ELSEVIER

International Journal of Pharmaceutics 183 (1999) 109–116

international journal of pharmaceutics

Photodegradation of amiloride in aqueous solution

Yuan Nian Benny Li, Douglas E. Moore *, Bruce N. Tattam

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

Received 26 October 1998; received in revised form 27 January 1999; accepted 28 January 1999

Abstract

The photodegradation of amiloride hydrochloride in deaerated aqueous solution at 30°C in the pH range 4.5–11.0 was studied by spectrophotometry and reversed-phase HPLC. The neutral form of the drug present in alkaline solution degraded approximately 3-fold faster than the cationic form. The quantum yields for photodegradation of neutral amiloride and its conjugate acid were determined using ferrioxalate actinometry to be 0.023 ± 0.002 and 0.009 ± 0.001, respectively. The initial photoreaction involves dechlorination of amiloride and the major product is *N*-amidino-3,5-diamino-6-hydroxylpyrazine-carboxamide, established by UV, ^{1}H and ^{13}C NMR, and chemical ionization-mass spectrometry. The mechanism of photolysis is postulated to involve cation radical formation that facilitates the dechlorination step. The photosensitizing activity of amiloride hydrochloride was tested by measuring (a) the rate of oxygen uptake in the presence of singlet oxygen substrates, 2,5-dimethylfuran or L-histidine, and (b) the rate of free radical polymerization of acrylamide, at 30°C in aqueous solution. Photosensitization by amiloride was concluded to occur predominantly via singlet oxygen rather than a free radical mechanism. However, amiloride is a much weaker photosensitizer than other diuretics such as frusemide and hydrochlorothiazide, tested under the same experimental conditions.

Keywords: Photodegradation; Amiloride; Photosensitization; Photodechlorination

1. Introduction

Amiloride (3,5-diamino-*N*-(diaminomethylene)-6-chloropyrazine-carboxamide monohydrochloride) (Fig. 1) is an orally administered potassium-sparing diuretic agent that is widely used therapeutically mainly in combination formulations with hydrochlorothiazide (Vidt, 1981). Among the side effects associated with the combination formulation are adverse photosensitivity reactions in patients, observed as an exaggerated sunburn-like reaction to sunlight (Magnus, 1977). Such responses are also associated with hydrochlorothiazide when used alone. As an ap-

* Corresponding author. Tel.: +61-2-9351-2334; fax: +61-2-9351-4391.

E-mail address: demoore@pharm.usyd.edu.au (D.E. Moore)

0378-5173/99/$ - see front matter
PII: S0378-5173(99)00035-6

proach to understanding the mechanism of the photosensitivity responses, the photochemistry of hydrochlorothiazide has been extensively investigated (Moore, 1977; Moore and Tamat, 1980; Tamat and Moore, 1983). It was demonstrated that hydrochlorothiazide undergoes photolytic dechlorination by a free radical mechanism, as well as being able to photosensitize oxidation by singlet oxygen production. The free radical process is the likely first step in the initiation of a photobiological effect (Tamat and Moore, 1983). On the other hand, the photodegradation and photosensitization properties of amiloride have not been fully investigated, even though photosensitivity responses have been documented (ADRAC, 1987; Thestrup-Pedersen, 1987). The potential of amiloride to participate in photochemical processes was evident from a laser flash photolysis and pulse radiolysis study which demonstrated that the primary process occurring upon photoexcitation of amiloride in aqueous solutions is photoionization yielding a solvated electron and an amiloride cation radical (Hamoudi et al., 1984). In this paper we report on both the photodegradation pathway and photosensitization capability of amiloride in aqueous solution to relate to its capacity to produce a photobiological effect.

2. Experimental

2.1. Materials

Amiloride hydrochloride was kindly supplied by Merck Sharp and Dohme (Sydney, Australia). A single peak was found by HPLC analysis so the compound was used without further purification. L-Histidine (Sigma, Sydney, Australia) was used as received. 2,5-Dimethylfuran (Fluka, Switzerland) was purified by twice distilling at room temperature under vacuum. Acrylamide (BDH Chemicals, Melbourne, Australia) was twice recrystallized from distilled chloroform before use. Potassium ferrioxalate for actinometry was prepared as described by Calvert and Pitts (1966). The buffers were prepared in a total concentration of 0.05 M from the following AR grade components: pH 2–5, acetic acid–sodium acetate; pH 5–8, potassium dihydrogen orthophosphate–disodium hydrogen phosphate; pH 8–9, borax–hydrochloric acid; pH 10, sodium bicarbonate–sodium carbonate; pH 11–12, disodium hydrogen phosphate–sodium hydroxide.

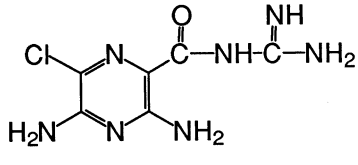

Fig. 1. Chemical structure of amiloride.

2.2. Procedure

A medium pressure mercury arc lamp (Hanovia 125 W) with a 2 mm Pyrex glass filter was used to irradiate solutions in a cylindrical quartz cuvette (20 mm path length) as described previously (Moore, 1987). Amiloride hydrochloride stock solution (0.5 mM) in water was accurately diluted 10-fold with buffer solution, then irradiated after bubbling with nitrogen for 15 min. UV-visible spectra of the photolysis mixture were recorded with a Perkin-Elmer (Lambda 5) UV/vis spectrophotometer after various times of irradiation. HPLC analysis of the photolysed solution was performed with an LKB (Bromma, Sweden) model 2150 pump, a Rheodyne 20 (μl loop injector, a fully end-capped reversed-phase column (Merck RP-Select B, 125×4.6 mm) and a Shimadzu SPD-6A UV detector operated at 243 nm or a Hewlett-Packard model HP 1040A photodiode-array detector. The mobile phase, acetonitrile–water (10:90 v/v), was delivered at a flow rate of 1 ml/min.

The potassium ferrioxalate actinometer (Calvert and Pitts, 1966) was used to determine the quantum yield for amiloride photodegradation in buffered aqueous solutions by the two-cell method as previously described (Moore, 1987). The degradation of amiloride was quantified by HPLC analysis.

2.3. Photosensitization experiments

Amiloride stock solution (1 ml, $2.5 10^{-3}$ M) was added to 50 ml of air-saturated buffer solution containing either L-histidine or 2,5-dimethylfuran (2 mM). The solution was transferred to a cylindrical glass reaction vessel (volume 30 ml) and irradiated at 30°C with a 125 W mercury arc. The rate of oxygen uptake by the solution was measured with a polarographic O_2 electrode (Radiometer, Copenhagen, Denmark). Polymerization of acrylamide (0.125 M) photosensitized by amiloride (5×10^{-4} M) was performed by dilatometry in the same reaction vessel with a measuring capillary replacing the O_2 electrode. The apparatus and procedure for photooxidation and photopolymerization have been described previously in more detail (Moore and Burt, 1981).

2.4. Isolation and identification of photodegradation products

An unbuffered aqueous solution of amiloride hydrochloride (300 mg/l) was maintained under a continuous flow of nitrogen gas and irradiated in a Hanovia 1 l photochemical reactor (125 W medium pressure mercury arc). After irradiation for 8 h the solution was reduced to approximately 5 ml on a rotary evaporator under reduced pressure at 35°C. The resulting concentrated solution was applied to a short bed preparative chromatographic column (100×20 mm i.d.) filled with reversed-phase packing material (Merck RP-select B, 5 μm). The photoproduct was separated from the parent drug by elution with 100 ml 5% acetonitrile in water. All solutions were protected from light during the separation procedure.

Mass spectra were obtained with a Finnigan MAT TSQ 46 (Finnigan, San Jose, CA, USA) operated in CI mode. The ^1H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 MHz (Palo Alto, CA, USA). The IR spectrum was obtained with a Bio-Rad FTS 20/80 spectrometer.

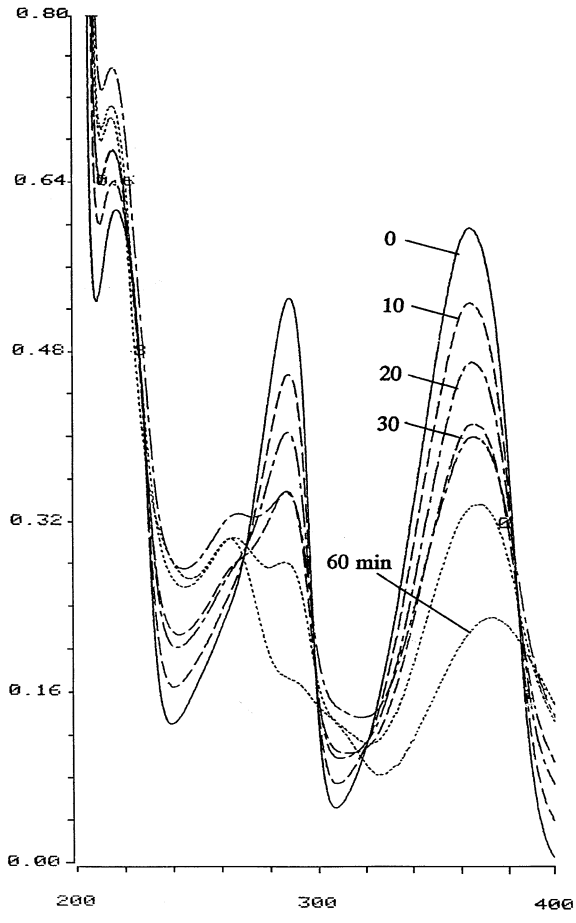

Fig. 2. Absorption spectra of amiloride (50 μM) in carbonate buffer (pH 10). The spectra were recorded after irradiation after successive 10 min intervals.

3. Results and discussion

The UV spectrum of amiloride (50 μM) in solution buffered to pH 10 gradually changed as it was irradiated in the spectrophotometer cuvette through the glass filter (Fig. 2). The presence of isosbestic points at 265 and 320 nm suggests a single transformation in the chromophore in the early stages of irradiation. After 50 min photolysis, the spectrum began to distort the isosbestic points, providing evidence of secondary photodegradation. At this stage approximately 50% of the original amiloride had degraded, as determined by HPLC analysis (Fig. 3). Using a diode array detector the spectrum of the major

photoproduct can be seen to resemble that of amiloride, but is less intense. The same major product was formed over the pH range 4–11.

3.1. Degradation kinetics

Semi-logarithmic plots of the amiloride concentration remaining (determined by HPLC analysis) versus time were linear for the early stages of the reaction, indicating that the photodegradation of amiloride in aqueous solution follows apparent first-order kinetics, as expected for the dilute solutions used (Moore, 1990). The neutral form of amiloride undergoes photodegradation about three times more efficiently than does the cationic form.

The values of the apparent first order rate constants will vary according to the irradiation apparatus used. The true constant of a photochemical reaction is the quantum efficiency (ϕ) (Moore, 1990) which was determined for each of the acid and base forms of amiloride. The maximum conversion, or fraction of amiloride reacted, was kept below 15% to avoid sensitization or quenching of the reaction by the photoproducts. The quantum yield for the photodegradation of the neutral form of amiloride at pH 10.4 was measured to be 0.023 ± 0.002, significantly higher than that of the protonated form (0.009 ± 0.001) at pH 4.0. The relative quantum efficiencies are shown in Fig. 4 and increase with increasing pH with an inflection that is close to the reported pK_a of 8.7 (Mazzo, 1986). The observed quantum efficiency can be expressed as a function of a_H the activity of hydrogen ion in solution according to Eq. (1):

$$k_{obs} = (a_H \varphi_{acid} + K_a \varphi_{base})/(a_H + K_a) \quad (1)$$

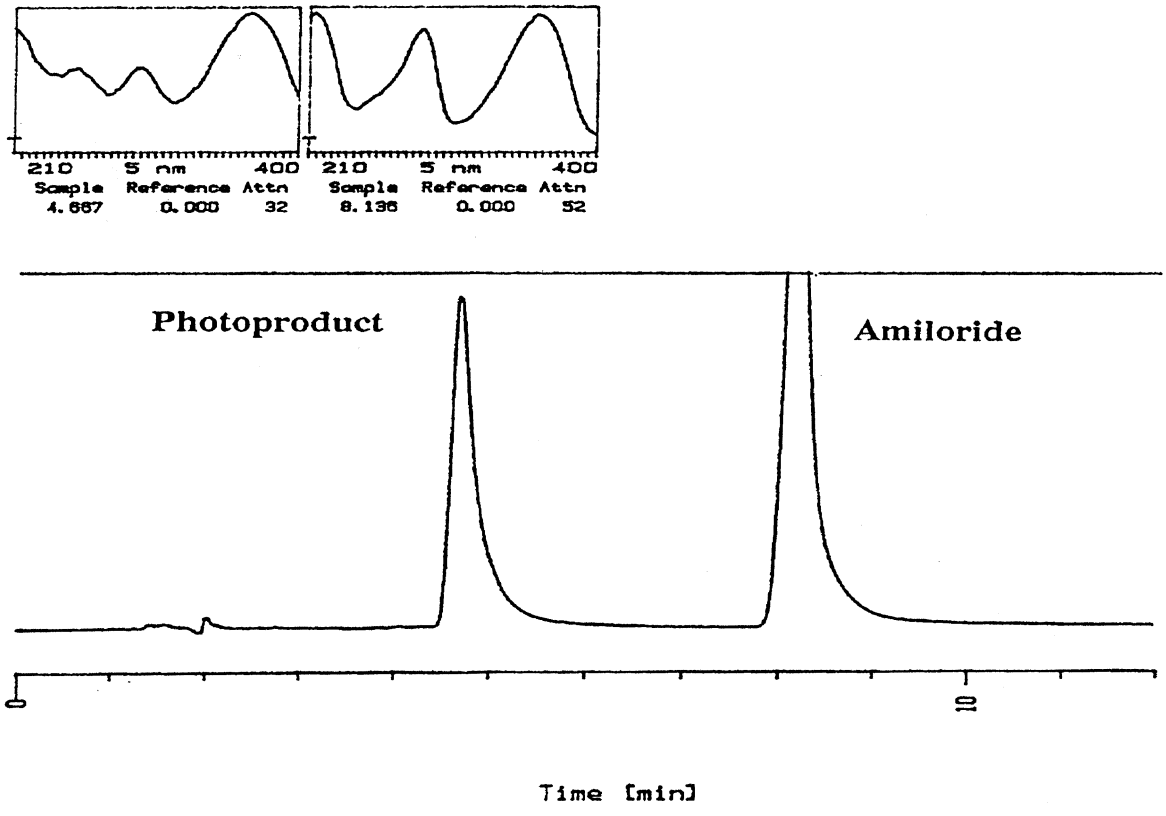

Fig. 3. Liquid chromatogram and UV absorption spectra of amiloride and its major photodegradation product.

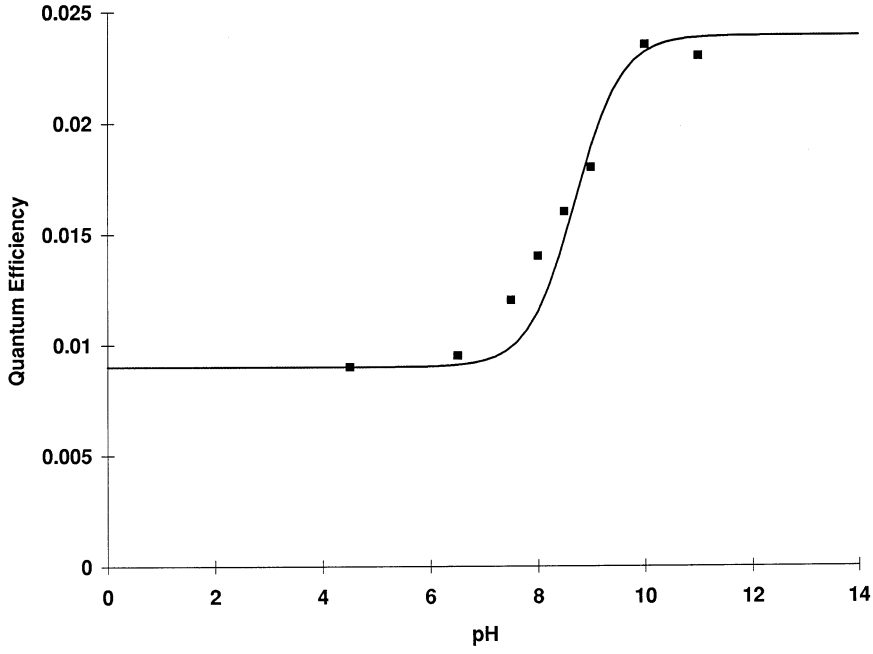

Fig. 4. pH-rate profile for the photodegradation of amiloride in aqueous solution. (Theoretical curve calculated according to $\varphi_{obs} = (a_H\varphi_{acid} + K_a\varphi_{base})/(a_H + K_a)$. (■) Experimental data.

where φ_{acid} and φ_{base} represent the quantum efficiencies for photodegradation of the acid and base forms of amiloride, respectively. The experimental data show a reasonable agreement with Eq. (1).

The flash photolysis study on amiloride in aqueous solutions (Hamoudi et al., 1984) had shown that the yield of the hydrated electron from photoionization was also pH-dependent, being higher at pH 10, as would be expected since removal of an electron from a cation is clearly more difficult than from a neutral species. Thus the primary photoprocess leading to degradation is confirmed to be the photoionization of amiloride.

3.2. Isolation and identification of the photoproduct

The product was isolated by preparative chromatography and identified by use of mass spectrometry and NMR spectroscopy. The CI-MS spectrum of the photolysis product with methane as reagent gas displayed a quasi-molecular ion at m/z 212 ($[M + H]^+$) with a major fragment peak at m/z 195. This suggests an empirical formula of $C_6H_9N_7O_2$ with the presence of an OH but the absence of the $-$Cl substituent in the product. In addition, an increase in free chloride ion was detected in the irradiated solution by titration with standard silver nitrate solution, in a similar fashion to the photodechlorination of hydrochlorthiazide and frusemide (Moore and Tamat, 1980). The final yield of chloride ion was quantitatively related to the original amiloride concentration.

$^1H-$ and ^{13}C NMR data of the product and amiloride in DMSO are presented in Table 1. In the ^1H NMR spectrum, signals appearing at δ 9.65 ppm (1H), δ 7.39 ppm (2H), δ 7.08 ppm (2H) 6.49 ppm (2H) disappeared on addition of D_2O and were assigned to the aromatic-NH_2 and the -NH- groups, suggesting that $(H_2N)_2$-$C_4H_4N_2$-CO- and *N*-amidino group remained intact. The observation that these signals are more downfield for the product compared to amiloride may be attributable to the effects of the sample concentration, temperature or pH on the signals due to H attached to N (Mazzo, 1986). The ^{13}C NMR data of the product showed that the C_6 (δ 170.5 ppm)

Table 1
^1H and ^{13}C NMR spectral assignments for amiloride (AML) and photolysis product

Number of H or C	δ ^1H (ppm)		δ ^{13}C (ppm)	
	AML	Product	AML	Product
2			109.1	98.4
3			154.1	145.3
5			155.8	146.9
6			119.7	170.5
7			165.0	161.6
9			154.9	154.9
NH	10.6	9.7		
Aromatic -NH_2	8.7; 8.6	7.3; 7.1		
-C-(NH_2)	6.5	7.3		

and C_2 (δ 98.4 ppm) in the molecule exhibited resonances significantly distinct from those of amiloride. An explanation is that the OH substituent exists predominantly in the keto tautomeric form in aqueous solution (Fig. 5). The same prototropic tautomerism has been observed for a number of monofunctional heterocyclic derivatives (Katritzky, 1985). Furthermore, the chemical shift of the carbonyl carbon in a nitrogen heterocycle is generally around 170 ppm (Crews et al., 1998), confirming the keto structure. Oxygen is an electron donor that increases the electron shielding of *ortho* carbon, C_3, and *para* carbon, C_4 with conjugative effects, resulting in high field chemical shifts of those carbon atoms at δ 145 ppm and δ 146 ppm. The resonances at δ 161.6 (C_7) and δ 154.9 (C_9) were similar to those of amiloride, verifying the ^1H NMR data of the intact *N*-amidino group. The evidence allowed the assignment of the product as *N*-amidino-3,5-diamino-6-hydroxylpyrazine-carboxamide.

The photoproduct identified in aqueous solution indicates that amiloride (Ar–Cl) undergoes decomposition by a dechlorination pathway, leading solely to a substitution product (Aryl–OH) with the OH arising from the solvent water. The stable form of the product appeared to be the tautomer shown in Fig. 5. This very simple product pattern differs markedly from that in the photodechlorination of frusemide and hydrochlorothiazide where the reduction product (Ar–H) was dominant over the substitution product, but photohydrolysis occurred as well. The precursor of the substitution product in photodechlorination is postulated to be a cation radical in an ion-pair exciplex formed from the excited state and ground state molecules (Grimshaw and de Silva, 1981). A cation radical has been clearly identified in flash photolysis of amiloride (Hamoudi et al., 1984). Since it is only the substitution product that is observed from amiloride, we postulate that the degradation occurs directly from the cation radical of amiloride, i.e. an exciplex ion-pair is not involved as has been proposed for frusemide, hydrochlorothiazide and diclofenac where both reduction and substitution products are formed (Moore et al., 1990). The lack of an effect by oxygen on the photodegradation of amiloride suggests that it is a nucleophilic attack of water on the radical cation that leads to the major product.

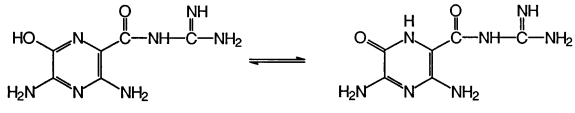

Fig. 5. Tautomeric forms of amiloride photoproduct.

3.3. Photosensitization studies

The photooxidation and photopolymerization experiments were undertaken to determine the relative ability of amiloride to generate reactive singlet oxygen and free radicals, respectively. The oxygen uptake rates measured for the UV irradiated amiloride solutions in the absence and presence of singlet oxygen substrates are recorded in Table 2, together with the rates of photopolymerization of acrylamide sensitized by amiloride. Each rate value is the mean of at least three determinations with a maximum S.D. of 5%. Amiloride by itself does not take up oxygen to any significant extent, indicating that there is no self-photooxidation of amiloride. In the presence of dimethylfuran or histidine, the rates of oxygen uptake show an increase with pH, similar to the photodegradation of amiloride. There was almost no dark reaction observed, and the oxygen uptake

Table 2
Oxidation of 2,5-dimethylfuran (DF) and L-histidine (His) and polymerization of acrylamide photosensitized by amiloride in aqueous solutions at 30°C

Buffer system	pH	Rate of oxygen uptake ($\mu M\ min^{-1}$)			Rate of polymerization ($mM\ min^{-1}$)
		AML	AML + DF	AML + His	
Acetate	5.0	0.02	0.72 ± 0.06	0.44 ± 0.06	0.19
Phosphate	7.0	0.03	0.86 ± 0.03	0.46 ± 0.04	0.23
Carbonate	10.0	0.04	1.16 ± 0.07	0.73 ± 0.07	0.68

was linear with time, i.e. zero order kinetics applied, indicating that the absorption of light by amiloride was the rate-limiting factor in the photosensitized oxidation in aqueous solutions. Additionally, amiloride was not an efficient photoinitiator of polymerization (Table 2), although the rate in alkaline solution was greater than that observed in acidic and neutral solutions.

4. Conclusions

In quantum efficiency terms, amiloride cannot be regarded as a significant photosensitizer since it absorbs strongly at the maximum output wavelength of the lamp used in these experiments, yet gives relatively minor rates compared to other photosensitizers under the same conditions (Moore and Chappuis, 1988; Zhou and Moore, 1997; Moore and Wang, 1998). However, because it is capable of absorbing the UVA radiation of both sunlight and artificial light, the adverse clinical photobiological responses must be related to the photochemical behaviour reported here for amiloride itself, in particular, the singlet oxygen photooxidation pathway, and the free radicals obtained following homolysis of the C–Cl bond. The photoproduct would not be expected to play a significant role since it is formed only slowly under the intense irradiation conditions used in the experiments described here, and it has lesser absorption characteristics.

Acknowledgements

This study was supported by the Australian Research Council Small Grants Scheme, and The Pharmacy Research Trust of NSW.

References

Adverse Drug Reactions Advisory Committee (ADRAC), 1987. A sunburnt country revisited. Adverse Drug Reactions Bulletin, Australian Department of Health, Canberra, pp. 372-377.

Calvert, J.G., Pitts, J.N., 1966. Photochemistry. Wiley, New York, p. 548.

Crews, P., Rodriguez, J., Jaspars, M., 1998. Organic Structure Analysis. Oxford University Press, New York, pp. 87–95.

Grimshaw, J., de Silva, A.P., 1981. Photochemistry and photocyclization of aryl halides. Chem. Soc. Rev. 10, 181–203.

Hamoudi, H.I., Heelis, P.F., Jones, R.A., et al., 1984. A laser flash photolysis and pulse radiolysis study of amiloride in aqueous and alcoholic solution. Photochem. Photobiol. 40, 35–39.

Katritzky, A.R., 1985. Heterocyclic Chemistry. Pergamon, New York, pp. 47–51.

Magnus, I.A., 1977. Dermatological Photobiology. Blackwell, London, pp. 211–235.

Mazzo, D.J., 1986. Analytical Profiles of Drug Substances, vol. 15. Academic Press, New York, pp. 1–34.

Moore, D.E., 1977. Photosensitization by drugs. J. Pharm. Sci. 66, 1282–1284.

Moore, D.E., 1987. Principles and practice of drug photodegradation studies. J. Pharm. Biomed. Anal. 5, 441–453.

Moore, D.E., 1990. Kinetic treatment of photochemical reactions. Int. J. Pharm. 63, R5–R7.

Moore, D.E., Burt, C.D., 1981. Photosensitization by drugs in surfactant solutions. Photochem. Photobiol. 34, 431–439.

Moore, D.E., Chappuis, P.P., 1988. A comparative study of the photochemistry of the non-steriodal anti-inflammatory drugs. Photochem. Photobiol. 47, 173–180.

Moore, D.E., Tamat, S.R.., 1980. Photosensitization by drugs: photolysis of some chlorine-containing drugs. J. Pharm. Pharmacol. 32, 172–177.

Moore, D.E., Wang, J., 1998. Electron transfer mechanisms in photosensitization by the anti-inflammatory drug benzydamine. J. Photochem. Photobiol. 43, 175–180.

Moore, D.E., Roberts-Thomson, S., Dong, Z., Duke, C.C., 1990. Photochemical studies on the anti-inflammatory drug dichlofenac. Photochem. Photobiol. 52, 685–690.

Tamat, S.R., Moore, D.E., 1983. Photolytic decomposition of hydrochlorothiazide. J. Pharm. Sci. 72, 180–183.

Thestrup-Pedersen, K., 1987. Adverse reactions in the skin from anti-hypertensive drugs. Dan. Med. Bull. 34 (Suppl. 1), 3–5.

Vidt, D.G., 1981. Mechanism of action, pharmacokinetics, adverse effects, and therapeutic uses of amiloride hydrochloride, a new potassium-sparing diuretic. Pharmacotherapeutics 1, 179–186.

Zhou, W., Moore, D. E., 1997. Photosensitizing activity of the antibacterial drugs sulfamethoxazole and trimethoprim. Photochem. Photobiol. 39, 63–72.