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# Structural characterization of photoproducts of pyrimethanil

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## Introduction

Pyrimethanil is an anilino-pyrimidine fungicide particularly active against gray mold (*Botrytis cinerea*) and pear scab (*Venturia inaequalis* and *Venturia pirina*) on grapes, strawberries, tomatoes, fruits, vegetables and ornamental plants in greenhouses and open field situation.[1–5] According to the European Food Safety Authority, pyrimethanil has no evident mutagenic, genotoxic or carcinogenic potential, but a short-term toxicity study on rats and mice has shown an increase in liver weight accompanied by changes in the histopathology of the liver and thyroid.[6] To avoid these side effects, the concentration of pyrimethanil has been limited by legislation. The European Commission set the Maximum Residue Level of pyrimethanil at 10 mg/l in citrus fruits, 5 mg/l in pome fruits, strawberries, table and wine grapes and 0.05 mg/l in tree nuts (EC/600/2010).[7] It is thus important to increase our knowledge regarding the concentration of pyrimethanil in environmental matrices, its degradability and the factors affecting it. One of these factors is the photo alteration by sunlight, which is known to play a significant role in the degradation of this compound, due to the prolonged half-life (77 days approximately) of pyrimethanil in the environment.[8] Previous studies on the degradation of pyrimethanil were carried out mainly in a waste water treatment context and focused on the photo catalytic degradation using various salts as catalysts. Agüera *et al.*[9] used $TiO_2$, Vanni *et al.*[10] and Anfossi *et al.*[11] added iron III, Navarro *et al.*[12] added ZnO and Gomis *et al.*[13] used thiopyrylium. Irradiation was carried out using a mercury or xenon lamp or direct sunlight. These studies have showed that a long irradiation time (between 150 and 1400 min, depending on the conditions and catalyst) is required for total disappearance of pyrimethanil. Degradation of pyrimethanil in water using the technique of photo-Fenton was investigated by Sirtori *et al*. They reported that the addition of sodium chloride accelerated the reaction and characterized four chlorinated photo-Fenton by-products.[14] The aim of the present study was the identification of UV–visible photo transformation products of pyrimethanil in water. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and gas chromatography coupled with multi stage mass spectrometry (GC-MS$^n$) were used for analysis, with the aim of covering a large range of polarities for the detection of the potential transformation products. Elucidation of the structures of photoproducts was carried out performing high-resolution measurements and collision-induced dissociation (CID) experiments. Photolysis mechanisms have also been proposed to explain the formation of photo products of pyrimethanil in water.

## Material and methods

Pure pyrimethanil (99%) and chromatographic grade solvents (99.99% purity), methylene chloride (DCM), acetonitrile (ACN) and formic acid (FA) were purchased from Sigma Aldrich (St Quentin Fallavier, France). Taking into account the low solubility of pyrimethanil in water (121 mg l$^{-1}$ at 25 °C), a first solution was prepared at 100 mg l$^{-1}$ in ACN. This solution was then diluted to prepare working solutions in water at a concentration of 5.0 mg l$^{-1}$ (i.e. 5% ACN/95% $H_2O$). This high concentration was necessary to allow multi stage mass spectrometry experiments on the photo products of low abundance. According to the ROMIL technical report, ACN does not absorb UV light since its transmission at 200 nm is about 100%.[15] Consequently, ACN is expected not to affect the nature of the photoproducts obtained during irradiation. This is the main reason why it has been currently used to dissolve pyrimethanil in previous studies devoted to photolysis. For instance, Gomis *et al.* studied photocatalytic degradation pathways of pyrimethanil in pure ACN.[13] Photolysis experiments were carried out in a self-made reactor equipped with a high-pressure mercury lamp (HPL-N 125 W/542 E27 SC; Phillips, Ivry-sur-Seine, France) delivering radiation at wavelengths ranging from 200 nm to 650 nm. The absorbance spectrum of pyrimethanil shows an absorption maximum between 260 and 280 nm.[16] According to manufacturer data, the incident radiation flux was 6200 lm. The reactor consists in six quartz tubes of 120 ml disposed in a circle around the lamp and immerged into a sonicator (AL04-12, Advantage-Lab, Switzerland) filled with deionized water. The solution of pyrimethanil was degassed with a nitrogen stream prior to irradiation and was sonicated during irradiation so that free oxygen is not expected to be dissolved in water in significant amounts. During experiments, the reactor was cooled by water circulation to maintain a constant temperature of 25 ± 3 °C. For each experiment, 30 ml of a solution of pyrimethanil was used.

LC-MS/MS analysis was performed on a 2690 series HPLC instrument coupled with a quadrupole time-of-flight 'Premier' mass spectrometer equipped with a Z-spray electrospray ionization (ESI) source (Waters Technologies, Saint Quentin en Yvelines, France). Compounds were separated on a T 3.3 µm analytical column C18 2.1 × 150 mm (Atlantis, France). The

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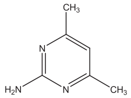
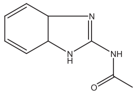
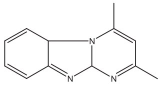
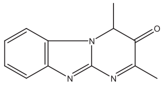
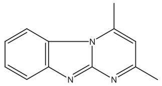
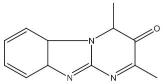
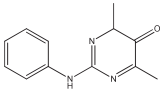
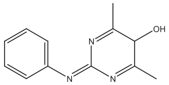
**Table 1.** Retention times, relative intensities, experimental m/z, CID transitions and proposed structures for the phototransformation products of pyrimethanil

| Compound | Retention time (min) | Relative intensity[a] | Experimental m/z of $MH^+$ | Error (ppm) | DBE[b] | Elemental composition | CID transitions (m/z) | Proposed structure | References[c] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 2.12 | 10.2 | 124.0870 | −4.0 | 3.5 | $C_6H_{10}N_3$ | 124 → 83 (−41)<br>124 → 107 (−17)<br>107 → 67 (−40) |  | [9,11,13,18,19] |
| 2 | 2.58 | 8.0 | 178.0964 | 3.4 | 5.5 | $C_9H_{12}N_3O$ | 178 → 94 (−84)<br>178 → 136 (−42)<br>136 → 119 (−17) |  | |
| 3 | 3.30 | 3.0 | 200.1182 | −3.0 | 7.5 | $C_{12}H_{14}N_3$ | 200 → 159 (−41)<br>159 → 143 (−16)<br>143 → 117 (−26) |  | |
| 4 | 3.30 | 17.5 | 214.0990 | 4.7 | 8.5 | $C_{12}H_{12}N_3O$ | 214 → 171 (−43)<br>214 → 143 (−71)<br>214 → 186 (−28)<br>186 → 117 (−69)<br>186 → 159 (−27) |  | |
| 5 | 3.34 | 9.8 | 198.1127 | −1.5 | 9.0 | $C_{12}H_{12}N_3$ | 198 → 182 (−19)<br>198 → 171 (−27)<br>198 → 157 (−41)<br>157 → 117 (−40) |  | |
| 6 | 5.97 | 9.0 | 216.1146 | 4.2 | 7.5 | $C_{12}H_{14}N_3O$ | 216 → 198 (−18)<br>216 → 77 (−139)<br>216 → 188 (−28)<br>188 → 117 (−71) |  | |
| 7 | 17.70 | 25.0 | 216.1120 | −7.9 | 7.5 | $C_{12}H_{14}N_3O$ | 216 → 77 (−139)<br>216 → 123 (−93)<br>216 → 188 (−28)<br>188 → 172 (−16)<br>188 → 160 (−28) |  | [9,11,14] |
| 8 | 18.06 | 9.9 | 216.1147 | 4.6 | 7.5 | $C_{12}H_{14}N_3O$ | 216 → 198 (−18)<br>216 → 199 (−17)<br>199 → 77 (−122)<br>199 → 122 (−77)<br>216 → 186 (−30)<br>186 → 160 (−26) |  | |
| Pyrimethanil | 18.60 | – | 200.1184 | 0.1 | 7.5 | $C_{12}H_{14}N_3$ | 200 → 77 (−123) | | |

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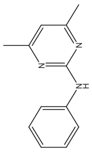
| | |
|---|---|
| 200 → 107 (−93)<br>200 → 143 (−57)<br>200 → 183 (−17)<br>183 → 167 (−16)<br>167 → 117 (−50) |  |

[a] Percentage of the sum of all the photoproduct abundances (determined on the total ionic current) for 3 hs of irradiation

[b] Double bond equivalency

[c] References of the articles in which the proposed structure has already been reported

HPLC solvents were ACN with 0.1% FA (A) and water with 0.1% FA (B). 10 µl were injected with a mobile phase flow of 0.2 ml min$^{-1}$. The following program of linear gradient was applied: 30% of A for 11.0 min and 30–100% of A from 11.1 to 20.0 min. The column was reconditioned with 30% of A from 20.1 to 30.0 min. Ions were recorded in positive ion mode in the 50–750 domain. The ESI cone and capillary voltages were set at 20 V and 3 kV, respectively. Extraction cone and ion guide voltages were set at 3 V and 4 V, respectively. Source and desolvation temperature were fixed at 120 °C and 450 °C. Nebulization and desolvation were performed using nitrogen gas. The cone gas flow was 70 l h$^{-1}$, and the desolvation gas flow was 700 l h$^{-1}$. For CID experiments, argon was used as collision gas with a flow of 0.28 ml min$^{-1}$. The electrostatic mirror was used in the W-mode for accurate m/z measurements. GC-MS analyses were carried out on a Varian 450GC gas chromatograph coupled with a Varian 240MS ion trap mass spectrometer; operating conditions were reported in a recent paper.[17]

## Results and discussion

This study is the first to report by-product structures obtained by UV–visible irradiation of pyrimethanil without added catalyst. Most of the previous studies devoted to pyrimethanil photolysis used solar irradiation,[18] a neon lamp[19,20] or a xenon lamp with cutoff of irradiation under 290 nm.[21] Thus, based on the UV–visible spectra of pyrimethanil (maximum absorption between 260 and 280 nm[16]), negligible photodegradation was expected. In the present study, the light has not been filtered, thus explaining why phototransformation products were observed.

The relative abundances of by-products were determined from times ranging from 15 min up to 8 h of irradiation. All the photoproducts were detected in the solution irradiated for 3 h; this solution was thus selected to carry out CID and high-resolution experiments on photoproducts. At this time, the degradation yield of pyrimethanil, estimated on the basis of total ion currents, was about 60%. Eight photoproducts were detected in LC-MS. Retention times, exact m/z ratios of molecular ions and CID main fragment ions are reported in Table 1. Only compound 1 and aniline in trace amounts were detected in GC-MS; they were easily characterized owing to the NIST spectra data base with matching scores greater than 94%.[22]

The first step of the transformation process was assumed to correspond to the formation of a radical cation by electron removal, as postulated in previous studies.[17,21,23] The secondary amine and all the double bonds of the system constitute likely ionization sites: electron removal leads in all cases to a highly delocalized radical cation. In aqueous solution, the radical cation can be captured by a water molecule. The resulting species can eliminate a proton to give a solvated free radical, which can in turn undergo subsequent photoionization to lead to a cation with an even number of electrons.

Three compounds referred as 6, 7 and 8 were detected with a molecular weight of 215 amu at retention times of 5.97, 17.70 and 18.06 min, respectively. They likely result from hydroxylation of pyrimethanil. Under CID, 7 and 8 provided an ion at m/z 77. This ion, also detected for pyrimethanil, corresponds to the benzene ring ionized $C_6H_5^{+\cdot}$. It suggests that the benzene part of the molecule was not affected in the formation of both compounds 7 and 8. $H_2O$ addition on ionized pyrimethanil followed by proton elimination leads to

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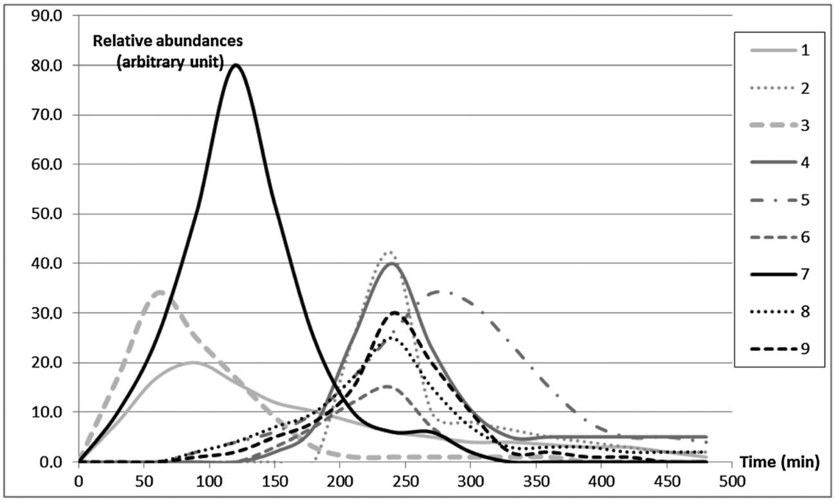

**Figure 1.** Relative abundances of photoproducts as a function of the irradiation time.

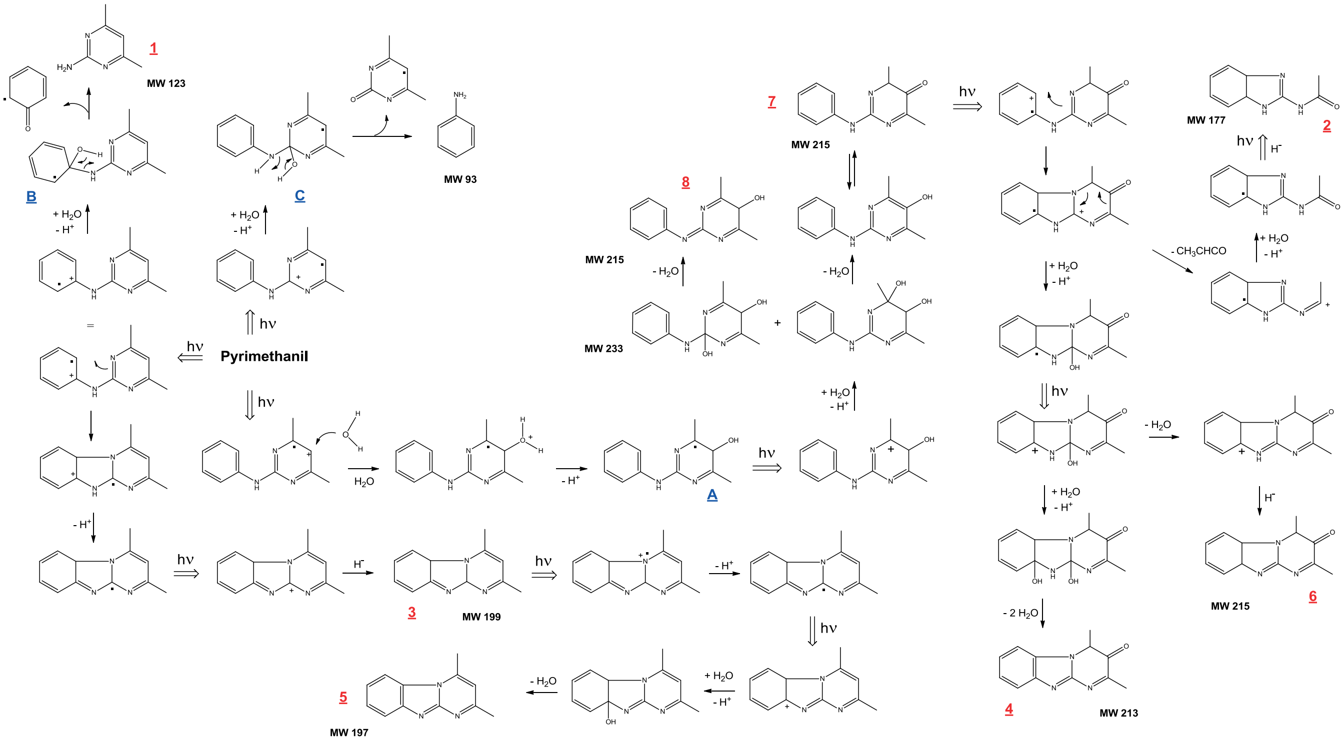

**Figure 2.** Mechanisms postulated for the formation of compounds 1 to 8 from irradiated pyrimethanil.

the free radical referred as A in Fig. 2. Subsequent photoionization of A leads to a cation with an even number of electrons on which water addition followed by proton elimination leads to two isomeric structures with MW = 233 amu. These compounds were not detected in this work, but they were reported in a previous study devoted to the photo catalytic degradation of pyrimethanil.[11] Water elimination from each of these structure leads to compounds 7 and 8 (Fig. 2). 7 corresponds to the only structure which can eliminate CO in mass spectrometry, while 8 can eliminate HO· and $H_2O$, leading to a stable final state in both cases, and also $H_2CO$ (see Table 1). A cyclic structure has been postulated for 6 given its short elution time in comparison with 7, 8 and pyrimethanil. Indeed, Hamilton reported that the elution times in reverse-phase HPLC are correlated to the number and the size of the rings and that cyclic structures elute before their acyclic analogues.[24] In mass spectrometry, the pseudo molecular ion $6H^+$ eliminates CO, as $7H^+$ does. Furthermore, Fig. 1 shows that the formation of 6 follows the disappearance of 7, thus suggesting that 6 likely arises from degradation of 7. A mechanism involving photo ionization of 7 followed by cyclization and water molecule additions has been postulated for the formation of 6 (see Fig. 2, right hand).

Given that kinetics data for compound 4 (213 amu) match those for 6 (see Fig. 1) and that $4H^+$ also eliminates CO, 4 was assumed to result from dissociation of 7 by a mechanism

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analogous to that postulated for the formation of 6 (Fig. 2, right hand). The formation of compound 2 (MW 177) may be also rationalized by a mechanism following photoionization of 7, in competition with that postulated for the formation of 6 and involving a ring aperture induced by the positive charge (Fig. 2, right hand).

Structures B and C in Fig. 2 result from a water molecule addition on ionized pyrimethanil followed by proton elimination. Compound 1, also characterized in GC-MS (see above), results from elimination of the cyclohexa-2,4-dienone radical from B, while the loss of the radical pyrimidine radical from C leads to aniline; both compounds have been detected in a previous study.[9] Isomeric forms of B and C have also been considered; they are not displayed in Fig. 2 as they lead, according to the mechanisms postulated above, to molecules which are not detected.

Kinetics data show that 3 (eluted at 3.3 min) is among the first compounds that are formed under irradiation. Cyclization of ionized pyrimethanil followed by proton elimination leads to a free radical, whose subsequent photoionization followed by hydride addition leads to 3. The structure postulated for 3 is in good agreement with the consecutive losses of $CH_3CN$, $CH_4$ and CN observed under collisional activation of the ion $3H^+$. Kinetics data (see Fig. 1) show that compound 5 seems to be produced from 3. Furthermore, given their very close retention times (3.30 min for 3 *vs* 3.34 min for 5) and their difference of two mass units (MW = 199 for 3 *vs*. MW = 197 min for 5), 5 was postulated to result from dehydrogenation of 3 after photoionization of the latter according to the mechanism depicted in Fig. 2.

## Conclusion

In conclusion, eight photo products were identified by HPLC-MS/MS. Among them, compound 1 was the only one which was also detected by GC-MS, with traces amounts of aniline. With the exception of compounds 1 and 7, the structures proposed for photoproducts on the basis of mass spectra interpretation have not been reported in previous studies.

*Yours,*

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