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Perinaphthenone phototransformation in a model of leaf epicuticular waxes



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ABSTRACT

Perinaphthenone (1*H*-phenalen-1-one, PN) is a reference photosensitizer producing singlet oxygen with a quantum yield close to one in a large variety of solvents. It is also the basic structure of a class of photo-toxic phytoalexins. In this work, the PN photoreactivity was studied for the first time in a paraffinic wax, used as model of leaf epicuticular waxes. The PN photodegradation was monitored by UV–Vis spectros-copy. The triplet excited state, singlet oxygen and the hydroxyperinaphthenyl radical were detected by diffuse reflectance laser flash photolysis, near infrared phosphorescence and by EPR spectroscopy, respectively. The PN phototransformation was found to be fivefold faster in the wax than in *n*-heptane under steady-state irradiation. The hydroxyperinaphthenyl radical formation was observed in aerated irradiated paraffin wax while in *n*-heptane solution the radical was observed only in the absence of oxygen. These results show that under continuous irradiation, PN is much more easily phototransformed in a solid environment than in solution. Several photoproducts were identified, in particular phenalanone, PN dimers, and oxidized PN-alkanes adducts. Finally, when *pyrethrum* extract is added into the wax along with PN, the hydroxyperinaphthenyl radical concentration was increased by a factor of 2.4. Such photochemical reactions may occur when systemic pesticides enter the plant cuticle.

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1. Introduction

Plants synthesize phytoalexin substances for microbial and often oxidative protection [1-3]. These toxins are produced *de novo* into the leaf cells [4] and have been identified from higher plant tissues into the upper epidermis and in the leaf epicuticular waxes [5–7]. Some phytoalexins are phototoxic due to their ability to produce singlet oxygen under solar irradiation [8–10]. Many of these natural products are based on the perinaphthenone (1*H*-phenalen-1-one, PN) skeleton and have been isolated from plants and fungi [11,12]. Therefore, the photochemical reaction mechanisms of PN are similar to those of some phytoalexins. PN is used as a reference photosensitizer in photochemistry and photobiology [13]. The singlet oxygen production quantum yield of this molecule is close to one over a large solvent range [13–16]. Nonetheless, in hydrogen donating solvents, PN can be photodegraded [16,17]. The plant leaf epicuticular waxes are mainly composed of long chain alkanes and of other compounds such as esters, ketones and alcohols [18,19]. Some of them are hydrogen donors. In this study paraffin wax films are used as a model of the leaf epicuticular waxes. Previous studies were performed with this model at the wax surface [20–22]. This model allows a first approach of the photochemical processes occurring in the waxes of a broad variety of plants. Indeed, paraffin wax is a weak hydrogen donating medium but PN phototransformation is expected because of the degradation observed in N,N'-dimethylacetamide and 1,4-dioxane [16]. In fact, in these media the competition between singlet oxygen production and PN transformation is not trivial.

The ability of phytoalexin to act as a singlet oxygen sensitizer and more generally the photochemistry of phytoalexins can be inconvenient for crop treatments [23,24]. Indeed, some pesticides are oxidized by singlet oxygen [25,26] and these reactions could occur at the leaf surface and within the leaf epicuticular waxes. Moreover, to our knowledge, no study has been performed on the possible interaction between the PN triplet excited state and pesticides absorbed by plants (systemic pesticides) which enter the cuticle. Under these conditions, pesticide molecules are

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expected to be in the close environment of phytoalexins that could induce their degradation.

For the first time, PN photochemistry is studied in a model of leaf epicuticular waxes. The goal of this study is to give a first approach of the PN photoreactivity in a solid medium composed of long-chain alkanes and to evaluate the competition between singlet oxygen production and phototransformation. Additionally, identification of photoproducts was performed. Finally, *pyrethrum* extract, a natural pesticide, was added to the paraffin wax film containing PN to obtain evidence of an interaction between the PN triplet excited state and this pesticide.

2. Materials and methods

2.1. Chemicals

Perinaphthenone (97%), *n*-heptane (99%), methanol (\geq 99.9%), acetonitrile (\geq 99.9%), paraffin wax (mp 53–57 °C) and silica gel (Davisil grade 634, 100–200 mesh, 60 angstrom, purity \geq 99%) were all purchased from Aldrich (Saint-Quentin Fallavier, France). Paraffin wax is a mixture of long-chain alkanes (mainly C₂₁–C₃₄). Pestanal pesticides tembotrione and *pyrethrum* extract were both purchased from Sigma–Aldrich.

2.2. Sample preparations

All the samples were prepared in a sodium lamp light room $(\lambda_{irr} = 589 \text{ nm})$ to avoid photochemical reactions during handling. Stock solutions of PN in n-heptane were prepared at concentrations ranging from 10^{-3} to 10^{-5} M and stored at room temperature. Paraffinic wax films containing PN were prepared as follows: 72 mg of wax and 16.54 mg of PN were mixed and heated at 100 °C under magnetic stirring to obtain a homogeneous liquid. Subsequently, the mixture was poured on aluminum foil and pressure was applied using a plate to produce the films. The 10^{-3} M PN solid concentration in the paraffin wax films was calculated based on a wax density of 0.77 g mL⁻¹. Less concentrated films were obtained by dilution with pure paraffinic wax. In all cases, films containing PN were rinsed with acetonitrile to remove any PN molecules remaining at the film surface. For UV-Visible spectroscopy, PN was mixed with cellulose at a 1/100 ratio in weight. For laser flash photolysis paraffin wax films containing PN were directly used without further modification. Silica gel samples containing adsorbed PN were used to monitor the triplet decay in the absence of oxygen. They were prepared as follows: 2 g of silica gel were dried by heating at 120 °C under vacuum for 2 h to a pressure of 5×10^{-5} mbar. Then, a PN solution at a concentration of 100 µM in methanol was added to the silica. The solvent was evaporated under vacuum at room temperature. Then, the sample was maintained under vacuum for 4 h to ensure removal of trace methanol, water, and air. Finally, the samples were sealed under vacuum in a quartz cuvette. For EPR analyses the liquid mixture containing wax and PN was directly poured inside Pyrex tubes. Pesticides were added to the hot liquid wax-PN mixture under magnetic stirring at amounts calculated to obtain a final concentration of 10^{-3} M for tembotrione and 4.3 μ L ml⁻¹ for the pyrethrum extract.

2.3. UV-Vis spectroscopy

Liquid state absorption spectra were recorded using a Cary 3 UV–Visible spectrometer (Varian). Baselines and spectra were recorded at room temperature in the 800–250 nm spectral range with a 1-nm resolution and a 600 nm min⁻¹ scan rate. Solid state UV–Visible spectra were recorded using a DRA-CA-30I integrating

sphere accessory (Varian) and a BaSO₄ reflectance standard (Spectralon). PN mixed with cellulose was poured into a circular quartz cell of 1-cm thickness and then the cell was placed in the diffuse reflectance port of the sphere accessory. UV–Vis absorption spectra of paraffin wax films containing PN were recorded by positioning the film in the transmission port of the sphere accessory. The signal intensity measured in percentage of transmittance was converted to absorbance from the following equation:

$$A(PN) = \log\left(\frac{I_t(Pa)}{I_t(PN + Pa)}\right)$$
(1)

where A(PN) is the PN absorbance, $I_t(Pa)$ and $I_t(Pa + PN)$ are the transmitted light through the pure paraffin and paraffin containing PN samples, respectively. For PN dissolved in *n*-heptane, the molar absorption coefficient was calculated from UV–Vis absorption spectra of 10^{-4} , 2×10^{-5} , and 10^{-5} M solutions. In the case of paraffin wax containing PN, the molar absorption coefficient was calculated from transmission spectra of four different films containing 10^{-4} M of PN. The film thickness was measured using a MI20 Micrometer (Messmer Instrument Ltd). The average film thickness was $500 \pm 100 \,\mu$ m. Molar absorption coefficient calculations of paraffin wax films containing PN were based on the Beer–Lambert law [27].

2.4. Photodegradation quantum yield and rate constants measurements

All the samples were irradiated at 366 nm (room temperature) using a mercury lamp (Oriel, 200 W) equipped with a monochromator (Photomax). The average photon fluence rate ($E_{\rm p,o}$ = 2.0 \times 10¹⁵ cm⁻² s⁻¹) was measured using a QE65000 UV-Visible spectrometer (Ocean Optics). Air-saturated solutions were irradiated in a cubic quartz cell of 1-mm pathlength. The decay of PN was monitored by UV-visible spectroscopy. The paraffin wax films containing PN were placed on a sample holder that allowed reproducible positioning of the sample for irradiation and UV-Visible analysis. The ~500 µm thickness films were irradiated under the same conditions as the solutions. Irradiation experiments were carried out in triplicate. Quantum yields of PN photodegradation ($\Phi_{\rm deg}$) were determined in air-saturated *n*-heptane and paraffin wax. The $\Phi_{\rm deg}$ values were calculated from the following equation:

$$\Phi_{\rm deg} = \frac{\Delta[\rm PN]}{P_a \cdot \Delta t} \cdot 10^{-3} \cdot N_A \cdot \ell \tag{2}$$

where Φ_{deg} is the PN photodegradation quantum yield, Δ [PN] (mol L⁻¹) is the loss of PN concentration after irradiation, P_a (cm⁻² s⁻¹) is the photon fluence rate absorbed by the sample, Δt (s) is the irradiation duration, N_A is the Avogadro number, and ℓ (cm) is the sample pathlength. Calculation of Φ_{deg} was based on a concentration loss of not more than 10% of the initial concentration.

2.5. LC-MS analyses

The MS system employed consisted of an LC/QTOF equipped with an orthogonal geometry Z-spray ion source (Waters/Micromass, Manchester, UK). For UV detection, a photodiode array detector Waters Alliance 2695 system was used. A volume of 40 μ L was injected on a reversed-phase column (Kinetex, Phenomenex, C18, 2.6 μ m, 100 mm \times 2.1 mm). A flow rate of 0.2 mL min⁻¹ was set. The binary solvent system used was composed of acetonitrile (ACN) and milli-Q water. The gradient elution started with 5% ACN and reached 95% ACN in 15 min linearly. These conditions were kept during 25 min. The desolvation chamber and ion source block temperatures were set at 180 °C and 100 °C, respectively. N₂ was used as the nebulizer gas (35 L h⁻¹) and the desolvation gas (250 L h⁻¹). The electrospray interface was operating in positive

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