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Note

High-performance liquid chromatographic determination of ketoprofen degradation products

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Ketoprofen (I) is a non-steroidal anti-inflammatory drug[1,2], the pharmacological[3] and clinical[4] properties of which are well known. In a previous paper[5] we reported a high-performance liquid chromatographic (HPLC) assay for ketoprofen and related impurities. During this work we noted the formation of products arising from the photochemical degradation of ketoprofen. This paper describes the isolation of these compounds and their determination by reversed-phase HPLC.

EXPERIMENTAL

Materials

Ketoprofen samples (single-dose vials of lyophilized ketoprofen) were obtained from different commercial sources.

The degradation products were obtained as follows. A solution of ketoprofen sodium salt (pH 6.8) (300 mg) in water (300 ml) was irradiated with UV light (254 nm) for 1 h. After addition of 1 *M* sodium hydroxide solution (10 ml), the mixture was extracted with diethyl ether (3 × 30 ml); the combined extracts were washed with water, dried over anhydrous sodium sulphate and evaporated. The residue was treated with pyridine (4 ml) at 100°C for 60 min and, after evaporation, the mixture was chromatographated on silica gel 40 [50 × 2 cm I.D. column, cyclohexane–ethyl acetate (83:17, v/v) eluent] to give II [λ_{max} (ethanol) 254.4 nm; IR, ν_{max} 1660, 1595, 1455, 1380 cm^{-1}; NMR, δ 1.26 (t, 3H) 2.72 (q, 2H), 7.25–8.15 (m,9H), analysis $C_{15}H_{14}O$], III [λ_{max} (ethanol) 255.4 nm; IR ν_{max} 3400, 1660, 1460, 1360 cm^{-1}; NMR, δ 1.54 (d, 3H), 4.85–5.15 (q, 1H), 7.25–8.15 (m, 9H), analysis $C_{15}H_{14}O_2$] and IV [λ_{max} (ethanol) 230 nm, IR, ν_{max} 1685, 1660, 1595, 1445, 1355 cm^{-1}; NMR, δ 2.58 (s, 3H), 7.34–8.50 (m, 9H), analysis $C_{15}H_{12}O_2$].

Acetonitrile and water were of HPLC grade and the other reagents used were commercial reagent grade.

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Solutions

Ketoprofen sodium salt samples (100 mg) were dissolved in 2.5 ml of water and kept at room temperature in daylight for different times (up to 1 h).

II, III and IV were dissolved in ethanol to give a final concentration of 0.2 mg/ml.

Chromatographic conditions

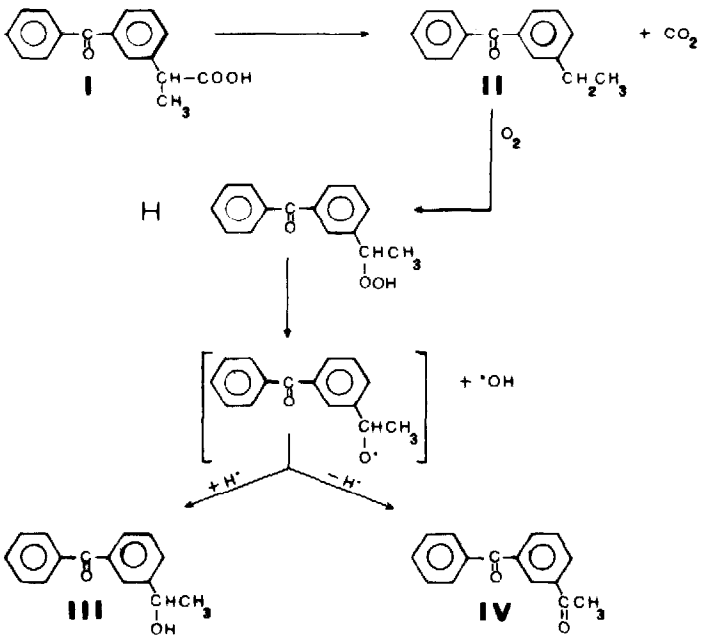
Thin-layer chromatography (TLC) was performed on silica gel pre-coated 60 F_{254} (Merck) plates using cyclohexane–ethyl acetate (70:30, v/v) as eluent.

HPLC was performed with a Waters M-6000 pump fitted with a Hibar Li-Chrocart RP C_{18} column (12.5 cm × 4 mm I.D., 7 μm particle size) (Merck) and a Model U6K injector (Waters Instruments).

The eluate was monitored with a Waters M 440 absorbance detector at 254 nm (0.02 a.u.f.s.); the output was measured on a Waters M 740 data module. The eluents were 0.01 *M* potassium monohydrogen phosphate–acetonitrile, (63:37, v/v), pH 2.8 and pH 7.1, and (50:50, v/v), pH 7.1. The flow-rate was 2 ml/min.

RESULTS AND DISCUSSION

Ketoprofen undergoes degradation when irradiated by UV light or sunlight according to Scheme 1. The overall reaction was followed by TLC, the R_F valves being 0.84, 0.52, 0.32 and 0.53 for II, the hydroperoxide, III and IV, respectively. The conversion of II into III and IV was ascertained by direct irradiation of II on



Scheme 1. Photochemical degradation of ketoprofen. UV wavelength, 254 nm. I = Ketoprofen; II = (3-benzolphenyl)ethane; III = (3-benzoylphenyl)ethanol; IV = (3-benzoylphenyl)ethanone; H = (3-benzoylphenyl)ethyl hydroperoxide.

the plate followed by chromatographic elution. An analogous procedure was performed to show that III when irradiated can yield IV. The hydroperoxide spot reacted positively with an acidic spray of potassium iodide, which is commonly used to detect hydroperoxides[6]. Own to the low stability of the hydroperoxide, it was converted into a mixture of III and IV by treatment with pyridine. II, III and IV were then easily separated by column chromatography and characterized by chemical and spectroscopic methods.

An authentic sample of (3-benzoylphenyl)ethanone was obtained[7] for comparison with IV. Both samples gave proton NMR spectra having the same pattern, and the same IR and UV spectra. The two samples gave also the same R_F valves and HPLC retention times. The structure of III as (3-benzoylphenyl)ethanol was assigned from spectroscopic data and from its oxidative conversion (potassium bichromate–dilute sulphuric acid) to the ketone IV. Quantitative elemental analysis and spectroscopic data indicated the structure of II to be (3-benzoylphenyl)ethane.

Ketoprofen solutions were first analysed by TLC, then reversed-phase HPLC was applied to assay the degradation. The eluent [0.1 *M* potassium monohydrogen

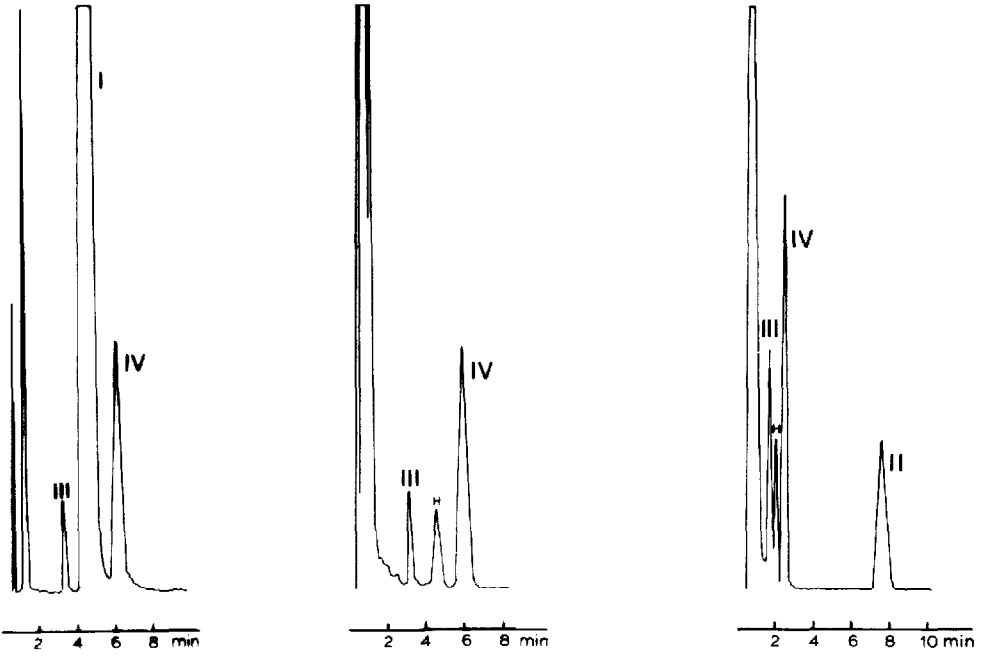


Fig. 1. Chromatogram of irradiated ketoprofen. Eluent: 0.1 *M* potassium monohydrogen phosphate–acetonitrile (63:37), pH 2.8. I = Ketoprofen; III = (3-benzoylphenyl)ethanol; IV = (3-benzoylphenyl)-ethanone.

Fig. 2. Chromatogram of irradiated ketoprofen. Eluent: 0.1 *M* potassium monohydrogen phosphate–acetonitrile (63:37), pH 7.1. III = (3-Benzoylphenyl)ethanol; H = (3-benzoylphenyl)ethyl hydroperoxide; IV = (3-benzoylphenyl)ethanone.

Fig. 3. Chromatogram of irradiated ketoprofen. Eluent: 0.1 *M* potassium monohydrogen phosphate–acetonitrile (50:50), pH 7.1. II = (3-Benzoylphenyl)ethane; III = (3-benzoylphenyl)ethanol; H = (3-benzoylphenyl)ethyl hydroperoxide; IV = (3-benzoylphenyl)ethanone.

phosphate acetonitrile (63:37, v/v), pH 2.8] proposed for the purity assay of ketoprofen was unsatisfactory, as the peak of ketoprofen (retention time, R_t = 4.7 min) overlapped that of the hydroperoxide, and II was also retained (Fig. 1).

With the same eluent at pH 7.1 ketoprofen was eluted in the front and the hydroperoxide peak (R_t = 4.6 min) was detected (Fig. 2). This peak disappeared in samples heated in the presence of puridine, thus confirming its peroxidic structure.

By keeping constant the pH and increasing the percentage of acetonitrile to 50%, the retention times were reduced to half and also II was eluted with T_t = 7.48 min (Fig. 3). This system was then chosen for the analysis of commercial samples. The method showed a linear UV (254 nm) response for II–IV between 50 and 600 ng injected with a correlation coefficient of 0.9994. The relative standard deviation was 0.54% (n = 5).

Assay results for commercial samples stored protected from the light indicated that very little (less than 0.05%) degradation of ketoprofen had occurred over a 24-month period. The amounts of II–IV increased greatly when the solution of the samples were left in daylight, and reached a total value of 2% over a 60 min period. Within this range the percentages of II, III and IV were 43%, 18% and 36%, respectively. Under the same conditions ketoprofen in the form of a crystalline powder was stable.

In conclusion, the HPLC procedure described here allows the photochemical degradation of ketoprofen to be monitored and can be applied successfully to routine process control.

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