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Photochemistry of naproxen in the presence of β-cyclodextrin

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Abstract

Irradiation of naproxen (**1**) in the presence of β-cyclodextrin (CD) leads to photodecarboxylation products with ethyl (**2**), 1-hydroxyethyl (**3**) and acetyl (**4**) side chains. The presence of CD does not protect against photodegradation, but rather results in a more rapid disappearance of the drug. The most important change associated with CD is the marked predominance of alcohol **3** over ketone **4**. Since **3** is more cytotoxic than **4**, the reduced photohaemolytic activity of **1** in the presence of CD must be attributed to the sequestering and stabilization of the radical intermediates by complexation, rather than to the nature of the stable photoproducts. © 1997 Elsevier Science S.A.

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1. Introduction

The photosensitivity disorders elicited by several drugs of the 2-arylpropionic acid group constitute a common type of side effect associated with the widespread clinical use of non-steroidal anti-inflammatory agents [1,2]. It has been reported that the 2-arylpropionic acids naproxen, benoxaprofen and ketoprofen form water-soluble complexes with β-cyclodextrin (CD) [3–8], which are less active than the non-complexed compounds in the photohaemolysis assay, an observation which points to the possibility of using CD complexation as a potentially valid tool in alleviating drug phototoxicity [3,5,7]. At present, the reason for this effect is rather unclear. Although some basic hypotheses have been advanced, a systematic comparative study aimed at establishing the different photoproduct selectivities achieved in the presence and absence of CD is still lacking. In this work, we have undertaken such a study on naproxen, whose CD complex exhibits a markedly reduced photohaemolytic activity.

2. Experimental details

2.1. General irradiation procedures

2.1.1. Irradiation in the presence of CD

Irradiation of naproxen (**1**) was carried out using Pyrex-filtered light from a 125 W medium pressure mercury lamp. The drug (approximately 30 mg) was added to distilled water (100 ml) containing 850 mg of CD and the mixture was stirred to afford a clear solution. In the absence of CD, most of the drug remained undissolved. The resulting solution was distributed in three Pyrex test tubes. After bubbling with oxygen or argon for 10 min, the tubes were placed surrounding a centrally positioned quartz cooling jacket containing the lamp and irradiated for 1 h. For comparison, parallel experiments were carried out using phosphate-buffered saline (PBS, pH 7.2, 30 ml) instead of water as solvent. The combined photolysates were extracted three times with ether (3×25 ml). The organic phase was dried over $MgSO_4$ and concentrated to dryness under reduced pressure.

2.1.2. Irradiation in micellar systems

A PBS solution (5 ml) containing naproxen and sodium dodecyl sulphate (SDS) (500 mg) was stirred for 5 min, and the resulting mixture was irradiated under oxygen or argon for 1 h using the above-mentioned procedure. Subsequently, $BaCl_2$ (saturated aqueous solution) (2 ml) and methanol (5 ml) were added. The precipitate was filtered in vacuo and the liquid phase was extracted with $CHCl_3$ (3×5 ml). The resulting organic solution was washed with water (3×10 ml), dried ($MgSO_4$) and concentrated to dryness.

2.1.3. Irradiation in the presence of red blood cells (RBCs)

Fresh human erythrocytes (2 ml), obtained from the Centro de Transfusiones de la Comunidad Valenciana, were added to PBS (20 ml). Subsequently, naproxen (20 mg) was added and the mixture was irradiated under oxygen. After

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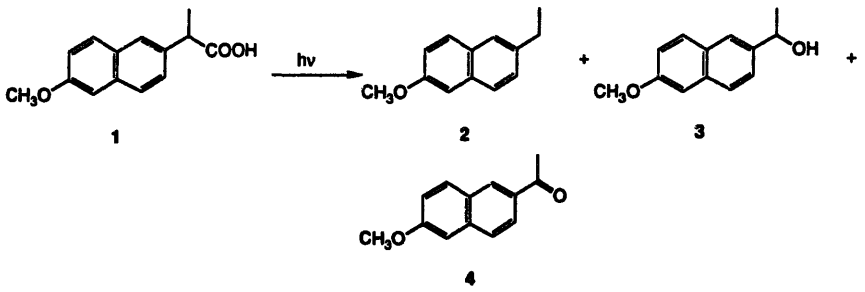


Scheme 1.

extraction with ether (3×50 ml), the organic phase was dried and evaporated.

2.2. Analysis of the photomixtures

The oily residues were analysed by gas chromatography/mass spectrometry (GC/MS) and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. The product selectivities were determined using the relative intensities of the most significant protons of the side chain: the methylene group of **2** (quartet at δ=2.8), the methine group of **3** (quartet at δ=5.1) and the methyl group of **4** (singlet at δ=2.7).

3. Results and discussion

Irradiation of naproxen (**1**) was performed in the presence of CD in either aerobic or anaerobic conditions using distilled water or PBS as solvent. In all cases, the reaction mixtures contained varying amounts of unreacted starting material, together with the photodecarboxylation products with ethyl (**2**), 1-hydroxyethyl (**3**) and acetyl (**4**) side chains. The product distribution was found to be strongly dependent on the reaction conditions (Scheme 1 and Table 1).

In general, the photoreactivity of naproxen was much higher under aerobic conditions, an observation which is in good agreement with previous results [9,10]. Surprisingly, the presence of CD did not protect against photodegradation, but rather resulted in a more rapid disappearance of the drug. Preliminary studies on the photodegradation of CD–naproxen complexes followed the reaction course through the appearance of the ketonic photoproduct **4** and the concomitant increase in absorbance at 312 nm [7]. This suggested a reduced photodegradation based on the assumption that **4** was the only significant product in aerated aqueous solution, which is nearly correct in the absence of CD (see Table 1, entry 2). However, our results show that the major photoproduct of the complexed drug is the alcohol **3** (see Table 1, entries 4 and 6), which displays a UV absorption spectrum similar to that of naproxen (Fig. 1). This explains the small variations observed in the UV spectra in spite of the considerable extent of photodecarboxylation.

It is interesting to note that **3** is the most toxic photoproduct of naproxen; for instance, its cytotoxicity to cultured hepatocytes using as endpoint the loss of activity of succinyl dehydrogenase (MTT test) is much higher than that of ketone **4** [9]. Hence the diminished phototoxicity of the CD-complexed drug cannot be attributed to the nature of the stable photoproducts, but instead must be associated with the involvement of different short-lived intermediates.

In principle, compound **3** can be formed by either oxygen trapping of the benzylic radical **5** (i) or by reaction of the corresponding benzylic cation **7** with water (ii) (Scheme 2). To ascertain whether the latter mechanistic pathway is the origin of the alcohol **3**, the reaction was performed in ^{18}O-labelled water (10% isotopic enrichment). Analysis of the photomixture by GC/MS clearly showed that there was no incorporation of ^{18}O in the product **3**, as indicated by a lack of a peak with m/z=204 (M^++2). This strongly suggests

Table 1
Photolysis of naproxen (**1**) under different reaction conditions

Entry	Conditions	Recovered **1** (%) [a]	Product yield (%) [a]		
			2	**3**	**4**
1	H_2O/Ar [b]	92	4	4	0.1
2	H_2O/O_2 [b]	59	0.1	7	34
3	$CD/H_2O/Ar$	76	8	12	4
4	$CD/H_2O/O_2$	26	13	52	9
5	CD/PBS/Ar	83	8	8	1
6	$CD/PBS/O_2$	52	15	30	3
7	SDS/PBS/Ar	97	1	–	2
8	$SDS/PBS/O_2$	53	4	32	11
9	RBC [c]/PBS/O_2	74	4	12	10

[a] Determined by ^{1}H NMR and GC/MS analysis of the photomixtures.
[b] Data taken from Ref. [9].
[c] Red blood cells.

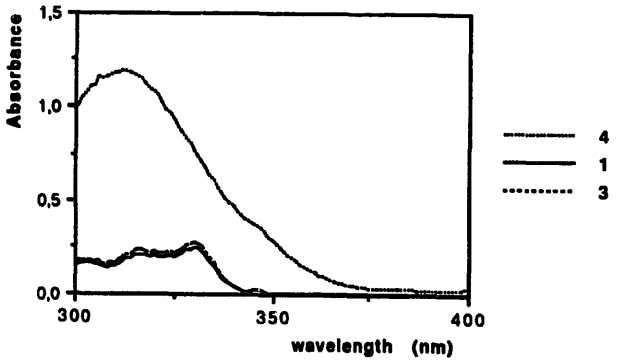
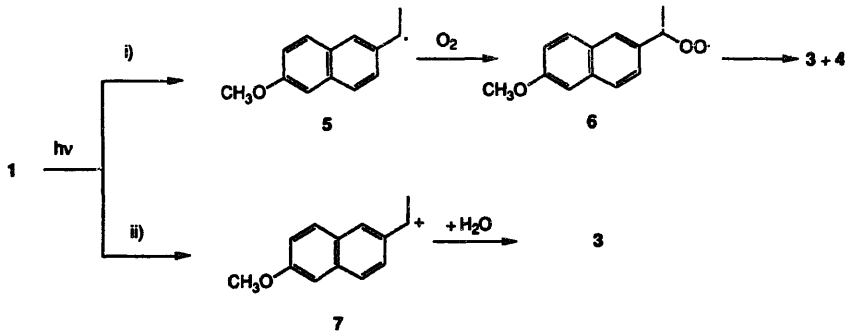


Fig. 1. UV absorption spectra of naproxen (**1**) (———) and its photoproducts **3** (- - -) and **4** (· · ·).



Scheme 2.

that route (i) involving oxygen trapping of the radical is operating. Indeed, significant amounts of the alcohol were obtained only when oxygen was present in the reaction medium (Table 1, entries 4 and 6).

According to previous observations, the lifetimes of benzylic radicals are affected by their encapsulation within CD cavities [11,12]. The subsequent steps leading to the major product **3** are closely related to well-established processes in peroxide chemistry [13]. In this case, the hydrogen source must be the CD matrix, as its C–H bonds are easier to cleave than the OH bonds of water.

In view of the above results, it appears that the reduced photohaemolytic activity of naproxen in the presence of CD can be attributed to the different behaviour of the complexed radical intermediates, rather than to an increased drug photostability. Efficient sequestering of these transient species by CD prevents their interaction with cell membranes, which is a prerequisite for the induction of photobiological damage. Additional experiments were carried out using an SDS micellar environment. The results (Table 1, entries 7 and 8) show that the extent of photodegradation is again markedly higher under aerobic conditions and the product selectivity (alcohol **3** as major product) is similar to that achieved using CD complexation.

It was wondered whether the above constraining media (CD and SDS) are good model systems to reproduce the photochemical behaviour of naproxen under the conditions used in the photohaemolysis experiments. Hence we tried to photolyse the drug in the presence of RBCs using oxygen-saturated PBS as solvent. In spite of some experimental difficulties (e.g. strong light screening by the RBC preparation), which resulted in comparatively low conversions, it became evident that the **3**/**4** ratio (approximately 1.2) was significantly higher than that obtained in the absence of cells (0.2). A similar trend was observed with SDS and CD.

4. Conclusions

The inclusion of naproxen (**1**) within CD has a marked influence on its photochemical behaviour. The most important change is associated with the predominance of alcohol **3** over ketone **4**, which is the major photoproduct in the absence of CD. Since **3** is more cytotoxic than **4**, the reduced photohaemolytic activity of **1** in the presence of CD must be attributed to the sequestering and stabilization of the radical intermediates and/or photoproducts by complexation, rather than to a decreased photoreactivity.

Acknowledgements

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