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Nifuroxazide Photodecomposition: Identification of the (Z)-isomer by ^{1}H-NMR Study

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Abstract. The objective of this work was to identify the transformation product of nifuroxazide which appeared during stability studies of the drug in hard-capsules using high-performance liquid chromatography. The impurity appears on exposure to light, and test samples contained 21.5% of the impurity after two hours of exposure to light at room temperature. Mass spectrometry, infrared and nuclear magnetic resonance spectroscopy indicated the occurrence of a geometric isomer of nifuroxazide. In general, ^{1}H-^{1}H NOE difference experiments after irradiation were in agreement with a photochemical Z/E isomerisation of nifuroxazide following exposure to ambient light.

Key words: Nifuroxazide stability; photoisomer; (E) and (Z) isomers; identification; ^{1}H-NMR spectroscopy.

Nifuroxazide, a nitrofuran derivative, [4-hydroxybenzoic acid ((5-nitro-2-furanyl)methylene)hydrazide], is an antibacterial agent used in the treatment of colitis and acute bacterial diarrhoea. Although this drug is widely used, literature data on nifuroxazide are scanty.

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Our preliminary studies of nifuroxazide stability in hard-capsules, using high-performance liquid chromatography (HPLC), revealed the rapid formation of an unknown compound. This degradation product required identification. The occurrence of a photodecomposition product in methanol and acetonitrile was noted for nifuroxazide [1]. These authors mentioned identification of an isomer, but results have not been published. Initial studies revealed a great difference in the concentration of this impurity between samples kept under ambient light and those left in the dark. At room temperature and after two hours of exposure to light, test samples contained 21.5% of the impurity. Restoring these samples in the dark led to nifuroxazide recovery. This suggested that the impurity was a product of phototransformation, that the reaction was reversible, and that the transformation product was the thermodynamically less stable form.

Experimental

Reagents

Nifuroxazide was purchased from Deshors (Paris, France). Deuterated (d_6)-dimethyl-sulfoxide and high purity grade methoxyethanol, acetonitrile and potassium bromide were obtained from SDS (Peypin, France).

Instruments and Methods

HPLC determinations were conducted on a Merck-Hitachi instrument equipped with a diode array UV detector with a 7 nm band width, using a Lichrospher 100 RP 18 (5 µm) column from Merck (Darmstadt, Germany), and acetonitrile-water (adjusted at pH 3.0 with phosphoric acid, 85:15 v/v) was used as eluent at 2 mL min^{-1}.

Mass spectra were recorded using a Hewlett-Packard 5989 A mass spectrometry (MS) system (Avondale, PA, USA) in the electron impact (EI) mode. Gas chromatographic (GC) separation was performed on an HP 5890 series II, using an HP 5 fused silica capillary column (30 m × 0.25 mm film thickness). The temperature of the oven was first programmed to rise from 80 °C (1.5 min) to 130 °C at 20 °C min^{-1} and then to 280 °C (5 min) at 35 °C min^{-1}. The injector and the transfer line were set at 250 °C and 280 °C, respectively.

Infrared spectra were recorded on a Nicolet 400 FT spectrometer, in KBr pellets. ^{1}H-NMR spectra were recorded in d_6-DMSO, using a Bruker AC 400 spectrometer, and ^{1}H-NMR NOE difference experiments were conducted on a Bruker 300 MSL spectrometer.

Results and Discussion

HPLC and MS Analysis

HPLC analysis of nifuroxazide solution kept under light showed well separated peaks for nifuroxazide and the phototransformation product with retention times of 15 and 20 min, respectively.

A phototransformed solution and a reference nifuroxazide solution were analysed using mass spectrometry. Direct introduction probe-mass spectrometry (DIP-MS) and gas chromatography-mass spectrometry (GC-MS) exhibited only one product. Also, the mass spectra were unchanged using reference nifuroxazide or a phototransformed solution. Both showed a slight abundance of the molecular ion at m/z 275 and a base peak at m/z 121, as well as ions at m/z 93, 65 and 51, which is characteristic of the phenol part. Further attempts to separate products using two derivatization methods, i.e. methylation using diazomethane and silylation using BSTFA, were unsuccessful, and both solutions appeared quite similar during GC-MS analysis. This supported the hypothesis of an intramolecular rearrangement and the formation of an isomeric photo product of nifuroxazide.

Isolation of the Nifuroxazide Transformation Product

In order to conduct IR and NMR spectroscopic analyses, higher levels of the phototransformation product had to be prepared. The phototransformation product was separated by precipitation after a 2.5 mg mL^{-1} solution of nifuroxazide in methoxyethanol (2 mL) had been exposed to ambient light. After five days of exposure, a brown precipitate formed. The solvent was discarded and the residue, evaporated to dryness at room temperature, contained ca. 96% of the transformation product as determined by HPLC.

IR Spectroscopy Analysis

Nifuroxazide or the brown precipitate was added to KBr. The main difference between the two spectra was a shift of the OH band from 3365 cm^{-1} (reference nifuroxazide) to 3400 cm^{-1} (brown precipitate), suggesting a more associated structure for the phenol function in the phototransformed product. The C=O band exhibited no difference and the hypothesis of a keto-enol equilibrium was discarded.

^{1}H-NMR Analysis

15 mg of either nifuroxazide or the nifuroxazide transformation product were dissolved separately in 0.5 mL of deuterated DMSO. The ^{1}H-NMR spectra of nifuroxazide and photoisomer recorded in d_6-DMSO displayed the same signals (multiplicity, number of protons), indicating identical proton distribution (Table 1). The possibility of s-cis and s-trans amide rotamers could be ruled out since the ^{1}H-NMR spectra of both compounds were unchanged when recorded at higher temperatures (60 °C and 100 °C).

The chemical shifts of the different protons of nifuroxazide and photoisomer were very close ($\Delta\delta = 0$ to 0.08 ppm), except for the imino (CH=N–) and nitrogen NH protons of the hydrazone function ($\Delta\delta = 0.5$ to 0.6 ppm). The main spectral differences between nifuroxazide and its photoisomer were therefore located in the CH=N–NH group, possibly reflecting a pair of

Table 1. ^{1}H-NMR chemical shifts assignments

Proton	Nifuroxazide δ (ppm)	Photoisomer δ (ppm)
H-2′, H-6′	6.9 (d, 2H, $J = 8.7$ Hz)	6.9 (d, 2H, $J = 8.5$ Hz)
H-3′, H-5	7.85 (d, 2H, $J = 8.7$ Hz)	7.93 (d, 2H, $J = 8.5$ Hz)
H-3	7.28 (d, $J = 3.95$ Hz)	7.34 (d, $J = 4$ Hz)
H-4	7.83 (d, $J = 3.95$ Hz)	7.90 (d, $J = 4$ Hz)
CH=N	8.41 (s, 1H)	7.74 (s, 1H)
OH	10.3 (s, 1H)	10.4 (s, 1H)
NH	12.1 (s, 1H)	11.6 (s, 1 H)

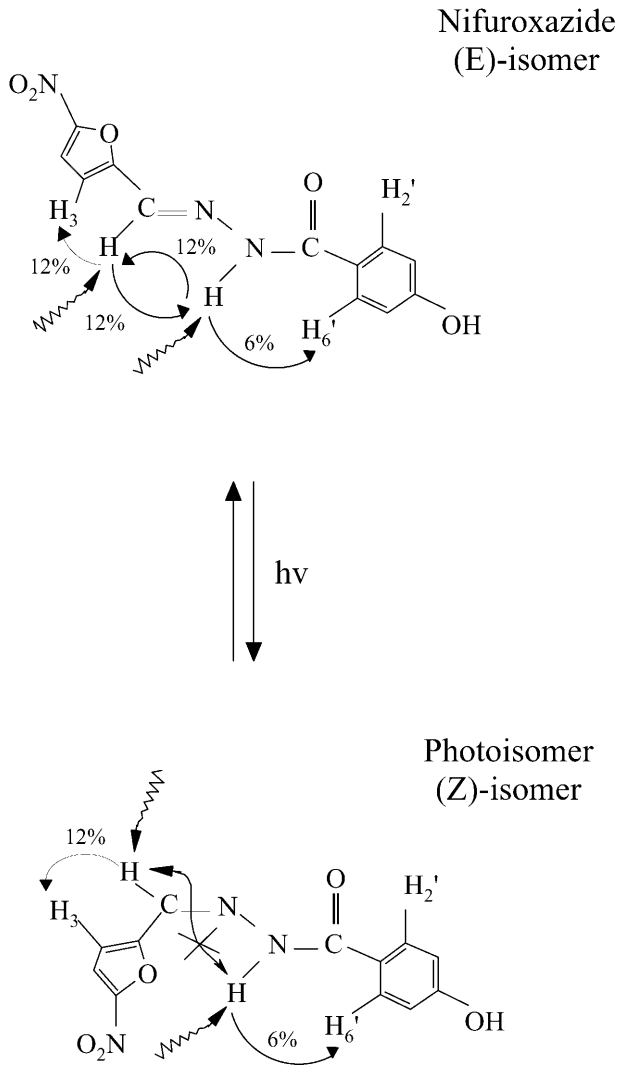

Fig. 1. ^{1}H-^{1}H NOE difference experiments irradiation in nifuroxazide and its photoisomer

Z and E isomers. This was confirmed by NOE difference experiments [2].

In ^{1}H-^{1}H NOE difference experiments, irradiation of the imino proton (CH=N, $\delta_H = 8.41$ ppm) showed enhancement only on the NH proton (ca. 12%, $\delta H = 12.1$ ppm) in nifuroxazide. Irradiation of the analogous proton of the photoisomer (CH=N, $\delta_H = 7.74$ ppm) had no effect on the signal of the NH proton (ca. 0%, $\delta_H = 11.6$ ppm) (Fig. 1). Hence, in nifuroxazide the imino (CH=N) and NH protons are cis to the C=N double bond. Hence, this is the E isomer, whereas in the photoisomer these same protons are trans to the C=N double bond, corresponding to the Z isomer.

Besides, the H3 proton showed a NOE (ca. 12%) with the imino (CH=N) proton, while the amido proton (NH) showed a NOE with the H2′ and H6′ aromatic protons (ca. 6%) in both compounds.

All these pieces of evidence strongly suggest the structure shown in Fig. 1 for the photodecomposition product corresponding to the photochemical Z/E isomerisation of nifuroxazide when stored under ambient light at room temperature.

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