Photolysis of NSAIDs. I. Photodegradation Products of Carprofen Determined by LC–ESI–MS

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Abstract

A solution of carprofen in methanol at a concentration of 2.74×10^{-2} mg/mL is subjected to photoirradiation using a Hanovia 200-W high-pressure Hg lamp for 9 h. In total, seven photodegradation products are separated, and their quasimolecular ions are subsequently determined online using a liquid chromatography (LC)–electrospray ionization (ESI)–mass spectrometry (MS) method. The high-performance LC consists of an Inertsil 5 ODS-80A (2.1- × 150-mm) column. The mobile phase is initially CH_3CN. NH_4OAc (20mM in de-ionized H_2O) is 43:57 (v/v), and after 14 min it is CH_3CN. NH_4OAc (20mM in de-ionized H_2O) is 54: 46 (v/v). The UV detector was set at 260 nm. The parameters of LC–MS for mass determination involves an atmospheric pressure ionization electron spray interface with a negative mode of polarity (ESI$^-$). The chemical structures of the degradants are elucidated based on the mass-to-charge ratio of the quasimolecular ions and the molecular weight changes by comparison with the parent drug (carprofen). The degradation proceeds via an initial dechlorination. A dechlorination or esterification reaction is competed with decarboxylation. This finding is in accordance with our previously reported result of first order photodecomposition kinetics for carprofen.

Introduction

Phototoxicity and photosensitivity disorders induced by carprofen (CPF) have been widely reported (1–7). CPF, 2-(6-chloro-2-carbazolyl)propanoic acid is a nonsteroidal anti-inflammatory drug (NSAID) that has an asymmetric carbon in the propionic acid group. It passes through a major photochemical pathway via dechlorination with a quantum yield (Φ) of approximately 0.37, although the commonly observed decarboxylation only represents a minor reaction route with a relatively low quantum yield of less than 0.01 (8). Moser et al., in an attempt to elucidate the phenomenon of drug–protein photobinding, found that the photobinding of CPF to human serum albumin appears to involve the formation of aryl radicals resulting from carbon-halogen (Cl) photocleavage (9).

Traditionally, contaminating impurities or the degradants produced during the stability test of drugs must undergo a tedious process of separations and purifications. Each constituent is then subjected to a series of spectroscopic identifications to decide its structure. Swift development of the combination of liquid chromatography (LC)–mass spectrometry (MS) was urgently needed. The advantage of LC–MS is that it allows the determination of the molecular weights of the components separated by high-performance LC (HPLC). Niessen thought that LC–MS had become the method of choice for analytical support in many stages of drug development within the pharmaceutical industry (10). Use of the atmospheric pressure ionization (API) interfaces, such as electrospray ionization (ESI), allows for online measurements of minor polar thermally unstable substances. The positive mode of ESI produces mainly $[MH]^+$ ions and the negative mode generates the $[M]^-$ quasimolecular ion. The chemical structure of each degradant can be inferred from the mass-to-charge ratio difference of the quasimolecular ions and CPF [i.e., by comparison of the degradant with the parent drug (CPF), the molecular weight change becomes the decisive factor of what functional group is most likely changed]. The signals of the fragments for each degradant also help to decide the reaction route (e.g., dechlorination or decarboxylation).

In this study, we used LC–ESI–MS for molar molecular weight determinations of photodegradation products derived from the photolysis of CPF in a methanol solution. We also elucidated the chemical structural of the degradants and unveiled photolytically the reaction scheme of this particular drug.

Experimental

Chemicals

Reagent-grade CPF was purchased from Sigma Chemical Co. (St. Louis, MO). LC-grade methanol was from J.T. Baker (Phillipsburg, NJ). LC-grade acetonitrile was the product of Labscan (Dublin, Ireland). Guaranteed-reagent-grade ammonium acetate was supplied by Merck (Darmstadt, Germany).

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Instruments

The HP series 1100 liquid chromatograph/mass selective detector (LC/MSD) (Hewlett-Packard, Palo Alto, CA) included an HPLC system equipped with an Intersil (Vercopak, Taipei, Taiwan) 5 ODS-80A column (150- × 2.1-mm i.d.). This instrument was equipped with an online built-in mass spectrometer. A Hanovia (Union, NJ) 200-W high-pressure mercury lamp was used as the light source for photolysis.

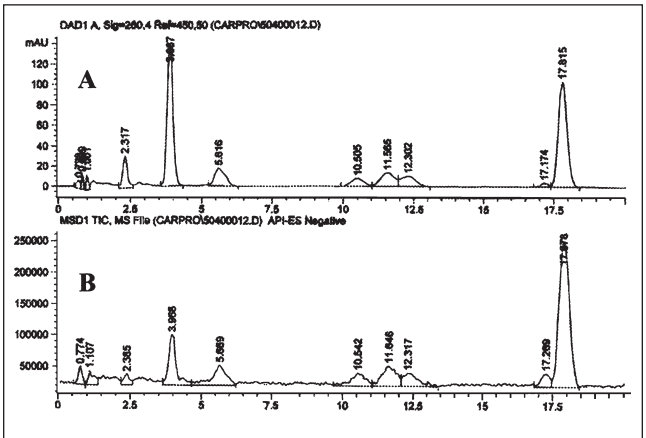


Figure 1. HPLC chromatograms: (A) photodegradants of CPF using a UV detector at 260 nm and (B) separation of photodegradants using an MS detector.

Sample preparation

An amount of 27.4 mg (1.00×10^{-4}M) of CPF was accurately weighed and placed in a 100-mL volumetric flask. Methanol was added to the mark and then stirred to ensure that the solution was homogeneous. Ten milliliters of the solution was pipetted and placed into another 100-mL volumetric flask, and once again methanol was added to the mark; the concentration of the solution became 2.74×10^{-2} mg/mL. Four milliliters of the preceding solution was pipetted into a 5-mL quartz sample vial and a stopper was inserted. The sample was prepared under ordinary atmospheric conditions, and it was then irradiated with a Hanovia 200-W high-pressure mercury lamp for 9 h with a distance between the lamp and the sample of 30 cm, a radiation area of 4.62 cm^2, and an intensity of 4800 lux.

Table I. LC–MS Information of CPF and Photodegradants

Compound	Retention time (min)	Quasimolecular ion and fragment (*m/z*)	Difference (*m/z**)	Attached a Cl?	Possible functional group gained or lost
CPF-1	2.39	$[M]^-$: 238 [fragmentation: 194	–34	No	–Cl
CPF	3.97	$[M]^-$: 272.0 fragmentation: 228	–	–	–
CPF-2	5.67	$[M]^-$: 244.0 fragmentation: 208	–28	Yes	–CO
CPF-3	10.54	$[M]^-$: 224.0 fragmentation: N.D.†	–48 (–34, –14)	No	–Cl $-C(=O)-OH \rightarrow -CH_2-OH$ $-C(=O)-OH \rightarrow -O-CH_3$
CPF-4	11.65	$[M]^-$: 242 fragmentation: N.D.	–30	Yes	$-CH(CH_3)-C(=O)-OH \rightarrow -C(=O)-CH_3$
CPF-5	12.32	$[M]^-$: 252.0 fragmentation: N.D.	–20 (–34 + 14)	No	–Cl $+CH_3$
CPF-6	17.27	$[M]^-$: 258.0 fragmentation: N.D.	–14	Yes	$-C(=O)-OH \rightarrow -CH_2-OH$ $-C(=O)-OH \rightarrow -O-CH_3$
CPF-7	17.88	$[M]^-$: 286.0 fragmentation: N.D.	+14	Yes	$+CH_3$

* $[M]^-_{CPF} - [M]^-_{CPF\text{-}X}$ in mass-to-charge ratio units.
† N.D., no data.

LC–MS analytical conditions

HPLC conditions

The LC–MS conditions for the separation of CPF photodegradants in methanol are described here. The column was an Intersil 5 ODS-80A (2.1 × 150 mm). The mobile phase consisted initially of CH_3CN–NH_4OAc (20mM in de-ionized H_2O) at a ratio of 43:57 (v/v), and then after 14 min, CH_3CN–NH_4OAc (20mM in de-ionized H_2O) of 54:46 (v/v). The UV detector was operated at 260 nm. The flow rate was 0.4 mL/min, and the injection volume was 10 µL.

We changed the composition of the mobile phase after 14 min in order to shorten the retention times of the upcoming components.

MS conditions

The parameters of the LC–MS for mass determination of CPF degradants are noted here. The interface was API electron spray. The polarity was in negative mode. The drying gas flow was 10 mL/min. The drying gas temperature was 350°C. The nebulizer gas pressure was 60 psi. The fragmentor voltage was 100 V and the capillary voltage was 3500 V. The scanning range was *m/z* 100–600 at 1.15 s/scan.

Results and Discussion

Parameter optimization of LC–MS for mass determinations

The HP series 1100 LC/MSD has 5 parameters that can be optimized. The choices we pursued in this study were as follows. (i) the API electron spray interface with a negative mode of polarity (ESI^-) was chosen because CPF contains a carboxylic group which can dissociate a proton to become a negatively charged carboxylate. (ii) CPF has a molecular weight of 273.7 g/mole, and the scanned range was *m/z* 100–600 at a rate of 1.15 s/scan. (iii–iv) Adjustments of the drying gas flow, drying gas temperature, and nebulizer gas pressure were determined by observing the actual sample passing through the flow injection analysis (FIA) by setting two fixed parameters and optimizing the third variable. The optimized parameters of LC–MS for mass determinations of CPF and its degradants were noted previously.

HPLC separation of the photodegradants of CPF

After photoirradiation of a methanolic solution of CPF for 9 h, seven degradants were separated by HPLC using a UV detector set at 260 nm (Figure 1A). The retention times are listed in Table I.

Chemical structure assignments

Table I also shows the mass-to-charge ratios of the quasimolecular ion and fragmentation data of MS for each degradant. CPF contains a chloro group at position 6. The natural abundance of isotope ^{37}Cl (24.47%) is approximately one-third that of ^{35}Cl (75.53%); as a result, the quasimolecular ion *m/z* 274 (M^-+2) signal of one-third intensity was observed in addition to *m/z* 272 (M^-) of CPF (two selected MSs of degradants are shown in Figure 2). This characteristic feature served as exclusive evidence for C–Cl cleavage (dechlorination) or for a Cl-attached degradant by careful examination of each MS. CPF-1, CPF-3, and CPF-5 all have the missing signals of isotope ^{37}Cl, (i.e., the three degradants formed during the photolytic process by the same initial dechlorination step). The structures of the remaining four degradants with their mass-to-charge ratio differences and the observable fragments related to the most probable variations in the propionic acid side chains were finally assigned (Table II). All seven degradants retained an intact carbazole ring during the photolytic process.

Characteristics of photoirradiation of CPF

A number of examples of photoinduced aromatic substitutions have been reported (11). In the case of haloaromatics, intersystem crossing followed by homolytic cleavages often predominates other mechanisms for photosubstitution. Dehalogenation reactions are common occurrences as reported by Turro (12). De Guidi et al. pointed out the C–Cl cleavage of CPF as early as in 1993 (6). The controversial phototoxic properties or photoinduced disorders may be attributed to the Cl radical moiety generated during the photolytic process. Dechlorination was also observed by Encinas et al. in a study of phototoxicity associated with diclofenac (13). The present LC–MS study provides solid proof of the dechlorination reaction route for CPF.

Solvent effects

The photolysis of CPF exhibited two specific solvent effects. First, we had previously reported methanol participation in

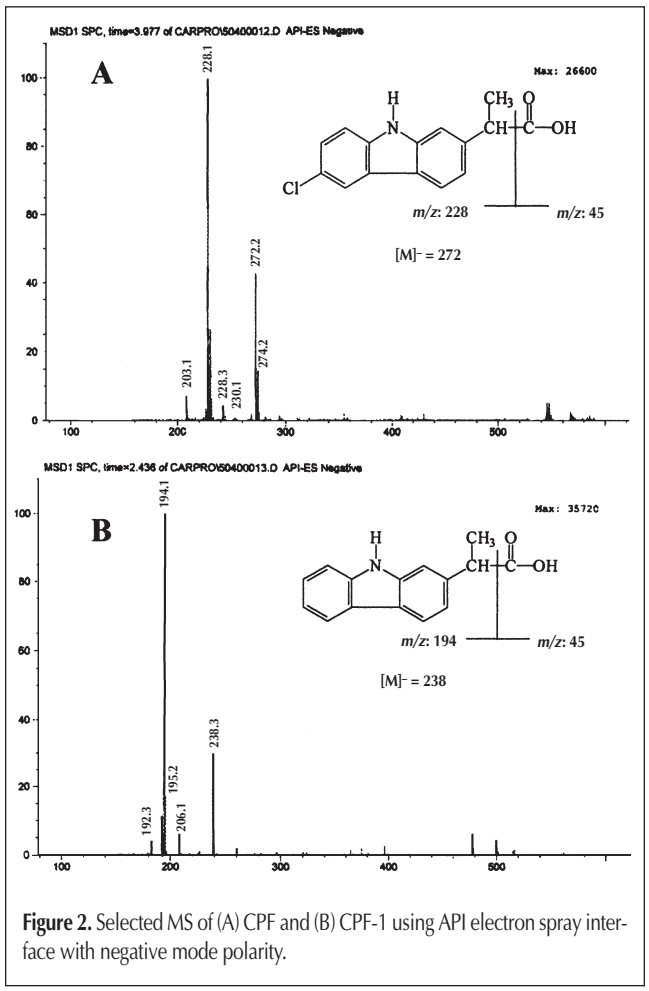


Figure 2. Selected MS of (A) CPF and (B) CPF-1 using API electron spray interface with negative mode polarity.

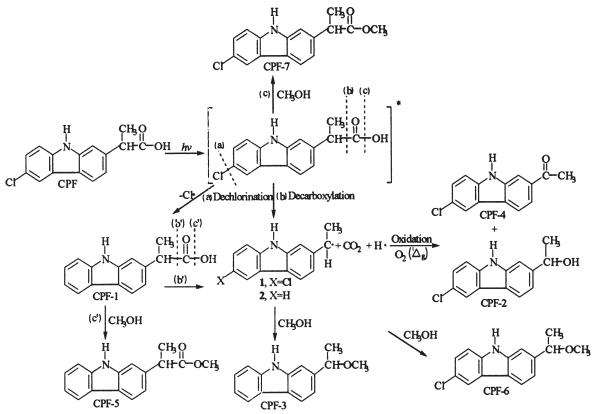


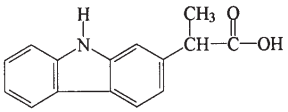
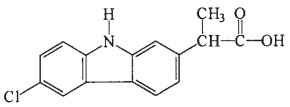
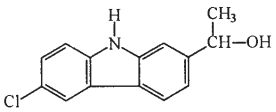
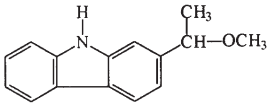
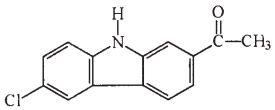
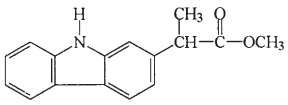
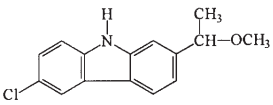
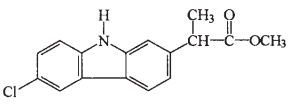
Figure 3. Reaction scheme of photoirradiation of CPF. Route (a), dechlorination; (b) and (b'), decarboxylation; and (c) and (c'), esterification.

esterification of indomethacin (14). A similar phenomenon occurred in the case of CPF. Immediately after the dechlorination reaction of CPF, CPF-1 was formed. Under acidic catalytic condition caused by the propionic acid groups, the methyl esters CPF-7 and CPF-5 formed next from CPF and CPF-1, respectively. Both CPF and CPF-1 then proceeded via parallel decarboxylation reactions to generate 2-carbazolyl ethyl radicals 1 and 2. After oxidation with singlet oxygen, radical 1 produced the oxidized products of CPF-2 (an alcohol) and CPF-4 (a ketone). No such oxidized products were formed from radical 2. Second, the formation of CPF-6 and CPF-3 was observed, and these are ether derivatives that use the methoxy radical (CH_3O) from the solvent (methanol) to intercept radicals 1 and 2.

A proposed reaction scheme for CPF

We previously reported a kinetic study of the photochemical decomposition of CPF in nine different organic or aqueous

Table II. Structure Elucidation Based on LC–MS Information

Compounds	Molecular weight (g/mol)	Chemical structure*	Quasimolecular ion and fragment (*m/z*)
CPF-1	239.3		[M]$^{-}$: 238 fragmentation: 194
CPF	273.7		[M]$^{-}$: 272 fragmentation: 228
CPF-2	245.7		[M]$^{-}$: 244 fragmentation: 208
CPF-3	225.3		[M]$^{-}$: 224 fragmentation: N.D.†
CPF-4	243.7		[M]$^{-}$: 242 fragmentation: N.D.
CPF-5	253.3		[M]$^{-}$: 252 fragmentation: N.D.
CPF-6	259.7		[M]$^{-}$: 258 fragmentation: N.D.
CPF-7	287.7		[M]$^{-}$: 286 fragmentation: N.D.

* International Union of Pure and Applied Chemistry names: CPF-1, 2-(2-carbazolyl)propionic acid; CPF-2, 2-(6-chloro-2-carbazolyl) ethyl alcohol; CPF-3, 2-(2-carbazolyl)ethyl methyl ether; CPF-4, 2-(2-carbazolyl) methyl ketone; CPF-5, 2-(2-carbazolyl)propionic methyl ester; CPF-6, 2-(6-chloro 2-carbazolyl)ethyl methyl ether; CPF-7, 2-(6-chloro-2-carbazolyl)propionic methyl ester.

† N.D., no data.

ethanolic solutions (15). Under photoirradiation, CPF follows first-order reaction rates. Although an initial dechlorination reaction is favored, the competing decarboxylation can also account for the kinetic results. In summary, a reaction scheme of the photoirradiation of CPF is depicted in Figure 3.

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