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Irradiation of fluvastatin in water Structure elucidation of photoproducts

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Abstract

Fluvastatin is easily transformed in water by solar irradiation. One day-light exposure induces a complete degradation of the drug and formation of a mixture of products. Different chromatographic processes led to the isolation of dihydrobenzocarbazole, benzocarbazole, azonane-2,7-dione and spiro[4.4]azononane derivatives. Structures of photoproducts were elucidated by spectroscopic means. Photocyclization and photooxygenation are the main reactions involved in the formation of the observed products.

Keywords: Fluvastatin; Benzocarbazole derivatives; Lactam; Photocyclization; Photooxygenation

1. Introduction

Risk associated with the introduction of pharmaceuticals and personal care products (PPCPs) into the aquatic environment has become an important issue in environmental research. These chemicals are considered emerging pollutants, because they are continuously introduced into the aquatic environment and have been detected in surface waters in many countries [1,2].

These substances enter into the aquatic environment due to the ineffectiveness of sewage treatment plants, and thus they are potential pollutants for the aquatic ecosystem, with adverse effects on aquatic organisms. Pharmaceuticals, in water, can be subject to abiotic transformations (hydrolytic and photochemical) leading to different products that in some cases are more persistent and more toxic than the parent compound [3–6]. Therefore, the presence and possible effects of these transformation compounds should be investigated, too. While the occurrence of pharmaceuticals in surface waters has been extensively reviewed, data on their fate in water are still limited.

In this context, we are interested in investigating the photochemical behaviour of some of the most commercialized drugs under environmentally relevant conditions, with particular attention to the isolation and identification of photoproducts. Recently our attention has been focused on synthetic statins [7,8]. Results obtained have shown an easy sunlight-induced photodegradation of these drugs giving rise to several photoproducts.

In this work, we have investigated the photochemical transformation processes of fluvastatin (**1**) in water and reported the structure elucidation of the main photoproducts. Fluvastatin sodium is a statin that acts as lipid-lowering agent and is widely used in the prevention of cardiovascular events. In a recent study, the photodegradation kinetics of fluvastatin was determined at λ 365 nm region (high-pressure mercury lamp, interference filter and Wood's filter) in methanol and water evidencing a fast degradation, mainly in the latter solvent [9].

2. Experimental

2.1. Chemicals

Fluvastatin sodium was obtained from KEMPROTEC Limited. Solutions and suspensions of the drug were prepared using

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Milli-Q water. All other solvents were of HPLC grade. Methylene blue and NaN_3 were obtained from Aldrich.

2.2. General procedures

HPLC experiments were carried out on an Agilent 1100 HPLC system equipped with an UV detector, the column used was a RP-18 column (Luna Prep C-18, 10 μm, 250 mm × 10 mm). Nuclear magnetic resonance (NMR) spectra were recorded at 500 MHz for [^{1}H] and 125 MHz for [^{13}C] on a Fourier Transform NMR Varian 500 Unity Inova spectrometer and at 400 MHz for [^{1}H] and 100 MHz for [^{13}C] on a Bruker AC 400 spectrometer. The carbon multiplicity was evidenced by DEPT experiments. The proton couplings were evidenced by ^{1}H–^{1}H COSY experiments. The heteronuclear chemical shift correlations were determined by HMQC and HMBC pulse sequences. ^{1}H–^{1}H proximities through space within a molecule were determined by NOESY. UV/vis spectra were recorded in MeOH on a Perkin-Elmer Lambda 7 spectrophotometer. IR spectra were recorded in $CHCl_3$ on a Nicolet 5700 FT-IR spectrometer. Low resolution electron impact mass spectra were obtained operating at 70 eV on a GC–MS (QP-5050A Shimadzu). A photoreactor (Helios Italquartz) equipped with a 500 W high-pressure mercury lamp (through a Pyrex glass filter, λ > 300 nm) was used for UV irradiation. Analytical TLC was performed on precoated Merck aluminum sheet (DC-Alufolien Kielselgel 60 F_{254}, 0.2 mm) or RP-18 F_{254} plates with 0.2 mm film thickness. The spots were visualized by UV light or by spraying with H_2SO_4–AcOH–H_2O (1:20:4). The plates were then heated for 5 min at 110 °C. Prep. TLC was performed on a Merck Kiesegel 60 F_{254} plates, with 0.5 or 1 mm film thickness.

2.3. Experimental procedure

2.3.1. Irradiation experiments

Experiments in the dark were conducted on solutions of the drug (10^{-4} M) in pure water and at pH 7 using buffered water with NaH_2PO_4/Na_2HPO_4. The preparations were kept in the dark for 1, 4 and 7 days, concentrated and the residues analyzed by TLC and ^{1}H NMR.

A solution of fluvastatin, 10^{-5} M, was exposed to sunlight and analyzed by UV every 15 min (Fig. 1).

Solutions of fluvastatin (40 mg, 10^{-4} M) were exposed to sunlight in pure water on June in Naples in open Pyrex flasks for 1 day-light. Irradiation mixtures were then dried under vacuum and the residue (40 mg) was separated by silica gel preparative TLC-chromatography (1 mm). Eluting with 50 ml of CH_2Cl_2/MeOH (95:5) with two drops of acetic acid (two runs) gave a fraction A (diastereomeric mixture **2**, 15 mg), photoproduct **3** (6 mg), photoproduct **4** (9 mg), and a fraction B (14 mg).

Fraction A was subjected to methylation with excess of diazomethane in ether solution. The resulting mixture was separated by silica gel TLC-chromatography eluting with toluene/ethyl acetate to afford pure diastereomeric methyl esters of **2a** (6 mg) and **2b** (7 mg).

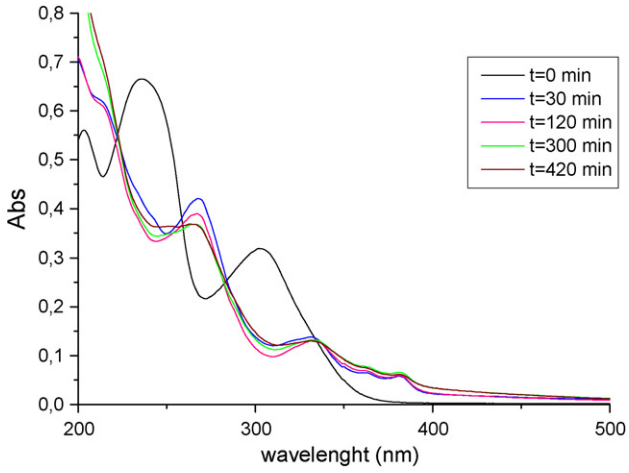

Fig. 1. Changes in UV spectra of water solution of fluvastatin (1.1×10^{-5} M) at different times of solar exposure.

Fraction B was a mixture of polar products that were separated by reverse-phase HPLC with a RP-18 column. The flow was set to 1.7 ml/min. The column was equilibrated with a mixture of A (H_2O containing 0.1% acetic acid)–B (methanol) 9:1 (v/v) and using the following program: an increase of B up to 50% in 11 min and a further increase to 100% in 11 min. The detector wavelength was set at 260 nm. Pure compounds **5a** (2 mg), **5b** (1 mg), **6a** (6 mg) and **6b** (5 mg) were obtained.

In order to evaluate the stability of the irradiation mixtures for longer times, solutions of fluvastatin (10^{-4} M) were exposed to sunlight in water for 10 days on June in Naples in open Pyrex flasks.

Irradiation experiments with the 500 W high-pressure mercury lamp in open Pyrex tube (distance of 15 cm from the lamp at r.t.) were conducted for different times (1, 2, and 4 h).

A solution of the drug (2 mg/20 ml, ca. 10^{-4} M) saturated with argon for 15 min was irradiated in a closed pyrex flask with the UV-lamp for 2 h.

A solution of drug (2 mg/20 ml, ca. 10^{-4} M) in the presence of NaN_3 (0.3 equiv.) was irradiated with UV-lamp in an open pyrex tube for 1, 2, and 4 h.

Compounds **2**, **3** and **4** (each 2 mg/20 ml) were irradiated in water in open pyrex tubes with the UV-lamp for different times.

A drug solution (2 mg/20 ml, ca. 10^{-4} M), after adding methylene blue (10^{-5} M) and bubbling oxygen for 15 min, was irradiated with 650 W halogen lamp for 10 min in a closed pyrex flask. Similar treatment was used starting from diastereomeric mixture **2**.

2.3.2. Compound *1*

White powder; UV spectrum shown in Fig. 1. IR $\nu_{max}(CHCl_3)$ 3676, 3588, 3300 br band, 2972, 1697, 1595, 1502, 1406, 1343 cm^{-1}. ^{1}H and ^{13}C NMR data are listed in Tables 1 and 2.

2.3.3. Compound *2* (mixture of diastereomers 1:1)

One isomer as methyl ester, i.e. **2a**: white powder; UV λ_{max} (CH_3OH) nm; 313 (log ε 4.1). IR $\nu_{max}(CHCl_3)$ 3693, 3604, 3484, 2923, 1726, 1601, 1503, 1459, 1370 cm^{-1}; EI–MS *m/z*