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A Photophysical and Photochemical Study of 6-Methoxy-2-naphthylacetic Acid, the Major Metabolite of the Phototoxic Nonsteroidal Antiinflammatory Drug Nabumetone

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ABSTRACT

Nabumetone is a phototoxic nonsteroidal antiinflammatory drug used for the treatment of osteoarthritis. However, nabumetone is considered a prodrug with its metabolite 6-methoxy-2-naphthylacetic acid the active form. Photophysical and photochemical studies on this metabolite have been undertaken. It undergoes photodecarboxylation in aerated aqueous and organic solvents. In addition to the accepted photodegradation pathway for related molecules, a new mechanism that implies generation of the naphthalene radical cation from the excited singlet and addition of O_2 prior to the decarboxylation process has been demonstrated. Evidence for the involvement of the excited singlet state in this mechanism have been obtained by steady-state and time-resolved fluorescence experiments. The fluorescence quenching by O_2 and the shorter singlet lifetime in aerated solvents support this assignment. Laser flash photolysis also supports this mechanism by showing the noninvolvement of the triplet in the formation of the naphthalene radical cation. Finally, the well-known electron acceptor CCl_4 acts as an efficient singlet quencher, enhancing the route leading to the radical cation, preventing intersystem crossing to the triplet and thus resulting in a dramatic increase in the yield of 6-methoxy-2-naphthaldehyde, the major oxidative decarboxylation product; this constitutes unambiguous proof in favor of the new mechanistic proposals.

INTRODUCTION

Many drugs are known to induce phototoxic responses after either systemic or topical application. The nonsteroidal antiinflammatory drugs (NSAID)† deserve special mention due to the incidence of photosensitivity disorders that are higher with NSAID than with other drugs (1–4).

In this context, nabumetone (NB) is an efficient NSAID (Fig. 1) used for the treatment of osteoarthritis (5). Nabumetone is a relatively selective cyclooxygenase-2 inhibitor (6,7) that shows less gastrointestinal side effects than other NSAID (8,9). However, NB was reported to be potentially phototoxic in human volunteers using an oral dosing protocol. Phototoxicity, consisting of wheal-and-flare reactions following exposure to UV radiation, was demonstrated (10). Nabumetone is considered a prodrug because it is metabolized by the liver to 6-methoxy-2-naphthylacetic acid (MNAA) that is actually the active form (Fig. 1).

Recently, a study on the transient intermediates generated from the photoexcitation of NB appeared in the literature (11). As the metabolite is the chemical entity able to reach the skin, we found it of interest to study the photophysical and photochemical properties of MNAA in order to understand the phototoxic effects of NB. In the present paper, we have dealt with two different aspects: (1) steady-state photolysis that provided insight into the photochemical properties and the photodegradation mechanisms of the metabolite and (2) a photophysical study using conventional and time-resolved techniques that provided information about the transient intermediates involved in the photodegradation pathways.

MATERIALS AND METHODS

Chemicals. Acetonitrile and methanol (HPLC grade) were purchased from J. T. Baker (Holland). Benzophenone was from Prolabo. All other chemicals used were of reagent grade and used as received. 6-Methoxy-2-naphthylacetic acid was synthesized from 6′-methoxy-2′-acetonaphthone (Aldrich) following a procedure described in the literature for related compounds (12,13).

Synthesis of MNAA. 6′-Methoxy-2′-acetonaphthone (1 g, 5 mmol) was added to a solution of thallium (III) nitrate (2.2 g, 5 mmol, 1 eq) in 12 mL of MeOH containing 2.5 mL of 70% perchloric acid. The mixture was stirred at room temperature for 6 h. The thallium (I) nitrate was filtered, and the filtrate was poured onto water, extracted with ethyl acetate (3 × 25 mL) and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure afforded the ester as a solid that was submitted to hydrolysis without further purification.

The crude ester (1.15 g) was added to a solution of 30% aqueous sodium hydroxide (7.5 mL) in methanol (25 mL) and heated under reflux, with stirring, for 4 h. The reaction mixture was poured onto water, acidified and extracted with ethyl acetate (3 × 25 mL). The

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†*Abbreviations:* GC, gas chromatography; MNAA, 6-methoxy-2-naphthylacetic acid; MS, mass spectrometry; NB, nabumetone; NSAID, nonsteroidal antiinflammatory drug; PBS, phosphate-buffered saline; PMT, photomultiplier tube.

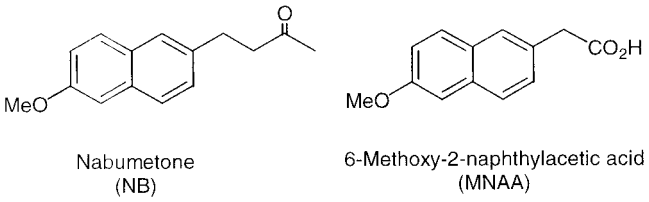


Figure 1. Chemical structures of NB and its major metabolite MNAA.

combined organic extracts were washed with water and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure gave the crude acid (0.9 g) that was purified by chromatography on silica gel using hexane–ether (3:7) as eluent and then repurified by preparative HPLC using methanol–water–acetic acid (60:39:1) as eluent.

Instrumentation. Gas chromatography/mass spectrometry (GC/MS) analyses were achieved with a FISONS HRGC 8000 spectrometer equipped with an HP-5 column (25 m × 0.32 mm). The ^{1}H-NMR spectra were measured by means of a Varian Gemini 300 MHz instrument. $CDCl_3$ was used as a solvent and the signal corresponding to trimethylsilane was used as the internal reference. Ultraviolet spectra were recorded on a Shimadzu UV/visible scanning spectrophotometer (2101PC) with a slit width of 5 nm. The HPLC analyses were performed on an HPLC Varian Systems equipped with a 9012Q pump and a photodiode array (Varian 9065). Samples were injected onto an analytical Kromasil 100 C18 column (Tracer, 25 × 0.4 cm, mean particle size 5 μm) using acetonitrile–water–acetic acid (50:49:1) as the mobile phase; flow rate 0.5 mL/min. Preparative HPLC purifications were performed on a Hitachi apparatus equipped with an L-6250 intelligent pump and an L-400 fixed wavelength UV detector at a wavelength of 320 nm. Samples were injected onto a Lichrosorb column (RP-18, 25 × 2.5 cm, mean particle size 7 μm) using methanol–water–acetic acid (60:39:1) as the mobile phase; flow rate: 10 mL/min.

Fluorescence measurements. Concentration was fixed by adjusting the absorbance of the solutions at the arbitrary value of 0.4 at the excitation wavelength (320 nm). The steady-state fluorescence was obtained with an FS900 Edinburgh Analytical Instruments apparatus, equipped with a 450 W xenon lamp. The time-resolved fluorescence determinations were performed with an FL900 Edinburgh Analytical Instrument apparatus using a hydrogen lamp (0.7 ns pulse width) as the excitation source. The samples were placed into quartz cells of 1 cm pathlength, and deoxygenation was made by bubbling nitrogen.

Laser flash photolysis measurements. A pulsed Nd:YAG SL404G-10 Spectron Laser Systems was used for the excitation at 266 or 355 nm. The single pulses were ~10 ns duration and the energy was ~10 mJ/pulse. A pulsed Lo255 Oriel xenon lamp was employed as detecting light source. The laser flash photolysis apparatus consisted of the pulsed laser, the Xe lamp, a 77200 Oriel monochromator, an Oriel photomultiplier tube (PMT) system made up of a 77348 side-on PMT, 70680 PMT housing and a 70705 PMT power supply. The oscilloscope was a TDS-640A Tektronix. The output signal from the oscilloscope was transferred to a personal computer. All the MNAA solutions studied had an absorbance of 0.5 at 266 nm and were degassed (when specified) by bubbling nitrogen.

Steady-state photolysis. Irradiations of the MNAA samples (1.5×10^{-3} *M*, 5 mL/tube) were performed by using the pyrex-filtered light from an OSRAM-HLQ 125 W medium-pressure Hg lamp located inside an immersion well photoreactor (Applied Photophysics model 3230). Parallel experiments were performed in acetonitrile and phosphate-buffered saline (PBS) aqueous solutions under both aerobic and anaerobic conditions. Eventually, different amounts of CCl_4 were added to the acetonitrile solutions. Photoreactions were monitored by reversed-phase HPLC using the conditions mentioned under instrumentation.

RESULTS AND DISCUSSION

Photophysical properties

Fluorescence. The absorption spectrum of MNAA was expected to extend into the biologically relevant UVA–UVB zone because this compound possesses a naphthalene chromophore. In fact, when the spectrum was measured in acetonitrile and PBS solutions it showed four bands with maxima at 220, 270, 320 and 330 nm.

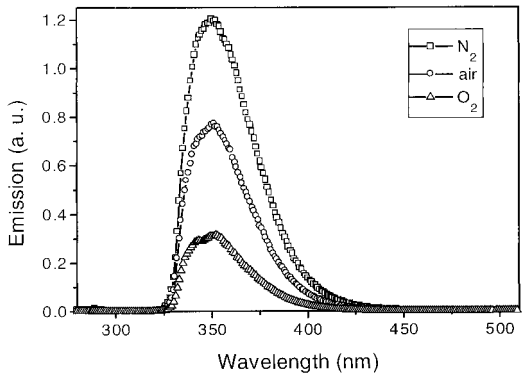


Figure 2. Emission spectra of MNAA in acetonitrile recorded after excitation at 320 nm under different conditions.

The emission spectrum of MNAA in both solvents showed a broad maximum at 351 nm; the excitation spectrum was essentially coincident with the absorption spectrum. From the intersection of the normalized excitation and emission spectra (336 nm) a singlet energy value of ~355 kJ mol^{-1} was estimated, identical to the E_{0-0} ~ 355 kJ mol^{-1} reported for NB in acetonitrile (11).

The fluorescence quantum yields were 0.50 in acetonitrile and 0.42 in PBS aqueous solution. The related NSAID naproxen in acetonitrile (Φ_{flu} 0.47) was used as a standard (11,14). The excited singlet state of the metabolite was found to be quenched by molecular oxygen. Thus, the Φ_{flu} of aerated MNAA acetonitrile solutions was only 0.35; it still dropped to 0.15 in oxygen-saturated solutions, a value significantly lower than the 0.50 obtained under N_2 (Fig. 2). Analogously, in PBS solutions the Φ_{flu} was 0.41 in aerated solutions and 0.39 in oxygen-saturated solutions (note that the apparently less effective quenching in PBS compared to the acetonitrile is mainly due to the different concentration of O_2 in the two solvents).

The quenching constant of the singlet state by oxygen in acetonitrile was calculated by means of time-resolved fluorescence spectroscopy. The lifetime (τ) of the singlet state was 13.6 ns under N_2, 8.0 ns in aerated solutions and 2.9 ns in oxygen-saturated solutions. From these data a value of $3.0 \times 10^{10}\ M^{-1}\ s^{-1}$ was calculated for the quenching constant by oxygen. Similarly, in PBS solutions the singlet lifetime was 10.3 ns under N_2, 10.1 ns in aerated solutions and 9.3 ns in oxygen-saturated solutions. From these data the calculated quenching constant by oxygen was $7.6 \times 10^9\ M^{-1}\ s^{-1}$.

Laser flash photolysis. Figure 3 shows the transient absorption spectra obtained after laser flash excitation at 266 nm of deaerated (A) and aerated (B) solutions of the NB metabolite in acetonitrile. The signal at 440 nm (Fig. 3A) with a lifetime of 4.3 μs was assigned to the triplet state by comparison with the literature (15). Another transient with two maxima at 380 and 610 nm and a lifetime of 3.7 μs was assigned to the naphthalene radical cation on the basis of the reported data for related molecules (16). Finally, a lon-

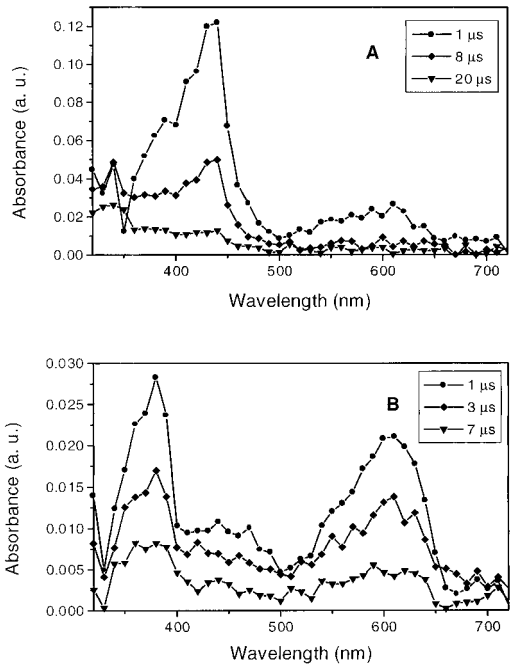


Figure 3. (A) Transient absorption spectra of a nitrogen-saturated acetonitrile solution of MNAA measured 1 μs, 8 μs and 20 μs after the pulse (266 nm). (B) Transient absorption spectra of an aerated acetonitrile solution of MNAA measured 1 μs, 3 μs and 7 μs after the pulse (266 nm).

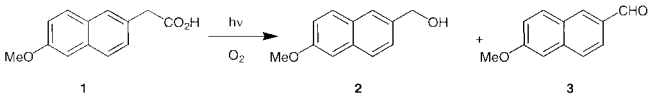
ger-lived species (25 μs) with a maximum at 340 nm could be associated with the formation of a benzylic radical; however, no further experiments were done to confirm unequivocally the nature of this band. Under oxygen-saturated conditions (Fig. 3B) the signal with maxima at 380 and 610 nm was the only remaining absorption after 1 μs.

When the laser flash photolysis of MNAA was performed in PBS solutions the same transient species were observed. The band at 440 nm assigned to the triplet state showed a longer lifetime (24 μs) in this aqueous solvent. However, the signal with the maxima at 380 and 610 nm had a lifetime (3.7 μs) similar to that found in acetonitrile. Again the band at 340 nm (51 μs) was observed as was the band corresponding to the solvated electron at 710 nm. In this solvent, the same quenching behavior as above was observed in the presence of O_2. As a result of the triplet state quenching, $^1O_2$ is probably formed, as described for related compounds (naproxen and NB) with a similar Φ_Δ of ~0.2 (11,17).

According to the literature, the naphthalene radical-cation in related molecules could arise from the excited singlet state (11). We tried to confirm whether this could also be true for MNAA by performing the laser flash photolysis experiment in the presence of benzophenone. Thus, selective excitation of benzophenone at 355 nm sensitized the formation of the triplet state of MNAA as the sole transient. Its decay was complete without formation of any other signal, confirming that the triplet state is not involved in the formation of the naphthalene radical cation.

Photochemistry

Products study. 6-Methoxy-2-naphthylacetic acid was found to be photolabile. Its irradiation in aerated PBS aqueous solutions (1.5×10^{-3} *M*) led to the alcohol **2** and the aldehyde **3** as major photoproducts (Scheme 1), being the conversion lower than 10% after 15 min. The same photoproducts were obtained when the irradiation was performed in aerated acetonitrile, although the extent of photodegradation was even lower in this solvent. Thus, the photoreactivity of the carboxylate form (predominating species in PBS) appears to be much higher than that of the free acid. No significant reaction was observed when deaerated solutions of MNAA were irradiated in any of the two solvents.

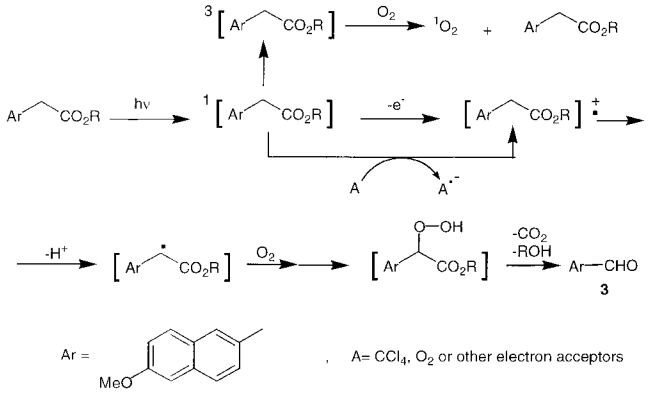


Scheme 1. Photodegradation products of MNAA under aerobic conditions.

Reaction mechanism. The enhancement of photodegradation observed in the presence of oxygen had been previously described for related molecules. Thus, in the case of naproxen the photodecarboxylation quantum yield was found to be 0.001 under deaerated conditions and 0.012 in aerobic medium (14). This could be explained by means of a new reaction mechanism that would operate in addition to the direct decarboxylation route established for related NSAID (18,19). The new mechanism could imply deprotonation of the naphthalene radical cation leading to a nondecarboxylated benzylic radical intermediate that would be trapped by O_2. Subsequent loss of CO_2 would result in the corresponding oxidative fragmentation compound 3 (Scheme 2).

In order to confirm this hypothesis two different series of experiments were undertaken following the rationale indicated below.

Chemical evidence for reaction via *radical cation*. First, methyl esters cannot undergo direct photodecarboxylation in organic solvents as the corresponding acids do. Thus, if methyl 6-methoxy-2-naphthylacetate were irradiated instead of the acid itself the direct mechanism would not be able to operate. As a matter of fact, irradiation of the ester (1.5×10^{-3} *M*) in aerated acetonitrile resulted in the formation of the expected aldehyde as the sole compound (~2% after 15 min). Therefore, formation of the aldehyde under the irradiation conditions indicates that the naphthalene radical cation has evolved to the benzylic radical prior to decarboxyl-



Scheme 2. Indirect mechanism postulated for the photodegradation of MNAA in the presence of O_2, CCl_4 or other electron acceptors.

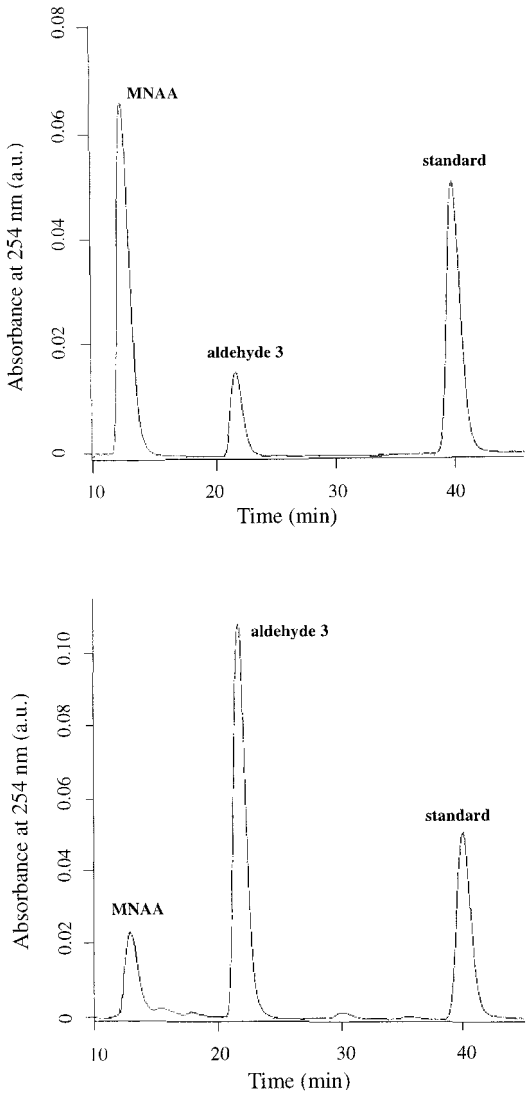


Figure 4. The HPLC elution profile recorded at 254 nm from an aerated (1 m*M*) solution of MNAA in acetonitrile after 2 min of irradiation. Upper trace, in the absence of CCl_4; lower trace, with added CCl_4 (65 m*M*). 2-Methoxynaphthalene (1 m*M*) was used as standard for integration.

ation. Trapping of this radical by O_2, subsequent formation of a peroxylactone and final loss of carbon dioxide explains formation of the aldehyde.

Second, carbon tetrachloride is known to act as an electron acceptor. Therefore, if the irradiation were performed in the presence of CCl_4, a significant enhancement of the photodegradation proccess through formation of the radical cation and irreversible evolution to the photoproducts would be expected. As anticipated, irradiation of MNAA (1.5×10^{-3} *M*) in aerated acetonitrile containing 65 m*M* CCl_4 resulted in a dramatic enhancement (~10 times) of the photodegradation, giving the aldehyde **3** as the only photoproduct (Fig. 4).

Photophysics of MNAA in the presence of CCl_4. The interesting results obtained upon addition of CCl_4 prompted us to study the photophysical properties of MNAA in the presence of CCl_4. Figure 5 shows the dramatic fluorescence quenching that occurs upon addition of increasing amounts

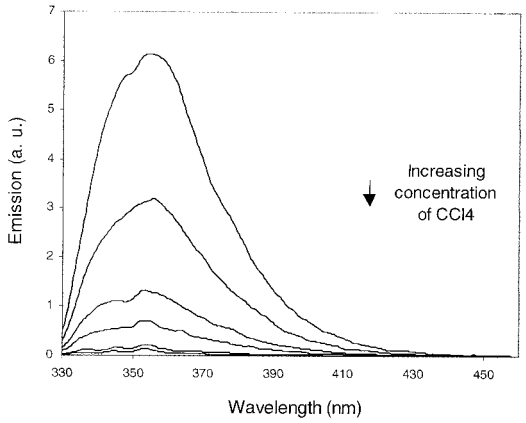


Figure 5. Fluorescence spectra of MNAA in acetonitrile upon addition of 0, 13, 26, 65, 258 and 517 m*M* CCl_4.

of CCl_4 to acetonitrile solutions of MNAA under anaerobic conditions. When the reciprocal emission intensities were plotted against the concentration of CCl_4, a linear relationship was observed; the calculated Stern–Volmer constant was 148.2 M^{-1}. Taking into account the fluorescence lifetime of MNAA in acetonitrile (see above), the estimated value of the fluorescence quenching rate constant was 1.1×10^{10} M^{-1} s^{-1}.

The effect of the added CCl_4 was also examined by means of laser flash photolysis. The most striking observation was a clear decrease in the amplitude of the transient signal associated with the triplet at 440 nm, concomitantly with a corresponding increase in the bands at 380 and 610 nm assigned to the radical cation. Figure 6 shows the ratio radical cation *versus* triplet as a function of the CCl_4 concentration. The transient lifetimes were not affected by CCl_4.

Overall, the photophysical properties in the presence of CCl_4 are consistent with quenching of the MNAA singlet by CCl_4 leading to the radical cation that competes favorably with intersystem crossing to the triplet.

CONCLUSIONS

6-Methoxy-2-naphthylacetic acid, the major metabolite of the phototoxic NSAID NB, has been found to be photoactive. Its photodegradation is enhanced in the presence of oxygen *via* a new photodegradation mechanism that involves

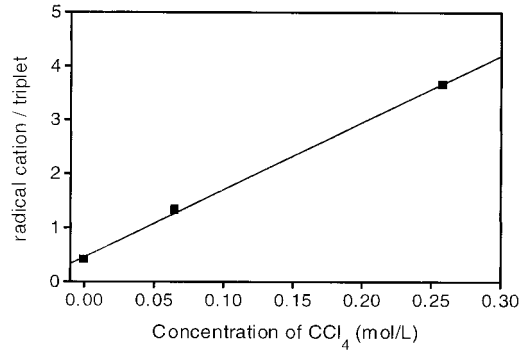


Figure 6. Plot of the ratio radical cation/triplet (measured by the relative intensities of the bands at 380 and 440 nm, respectively, in the transient absorption spectra) against the concentration of CCl_4.

formation of the naphthalene radical cation from the singlet state, followed by deprotonation to a benzylic radical that is trapped by O_2. This route could be enhanced in the presence of certain biomolecules acting as electron acceptors as CCl_4 does. Thus, a Type I photosensitization mechanism (in addition to the already suggested Type II pathway) should be taken into account to explain the photobiological properties of NB.

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