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Photolytic degradation of frusemide

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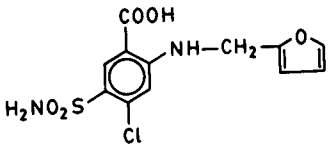
Irradiation with 365 nm u.v. light of frusemide (4-chloro-*N*-furfuryl-5-sulphamoylanthranilic acid) in methanol results primarily in photoreduction to *N*-furfuryl-5-sulphamoylanthranilic acid and photohydrolysis to 4-chloro-5-sulphamoylanthranilic acid (saluamine).

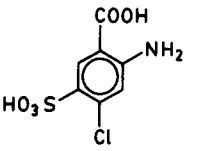
Frusemide, 4-chloro-*N*-furfuryl-5-sulphamoyl anthranilic acid (I), is a diuretic widely prescribed for the treatment of oedema, but is reported to cause adverse photosensitivity effects in-vivo (Magnus 1976). Previous work in this laboratory has demonstrated that frusemide has a high in-vitro photosensitizing capability, being able to initiate both excited-state energy transfer and free radical reactions (Moore 1977). The neutral form of frusemide was shown to be the principal photosensitizing species and the ionization of carboxyl function (pK_a 3·9) can be suppressed by incorporation into anionic surfactant micelles (Moore & Burt 1981). By this means, the apparent pK_a can be raised by about 2 pH units, thus enabling the major photoactive species to exist close to physiological conditions. An implication of this work is that the mechanism by which frusemide exerts its photobiologic effect is through sensitized damage to cellular membranes to which it becomes associated.



I

An alternative possible mechanism is that photodecomposition products from frusemide prove toxic to the biological system, as demonstrated, for example, by chlorpromazine (Kochevar & Lamola 1979).

The photodecomposition of frusemide has received relatively little attention. Rowbotham et al (1976) subjected frusemide to prolonged u.v. irradiation (48 h) in alkaline solution and could separate only 4-amino-5-carboxy-2-chlorobenzenesulphonic acid (VII) from an otherwise intractable tar. This product represented an oxidation of the $-SO_2NH_2$ group to $-SO_3H$, and hydrolysis of the furan ring.



VII

They concluded that the photochemical degradation of frusemide was a hydrolysis and a photochemical oxidation process. It was not clear, however, whether the hydrolytic step was light initiated or due to the alkalinity of the solution. Thermal degradation of frusemide occurs only in acidic solution at elevated temperatures with formation of saluamine (4-chloro-5-sulphamoylanthranilic acid) and furfuryl alcohol (Kovar et al 1974). A study of the kinetics of this reaction (Cruz et al 1979) suggested a mechanism whereby the amino nitrogen is protonated followed by nucleophilic attack on the furfuryl carbon.

On the other hand, Moore & Tamat (1980) found complete dechlorination to occur after only 1 h irradiation of frusemide in deoxygenated methanol and neutral aqueous solutions. The present work reports on the separation and identification of the photodecomposition products of frusemide in methanolic solution. Particular emphasis has been placed on determining the nature of the products formed in the early stages of the irradiation before secondary reactions occur.

MATERIALS AND METHODS

Frusemide and its hydrolysis product, saluamine (4-chloro-5-sulphamoylanthranilic acid) were kindly supplied by Hoescht Australia Ltd, Melbourne. Both compounds were indicated to be >99% pure by

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high performance liquid chromatography (h.p.l.c.). Trimethylanilinium hydroxide (TMAH) a methylating agent for gas chromatography was obtained as a 2 M solution in methanol (Pierce Chemical Co., Rockford, IL). All other chemicals and solvents were of analytical grade (Ajax Chemicals, Sydney).

A solution of frusemide (5×10^{-4} M) in methanol was irradiated with a medium pressure mercury lamp (365 nm) at 30 °C in the glass apparatus previously described (Moore & Tamat 1980). The solution was presaturated with nitrogen by bubbling for 60 min and the gas flow was maintained to stir the solution during the irradiation. The product mixture was sampled at various times and analysed by:

(i) U.v. spectrophotometry using a Varian model 634 after a 25-fold dilution with solvent.

(ii) Gas chromatography with a Hewlett-Packard model 5720A, flame ionization detector, nitrogen carrier gas and a 3 mm × 1 m silanized glass column packed with 100–120 mesh Gas-Chrom Q coated with 3% OV-1 (Applied Science Labs). The irradiated solution was concentrated tenfold and to 100 µl was added 2·5 µl of TMAH. Methylation occurred in the injection port at 280 °C. The column and detector temperatures were 235 and 320 °C, respectively. Selected samples were analysed by gas chromatography-chemical ionization mass spectrometry with a Finnigan 6110-9500 system using the column as above and methane carrier gas.

(iii) High performance liquid chromatography using an Altex model 330 with fixed wavelength u.v. detector (254 nm) and a Brownlee RP-8 10 µm column (4 × 250 mm). Separation was achieved using an isocratic solvent system of acetic acid, water and methanol (0·5:74·5:25). A semi-preparative scale column (7 × 250 mm) contained the same packing material. Fractions corresponding to the peaks were collected, freeze-dried and submitted for molecular weight determination by solid-probe mass spectrometry.

(iv) Testing for the presence of primary aromatic amines by the Bratton-Marshall reaction, i.e., diazotization and coupling with the colour reagent *N*-(1-naphthyl)-ethylene diamine (Connors 1967).

Quantum yields were estimated using the ferrioxalate actinometer and irradiation procedure described previously (Moore & Tamat 1980). Product analysis was by h.p.l.c. after calibration with authentic samples.

RESULTS AND DISCUSSION

The ultraviolet absorption spectra shown in Fig. 1 are of a frusemide solution in oxygen-free methanol,

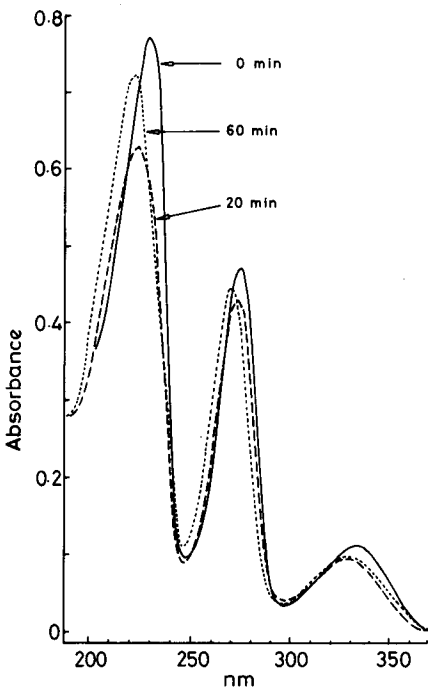

FIG. 1. U.v. absorption spectra of frusemide solution in oxygen-free methanol. The curves are labelled to indicate the times of irradiation.

after various times of irradiation. Only relatively minor changes are evident in the spectrum, although there is no clear isosbestic point to indicate a simple 1:1 transformation, such as is shown in the acid-catalysed hydrolysis of frusemide to form saluamine (Cruz et al 1979). However, in the course of the 60 min irradiation, the chlorine substituent is completely removed with a quantum yield of 0·40 ± 0·08, as determined by chloride ion potentiometry (Moore & Tamat 1980). Additionally samples taken at various times gave a positive reaction to the diazotization and coupling colour test for primary aromatic amines (Bratton-Marshall reaction). The amount of primary aromatic amine detected after 60 min irradiation represented only 40% of saluamine possible if complete hydrolysis occurred. Thus it was concluded that a combination of dechlorination and hydrolysis reactions was occurring as a result of u.v. irradiation. The relatively minor spectral changes reaffirm the ineffectiveness of the B.P. (1973) spectrophotometric assay as a determinant of frusemide purity, following exposure to light, as pointed out by Rowbotham et al (1976).

Therefore, the kinetics of the reaction were followed by h.p.l.c. analysis of the irradiated solu-

tions. The initial rate of photodegradation of frusemide was examined in oxygenated and deoxygenated solutions in methanol and aqueous buffers in the pH range 9–12. Plots of log (% frusemide remaining) vs time was linear and the apparent rate constants obtained from the slopes of such plots are given in Table 1. True rate constants for these processes require the quantum yield and the lifetime of the excited state. However when a constant source of irradiation was used, the values in Table 1 could be compared to ascertain the influence of other variables on the reaction. Clearly, the photodegradation is seen to be insensitive to the pH of the aqueous media, implying no alkaline catalysis of the hydrolytic component of the reaction. The effect of oxygen is to inhibit strongly the degradation in methanol but to cause a slight acceleration in the aqueous systems. In methanol, the oxygen inhibition is attributed to oxygen quenching of the triplet state of the molecule, thus accounting for the high efficiency of frusemide in sensitizing photo-oxidation reactions in methanol (Moore & Tamat 1980). On the other hand, the ionization of frusemide in aqueous systems lessens its capability as a photosensitizer (Moore & Burt 1981) but is seen here to result in more rapid degradation of the drug itself.

The products of photolysis of frusemide in methanol were examined by gas-chromatography-mass-spectrometry (g.c.-m.s.) and h.p.l.c.

Table 1. Apparent rate constants for the photodegradation of frusemide in various media at 30 °C.

Medium	10^2 k, min $^{-1}$ N_2 saturated	O_2 saturated
Methanol	4·6	0·32
pH 9·0	1·55	1·95
pH 10·0	1·70	—
pH 11·0	1·53	1·82
pH 12·0	1·60	—

Initial concentration of frusemide 5×10^{-4} M.

For g.c.-m.s., derivatization by direct flash alkylation with trimethylanilinium hydroxide (TMAH) was used. This reagent has been found successful for the derivatization of hydrochlorothiazide which also contains several acidic protons (Vandenheuvel et al 1975). Lindstrom & Molander (1975) reported a g.c. method for frusemide in plasma, involving a multistep procedure of extractive alkylation with methyl iodide, but this proved cumbersome and relatively inefficient for the separation of the photolysis mixture. The major difficulty of all g.c. methods for this class of compound lies with the necessity of achieving complete derivatization.

It was found that essentially complete methylation of frusemide could be achieved by mixing with a tenfold excess of TMAH in the injection port at 280 °C. The TMAH-treated samples of frusemide irradiated for various times were analysed by g.c.-m.s. in the chemical ionization mode with methane carrier gas, and molecular weights of the permethylated products determined as shown in Fig. 2. The *m/z* values correspond to trimethyl derivatives of the following compounds: saluamine (*m/z* = 292), *N*-furfuryl-5-sulphamoylanthranilic acid (*m/z* = 338) and frusemide (*m/z* = 372). The chromatograms show the dechlorinated compound to be the major product at short irradiation times. However, any compound which is inefficiently derivatized or unstable at the elevated temperatures used will not show up quantitatively in this system.

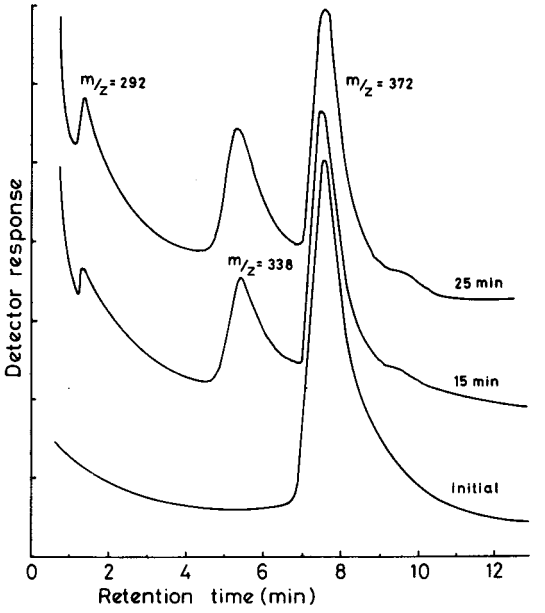

FIG. 2. Gas chromatograms of samples of frusemide which had been irradiated for the times indicated. The samples were treated with trimethylanilinium hydroxide as described in the text.

H.p.l.c. at room temperature (20 °C) of the photolysis mixture did not require prior derivatization and indicated the presence of a greater number of products than did g.c. Using an analytical scale reverse phase column and a mobile phase consisting of acetic acid, water and methanol (0·5:74·5:25), the chromatogram of a 10 min irradiated sample is as shown in Fig. 3. On scaling up to a semi-preparative system with the same stationary and mobile phases,

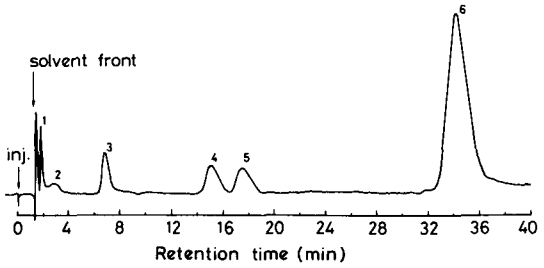

FIG. 3. Separation by h.p.l.c. of 5×10^{-4} M frusemide in oxygen-free methanol after irradiation for 10 min. Conditions are described in the text.

some resolution was lost, but it was possible to collect fractions corresponding to the major peaks. These were freeze-dried and molecular weights determined by solid-probe mass spectrometry as given in Table 2. Observation of the chromatograms as a function of time showed that the products listed in Table 2 were present in the first samples taken (1 min irradiation). Irradiation for times longer than 20 min yielded additional peaks which can be attributed to secondary breakdown, such as hydrolysis of the dechlorinated frusemide. Quantum yields in Table 2 were estimated after calibration of the h.p.l.c. response with frusemide (I) and saluamine (II). Since insufficient of the initial products III and IV could be isolated for calibration purposes, the assumption was used that the quantum yield for the loss of frusemide equalled the sum of those for formation of the initial product, i.e. $\phi_{-I} = \phi_{II} + \phi_{III} + \phi_{IV}$.

Table 2. H.p.l.c. Separation and mass spectral characterization of frusemide photolysis products after irradiation for 10 min in methanol.

H.p.l.c. peak (Fig 3)	Rt min	Mol. wt.	Bratton-Marshall reaction	Assignment (Fig. 4)	Quantum yield
1	1·8	216	+	V	—
2	3·6	246	+	VI	—
3	6·8	250	+	II	0·15
4	15·0	296	−	III	0·30
5	17·4	326	−	IV	
6	34·2	330	−	I	−0·45

The photolysis of frusemide in oxygen-free methanol is represented by Fig. 4. The observed product distribution indicated the major pathways in this system to be reduction (i.e., Cl replaced by H) and substitution (i.e. $-OCH_3$ from the solvent replaces Cl). This pattern has been observed for other simple chloroaromatic compounds (Soumillion & DeWolf 1981) and drugs such as chlorpromazine (Rosenthal et al 1978) and hydrochlorothiazide (Tamat & Moore 1983). In each of these examples the photoreaction is postulated to occur through the triplet state of the molecule. For frusemide the predominance of the triplet state as the major photoexcited species has been verified by the observation of efficient photosensitization of oxidation of substrates in methanol via a triplet state mechanism (Moore & Tamat 1980).

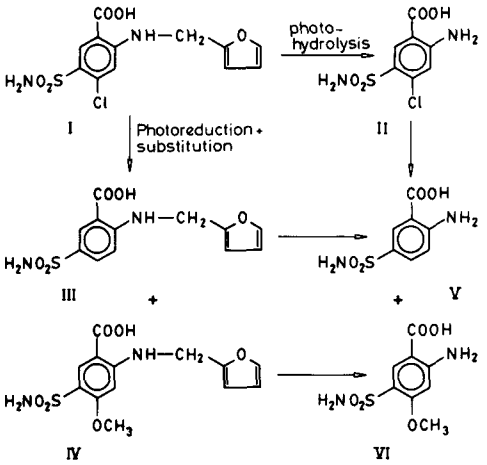

FIG. 4. Pathways of photodegradation of frusemide in oxygen-free methanol.

The existence of a proportion of photohydrolysis in the photodegradation is similar to the situation observed following irradiation of some other drugs susceptible to solvolysis, such as pentobarbitone (Barton et al 1980) indapamide (Davis et al 1979) and hydrochlorothiazide (Tamat & Moore 1983).

Frusemide has a secondary amine functional grouping and is therefore susceptible to acid catalysed hydrolysis and slow uncatalysed hydrolysis in neutral and alkaline solutions (Cruz et al 1979). We found solvolysis in methanol to be negligible in the dark in the course of the photolysis experiments.

Photohydrolysis of hydrochlorothiazide was attributed to the occurrence of photoionization as a primary photochemical process (Tamat & Moore 1983). By this means a cation radical is formed and the introduction of a positive charge renders the molecule more susceptible to nucleophilic attack by the solvent. Similarly, it is suggested that photoionization occurs to some extent in frusemide, although this remains to be proven by flash photolysis experiments. The positive charge is expected to be stabilized on the amine nitrogen and solvolysis is

facilitated in essentially the same manner as proposed for hydrolysis in acid solutions (Cruz et al 1979).

The significant conclusion from this study is that the photolysis of frusemide yields products of similar u.v. spectral characteristics which are, therefore, potential photosensitizers.

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