Photochemistry of 2-(2-Furyl)-benzimidazole (Fuberidazole)

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The photodegradation of 2-(2-Furyl)-benzimidazole (Fuberidazole **1**) has been reinvestigated employing advanced HPLC–UV/VIS technique and fluorescence emission and excitation spectroscopy in methanol at natural pH, in acidic medium and in aqueous solutions at pH 7 and 3, and four main products benzimidazole-2-carboxylic acid **3**, its methyl ester **2**, 1-methoxy benzimidazole **5**, methyl 4-oxo-2-benzimidazole crotonate **7** (*cis* and *trans* isomers) besides benzimidazole **4** and 2,2′-bibenzimidazole **6** and other side products have been isolated and characterized. The kinetics of the photodegradation process was followed independently by HPLC–UV and fluorescence emission showing a significant similarity of the curve habit; this allows to monitor a photodegradation at very low concentrations ($5 \cdot 10^{-5} - 5 \cdot 10^{-6}$ M). The quantum yield of disappearance of Fuberidazole has been determined.

Introduction

Efficient degradation and mineralization of chemicals used in agriculture is needed to minimize the environmental impact of these products, and photodegradation is, among the several degradation modes, one of the most important. Besides the more or less efficient degradation, it was also realized in the past the importance of knowing the chemical nature of final degradation products. A complete view of the problem must take into account the formation and disappearance of intermediate products. Such an approach is needed not only for environmental reasons (a very toxic intermediate compound could be very menacious for the environment, in spite of its transitory lifetime), but also for understanding the degradation mechanisms.

Photodegradation of 2-substituted benzimidazoles, the most important class of benzimidazoles used in agriculture, has been subject to some attention, in particulary some of us have been involved in first and basic photodegradation experiments on 2-(2-furyl)benzimidazole (Fuberidazole) [1, 2].

Fuberidazol has been marketed under the trade name Voronit^R and has been applied as a fungicide [3], as an antihelmintic [4] and against enteroviruses [5]. Although the importance of Fuberidazole has decreased meanwhile, especially this benzimidazole derivative has proved to be an excellent model compound for studying abiotic photodegradation processes in detail with simultaneous separation and identification of the individual photolysis products by HPLC–UV/VIS separation technique combined with a photodiode array detector, as well as for establishing the kinetics of the photoreaction both with HPLC–UV/VIS and with fluorescence spectroscopy; and this combination opens a novel analytical technique in abiotic photodegradation analysis.

The study of the photodegradation pattern of *in vitro* irradiated chemicals is an indispensable step for simulating *in vivo* photodegradations. In the light of this, we describe the photolysis of Fuberidazole in methanol solution to exemplify this approach to degradation studies. Furthermore, we present first results extending these studies to water.

Experimental

Fuberidazole has kindly been made available by the Bayer AG, D-W-5090 Leverkusen. The irra-

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diation was carried out in methanol and water as solvents in standard immersion photoreactors equiped with high pressure Hg lamps (Philips HPK 125 W) and pyrex filters (to cut-off wavelengths lower than λ = 313 nm. The different photoproducts were separated by a Waters HPLC-system with a RP_{18}-column (Nucleosil^R, Macherey-Nagel, 250 mm × 4 mm) and detected by a tunable UV-detector (Waters 484) and a photodiode array detector (Waters 990). For high-resolution mass spectrometry a A.E.I. Kratos MS 50 (70 eV, DE 150 °C) was used.

HPLC chromatograms were recorded using the following acetonitrile-water eluent gradient (flow: 1 ml/min):

Time (min)	Acetonitrile (%)	Water (%)
0	12	88
3	12	88
15	50	50
19	50	50
22	12	88
30	12	88

All HPLC-grade solvents used were purchased from Riedel-de Haën. Absorption spectra were recorded on a Perkin-Elmer Lambda 6 and fluorescence emission and excitation spectra on a Spex F111 Fluorolog. All emission and excitation spectra were corrected. For quantum yield measurements (10^{-5} to 10^{-6} M) light excitation at λ = 313 nm was provided by a medium-pressure Hg lamp from Müller Optik, the excitation wavelengths being selected with filters from Oriel. The incident light intensity 1.1×10^{-6} Einstein · min^{-1} was measured by ferric oxalate actinometry [6]. The photodegradation products were characterized by high resolution mass-spectrometry of the separated peaks, UV-spectra and comparison with authentical samples (preparative column chromatography and independent preparative synthesis).

Results and Discussion

Photodegradation kinetics monitored by HPLC

Fuberidazole **1** was photolyzed in methanol solutions at natural pH. The solution was monitored during irradiation time by HPLC. The area of the several chromatographic peaks observed at the absorption wavelength of λ = 254 nm was divided by the relative molar absorptivity to obtain proportionality with concentration, and plotted as a function of time (see Fig. 1).

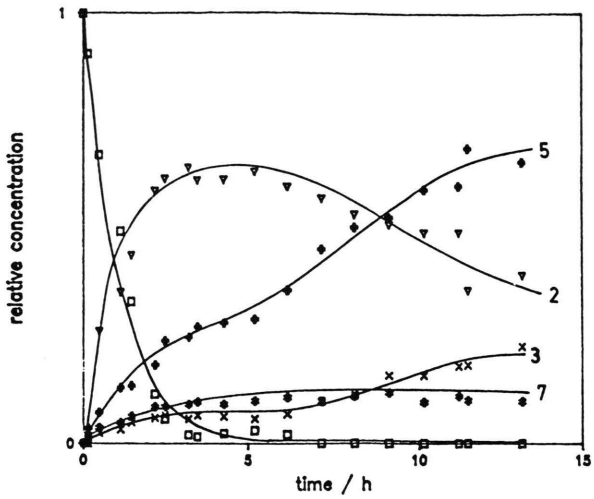


Fig. 1. Photodegradation of Fuberidazole in methanol, followed by HPLC. Normalized concentrations *versus* irradiation time:

□ – Fuberidazole

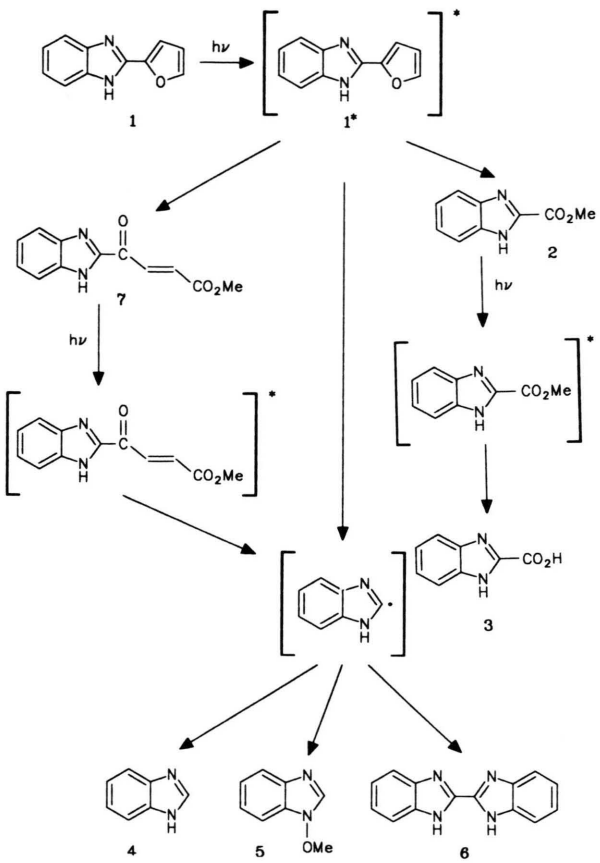
× – Product **3,** retention time of 3.75 min, benzimidazole-2-carboxylic acid

▽ – Product **2,** retention time of 16.18 min, methyl benzimidazole-2-carboxylate

\+ – Product **5,** retention time of 18.25 min, metoxy-1-benzimidazole

– Product **7,** retention time of 19.69 min, methyl-4-oxo-2-benzimidazole-crotonate

This shows the formation of four main products, which were identified as being benzimidazole-2-carboxylic acid (**3,** retention time of 3.75 min), methyl benzimidazole-2-carboxylate (**2,** retention time of 16.18 min), 1-methoxybenzimidazole (**5,** retention time of 18.25 min) and methyl 4-oxo-2-benzimidazolecrotonate (**7,** retention time of 19.69 min). Benzimidazole **4,** 2,2′-bibenzimidazole **6,** a *cis*-isomer of **7,** and a methanol adduct $C_{12}H_{10}N_2O_3 \cdot MeOH$ (not identified) were also detected in low concentrations. The observed products and their kinetic pattern can be account by Scheme 1. The photoproducts **2, 4,** and **7** have been found in our previous work employing column chromatography [1], while **3, 5, 6,** the *cis*-isomer of **7** and the methanol adduct have been only now detected by employing the much more sensitive HPLC-technique [2].



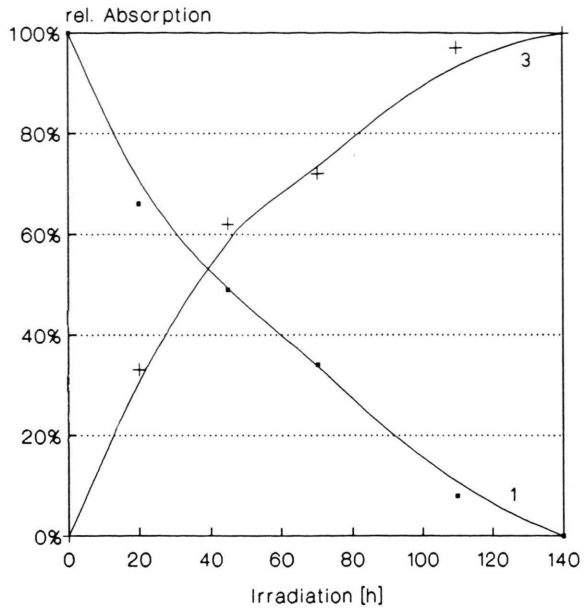


Fig. 2. Photodegradation of Fuberidazole in water, followed by HPLC. Normalized concentrations *versus* irradiation time:

■ – Fuberidazole **1**
\+ – Benzimidazole-2-carboxylic acid **3**

Irradiation of Fuberidazole **1** in H^+/MeOH was also performed. No substantial qualitative differences were observed, when compared with the same experience at natural pH, besides the different relative amount of the several photoproducts. A completely different result was obtained for the irradiation of Fuberidazole in water at pH = 3.0, as is shown in Fig. 2. Under these conditions, Fuberidazole **1** decays to give a main product, which was identified as being benzimidazol-2-carboxylic-acid.

Photodegradation kinetics followed by absorption and fluorescence emission

Absorption and particulary fluorescence emission are two powerful high sensitive analytical techniques to be used for routine analysis in kinetic studies of photodegradation of chemicals. For fluorescence emission the advantages result especially from the very low concentration limits, which fit perfectly the natural conditions, in which the environmental impact must be studied, simplicity of analysis and less time consuming.

Some disadvantages of fluorescence emission, for example a not sufficient selectivity, can be overcome by a combination of the results with those obtained by HPLC.

Methanolic solutions of Fuberidazole ($5 \cdot 10^{-5}$ to $5 \cdot 10^{-6}$ M) at natural pH, air degassed by nitrogen circulation, were irradiated with a wavelength of λ = 313 nm, and monitored by electronic absorption (high concentration limit), Fig. 3a, and fluorescence emission (low concentration limit), Fig. 3b.

The evolution of the fluorescence emission spectra as a function of time was obtained for an excitation wavelength of λ = 306 nm, where Fuberidazole is the largely dominant emissive species in solution, and for this reason, Fig. 3b shows the disappearance of the title compound. For different excitation wavelengths, other patterns are observed, including increase of fluorescence emission due to formation of photoproducts, as is shown in Fig. 3b for an excitation wavelenght of λ = 360 nm

and Fig. 3b for an excitation wavelength of λ = 272 nm.

Whenever it is not possible to select an excitation wavelength for which only one species is emissive (it depends on the overlap of its absorption spectra with other emissive species present in solution), an increase on selectivity can be achieved by choosing the appropriate emission wavelength (it depending on the fluorescence emission spectra overlap), allowing to measure exclusively the emission of the species studied.

In Fig. 4 is shown a practical example of the procedure described above. The Fuberidazole decay curve is distinctly obtained at the wavelengths of λ = 306, and 324 nm for excitation and emission, respectively. By a criterious choice of different excitation and emission wavelengths we were able to obtain curves for some other photoproducts.

In spite of the similarity of the curves describing the kinetic patterns of Fuberidazole photodegradation obtained both by HPLC and fluorescence

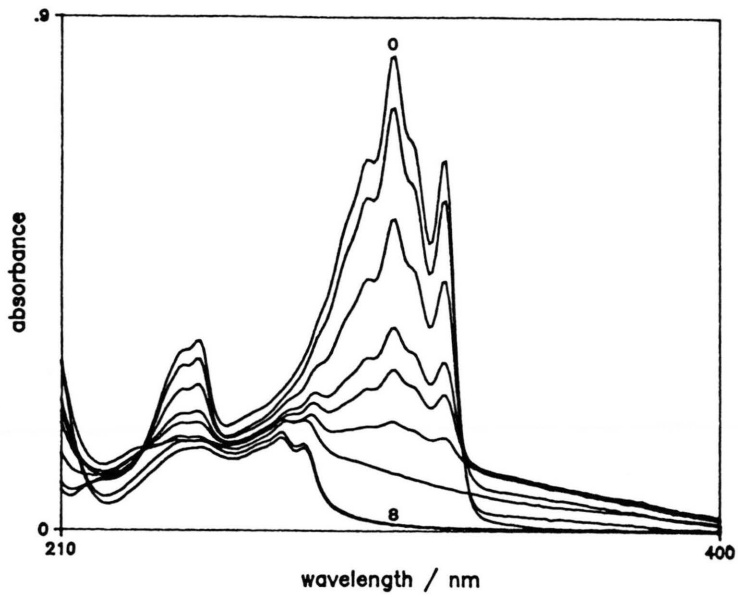


Fig. 3a. Photodegradation of Fuberidazole in methanol followed by absorption:

0 – 0 min
1 – 15 min
2 – 45 min
3 – 85 min
4 – 110 min
5 – 170 min
6 – 425 min
7 – 800 min
8 – 1230 min

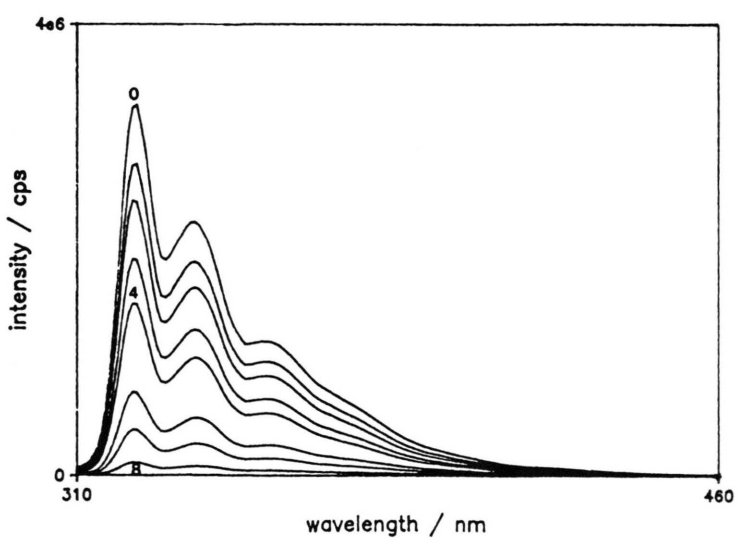


Fig. 3b I. Photodegradation of Fuberidazole in methanol followed by emission (excitation: λ = 306 nm):

0 – 0 min
1 – 20 min
2 – 40 min
3 – 65 min
4 – 95 min
5 – 155 min
6 – 195 min
7 – 240 min
8 – 390 min

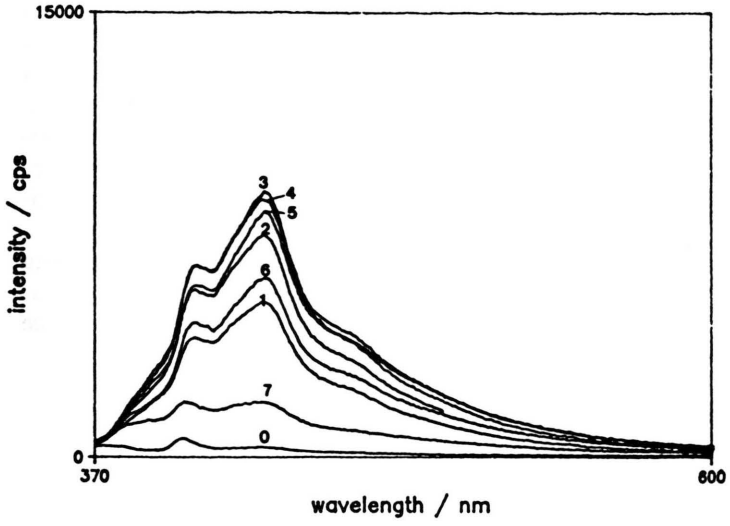


Fig. 3b II. Photodegradation of Fuberidazole in methanol followed by emission (excitation: λ = 360 mn):

0 – 0 min
1 – 40 min
2 – 65 min
3 – 95 min
4 – 155 min
5 – 195 min
6 – 240 min
7 – 390 min

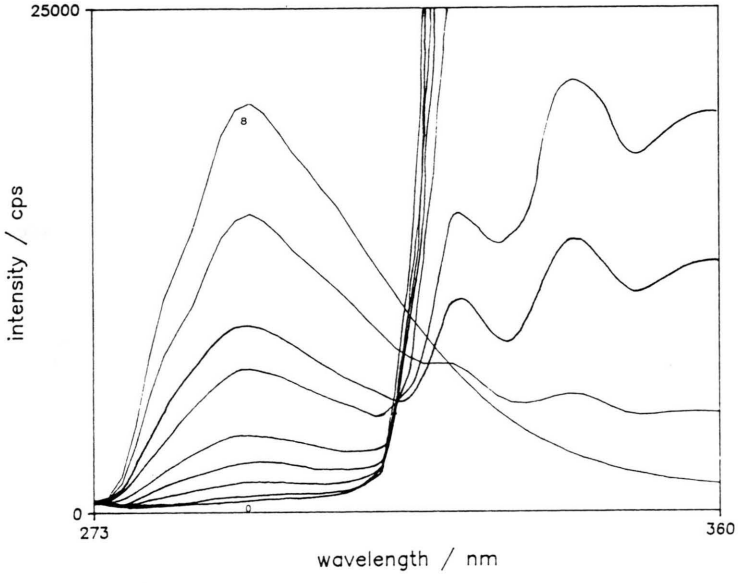


Fig. 3b III. Photodegradation of Fuberidazole in methanol followed by emission (excitation: λ = 272 nm):

0 – 0 min
1 – 20 min
2 – 40 min
3 – 65 min
4 – 95 min
5 – 155 min
6 – 195 min
7 – 240 min
8 – 390 min

emission, Fig. 1 and Fig. 4 respectively, some differences were observed which may be attributed to the different characteristics of the two analytical methods. The intermediate species described in Fig. 3 was followed by monitoring the excitation and emission wavelengths at λ = 360, and 435 nm in Fig. 4, respectively. Excitation spectra of this species were also obtained at the emission wavelength of λ = 460 nm (to avoid interference of other emissive products), and the spectra obtained is consistent with the formation of compound **7** or some of its isomers, Fig. 1.

The photoproduct observed by following the photochemical reaction at the excitation wavelength of λ = 272 nm, Fig. 3b (III), was represented in Fig. 4, at the maximum emission wavelength (λ = 295 nm). The shape of the emission spectra and also the excitation spectra (for a wavelength emission of λ = 295 nm) is consistent with benzimidazole **4** or 1-methoxy-benzimidazole **5.**

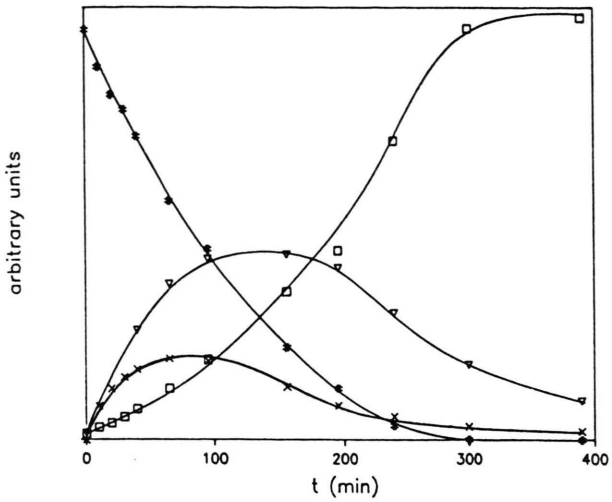


Fig. 4. Kinetic curves of Fuberidazole photodegradation followed by fluorescence emission:

– excitation λ = 306 nm, emission λ = 324 nm
▽ – excitation λ = 360 nm, emission λ = 435 nm
□ – excitation λ = 272 nm, emission λ = 295 nm
× – excitation λ = 340 nm, emission λ = 360 nm

A third photoproduct was observed by choosing the wavelengths of λ = 340, and 360 nm for excitation and emission, respectively. This product was not clearly identified due to the overlap of its emission spectra with those of Fuberidazole **1** and photoproduct **7,** while rough spectra of emission, obtained upon subtraction of the two interferences mentioned before, could suggest to be 2,2′-bibenzimidazole **6.**

The irradiation of Fuberidazole in aqueous solution at pH = 3.0 was as well followed by fluorescence emission, Fig. 5. In agreement with the results obtained by HPLC, we observed the disappearance of Fuberidazole and the formation of just one photoproduct, whose excitation spectra is consistent with the compound identified by HPLC to be benzimidazole-2-carboxylic acid **3.** In this particular case the two methods detect the same photoproducts and we can take profit of the very low concentration limits available, when fluorescence emission is used.

Quantum yields measurements

Quantum yields measurements for Fuberidazole disappearance in methanol, were obtained at λ = 313 nm (Table I).

Table I. Quantum yields for Fuberidazole disappearance in methanol at λ = 313 nm.

pH	O_2	N_2	Quantum yield [Φ]
Natural	+	–	1.3×10^{-4}
Natural	–	+	4.7×10^{-4}
Acidic ($HClO_4$)	–	+	4.9×10^{-3}

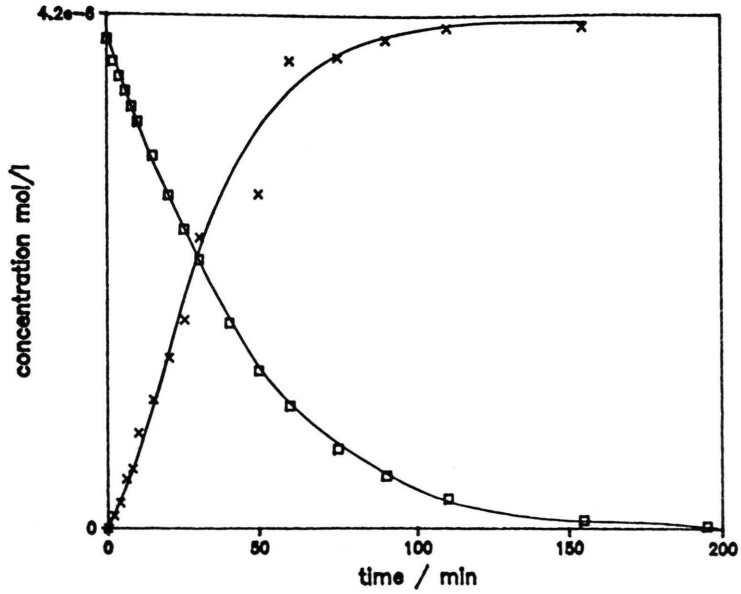


Fig. 5. Photodegradation of Fuberidazole in aqueous solution followed by fluorescence emission and UV-visible absorption:

□ – excitation λ = 314 nm, emission λ = 340 nm,
× – absorption λ = 273 nm

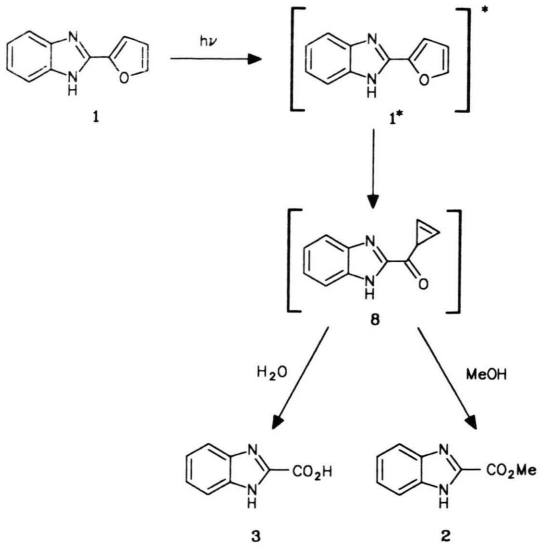
The results reported in Table I indicate a quenching by O_2 on the quantum yield for Fuberidazole disappearance, which is a strong indication to attribute the triplet as the reactive species. In air equilibrated acidic aqueous solutions we obtained a quantum yield Φ of 9.0×10^{-4}.

Photochemical mechanism

The chemical nature of the photoproducts resulting from the photodegradation of Fuberidazole, (all of them containing the benzimidazole ring), points to the furane ring moiety as the exclusively reactive part of the Fuberidazole molecule in the excited state. Another information arises from the kinetical pattern of the photodegradation curves, which show the formation of methyl benzimidazole-2-carboxylate **2** as the most important pathway of photodegradation. It is known from the photochemistry of furane and methyl substituted furanes, that cyclopropenyl ketone acts as an intermediate in the conversion to products [6, 7]. In that light a possible mechanism to account for the formation of methyl benzimidazole-2-carboxylate **2** could proceed through a similar cyclopropenyl ketone transient **8,** followed by reaction with methanol to give the final compound, see Scheme 2.

The other product, methyl-4-oxo-2-benzimidazole-crotonate **7** can be explained by a photoassiste ring cleavage. Benzimidazole **4,** 2,2′-bibenzimidazole **6** and 1-methoxybenzimidazole **5** are obviously formed by reaction of the benzimidazole radical. Such a radical can be formed directly from our proposed transient **8,** or through the secondary photochemical reactions.

When water is used as the solvent, a similar mechanism may be considered: the formation of benzimidazole-2-carboxylic acid **3** resulting from the attack of the transient cyclopropenyl ketone **8** by water.



Conclusions

Inspection of Fig. 1 indicates that for a long time upon complete disappearance of Fuberidazole, there is still a lot of degradated intermediate products. Particulary some of them may be considered to have a longer lifetime when compared with Fuberidazole, and their persistence in soils and natural waters is greater than the target molecule.

Such a behaviour illustrates our assumption, that the simple analysis of a photodegradation curve of a chemical is not sufficient to follow their environmental impact, and points to the need of more detailed studies.

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