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Photochemical decomposition of midazolam. II. Kinetics in ethanol

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Summary

The kinetics of the photodecomposition of midazolam was studied at 5 different midazolam concentrations. Silica capillary gas chromatography with a temperature program was used to quantify midazolam and its main degradation products. The photodecomposition exhibited apparent first-order kinetics at all concentrations and was dependent on the concentration: the logarithm of the reaction rate constant was directly proportional to the initial drug concentration.

Introduction

Midazolam (**I**), an imidazobenzodiazepine derivative, is a photosensitive compound. Recently the main photodecomposition products in ethanol were found to be the quinazoline derivative, 6-chloro-2-methyl-4-(2′-fluorophenyl)quinazoline (**II**), *N*-desalkylflurazepam (**III**) and a solvent addition product 7-chloro-2[(1-ethoxyethylimino) ethoxymethyl]-5-(2′-fluorophenyl)-3H-1,4-benzodiazepine (**IV**) (Fig. 1) (Selkämaa and Tammilehto, 1989). Preliminary studies showed midazolam to degrade much slower in aqueous solutions (pH 3.3) than in ethanol, but analyzed by TLC the products were mostly the same except for the missing solvent addition product.

Gas chromatography (GC) has proved to be a useful method for the assay of benzodiazepines. Numerous articles have been published on the quantification of midazolam and its metabolites in biological fluids on GC using an electron capture detector and packed glass columns (Coassolo et al., 1982; Ha et al., 1988 and references therein). The metabolites need to be derivatized because of their OH-moiety.

The aim of the present work was to develop a gas chromatographic method to study the kinetics of the photodegradation of midazolam and the effect of concentration on the reaction. Midazolam was irradiated in ethanolic solutions because photodecomposition is faster there than in aqueous solutions. The main degradation products were assayed.

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Experimental

Materials

Midazolam was kindly supplied by Hoffmann-La Roche (Basle, Switzerland). The degradation products of midazolam used as quantification standards were isolated from the irradiated ethanolic solution by flash chromatography (Selkämaa and Tammilehto, 1989). Identity and purity of the substances were verified by TLC and GC and by UV, IR, ^{1}H-NMR and ^{13}C-NMR spectra. The internal standard medazepam was also obtained from Hoffmann-La Roche and used as such. All solvents were of analytical grade.

Apparatus

The radiation source was a high-pressure mercury lamp, Original Hanau TQ150. The water-bath with a thermostat was a MGW Lauda WB 20 D. The gas chromatographic analyses were performed on a Carlo Erba Fractovap 4200 instrument equipped with a FID and a Merck-Hitachi D-2000 integrator. The silica capillary column was coated with SE-54; the length of the column was 15 m, the internal diameter 0.32 mm and the thickness of the phase layer 0.10 μm. Temperatures of the injection port and detector were 300 °C and the temperature program was 174–255 °C/7 °C · min^{-1}. The carrier gas was helium (0.6 kg/cm^2) and the split-ratio 1 : 20.

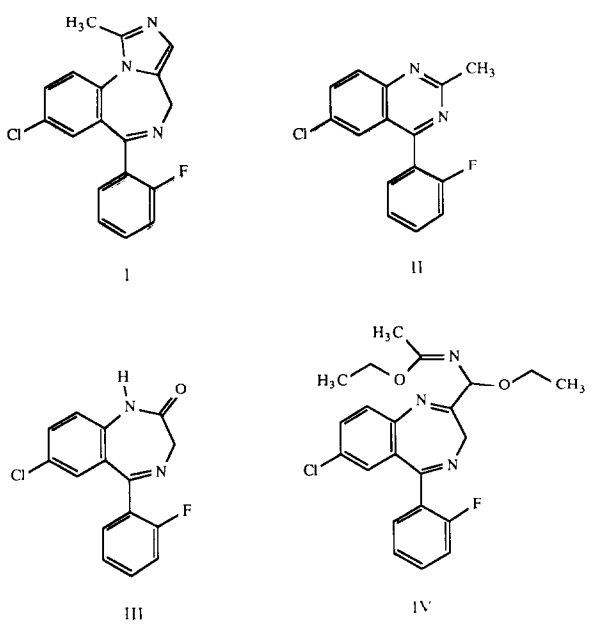

Fig. 1. Structures of midazolam (**I**) and its main photodecomposition products (**II**, **III** and **IV**).

Photodegradation of midazolam

Ethanolic midazolam solutions were prepared in 5 different concentrations: 5, 7.5, 10, 15 and 20 mM. Aliquots of each solution (3 ml) were placed in glass cuvettes and were exposed to a high-pressure mercury lamp at a distance of 1 cm, in a water-bath with thermostat set to 25 °C. Times of exposure varied between 5 min and 5 h. At each point of time 4 parallel samples were irradiated for the quantification of midazolam and two parallel samples for the quantification of the degradation products.

Preparation of the calibration curves

The calibration curves for the compounds were prepared in concentration ranges as follows: 0.06–0.65 mg/ml for compound **I**, 0.04–0.14 mg/ml for compound **II**, 0.04–0.20 mg/ml for compound **III** and 0.10–0.31 mg/ml for compound **IV**. Each standard was injected 6 times and the calibration curves were checked daily. The internal standard was medazepam and the ratios of the integrated areas of the peaks were used in the analysis.

Sample preparation for GC

Quantification of midazolam. One ml of the irradiated ethanolic solution, together with the internal standard medazepam in ethanol, was evaporated to dryness with a gentle nitrogen stream. Solutions of 5–10 mM were diluted to 5 ml with chloroform, and solutions of 15–20 mM were diluted to 10 ml. Final concentration of the internal standard was 0.20 mg/ml. One μl of each solution was injected into the gas chromatograph.

Quantification of the degradation products. One ml of the irradiated ethanolic midazolam solution was evaporated to dryness with a gentle nitrogen stream and 1 ml of the internal standard solution in chloroform (0.10 mg/ml) was added. One μl of this solution was injected into the gas chromatograph.

Results and Discussion

The photodecomposition kinetics of midazolam was studied by silica capillary gas chromatographic technique. The many products demanded the use of a high resolution analytical method, and silica capillary gas chromatography appeared to be most suitable. The SE-54 phase separated midazolam from its degradation products and the degradation products from each other (Fig. 2). Derivatization of the sample components was unnecessary because both the parent compound and the photodecomposition products are readily vaporized. A temperature program was essential, because an isocratic assay could not resolve all the peaks within a reasonable time. An internal standard method was used in the assay. Medazepam was separated well enough from the other components in the sample solution (retention time of medazepam 3.65 min). All the calibration curves proved to be strictly linear in the concentration range used (Table 1). The relative standard deviation for replicate samples was 2.6–7.7% (usually under 4%) ($n = 6$), which is acceptable for split-technique.

As seen in Fig. 3, the logarithm of the residual midazolam concentration is directly proportional to the irradiation time. The rate constants, the half-lives and the correlation coefficients for these

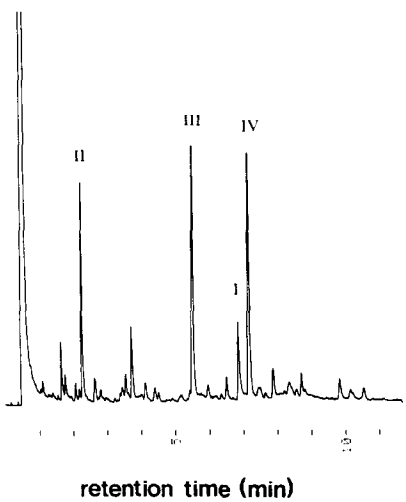

Fig. 2. Gas chromatogram of an irradiated solution of midazolam (7.5 mM) in ethanol (5 h, not thermostated).

TABLE 1

Calibration curves for I, II, III and IV

Compound	Calibration curve	Correlation coefficient
I	$y = -0.056 + 3.883x$	0.9998
II	$y = -0.023 + 11.721x$	1.0000
III	$y = +0.009 + 5.363x$	1.0000
IV	$y = -0.051 + 5.484x$	0.9995

apparent first-order kinetic reactions are presented in Table 2. According to Connors et al. (1986) photodegradation reactions obey approximate first-order kinetics in dilute solutions but may approach pseudo-zero-order kinetics in more concentrated solutions. In the case of the photodegradation of midazolam, apparent first-order kinetics was observed over the whole concentration range, which represented midazolam concentrations from 5 to 20 mM (1.6–6.5 mg/ml); the usual concentration in the injectable preparations is about 15 mM (5.0 mg/ml). A slight departure from linearity was observed in the 15 and 20 mM solutions. Conceivably, the increase in the colour intensity of the solutions at the high concentrations hinders UV absorption by the reacting molecules, thereby slowing down the rate of the reactions (Ahmad, 1982). The relative standard deviation for the parallel samples was usually between 3% and 10%, though in a few extreme cases it was 15–24%, especially when the exposure time was long. Samples were not mixed during the irradiation, which might explain the great variation for samples irradiated a long time; Allwood and Plane (1986) have nevertheless reported that,

TABLE 2

Apparent first-order kinetic data for the photodecomposition of midazolam

Concentration (mM)	Rate constant (min^{-1})	Half-life (min)	Correlation coefficient
5	0.0322	21.5	0.9853
7.5	0.0196	35.5	0.9881
10	0.0138	50.2	0.9923
15	0.0060	115.7	0.9635
20	0.0037	188.1	0.9615

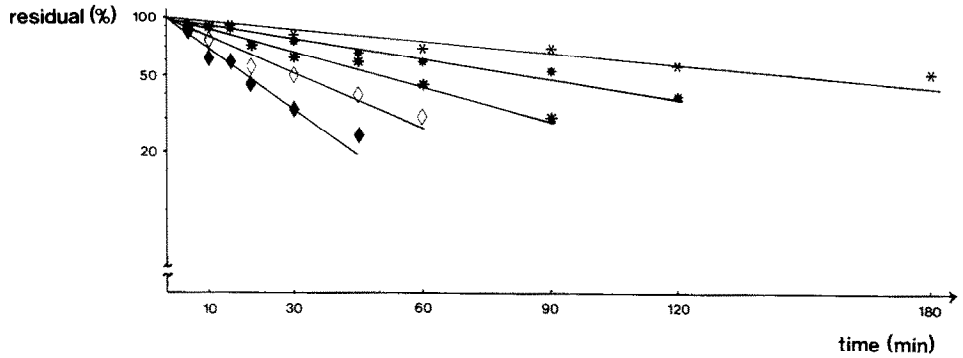

Fig. 3. Photodegradation of midazolam in ethanolic solutions. ◆, 5 mM; ◇, 7.5 mM; *, 10 mM; * , 15 mM; *, 20 mM.

in the photodegradation of vitamin A, mixing causes greater inter-sample variation but does not affect the mean degradation rate.

The rate of photodecomposition of midazolam is solely dependent on the concentration: the logarithm of the apparent first-order reaction rate constant is directly proportional to the initial concentration of the midazolam solution ($r = 0.9934$) (Fig. 4).

In general, apparent first-order kinetics has been demonstrated for the photodecomposition of drug compounds – for example, for adriamycin (Tavoloni et al., 1980), furosemide (Bundgaard et al., 1988), menadione (Abd El-Fattah and Daabis, 1977), nifedipine (Thoma and Klimek, 1985), sulphacetamide (Ahmad, 1982) and theophylline (Ishiguro et al., 1981). For ketorolac tromethamine (Gu et al., 1988) apparent first-order kinetics was observed at low concentrations (2 μg/ml),

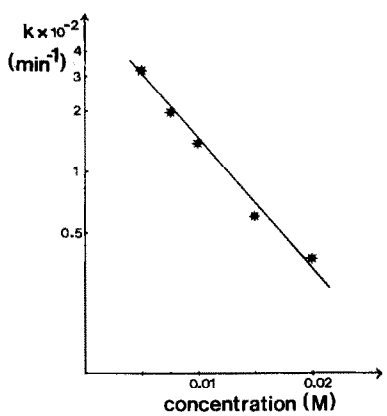

Fig. 4. Logarithm of the apparent first-order rate constant versus the initial concentration of the midazolam solution.

but non-first-order kinetics when the concentration is increased (10 μg/ml).

The photodegradation products were assayed in the 7.5 mM midazolam solution. All 3 main products were evident in the gas chromatogram from the start of the irradiation. The amount of the solvent addition product seemed to grow fastest at the beginning and decreased as the degradation proceeded. The amount of *N*-desalkylflurazepam calculated as mole percent relative to the initial midazolam concentration reached about 10% in 4 h. The quinazoline derivative was present in amounts of 2.3–3.2% (relative to initial midazolam) in 1–4 h samples, but was barely detectable in 5 h sample. The disappearance may have been due to the formation of further degradation products. The overall photodegradation was dependent on the temperature; the distribution of the products was different and their amounts were greater in the non-thermostated experiment. At this point no kinetic models can be presented for the formation of the degradation products.

Acknowledgement

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