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Photolysis of NSAIDs. II. Online LC-MS Determination of Photodegradants from Carprofen

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ABSTRACT

Carprofen in a 10% aqueous ethanol solution at a concentration of 0.10 mg/mL was subjected to photo-irradiation under sunlight for 24 hr. Four photodegradants were separated and subsequently followed an online determination of their quasi molecular ions using an LC-MS method. The HPLC consisted an Inertsil 5 ODS-80A (2.1 mm i.d. × 150 mm) column, and the mobile phase was initially CH_3CN: NH_4OAc (20 mM in D.I. H_2O) = 43: 57 (v/v). After 14 min, CH_3CN: NH_4OAc (20 mM in D.I. H_2O) was changed to 54: 46 (v/v). The UV detector was set at 260 nm. The parameters of LC-MS for mass determination were optimized with an API electron spray interface with negative mode of polarity (ESI^-). The chemical structures of the degradants were elucidated based on the m/z of the quasi molecular ions. Two degradants were found to proceed *via* an initial dechlorination with C-Cl cleavage. Dechlorination was observed to be competing with decarboxylation, i.e. either reaction is in accord with our previously reported result with a first-order photodecomposition kinetics of carprofen.

Key words: photolysis, carprofen, NSAIDs, LC-MS, dechlorination

INTRODUCTION

Carprofen (CP), 2-(6-chloro-2-carbazolyl)propanoic acid, is a NSAID that is widely used as an anti-inflammatory, analgestic and anti-pyretic agent[1,2]. It is frequently taken for the remedy of arthritis, osteoarthritis, and dysmenorrhea[3,4]. However, the photohemolytic and photosensitized allergic effects induced by CP have been occasionally reported[5-9].

Traditional methods involving isolation, and purification of trace components, such as degradants and impurities, are expensive and time-consuming. Therefore, the use of LC-MS profiling, an analytical method that permits rapid cataloging and identification of potential degradants, is an attractive alternative and serves to shorten the drug product development cycle time. One of the apparent advantages of LC-MS also allows us to determine the molecular weight for each component separable on HPLC. The use of electrospray ionization (ESI), a modern interface of atmospheric pressure ionization (API) technique, allows online-measurement of minor, polar and thermal unstable substances. The chemical structure of each degradant can be interpreted from the m/z differences of the quasi molecular ions. The shortcoming of LC-MS is that frequently only the mass of the unfragmented molecular ion can be obtained. By increasing the electrical voltage of the fragmentation zone, the effect of collision-induced dissociation is limited. In stress treatments of cefadroxil, Rourick et al. were able to determine the structures of degradants induced by acid, base, and heat using LC-MS[10]. Volk et al. utilized LC-MS profiling to elucidate the degradation products of paclitaxel[11].

In this study[12], we attempted to use LC-MS for molecular weight determinations of photodegradation products derived from the photolysis under sunlight of CP in 10% aqueous ethanol solution. We aimed at the chemical structural elucidation of the degradants to unveil the photolytical reaction scheme of this particular drug.

MATERIALS AND METHODS

I. *Chemicals*

Carprofen (CP) was purchased from Sigma Chemical (St. Louis, MO, USA). Absolute ethanol was supplied by Taiwan Tobacco and Liquor Corporation. Acetonitrile of LC grade was the product of Labscan (Dublin, Ireland). Ammonium acetate (NH_4OAc) of GA grade was supplied by Merck (Darmstadt, Germany).

II. *Instruments*

HP series 1100 LC/MSD (Palo Alto, CA, USA) included a high-performance liquid chromatograph equipped with an Inertsil 5 ODS-80A column (150 mm × 2.1 mm i.d., Vercopak, Taipei, Taiwan).

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III. *Sample Preparation*

An amount of 10 mg CP was accurately weighed and placed in a 100-mL volumetric flask. A 10% aqueous ethanol solution was added to mark and stirred to make the solution with a concentration of 3.65×10^{-4} mol/L (0.10 mg/mL). Four milliliters of the preceding solution was pipetted to a 5-mL quartz sample vial and stoppered. The sample was prepared under ordinary atmospheric conditions and it was then irradiated under sunlight for a total of 24 hr.

IV. *LC-MS Instruments and Analytical Conditions*

LC/MS for the separation of CP photodegradants in 10% aqueous ethanol solution, the HPLC contained an Inertsil 5 ODS-80A column (2.1 mm i.d. × 150 mm) and the mobile phase was initially set CH_3CN: NH_4OAc (20 mM in D.I. H_2O) = 43: 57 (v/v). After 14 min, CH_3CN: NH_4OAc (20 mM in D.I. H_2O) composition was changed to 54: 46 (v/v). The change in the composition of the mobile phase shortened the retention times of the upcoming components. The UV detector was monitored at 260 nm. The flow rate was 0.4 mL/min with a injection volume of 10 μgL.

The parameters of LC-MS for mass determination of CP degradants, the detailed MS conditions: API electron spray interface was adopted with negative mode polarity. Dry nitrogen gas flow was set at 10 L/min with the drying gas temperature maintained at 350°C. The nebulizer gas pressure was 60 psi, the fragmentor voltage 100 V, and capillary voltage 3500 V. The scanning range was at m/z 100-600 with 1.15 s/scan.

RESULTS AND DISCUSSION

I. *Parameters Optimization of LC-MS for Mass Determinations*

The determination of CP and the corresponding photodegradants was optimized by 5 parameters using HP series 1100 LC/MSD. The choices of the present study were: (1) Positive mode of ESI produces mainly $[MH]^+$, while negative mode generates $[M]^-$ quasi molecular ion. The API electron spray interface with negative mode of polarity (ESI^-) was chosen because CP contains a carboxylic group, which can dissociate a proton becoming a negatively charged carboxylate. (2) CP with a molecular weight of 273.7 g/mole, the scanned range was m/z 100-600 at a rate of 1.15 s/scan. The 3rd to 5th parameters were correlated that the adjustments of drying gas flow, drying gas temperature, and nebulizer gas pressure must be determined by observing the actual sample passing through flow injection analysis (FIA) by setting two fixed parameters and optimizing the third variable.

II. *HPLC Separation of the Photodegradants of CP*

The 10% aqueous ethanoic solution of CP was exposed to sunlight for 24 hr and a total of 4 degradants were separated on the HPLC chromatogram (Figure 1). The retention times are listed in Table 1.

III. *Chemical Structure Assignments*

Table 1 also shows m/z of quasi molecular ion and fragmentation data of MS for each degradant. CP contains a

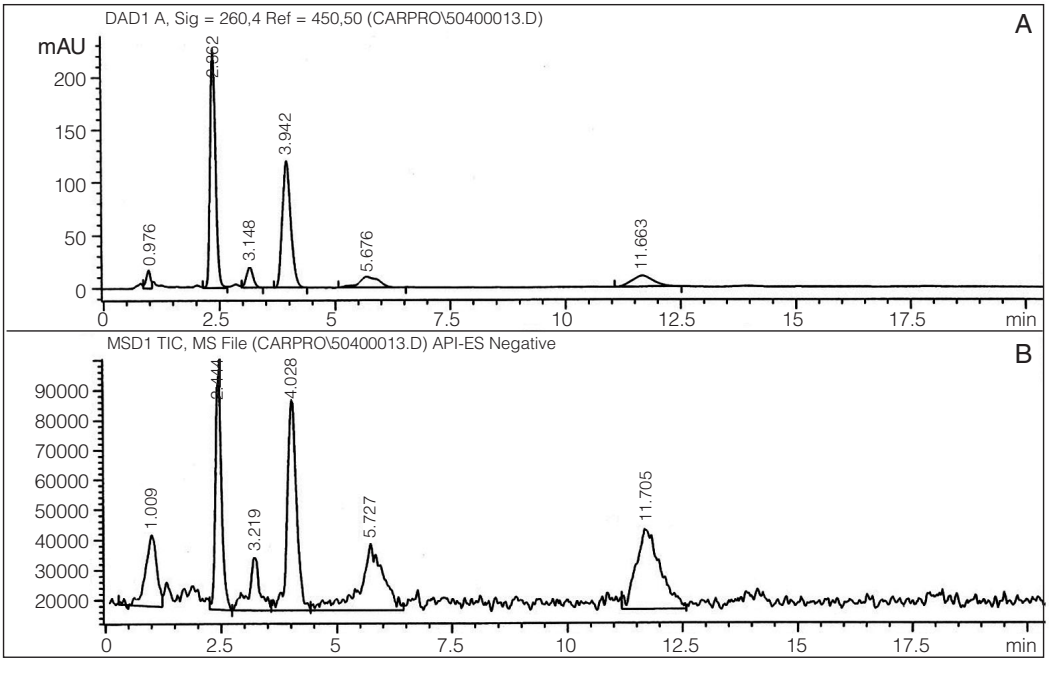
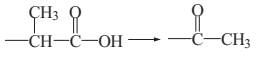


Figure 1. A: HPLC chromatogram; B: LC-MS of CP and photodegradants.

Table 1. LC-MS information of CP and photodegradants

Compound	Retention time (min)	Quasi molecular ion and fragment (m/z)	Difference in m/z*	Cl Attached ?	Possible functional group lost or gained
CP-1	2.44	$[M]^-$: 238 fragmentation: 194	-34	No	-Cl
CP-2	3.22	$[M]^-$: 210 fragmentation: N.D§	-62 (-28)	No	-Cl -CO
CP	3.97	$[M]^-$: 272 fragmentation: 228	—	—	—
CP-3	5.67	$[M]^-$: 244 fragmentation: 208	-28	Yes	-CO
CP-4	11.65	$[M]^-$: 242 fragmentation: N.D.§	-30	Yes	

*: $[M]^-_{CP} \sim [M]^-_{CP\text{-}X}$ in m/z unit. §N.D.: no data.

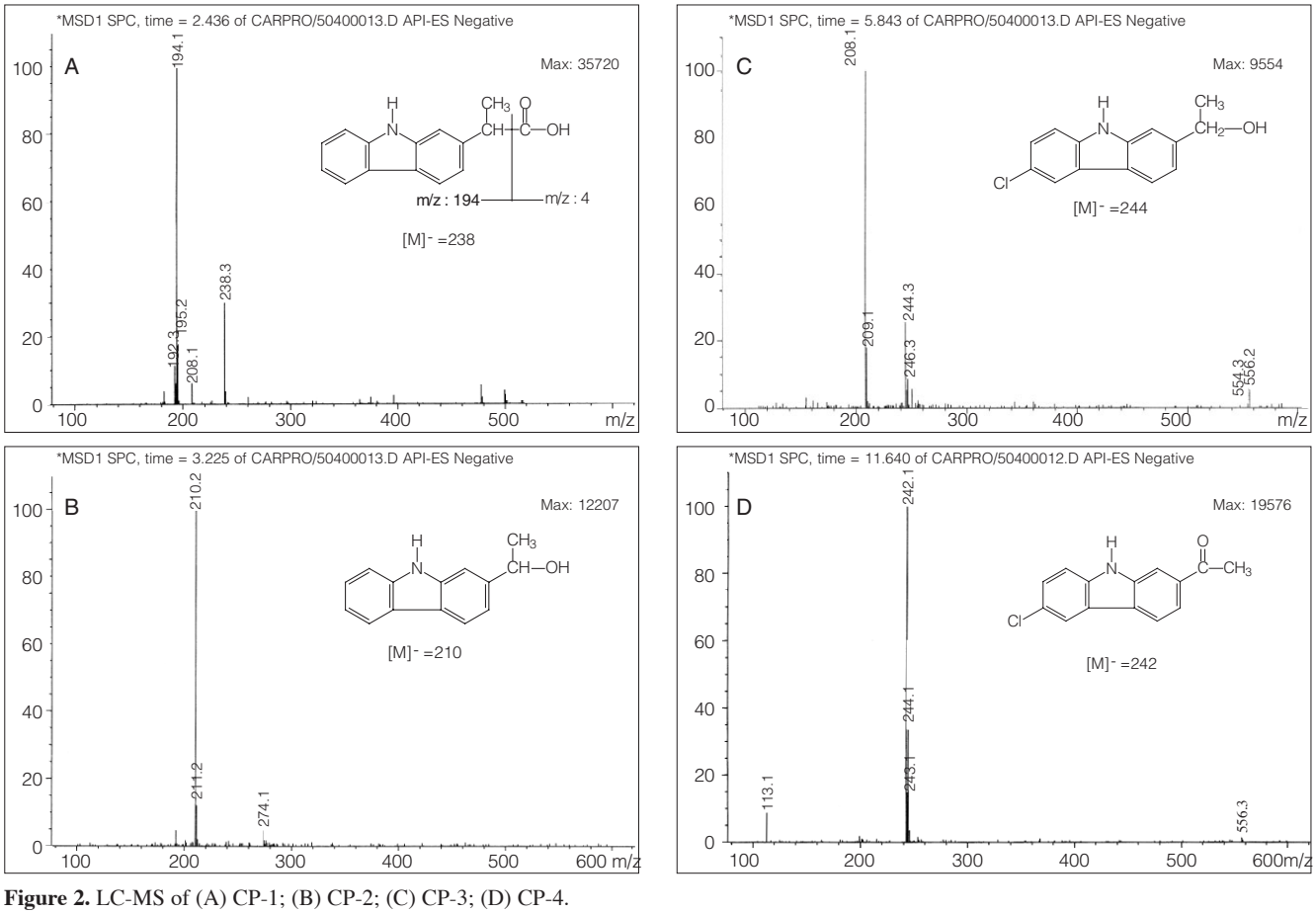


Figure 2. LC-MS of (A) CP-1; (B) CP-2; (C) CP-3; (D) CP-4.

chloro group at 6 position of a carbazole ring. Natural abundance of isotope ^{37}Cl (24.47%) is about one third of ^{35}Cl (75.53%). As a result, the quasi molecular ion m/z 274 (M^-+2) signal of one-third intensity was observed in addition to m/z 272 (M^-) of CP (MS of 4 degradants are shown in Figure 2). This characterictic feature becomes an exclusive evidence for C-Cl cleavage (dechlorination) or Cl-attached degradant by careful examination of each MS obtained. CP-1 and CP-2 have missing signals of isotope ^{37}Cl, i.e. the 2 degradants formed during the photolytic process by the same initial dechlorination step. The structure of the remaining 2 degradants, with their m/z differences and the observable fragments related to the most probable variations in the propionic acid side chains, were finally assigned (Table 2). All 4 degradants left carbazole ring intact during the photolytic process.

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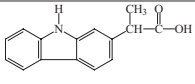
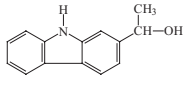
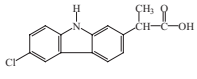
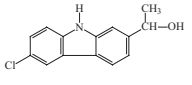
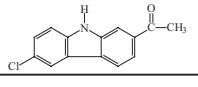
IV. *Characteristics of Photo-irradiation of CP*

A number of examples of photoinduced aromatic substitution have been reported[13]. In the case of haloaromatics, intersystem crossing followed by homolytic cleavages often predominates over other mechanisms for photosubstitution. Dehalogenation reactions are common occurrences as reported by Turro[14]. According to De Guidi et al., CP experiences the major photochemical pathway *via* dechlorination[15], i.e. in the presence of good hydrogen donors, such as methanol, etc., leads to the formation of dechlorinated primary product (CP-1). While the photobinding of CP to human serum albumin appears to involve the formation of aryl radicals resulting from carbon-halogen (Cl) photocleavage[16], 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[17]. The present LC-MS study has provided a solid proof of dechlorination reaction route for CP. A reaction scheme of the photo-irradiation of CP is depicted in Figure 3. Both CP and CP-1 then proceeded via a parallel decarboxylation to generate 2-carbazolyl ethyl radicals, **1** and **2**. From **1**, the oxidized products of CP-3 and CP-4 were produced followed by oxidation with singlet oxygen. From **2**, the oxidized product CP-2 was found. The solvent effects including esterification and methyl ether formation were not observed.

We had reported a kinetic study of photochemical decomposition of CP in nine different organic or aqueous ethanolic solutions[18]. Under photo-irradiation, CP follows a first-order reaction rates. Although an initial dechlorination reaction is favored, the competing decarboxylation could also account the kinetic results.

Table 2. Structure elucidation based on LC-MS information

Compound	Molecular weight (g/mol)	Chemical structure	Quasi molecule ion and fragment (m/z)
CP-1	239.3		$[M]^-$: 238 fragmentation : 194
CP-2	211.3		$[M]^-$: 210 fragmentation : N.D.*
CP	273.7		$[M]^-$: 272 fragmentation : 228
CP-3	245.3		$[M]^-$: 244 fragmentation : 208
CP-4	243.7		$[M]^-$: 242 fragmentation: N.D.*

*N.D.: no data.

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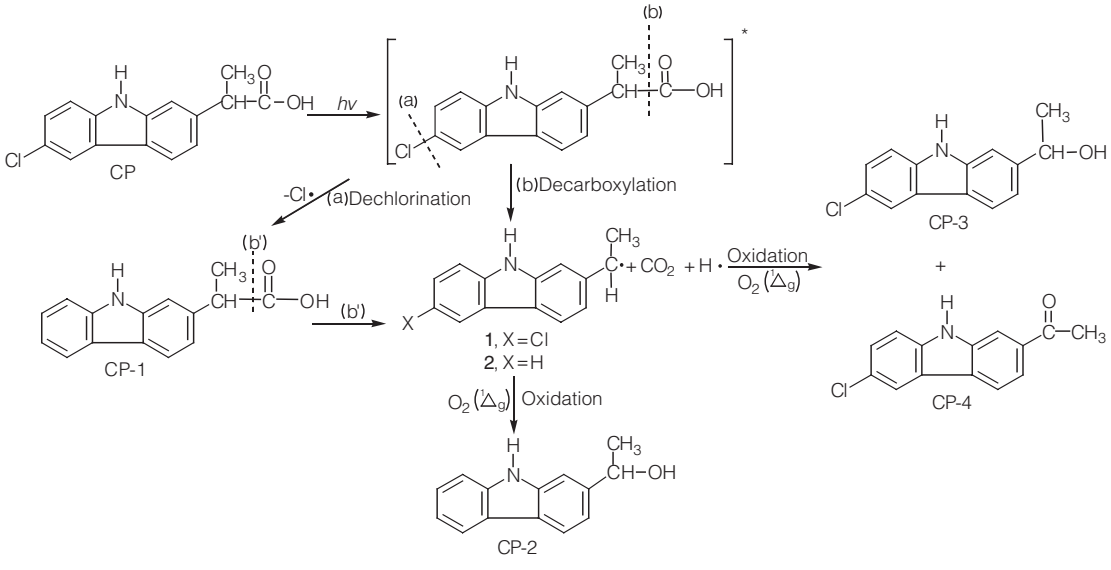

Figure 3. Reaction scheme of photo-irradiation of CP under sunlight.

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