J. Pharm. Pharmacol. 1980, 32: 172–177 Received June 8, 1979

Photosensitization by drugs: photolysis of some chlorine-containing drugs

DOUGLAS E. MOORE* AND SWASONO R. TAMAT

Pharmacy Department, The University of Sydney, Sydney NSW 2006, Australia

Irradiation with ultraviolet light of chlorpromazine, prochlorperazine, frusemide or hydrochlorothiazide in either aqueous or methanol solution yielded free chloride ion. Potentiometric methods were used to detect one mol of chloride ion produced per mol of drug irradiated in deoxygenated solution, with a concomitant equimolar appearance of hydrogen ion. Saturation of the solutions with oxygen strongly inhibited the photolysis reaction in methanol but only partially inhibited the production of Cl^- and H^+ in aqueous solution. The oxidation of 2,5-dimethylfuran is photosensitized by these drugs more effectively in methanol compared with aqueous solution. The photochemical behaviour is consistent with a photodissociative process occurring predominantly in methanol while photoionization predominates in aqueous media. No chloride ion was detected after extended irradiation of chlortetracycline, demeclocycline, chlordiazepoxide and hexachlorophane. The photolability of the chlorine in the compounds tested correlated with their ability to photosensitize oxidation by the Type I (free radical) mechanism.

Of the many drugs reported to cause adverse photosensitivity effects (Magnus 1976), a significant number contain one or more chlorine atoms as substituent(s). The photolabile nature of chlorine has been demonstrated in many chloroaromatic compounds (Sammes 1973). Similar photodechlorination of relevance to clinical photosensitization occurs with tetrachlorosalicylanilide (Davies et al 1975) and chlorpromazine (Grant & Greene 1972; Davies et al 1976; Nejmeh & Pilpel 1978; Rosenthal et al 1978; Bunce et al 1979).

It has been suggested that the free radical formed on dissociation of the C-Cl bond is able to combine readily with proteins and thus remain localized in the skin to prolong the photobiological effect (Grant & Greene 1972; Davies et al 1976).

Chlorine-containing drugs were among a number of chemicals shown to be capable of photosensitizing oxidation reactions in vitro (Moore 1977). Such photodynamic action is believed to be the initiating step in adverse photosensitivity action (Spikes 1977) and can occur by a Type I (free radical) and/or Type II (singlet molecular oxygen) mechanism. In the present investigation some chlorine-containing compounds implicated in photosensitivity reactions have been irradiated. Only those which photosensitize oxidation by the Type I mechanism yielded free chloride ion.

* Correspondence.

METHODS

Drug samples were as described previously (Moore 1977). All other chemicals were of analytical grade.

Drug solutions (5×10^{-4} M) in methanol or doubly distilled water were presaturated for 90 min with nitrogen or oxygen as desired in a cylindrical vessel constructed from Pyrex glass (diameter 75 mm, depth 30 mm, volume 135 ml) set in a thermostatted water bath at 30°C. The solution was then irradiated using a medium pressure mercury lamp (Hanovia, 125 W). The gas flow was maintained during irradiation, thus stirring the solution. After photolysis, aliquots were taken, diluted with water, and assayed potentiometrically using a Radiometer TTT 1 Automatic Titrator, with a saturated calomel reference electrode connected via an ammonium nitrate salt bridge to the titration vessel.

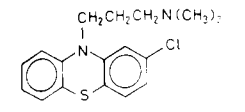
Chloride ion was assayed by titration with 0·025 M silver nitrate with a silver indicating electrode. Hydrogen ion was assayed similarly with 0·025 M sodium hydroxide and glass electrode as indicating electrode. Triplicate titrations were carried out for each ion for each irradiation and compared with titrations for unirradiated solutions.

The quantum yield for chloride ion production was determined using the ferri-oxalate actinometer (Calvert & Pitts 1966) and the double cell procedure described by Davies et al (1976). In this part of the work, silica cells were used with a Pyrex filter. The drug solutions were degassed on a vacuum line with three freeze-pump-thaw cycles.

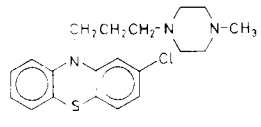
Rates of oxygen uptake at 30°C were determined in the presence and absence of 2,5-dimethylfuran using the polarographic oxygen electrode as described previously (Moore 1977) with drug concentration $1{\cdot}3 \times 10^{-4}$ M. Aqueous systems for these experiments were maintained at pH 7·0 with 0·05 M phosphate buffer.

RESULTS

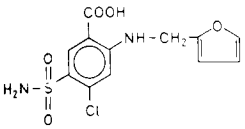
The u.v. absorption characteristics in the region above 300 nm are given in Table 1 for the substances tested. The concentration of irradiated drug solution (5×10^{-4} M) was chosen to give an absorbance of approximately 2 at the wavelength of maximum absorption (λ_{max}) in Table 1. While the major output of the medium pressure mercury lamp is 365 nm (relative intensity 100), there is a significant emission at 313 nm (rel. int. 50), transmitted to the extent of 56% by the 2 mm thick Pyrex glass of the irradiation vessel (Calvert & Pitts 1966).



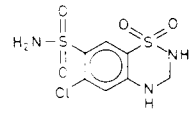
CHLORPROMAZINE



PROCHLORPERAZINE



FRUSEMIDE



HYDROCHLOROTHIAZIDE

Chlorpromazine

Davies et al (1976) found that irradiation of chlorpromazine in deoxygenated propan-2-ol solution yielded free chloride ion and concomitantly an equimolar amount of hydrogen ion with a quantum yield of 0·12. Bunce et al (1979) reported a value of 0·46 in degassed acetonitrile–water (4:1). We found the quantum yield to be the same within experimental error, 0·65 ± 0·15, in three different degassed solvents, viz., propan-2-ol, methanol and water. Fig. 1 shows the kinetics of Cl^- and H^+ production in methanol and water.

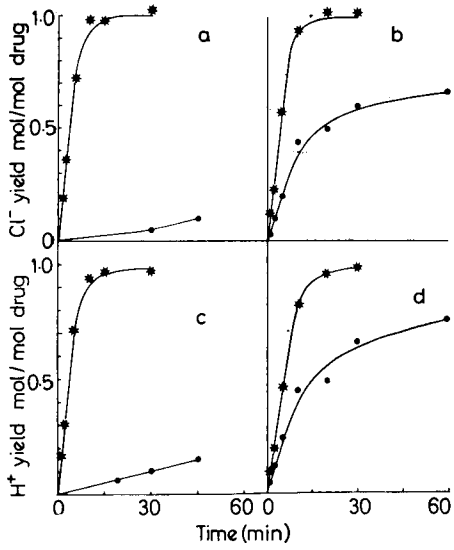


FIG. 1. Photolysis of chlorpromazine in solution (5×10^{-4} M) saturated with nitrogen (*) or oxygen (●). Chloride ion yield in (a) methanol (b) water, Hydrogen ion yield in (c) methanol (d) water.

When air or oxygen is introduced the production of chloride and hydrogen ions is inhibited, but not completely. The effect of oxygen is much smaller in aqueous solution. Incomplete deoxygenation would therefore have an effect dependent on the solvent and may account for the varying quantum yields reported from different laboratories. The solubility of oxygen in methanol (0·0096 M) is markedly greater than in water (0·0011 M) (Int. Crit. Tables) although one would not expect this to be a limiting factor at the drug concentration used, and in view of the continual bubbling of oxygen through the solution.

Table 2 shows the initial rate data for the consumption of oxygen from air-saturated solutions of chlorpromazine in the absence and presence of 2,5-dimethylfuran, an efficient acceptor for singlet molecular oxygen (Foote 1968). While the chlorpromazine by itself consumed approximately the same amount of oxygen in methanol and water, its ability to photosensitize oxidation is significantly less

Table 1. *U.v. absorption above 300 nm of some chlorine containing drugs.*

Compound	In methanol λ_{max}, nm	In methanol ε_{max}, m^2mol^{-1}	In aqueous buffer pH 7·0 λ_{max}, nm	In aqueous buffer pH 7·0 ε_{max}, m^2mol^{-1}
Chlorpromazine hydrochloride	308	450	307	430
Prochlorperazine maleate	312	466	308	488
Frusemide	343	568	330	580
Hydrochlorothiazide	318	320	318	320
Chlortetracycline hydrochloride	373	1350	368	1120
Demeclocycline hydrochloride	370	1510	372	1570
Chlordiazepoxide	310(sh)	740	308(sh)	860
	350(sh)	310	350	440
Hexachlorophane	300	634	302	680

(sh) means shoulder on main absorption peak of lower λ_{max}.
Errors: in λ_{max} ± 1 nm; in ε_{max} ± 3%.

Table 2. Rates of oxygen uptake by irradiated drug solutions.

	Rate of oxygen uptake μmol l^{-1} min^{-1}			
	Drug alone (0·13 mM)		Drug + DMF (1 mM)	
	in buffer	in methanol	in buffer	in methanol
Chlorpromazine	3·0	3·4	5·3	13·7
Prochlorperazine	1·4	3·0	2·5	25·1
Frusemide	0·1	5·5	0·1	81·2
Hydrochlorothiazide	0·4	0·9	0·4	5·0

in water solutions. In the irradiation of $1{\cdot}3 \times 10^{-4}$ M chlorpromazine in air-saturated buffer, the oxygen consumption initially followed a zero order rate before diminishing sharply. The amount of oxygen consumed in the zero order section was about 0·45 mol mol^{-1} of chlorpromazine. There was no apparent correlation with the amount of Cl^- produced in the same time (0·7 mol mol^{-1} chlorpromazine). The u.v. absorption spectrum of chlorpromazine in air-saturated buffer or methanol changed upon irradiation, but with a loss in definition. The minor peak at 307 nm became merged with the major peak at 280 nm. The solution developed a faint pink colour which disappeared on standing, indicating the presence of the semiquinone-type free radical intermediate leading to the chlorpromazine-5-oxide (Merkle & Dischер 1964). The spectral changes did not correlate with the oxygen consumption, suggesting the formation of more than one product. Davies et al (1976) reported that, in propan-2-ol, oxygen is not consumed nor is there any spectral change on irradiation of oxygen-saturated solutions.

Prochlorperazine

Chloride and hydrogen ions were produced on irradiation (Fig. 2), at approximately half the rate observed with chlorpromazine. The quantum yield for chloride ion formation was 0·15 ± 0·04 reflecting a higher absorbance of the solution. The pattern of inhibitory effects of oxygen and the spectral changes upon irradiation were similar to those observed with chlorpromazine. However, from Table 2 it can be seen that in methanol prochlorperazine is a stronger photosensitizer of dimethylfuran oxidation than is chlorpromazine.

Frusemide

Oxygen-free solutions of frusemide in methanol produced hydrogen and chloride ions concomitantly on irradiation with a quantum yield of 0·40 ± 0·08. The aqueous system studied contained 25% methanol because of the low solubility of frusemide in pure water. Nevertheless the same quantum yield and complete dechlorination was observed (Fig. 3). In oxygenated solutions, Cl^- production was inhibited more than H^+ production in methanol. In the water-methanol (3:1) mixture H^+ production appeared the same irrespective of the presence of oxygen.

The consumption of oxygen (Table 2) is close to zero in the aqueous system (contains 2% methanol) compared with an extremely efficient photosensitizing capability in methanol solution.

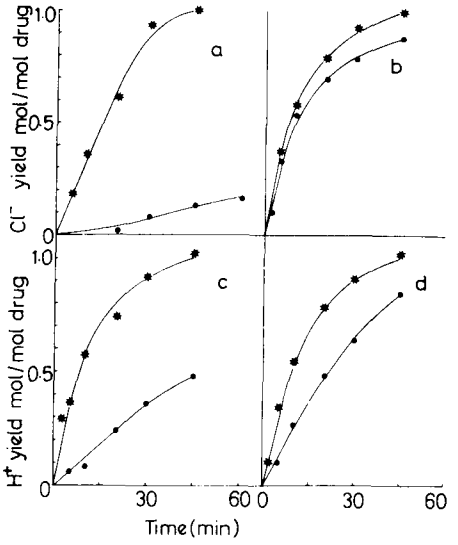


FIG. 2. Photolysis of prochlorperazine in solution (5×10^{-4} M) saturated with nitrogen (*) or oxygen (●). Chloride ion yield in (a) methanol (b) water. Hydrogen ion yield in (c) methanol (d) water.

Hydrochlorothiazide

This compound has a lower absorbance in the 300–400 nm region than the above compounds but nevertheless slowly decomposes on irradiation (Fig. 4). The quantum yield for Cl^- production was 0·18 ± 0·05 in degassed solutions. To emphasise the much slower rates for Cl^- and H^+ production from hydrochlorothiazide, Fig. 4 is drawn to 120 min by which time 0·5 mol Cl^- or H^+ has been formed per mol of drug. Continuous irradiation to 300 min revealed a total dechlorination of hydrochlorothiazide. The inhibition due to oxygen followed the same pattern as for chlorpromazine and prochlorperazine. Hydrochlorothiazide also showed a slight photosensitizing capability in methanol (Table 2) but not in aqueous systems.

Other compounds

Chlortetracycline, demeclocycline, chlordiazepoxide and hexachlorophane have absorption maxima above 300 nm but did not produce chloride nor

hydrogen ion upon irradiation in methanol or aqueous solution. These compounds show very slow rates of oxygen uptake upon irradiation (Moore 1977), but the photolysis reaction does not appear to involve dissociation of chlorine.

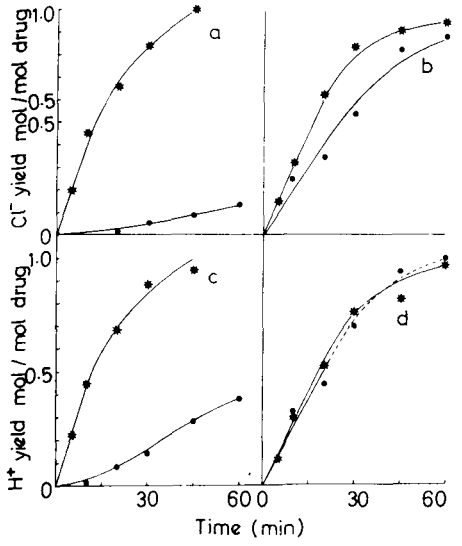


FIG. 3. Photolysis of frusemide in solution (5×10^{-4} M) saturated with nitrogen (●) or oxygen (*). Chloride ion yield in (a) methanol (b) water–methanol (3:1). Hydrogen ion yield in (c) methanol (d) water–methanol (3:1).

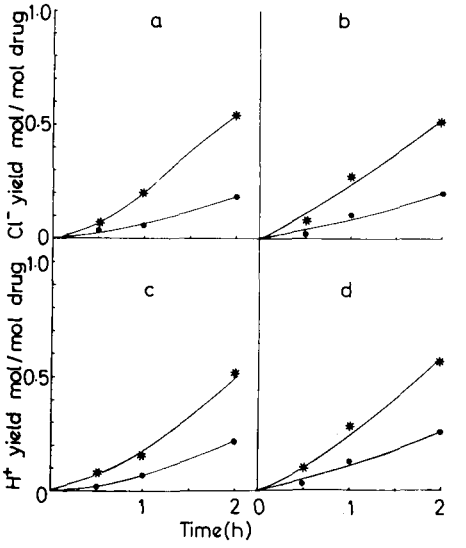


FIG. 4. Photolysis of hydrochlorothiazide in solution (5×10^{-4} M) saturated with nitrogen (*) or oxygen (●). Chloride ion yield in (a) methanol (b) water. Hydrogen ion yield in (c) methanol (d) water.

DISCUSSION

The photolability of the chlorine atom in chlorpromazine, prochlorperazine, frusemide and hydrochlorothiazide correlates with the ability of these compounds to photoinitiate polymerization of acrylamide as previously described (Moore 1977) On the other hand, chlortetracycline, demeclocycline, chlordiazepoxide and hexachlorophane neither give rise to free chloride nor initiate polymerization upon irradiation. This suggests that the product of the photochemical reaction initiates the polymerization, although it cannot be determined whether the initiation step is free radical or ionic in nature.

The fact that the quantum yield for chloride ion formation from each compound is similar in aqueous and methanol solutions might indicate that the primary photochemical process is the same in either solvent. However, the inhibitory effect of oxygen is markedly different in the two solvents, and it is unlikely that solubility differences can account for this. It is possible to understand the inhibition by oxygen in the case of chlorpromazine which has been the subject of considerable study, and it is probable that the mechanistic conclusions extend to the other compounds.

Recent flash photolysis experiments with chlorpromazine (Navaratnam et al 1978) show that the cation radical and the triplet state of chlorpromazine together with the hydrated electron are the species observed immediately upon irradiation. The relative yields of the cation radical and triplet state were found to depend on the nature of the solvent, with little or no photoionization occurring in propan-2-ol but large yields of electrons in aqueous media.

In oxygen-free solutions we find the HCl yield to be independent of the solvent used, and the other products are formed analogously:

(i) in propan-2-ol—promazine and 2-isopropoxypromazine (Davies et al 1976);
(ii) in methanol—promazine and 2-methoxypromazine (Rosenthal et al 1978);
(iii) in water—promazine and 2-hydroxypromazine (Grant & Greene 1972).

Davies et al (1976) proposed a direct homolysis of the triplet chlorpromazine

$$^3ClP \rightarrow Cl\cdot + P\cdot \qquad (1)$$

affording radicals which abstract hydrogen atoms from the solvent yielding the observed products.

On the other hand, electrons react with ground state chlorpromazine (Davies et al 1979)

$$e^- + Cl\,P \rightarrow Cl^- + P\cdot \qquad (2)$$

Thus the product profile is independent of the relative amount of chlorpromazine cation radical and triplet state initially formed.

Either the promazine radical ($P\cdot$) or the chlorpromazine cation radical (Cl $P\cdot$) would be capable of initiating polymerization reactions. We have observed polymerization photoinitiated by chlorpromazine in both methanol and aqueous systems (Moore & Burt to be published). The rate of polymerization in aqueous buffer (pH 7·0) is approximately 100 fold greater than in methanol indicating a greater reactivity or concentration of the radical species formed in water.

In the presence of oxygen the chlorpromazine triplet state energy is efficiently transferred with the formation of singlet molecular oxygen

$$^3\text{Cl } P + {}^3\text{O}_2 \rightarrow \text{Cl } P + {}^1\text{O}_2 \qquad (3)$$

This is verified by the subsequent oxidation of 2,5-dimethylfuran both in propan-2-ol (Davies et al 1976) and methanol (Moore 1977), and the strong inhibition of the Cl^- production.

In neutral aqueous solutions we observe only slight oxidation of 2,5-dimethylfuran photosensitized by chlorpromazine. This observation could be accounted for in terms of the low yield of chlorpromazine triplet state although singlet oxygen does display a shorter lifetime in aqueous media (Merkel & Kearns 1972). It is apparent that subsequent reactions of the chlorpromazine cation radical with oxygen lead to the sulphoxide in view of the product analysis (Felmeister & Discher 1964). To support this, the spectral changes we observed are similar to those occurring in the electrochemical oxidation of chlorpromazine (Merkle & Discher 1964).

Navaratnam et al (1978) showed that the half-life of the hydrated electron generated by irradiation of chlorpromazine in aqueous solution is diminished by oxygen saturation although the electron yield remains constant. Consequently the Cl^- yield through reaction (2) is only slightly affected by oxygen saturation.

It seems reasonable to extend the above explanation to other phenothiazines with 2-chloro substituents. Rosenthal et al (1978) found that 2-chlorophenothiazine upon irradiation in methanol was converted to phenothiazine (90%) and 2-methoxyphenothiazine (ca 2%). Bunce et al (1979) measured the quantum yield as 0·20 for photodechlorination of the same compound in acetonitrile-water, and concluded that the *N*-alkyl substituent is not a necessary requirement, but may accelerate the process of chlorine removal by an intramolecular electron transfer mechanism.

Although the full interpretation of the photoreactivity of frusemide and hydrochlorothiazide must await full product analysis and the identification of transient species by flash photolysis, it is probable that the nature of the dechlorination is similar to that occurring with the phenothiazines. Of particular interest to us is the inability of frusemide to photosensitize oxidation in aqueous solution while performing very effectively in methanol. Hydrochlorothiazide, on the other hand, shows a relatively slow reaction in all systems, which may be interpreted in part as due to a lesser absorption of the output of the u.v. lamp used for these experiments.

An earlier study of the photochemical degradation of frusemide in oxygenated buffer at pH 10 (Rowbotham et al 1976) using an irradiation time of 48 h concluded on the basis of spectral changes at around 270 nm that the product was 4-chloro-5-sulphoanthranilic acid, i.e., the chlorine remained intact. Associated with the photodechlorination, we have observed slight spectral changes at 271 nm of a similar nature and therefore believe Rowbotham et al to be erroneous in their product identification. Notwithstanding, we agree that the B.P. 1973 spectroscopic assay for parenteral solutions of frusemide is inappropriate to solutions which have been exposed to u.v. light. The same conclusion need not be extended to hydrochlorothiazide, as significant spectral changes at 273 and 323 nm (the B.P. specified wavelengths) were observed on photodechlorination.

Of the other four compounds studied, it is perhaps surprising that the chlorine substituents were stable to irradiation, particularly in view of the facile dechlorination observed for many *o*-, *m*- and *p*-chlorophenols (Pinhey & Rigby 1969). In chlortetracycline and demeclocycline the chlorine is bound to the hydroxylated aromatic residue which is considered to be responsible for the u.v. absorption at 370 nm (McCormick et al 1957; Mitscher et al 1968). Hexachlorophane similarly is phenolic in nature although its u.v. absorption barely extends into the range of radiation reaching the reaction vessel. In the case of chlorodiazepoxide the absorption quoted is strongly pH dependent and thus is probably not associated with the aromatic residue carrying the chlorine substituent.

Although the detailed nature of the mechanism by which the compounds studied here cause clinical photosensitivity reactions is by no means resolved, the photolability of the aromatic chlorine substituent

and the accompanying generation of free radicals in situ would appear to be an important precursor. If the photo-allergic reaction is initiated by free radicals as has been suggested, then it is clear why chlorpromazine is more strongly implicated in this regard. When the drug is located in a region where the concentration of oxygen is significant, the polarity of that environment would appear to determine whether the photoreaction occurs predominantly via free radical formation or molecular oxygenation of suitable oxidizable species.

Acknowledgements

Drug samples were kindly donated by May & Baker Australia, Hoechst Australia, Lederle Laboratories and Ciba-Geigy Australia.

S.R.T. is a recipient of a Colombo Plan Scholarship.

REFERENCES

Bunce, N. J., Kumar, Y., Ravanal, L. (1979) J. Med. Chem. 22: 202–204

Calvert, J. G., Pitts, J. N. (1966) Photochemistry, Wiley, New York, pp 696, 748, 783

Davies, A. K., Hilal, N. S., McKellar, J. F., Phillips, G. O. (1975) Br. J. Derm. 92: 143–147

Davies, A. K., Navaratnam, S., Phillips, G. O. (1976) J. Chem. Soc. Perkin Trans. 2: 25–29

Davies, A. K., Land, E. J., Navaratnam, S., Parsons, B. J., Phillips, G. O. (1979) J. Chem. Soc. Faraday Trans. 1, 75: 22–35

Felmeister, T., Discher, C. A. (1964) J. Pharm. Sci. 53: 756–762

Foote, C. S. (1968) Accounts Chem. Res. 1: 104–110

Grant, F. W., Greene, J. (1972) Toxicol. Appl. Pharmacol. 3: 71–74

McCormick, J. R. D., Fox, S. M., Smith, L. L., Bitler, B. A., Origoni, V. E., Muller, W. H., Winterbottom, R., Doerschuk, A. P. (1957) J. Am. Chem. Soc. 79: 2849–2858

Magnus, I. A. (1976) Dermatological Photobiology, Blackwell, Oxford, pp. 213–215

Merkel, P. B., Kearns, D. R. (1972) J. Am. Chem. Soc. 94: 1029–1030

Merkle, F. H., Discher, C. A. (1964) J. Pharm. Sci. 53: 620–623

Mitscher, L. A., Bonacci, A. C., Sokoloski, T. D. (1968) Antimicrob. Agents Chemother.: 78–86

Moore, D. E. (1977) J. Pharm. Sci. 66: 1282–1284

Navaratnam, S., Parsons, B. J., Phillips, G. O., Davies, A. K. (1978) J. Chem. Soc. Faraday Trans. 7, 74: 1811–1819.

Nejmeh, M., Pilpel, N. (1978) J. Pharm. Pharmacol. 30: 748–753

Pinhey, J. T., Rigby, R. D. G. (1969) Tetrahedron Lett.: 1271–1274

Rosenthal, I., Ben-Hur, E., Prager, A., Riklis, E. (1978) Photochem. Photobiol. 28: 591–594

Rowbotham, P. C., Stanford, J. B., Sugden, J. K. (1976) Pharm. Acta Helv. 51: 304–307

Sammes, P. G. (1973) in: Patai, S. (ed) The Chemistry of the Carbon-Halogen Bond, Wiley-Interscience, New York, chapter 11

Spikes, J. D. (1977) in: Smith, K. C. (ed) The Science of Photobiology, Plenum, New York, pp 87–112