

Photostability and phototransformation pathway of an benzimidazolic fungicide

A. Boudina¹, A. Baaliouamer¹, C. Emmelin², J.M Chovelon²

¹Laboratoire d'Analyse Organique Fonctionnelle, Faculté de Chimie, USTHB, BP 32 El-Alia, Bab-Ezzouar, Alger, Algérie

²Institut de Recherche sur la Catalyse et l'Environnement, (IRCELYON), UMR CNRS 5256, Université de Lyon I, 43, Boulevard du 11 novembre 1918, 69622, Villeurbanne Cedex France.

Abstract. Carbendazim or methyl-2-benzimidazole carbamate (MBC) is the most widely used active ingredient in the benzimidazole carbamate class of fungicides. It is a systemic fungicide with both protective and curative activities against a wide range of fungal diseases. Carbendazim has been shown to be the hydrolysis product of benomyl and thiophanate-methyl and in turn, it is the active substance of these two compounds. In the first part, the transformation of the fungicide carbendazim induced by the UV photolysis was studied in dilute aqueous solution under various conditions (i.e. in the presence of air, at various pH). In the second part, the kinetics of the photodecomposition was carried out using high-performance liquid chromatography (HPLC)-diode array. The study of the reaction kinetics yielded a first order rate constant, $k = 0.0281 \pm 0.0006 \text{ min}^{-1}$, and a half-life $t_{1/2} = 24 \pm 1 \text{ min}$ for MBC photolysis. The degradation products were identified by high performance liquid chromatography mass spectrometry (HPLC-MS).

Keywords: benzimidazole carbamate, photodegradation, HPLC-DAD, HPLC-MS

1. Introduction

Benzimidazole fungicides were introduced in the 1960s and their use has considerably increased. Actually, they are efficient at low doses and they inhibit the development of a wide variety of fungi. Indeed, these fungicides play a crucial role in modern agriculture and act by either inhibiting cell division [1]. Among these systemic fungicides, the most used are thiabendazole, fuberidazole, benomyl, thiophanate-methyl and carbendazim [2]. It has been found that excessive application of this fungicide could be harmful for healthy plants; such treatment would sharply reduce the capacity of these plants to respond against pathogen attack [3,4]. Carbendazim has been shown to be the hydrolysis product of benomyl and thiophanate-methyl and in turn, it is the active substance of these two compounds (Fig. 1). Because of the extensive use of these fungicides, they are considered to be pollutants of water resources. Studies on the photo-transformation of carbendazim have already been reported [5,6], but only one has been performed in pure dilute aqueous solution [6]. This last study only focused on carbendazim photolysis under polychromatic irradiation. No indication was given concerning the identification of degradation products. The degradation of carbendazim in the environment has received extensive attention, where photodegradation could be a potential process of the fungicide destruction [6,7].

Here, we report a completed study of the induced transformation of carbendazim by UV light. Accelerated degradation of this pesticide in water has been investigated under atmospheric conditions using a UV light source (high pressure mercury arc lamp).

¹⁺ Corresponding author. Tel.: + (213 21247311); fax: +(213 21247311).
E-mail address: (aboudina36@yahoo.com).

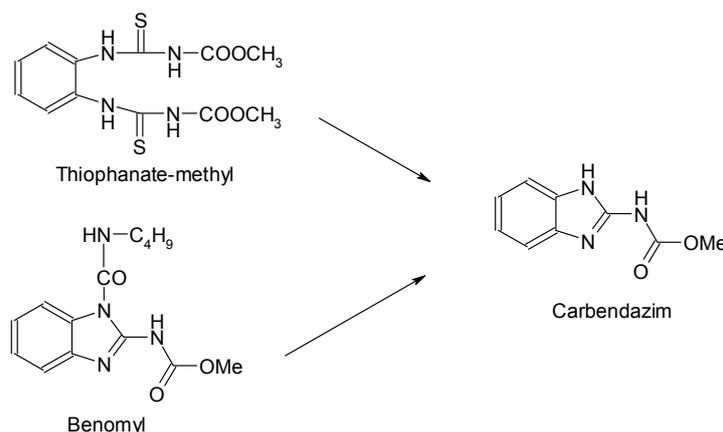


Fig. 1: Principle photochemical transformation of methyl-thiophanate and benomyl.

2. Materials and Methods

2.1. Fungicide

Carbendazim or MBC (IUPAC: methyl benzimidazol-2-ylcarbamate). *Mol. Wt.* = 191.19 g mol⁻¹. Its solubility, at 24 °C, in water is 8 mg L⁻¹ at pH = 7 [8]. MBC with a purity > 99.9% (w/w) was used for the photodegradation experiments were obtained from FLUKA-RIEDEL DE HAËN (SIGMA-ALDRICH CHIMIE). Standards of the 2-aminobenzimidazole (2-AB) (purity >99.9 %) used for product identification were obtained from the same company.

2.2. Solvents

The solvents used for HPLC analysis were methanol, HPLC grade from MERCK, and ultra pure water for making MBC solutions was purified with a MILLIPORE-MILLI Q system.

2.3. Photochemical procedures

Two photochemical reactors were used. The first reactor was a 50 mL cylindrical vessel made of Pyrex with a quartz window in front of a HPK 125 W arc lamp. It was used to accelerate the rate of photochemical degradation for kinetic studies and photoproducts identifications. Experiments were carried out at 19 ± 1°C.

2.4. Kinetic experiments

Samples from the aqueous solution of carbendazim ($C = 2.2 \cdot 10^{-5}$ mol L⁻¹) were taken at regular time intervals for HPLC-DAD analysis without pre-concentration. The same procedure for hydrolysis experiments was carried out, but samples were kept in the dark.

2.5. HPLC- DAD analysis conditions

HPLC analyses were conducted using a SHIMADZU instrument, VP series, equipped with a photodiode array detector (DAD). Two types of columns were used: a Hypersil BDS C18 column (5µm, 125 x 4mm) for the MBC disappearance kinetic studies and a C4 column (5µm, 250 x 4mm) Kromasil TOUZART & MATIGNON, for the photoproducts separation. The flow rate of isocratic elution (methanol/water; 40 + 60; % v/v) was 1 mL.min⁻¹ and the injection volume was 20 µL.

2.6. Solid-Phase Extraction

As the resulting photoproducts formed had very low concentrations, a pre-concentration step was required prior to HPLC-MS analysis. For pre-concentration, each sample was first extracted on solid phase extraction (SPE) cartridges ISOLUTE ENV+ packed with 25 mg of highly-linked styrene-divinylbenzene copolymer and preconditioned with 3 mL each of methanol then de-ionized water. The pre-conditioned sorbents were not allowed to dry before irradiated samples of AZX were passed through them using a Varian vacuum manifold. The pre-concentrated analytes were then eluted from the cartridges using 1 mL of methanol.

2.7. HPLC-MS:

Photoproduct identification was performed using HPLC/MS apparatus (HEWLETT PACKARD HP 1100 SERIES LC-MSD) equipped with the C4 column Kromasil (thermostated to 40 °C) was used with the same operating conditions as the HPLC-DAD analysis. The injection volume was 2 μ L.

The MS detection was performed using an ESI (Electrospray Ionisation) in both positive and negative modes. A capillary potential of 300 V; an N₂ drying gas flow of 13 L min⁻¹, and a pressure of 55 psi were maintained for ESI analysis.

3. Result and Discussion

3.1. Kinetics studies of AZX

Fig. 2 shows the variation of MBC concentrations in (1) dark controls and (2) under HPK irradiation as a function of time. As can be seen, dark controls were stable over the kinetic period indicating that hydrolysis is not a factor in the carbendazim degradation. The photolysis shows a complete disappearance of MBC after 120 min for an initial concentration of 2.2 10^{-5} mol L⁻¹. Degradation data are well-fitted by an exponential curve suggesting a first-order model. The linear plot of Ln C/C₀ versus irradiation time ($r^2 = 0.9929$) allows us to determine the first-order rate constant, $k = 0.0281 \pm 0.0006$ min⁻¹, and a half-life $t_{1/2} = 24 \pm 1$ min for MBC photolysis.

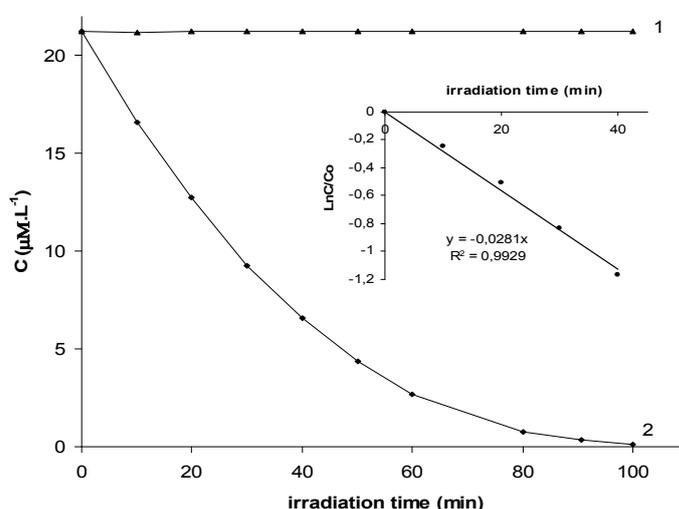


Fig. 2. (1) Evolution of MBC in dark and (2) during the irradiation using the (HPK arc lamp). The inset shows the first-order linear transforms $\ln(C/C_0) = f(t)$ of carbendazim degradation.

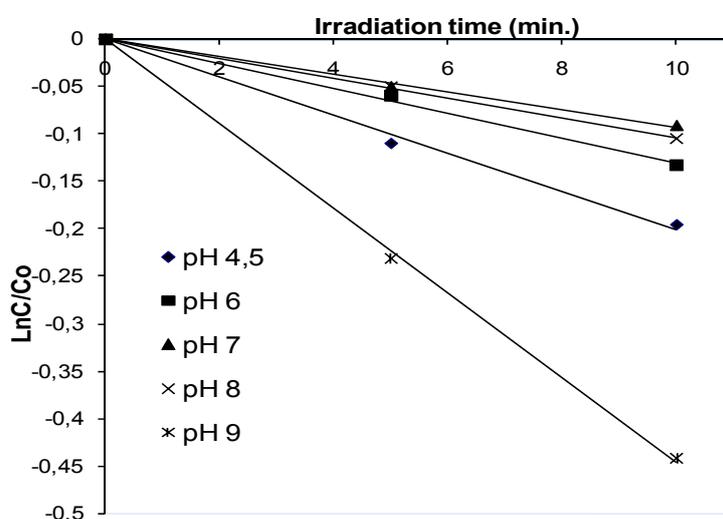


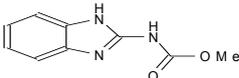
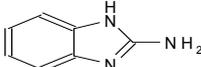
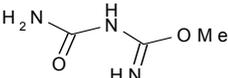
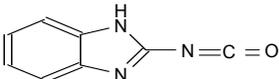
Fig. 3. Kinetics of the photodegradation of MBC in aqueous solutions at different pH

The variation of MBC concentration with the irradiation time (using HPK arc lamp) at different pH levels: 4.5; 6; 7; 8; 9 have studied. At all pH values of buffered water, carbendazim photodegradation follows a first-order reaction kinetics with respect to the pesticide concentration (Figure 3). The kinetic constant (k) was calculated from the curve $\ln(C/C_0)$ vs time. As was previously reported [6], the pH influence is particularly significant in alkaline medium, the kinetic constant increases from $7 \cdot 10^{-3} \text{ min}^{-1}$ at pH 7 to $45 \cdot 10^{-3} \text{ min}^{-1}$ at pH 9, whereas no degradation was observed in the dark. We suggested that the photolysis activation in alkaline mediums could be due to a higher quantity of deprotonated species ($\text{pK}_a = 4.3$) which could be more sensitive towards UV irradiation. Our results confirm the alkaline medium results but for the acidic pH value a slight increase of the constant (k) from $3 \cdot 10^{-3} \text{ min}^{-1}$ to $17 \cdot 10^{-3}$ was observed when the pH drops from 7 to 5.

3.2. Analysis and identification of the carbendazim photoproducts in pure water (HPK arc lamp irradiation, quartz window reactor)

The aqueous solution of carbendazim is stable in the dark but less under UV irradiation. A degradation product of MBC has already been identified in water [8,9]: the 2-aminobenzimidazole (or 2-AB). HPLC-MS analyses have been carried out to identify other possible photoproducts. From the total ion current (TIC) chromatogram obtained after 120 min of MBC irradiation (HPK lamp) in pure water, typical fragments of photoproducts detected by ESI in positive mode are summarised in table 1. Concerning the molecular peaks and fragments detected by HPLC-MS, different structures for the photoproducts can be envisaged.

Table 1: Retention times (R_t), pseudo-molecular peaks ($M+H$)⁺ and fragments detected with ESI in positive mode.

Compound	R_t (min)	Mass (m/z) ($M+H$) ⁺ amu	Mass fragments (m/z) amu	UV Data (nm)
MBC (A) 	6,4	192	160 (M - CH ₃ OH)	$\lambda = 210; 230$ (shoulder); 280
2-AB (B) 	5,6	134	127; 118 (M - NH ₂)	$\lambda = 205; 273;$ 280
(C) 	2,8	118	101 (M - NH ₃)	$\lambda = 215$
(D) 	3,2	160	133 (M - HCN); 118 (M - NCO)	$\lambda = 215$ (shoulder); 230

In our HPLC-conditions MBC appeared at a retention time (R_t) of 6.36 min ($M+H^+=192$) and 2-AB is detectable at $R_t= 5.64$ min and characterized by a molecular ion peak at m/z 134 [$M+H^+$] (photoproduct B). The injection of the analytical standard confirmed this result. At $R_t= 2.86$ min the mass spectrum of photoproduct C, showed a molecular ion peak at m/z 118 [$M+H^+$] and a fragment ion peak at 101 (which could correspond to the loss of NH₃ fragment [118-NH₃]). With regard to the results previously published on MBC photolysis in methanol (Abdou et al., 1985) this mass spectrum could correspond to the monocarbomethoxy-guanidine [CH₃OCH(NH)NHCONH₂]. A short retention time is compatible with the polarity of this product and the fragment ion peak at 101 is in agreement with the envisaged structure. At $R_t= 3.24$ min the mass spectrum of the photoproduct D showed a protonated molecular ion peak [$M+H^+$] at m/z 160 and a characteristic ion fragment peak at m/z 133. This fragment could be attributed to the molecular peak after HCN loss (160-HCN). The appearance of an analogous compound during the photolysis of the fungicide vinclozolin in water and methanol-water solutions [10] suggested a benzimidazole isocyanate structure (photoproduct D). The formation of compound B from D is possible because the isocyanate can react with water to yield the corresponding carbamic acid with CO₂ losses yielding the amine compound.

4. Conclusion

MBC in aqueous solution absorbs light at wavelengths higher than 290 nm and can therefore be photodegraded in the environment. As was previously reported, the pH influence is particularly significant in alkaline medium, the kinetic constant increases from $7 \cdot 10^{-3} \text{ min}^{-1}$ at pH 7 to $45 \cdot 10^{-3} \text{ min}^{-1}$ at pH 9, whereas no degradation was observed in the dark.

The main obtained photoproducts, 2-aminobenzimidazol, derives from the hydrolysis of MBC. However, this study shows that the 2-AB is not the only photoproduct formed during carbendazim irradiation, others photoproducts have been tentatively identified in this study which could be be originated from the photolytic degradation of this benzimidazolic fungicide.

5. References

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