoxide did not appear to sensitize the photooxygenation of dimethylfuran. In acidified methanol solution, the absorption spectra of both diazepam and chlordiazepoxide changed dramatically and photosensitization occurred, although it was complicated by acid-catalyzed hydrolysis.

Results for the tetracyclines reported previously (5) were obtained with a different reaction vessel. The values in Table III for tetracycline and demeclocycline were redetermined so that these compounds could be compared more readily as photosensitizers.

Other compounds tested in this system and found to have zero reactivity were warfarin sodium, dicumarol, and sodium aminosalicylate. Many more substances with absorption in the near UV region could be examined. One difficulty to overcome is the low solubility in the reaction system, although some heterogeneous techniques have been developed for such a situation (14).

The conclusion to be drawn from the results in Tables I–III is that every compound listed except chlordiazepoxide appears capable to a greater or lesser extent of photosensitizing oxygenation of dimethylfuran by the Type II mechanism involving singlet molecular oxygen. This finding does not necessarily preclude the possibility of direct energy transfer between the sensitizer and dimethylfuran, a process that can occur at these higher concentrations of the acceptor (15).

Photopolymerization—The observation that many compounds absorbed oxygen when irradiated in the absence of substrate is indicative of one or both of the following possibilities. Either (*a*) the drugs act as photosensitizers for their own oxygenation *via* a singlet oxygen mechanism, the rate being small because of the relatively low concentration of drug in solution, or (*b*) the drugs, on irradiation, yield free radicals that are scavenged by oxygen molecules with a 1:1 stoichiometry.

The generation of free radicals is well known in the phenothiazine derivatives (11), so these compounds served as references for comparison of other series. The measurement of oxygen uptake using an autoxidizable substrate such as benzyl alcohol or benzaldehyde is conveniently done in the apparatus but does not give an unambiguous indication of free radical formation because of the possible simultaneous occurrence of mechanism *a* or *b*.

For these reasons, the monomeric substrate acrylamide was used in deaerated conditions as a detector of free radicals. All compounds (Tables I–III) were tested with acrylamide, and Table IV lists those that photosensitized the polymerization. The polymerization reaction was evident in two ways: by the appearance of turbidity in the reaction mixture due to the insolubility of polyacrylamide and by the contraction in volume of the reaction mixture observed in the dilatometer capillary. The turbidity meant that only initial rates could be measured from the initial linear portion of the contraction–time plot. No reaction was observed when the acrylamide solution was irradiated by itself.

The fact that molecules such as anthracene show a very high photosensitizing capability with dimethylfuran but are unreactive with acrylamide indicates that the photopolymerization system is a reasonable test of radical formation and not just energy transfer.

The phenothiazines and thiazides are the only groups of drugs tested for which free radical formation appears to occur on irradiation. Within the phenothiazine series, promethazine and promazine displayed a reactivity out of proportion to their absorbance at 365 nm. Davies *et al.* (6) suggested that irradiation of chlorpromazine promotes carbon–chlorine bond fission, as shown by titration of the free chloride ion formed in the solution. The high reactivity of promethazine and promazine, which do not contain chlorine in the 2-position, suggests that other mechanisms of radical formation are possible. Further work, including an electron spin resonance study of the radicals, is required for clarification of the reaction mechanisms.

CONCLUSIONS

Phenothiazine and thiazide derivatives are capable of photosensitization by both Type I (free radical) and Type II (singlet oxygen) mechanisms. Reports of *in vivo* photosensitivity (2) indicate that both photoallergic and phototoxic reactions have been seen with these groups of drugs. Anthracene and the tetracyclines, which have been shown to participate only in a Type II mechanism, are reported to have the more direct phototoxic effect only. Some adverse reactions to quinine are optical in nature (16) but have not been specifically defined as photosensitivity.

Of particular interest is the thiazide-like substance furosemide, which shows a photosensitizing capability similar to the phenothiazines. Perhaps the reported skin rash reaction among patients taking furosemide (17) has a photosensitivity component. The spectral properties of the substances involved in this study are likely to vary depending on the polarity of the system in which they are incorporated. Nevertheless, it is unlikely that window glass will afford complete protection from photosensitivity reactions due to the compounds listed in Tables I–III.

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