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PHOTOCHEMICAL AND PHOTOBIOLOGICAL PROPERTIES OF KETOPROFEN ASSOCIATED WITH THE BENZOPHENONE CHROMOPHORE

FRANCISCO BOSCÁ[1], MIGUEL A. MIRANDA*[1], GERMANO CARGANICO[2] and DAVID MAULEÓN[2]
[1]Departamento de Química/Instituto de Tecnología Química UPV-CSIC, Universidad Politécnica de Valencia, Camino de Vera s/n, Apartado 22012, E-46071 Valencia, Spain and
[2]Departamento de Investigación, Laboratorios Menarini, S.A., Calle Alfonso XII, 587, E-08912 Badalona (Barcelona), Spain

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Abstract—Irradiation of ketoprofen in neutral aqueous medium gave rise to 3-ethylbenzophenone as the major photoproduct. Its formation is justified *via* protonation of a benzylic carbanion or hydrogen abstraction by a benzylic radical. Minor amounts of eight additional compounds were isolated. Four of them are derived from the benzylic radical: 3-(1-hydroperoxyethyl)benzophenone, 3-(1-hydroxyethyl)benzophenone, 3-acetylbenzophenone and 2,3-*bis*-(3-benzoylphenyl)butane. The other four products involve initial hydrogen abstraction by the excited benzophenone chromophore of ketoprofen: 1,2-*bis*-(3-ethylphenyl)-1,2-diphenyl-1,2-ethanediol, 2-(3-benzoylphenyl)-1-(3-ethylphenyl)-1-phenylpropan-1-ol, α-(3-ethylphenyl)phenylmethanol, 1,2-*bis*-[3-(2-hydroxycarbonylethyl)phenyl]-1,2-diphenyl-1,2-ethanediol. The latter process was found to mediate the photoperoxidation of linoleic acid through a type I mechanism, as evidenced by the inhibition produced by the radical scavengers butylated hydroxyanisole and reduced glutathione. The major photoproduct, which contains the benzophenone moiety but lacks the propionic acid side chain, also photosensitized linoleic acid peroxidation. Because lipid peroxidation is indicative of cell membrane lysis, the above findings are highly relevant to explain the photobiological properties of ketoprofen.

INTRODUCTION

Nonsteroidal anti-inflammatory 2-arylpropionic acids have been widely used in clinical practice. Although these drugs present a very favorable benefit/risk ratio, several members of the group have been involved in adverse photosensitivity reactions.[1,2] Benoxaprofen, which was withdrawn from the market shortly after its introduction, presented a high incidence of phototoxic reactions.[3,4] Although ketoprofen (KP)† has a much lower photosensitizing potential, it has also been able to elicit photocontact dermatitis in a number of cases.[5–8] Screening tests such as photohemolysis[9] or photo-basophil–histamine release[10] have shown the *in vitro* phototoxicity of this drug.

Trying to explain the above observations, the photochemistry of KP has been previously investigated in buffered aqueous media under aerobic conditions.[11,12] Formation of the isolated products (**2–5**) (Fig. 1) has been justified on the basis of initial photodecarboxylation, through the key intermediacy of a benzylic radical. This short-lived species together with photoproducts **3**, **4** and superoxide anion are thought to be responsible for the KP-induced photohemolysis.[11]

The present work shows that the benzophenone chromophore of KP is photochemically active, as evidenced by the isolation of four photoproducts (**7–10**) derived from ketyl radical intermediates, in addition to the decarboxylation products previously reported (**2–5**) and the dimer **6** (Fig. 1). This new photochemistry of KP is associated with the hydrogen-abstracting ability of the excited carbonyl moiety. Its relevance to the photobiological properties of the drug is strongly suggested by experiments on the KP-photosensitized formation of linoleic acid hydroperoxides.

MATERIALS AND METHODS

Chemicals. Ketoprofen was provided by Laboratorios Menarini S.A. (Badalona, Spain). Linoleic acid (LA), reduced glutathione (GSH) and butylated hydroxyanisole (BHA) were purchased from Sigma (St. Louis, MO, USA). Monodeuterated methanol (MeOD) was from Aldrich (Steinheim, Germany).

Photolysis of KP. The drug was exposed to natural sunlight, dissolved in 500 mL aqueous phosphate-buffered saline (PBS: phosphate buffer; 50 m*M*, pH 7.2, 0.9% NaCl), under oxygen atmosphere. Irradiations were performed at different initial concentrations of KP (10^{-2}, 10^{-3} and 10^{-4} *M*). In the case of 10^{-2} *M* KP concentration, gentle warming and sonication were necessary to obtain clear solutions. The progress of the reaction was monitored by taking aliquots after different irradiation times. These aliquots were acidified and extracted with CH_2Cl_2. The resulting extracts were analyzed by thin-layer chromatography (TLC), high performance liquid chromatography (HPLC) and gas chromatography.

Alternatively, irradiations of KP solutions (10^{-2}, 10^{-3} and 10^{-4} *M* in PBS or methanol, eventually in the presence of equimolar amounts of NaOH) were also carried out in a pyrex immersion-well photoreactor (Applied Photophysics, parts no. 3230 + 3307), with an OSRAM HQL 125 W medium-pressure Hg lamp for 1 h, under oxygen atmosphere. Parallel experiments were done irradiating the sodium salt of KP in monodeuterated methanol.

Isolation and identification of the photoproducts. The aqueous photomixtures were extracted with CH_2Cl_2 and the organic phases were dried over anhydrous $MgSO_4$ and evaporated at reduced pressure. Isolation of the photoproducts was attempted by column chromatography using hexane/ethyl acetate (2/1) as eluent. Six fractions were obtained. The first one was a mixture of the ethyl derivative **2**[11] and its hydrodimer **7**. These compounds were separated by HPLC using hexane/ethyl acetate (30/1) as mobile phase. The second fraction contained the dimers **6** and **8**, as well as the reduced ethyl

*Author to whom correspondence should be addressed.
†*Abbreviations:* BHA, butylated hydroxyanisole; GC, gas chromatography; GSH, reduced glutathione; HPLC, high-performance liquid chromatography; KP, ketoprofen; LA, linoleic acid; MS, mass spectrometry; PBS, phosphate-buffered saline; TLC, thin-layer chromatography.

derivative **9**.[13] This fraction was resolved into its pure components by HPLC (mobile phase: hexane/ethyl acetate 20/1). The last four fractions were constituted by single products, identified as the acetyl (**5**),[11] 1-hydroperoxyethyl (**3**)[11] and 1-hydroxyethyl (**4**)[11] derivatives, together with the KP hydrodimer **10**.

All the structures were assigned by means of IR spectrophotometry, NMR spectroscopy (^1H and ^{13}C), UV-vis spectrophotometry and mass spectrometry (MS). Infrared absorptions (cm^{-1}) are given only for relevant functional groups.

3-Ethylbenzophenone (**2**). IR (CCl_4): ν = 1655 (C=O); ^1H-NMR ($CDCl_3$): δ = 7.85–7.20 (m, 9H, aromatic), 2.65 (q, J = 7 Hz, 2H, CH_2), 1.25 (t, J = 7 Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): δ = 198.0, 145.1, 138.4, 138.2, 132.9, 132.7, 130.6, 129.9, 128.9, 128.7, 128.2, 29.0, 15.7; MS: m/z (%) = 210 (M^+, 55), 181 (25), 165 (5), 133 (100), 105 (65), 77 (50), 51 (12); UV-vis (CH_3OH): λ (log ϵ) = 254 (4.2), 206 (4.4).

3-[1-^{2}H]ethylbenzophenone (**2D**). ^1H-NMR ($CDCl_3$): δ = 7.85–7.20 (m, 9H, aromatic), 2.70 (q, J = 7 Hz, 1H, CHD), 1.26 (d, J = 7 Hz, 3H, CH_3); MS: m/s (%) = 211 (M^+, 36), 181 (19), 134 (100), 106 (21), 105 (72), 78 (21), 77 (75).

3-(1-Hydroperoxyethyl)benzophenone (**3**). IR (CCl_4): ν = 3520, 3300 (OH), 1655 (C=O); ^1H-NMR ($CDCl_3$): δ = 8.00–7.30 (m, 9H, aromatic), 5.20 (q, J = 7 Hz, 1H, CH), 1.50 (d, J = 7 Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): δ = 197.7, 142.5, 138.0, 137.7, 132.8, 130.2, 130.2, 130.1, 128.8, 128.7, 128.6, 83.3, 20.2; MS: m/z (%) = 242 (M^+, 1), 226 (M^+-O, 3), 224 (M^+-H_2O, 28), 209 (M^+-OOH, 70), 181 (11), 165 (2), 133 (9), 105 (100), 91 (9), 77 (84), 51 (22), 43 (12); UV-vis (CH_3OH): λ (log ϵ) = 252 (4.2), 206 (4.4).

3-(1-Hydroxyethyl)benzophenone (**4**). IR (CCl_4): ν = 3600, 3440 (OH), 1655 (C=O); ^1H-NMR ($CDCl_3$): δ = 7.95–7.25 (m, 9H, aromatic), 4.95 (q, J = 7 Hz, 1H, CH), 1.50 (d, J = 7 Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): δ = 198.1, 147.0, 138.3, 138.1, 133.2, 130.7, 130.2, 129.8, 129.0, 128.9, 127.5, 70.2, 25.6; MS: m/z (%) = 226 (M^+, 5), 211 (M^+-CH_3, 30), 183 (33), 165 (3), 133 (18), 105 (100), 77 (93), 51 (24); UV-vis (CH_3OH): λ (log ϵ) = 253 (4.2), 206 (4.4).

3-Acetylbenzophenone (**5**). IR (CCl_4): ν = 1695, 1665 (C=O); ^1H-NMR ($CDCl_3$): δ = 8.50–7.40 (m, 9H, aromatic), 2.70 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): 198.2, 196.7, 138.7, 137.8, 137.6, 134.6, 133.5, 132.4, 130.6, 130.3, 129.3, 129.1, 27.0; MS: m/z (%) = 224 (M^+, 45), 209 (M^+-CH_3, 90), 181 (12), 165 (1), 147 (20), 105 (100), 91 (10), 77 (65), 51 (18), 43 (13); UV-vis (CH_3OH): λ (log ϵ) = 250 (sh), 230 (4.5), 203 (4.3).

2,3-*Bis*-(3-benzoylphenyl)butane (**6**). IR (CCl_4): ν = 1655 (C=O); ^1H-NMR ($CDCl_3$): δ = 8.00–7.20 (m, 18H, aromatic), 3.00 (m, 2H, CH), 1.40, 1.15 (d + d, J = 7 Hz, 6H, CH_3); ^{13}C-NMR ($CDCl_3$): δ = 197.2, 197.0, 146.4, 138.0, 137.9, 137.8, 137.7, 132.6, 132.5, 132.0, 131.9, 130.2, 130.1, 129.7, 129.2, 128.5, 128.4, 128.3, 128.1, 47.0, 46.9, 20.6, 19.1; MS: m/z (%) = 418 (M^+, 0), 209 (M^+/2, 22), 149 (24), 105 (36), 86 (27), 84 (50), 81 (52), 77 (22), 69 (100), 55 (28). UV-vis (CH_3OH): λ (log ϵ) = 254 (4.8), 212 (4.9).

1,2-*Bis*-(3-ethylphenyl)-1,2-diphenyl-1,2-ethanediol (**7**). IR (CCl_4): ν = 3540 (OH); ^1H-NMR ($CDCl_3$): δ = 7.60–7.00 (m, 18H, aromatic), 3.25 (s, 2H, OH), 2.60 (q, J = 7 Hz, 4H, CH_2), 1.16, 1.15 (t + t, J = 7 Hz, 6H, CH_3); ^{13}C-NMR ($CDCl_3$): δ = 145.2, 144.9, 143.9, 129.6, 129.0, 128.8, 128.0, 127.6, 127.2, 126.9, 83.7, 29.4, 16.0; MS: m/z (%) = 422 (M^+, 0), 211 (M^+/2, 65), 181 (3), 165 (2), 133 (18), 105 (100), 77 (38); UV-vis (CH_3OH): λ (log ϵ) = 205 (4.8).

1,2-*Bis*-(3-[1-^{2}H]ethylphenyl)-1,2-diphenyl-1,2-ethanediol (**7D**). ^1H-NMR ($CDCl_3$): δ = 7.60–7.00 (m, 18H, aromatic), 3.15 (s, 2H, OH), 2.55 (q, J = 7 Hz, 2H, CHD), 1.10, 1.11 (d + d, J = 7 Hz, 6H, CH_3); MS: m/z, (%) = 212 (M^+/2, 30), 181 (18), 134 (11), 105 (100), 77 (53).

2-(3-Benzoylphenyl)-1-(3-ethylphenyl)-1-phenylpropan-1-ol (**8**). IR (CCl_4): ν = 3590, 3480 (OH), 1655 (C=O); ^1H-NMR ($CDCl_3$): δ = 7.80–6.80 (m, 18H, aromatic), 3.95 (q + q, 1H, CH), 2.65, 2.45 (q + q, J = 7 Hz, 2H, CH_2), 2.55 (s, 1H, OH), 1.35 (d + d, J = 7 Hz, 3H, CHCH_3), 1.20, 1.00 (t + t, J = 7 Hz, 3H, CH_2 CH_3); ^{13}C-NMR ($CDCl_3$): δ = 197.7, 146.7, 144.9, 144.3, 143.3, 138.3, 137.4, 134.2, 132.9, 132.3, 130.6, 129.0, 128.8, 128.4, 127.4, 127.0, 126.4, 126.3, 125.9, 124.2, 123.9, 81.1, 48.2, 29.3, 16.9, 15.9; MS: m/z (%) = 420 (M^+, 1), 212 (11), 211 (58), 210 (33), 209 (7), 165 (4), 133 (16), 105 (100), 77 (38); UV-vis (CH_3OH): λ (log ϵ) = 237 (4.3), 206 (4.9).

α-(3-Ethylphenyl)phenylmethanol (**9**). IR (CCl_4): ν = 3600, 3450 (OH); ^1H-NMR ($CDCl_3$): δ = 7.60–7.20 (m, 9H, aromatic), 5.85 (s, 1H, CH), 2.70 (q, J = 7 Hz, 2H, CH_2), 1.20 (t, J = 7 Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): δ = 144.7, 144.3, 144.2, 128.7, 128.7, 127.7, 127.3, 126.9, 126.4, 124.2, 76.4, 28.9, 15.5; MS: m/z (%) = 212 (M^+, 25), 183 (23), 165 (10), 133 (56), 107 (34), 106 (53), 105 (100), 91 (23), 79 (56), 77 (43); UV-vis (CH_3OH): λ (log ϵ) = 206 (4.3).

1,2-*Bis*-[3-(2-hydroxycarbonylethyl)phenyl]-1,2-diphenyl-1,2-ethanediol (**10**). IR (KBr): ν = 3250 (OH), 1700 (C=O); ^1H-NMR ($CDCl_3$): δ = 7.50–7.00 (m, 18H, aromatic), 4.10 (s, 1H, OH), 3.55 (q, J = 7 Hz, 2H, CH), 3.20 (s, 1H, OH), 1.30 (d + d, J = 7 Hz, 6H, CH_3); ^{13}C-NMR ($CDCl_3$): δ = 181.1, 145.1, 144.5, 139.3, 129.1, 129.0, 128.6, 128.2, 127.9, 127.6, 127.0, 83.5, 45.6, 18.3, 18.1; MS: m/z (%) = 510 (M^+, 0), 256 (M^+/2 +1, 6), 255 (M^+/2, 26), 209 (24), 177 (19), 165 (11), 133 (6), 105 (100), 77 (72). UV-vis (CH_3OH): λ (log ϵ) = 205 (4.8).

Photoperoxidation of linoleic acid in the presence of KP and its photoproducts. Solutions of LA (10^{-3} *M*) in PBS, containing either KP (10^{-5} *M*) or each of the compounds **2–10**, were irradiated through pyrex with a 400 W mercury lamp for 1 h, keeping the temperature at 37°C by means of a thermostated bath. The reaction was monitored by UV-vis spectrophotometry, following the appearance and subsequent increase of a new absorption maximum at λ = 233 nm, due to the conjugated dienic hydroperoxides derived from LA.[2,14] The controls were solutions of LA without KP or its photoproducts, as well as solutions of the latter (compounds **1–10**) without LA, at the same concentrations. Controls were irradiated under identical conditions, and a set of duplicate samples was not irradiated. This test was repeated using KP in the presence of BHA, 10^{-4}–10^{-6} *M*, or GSH, 10^{-3} *M*, as radical scavengers.

RESULTS

Photochemistry of KP

When a 10^{-2} *M* solution of KP in buffered aqueous medium was exposed to sunlight, an almost complete consumption of the drug was observed after 1 h. Owing to the lower solubility of the photoproducts, the solution became unclear and then an oily phase was formed. Column chromatography of the photomixture, eventually followed by HPLC repurification, allowed separation of nine different photoproducts. Their structures were assigned on the basis of the IR, NMR (^1H and ^{13}C), MS and UV-vis spectral data (Fig. 1). Four of them (**2–5**) had been previously identified as photoproducts of KP, while the remaining products (**6–10**) were unknown. Their relative ratios depended upon the irradiation time: the major photoproduct was always 3-ethylbenzophenone (**2**), but its yield reached a maximum after 40 min, progressively decreasing on further photolysis. Conversely, the percentages of the other photoproducts increased with time until *ca* 2 h; more prolonged irradiation resulted in substantial polymerization (data not shown).

The product distribution was also influenced by the initial KP concentration. Table 1 shows the results obtained for drug solutions in the range 10^{-2}–10^{-4} *M*, after the irradiation times required in each case to achieve complete consumption. These experiments were monitored independently by HPLC, which allowed quantification of all products except the KP hydrodimer (**10**). A clear observation was that dimeric products were not formed upon photolysis of KP under the highest dilution.

In order to obtain reliable data for the mechanistic interpretations and to minimize the complications associated with insolubilization of the photoproducts, the photolysis of 10^{-3} and 10^{-4} *M* KP solutions were examined at lower conversions, under aerobic as well as anaerobic conditions. Figure

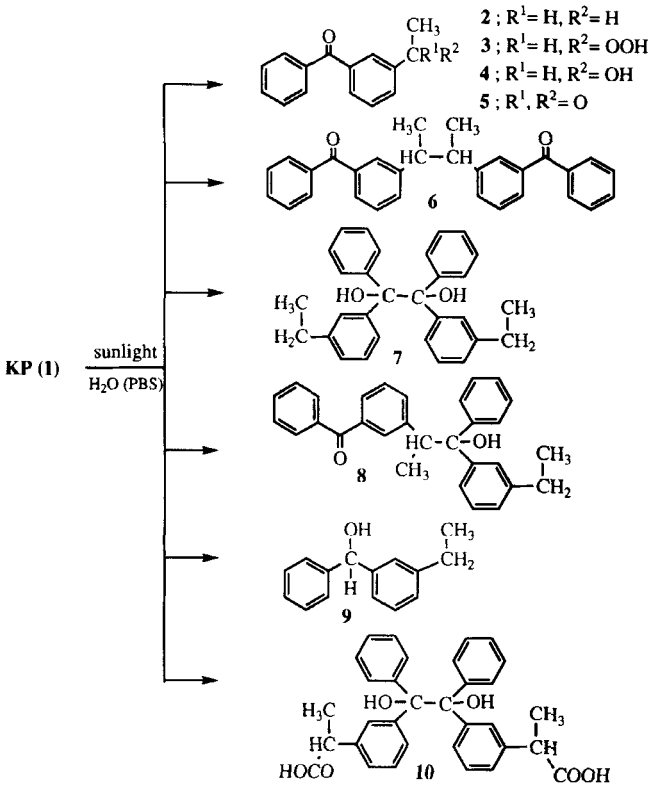

Figure 1. Photodegradation of KP (**1**) in neutral aqueous medium induced by sunlight. Besides the known photodecarboxylation products (**2–5**),[11,12] the dimer **6** and several photoproducts involving benzophenone photochemistry (**7–10**) are formed.

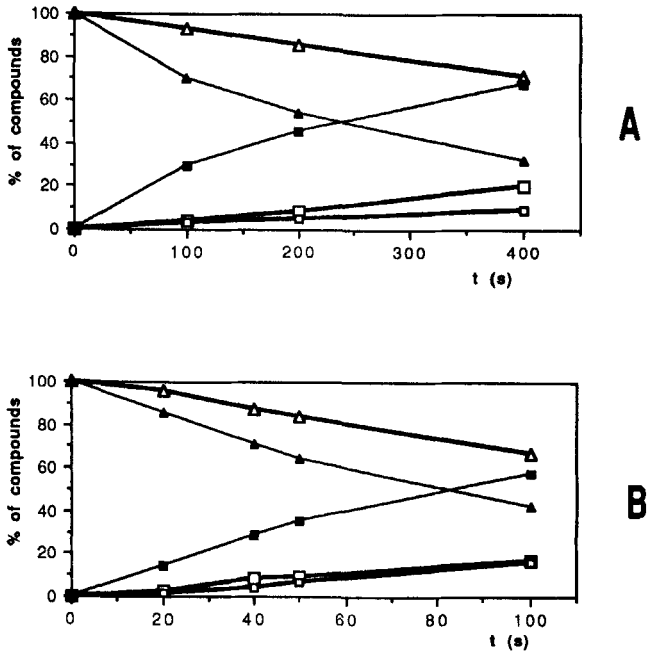

Figure 2. Time course of the sunlight-induced photolysis of KP in PBS, at 10^{-3} (A) and 10^{-4} (B) *M* concentration, under aerobic (air = Ox) and anaerobic (argon = Ar) conditions. The amounts of remaining drug **1** (△ = Ox, ▲ = Ar) and its photoproducts **2** (□ = Ox, ■ = Ar) or **3** + **4** + **5** (□ = Ox, ■ = Ar) are indicated at different early stages of the reaction.

2 shows that the initial rates of KP photodegradation were higher under argon, and that the oxygenated photoproducts **3**, **4** and **5** were present in the reaction mixtures from the very early stages of irradiation.

Besides examining the behavior of KP toward sunlight, a point of high interest in connection with the reported *in vivo* photosensitizing potential of this drug, a similar experiment was carried out using the more reproducible pyrex-filtered light of a medium-pressure mercury lamp. The results (Table 2) were qualitatively comparable, although the times required to produce given conversions with sunlight were roughly twice those employed with the artificial light source. It is evident from Tables 1 and 2 that as a general rule complex photomixtures were obtained in PBS. By contrast, when methanol was used as solvent, KP was cleanly and efficiently transformed into its hydrodimer **10**, while the sodium salt $\mathbf{1}^-$ was converted into its double-decarboxylated analogue **7**, together with minor amounts of **2**.

In a parallel experiment, the sodium salt ($\mathbf{1}^-$) was irradiated in monodeuterated methanol (10^{-2}, 10^{-3} and 10^{-4} *M*) to determine the origin of the benzylic hydrogen incorporated to **2** and/or **7**. Analysis of the photomixtures by MS evidenced in all cases the formation of the deuterated compounds **7D** and **2D**.[15] As expected, the ratio **7D**/**2D** decreased with decreasing concentration of the initial KP solutions.

Table 1. Irradiation of KP with sunlight in PBS at different concentrations

KP (1) [*M*]	Photoproducts (%)* 2	3	4	5	6, 8, 9	7	t (½)† (min)	Irradiation time‡ (min)
10^{-4}	46	5	15	28	<1	—	3	12
10^{-3}	60	<1	5	15	10	<1	11	40
10^{-2}	35	<1	6.5	4.5	11	3.8	25	90

*Determined by HPLC. Compounds **6**, **8** and **9** could not be separated from each other, while compound **10** was not detected under the employed conditions.
†Time required to achieve 50% photodegradation of KP.
‡Irradiation time to obtain the indicated photoproduct distribution, which corresponds to complete consumption of KP.

Table 2. Products obtained after 1 h irradiation of KP at 10^{-2} *M* concentration in different solvents

Solvent	Photoproducts (%)* 2	3	4	5	6	7	8	9	10
PBS†	46	1.2	6.1	3.5	2.8	2.4	5.6	1	7.6
PBS‡	29	—	3.1	2.6	3.2	4.3	6.3	<1	8.3
MeOH‡	<1	—	—	—	—	<1	—	—	86
MeOH/ NaOH‡	4	—	—	<1	—	83	—	—	—

*Isolated after chromatographic separation.
†Irradiated with sunlight.
‡Irradiated with pyrex-filtered light of a medium pressure mercury lamp.

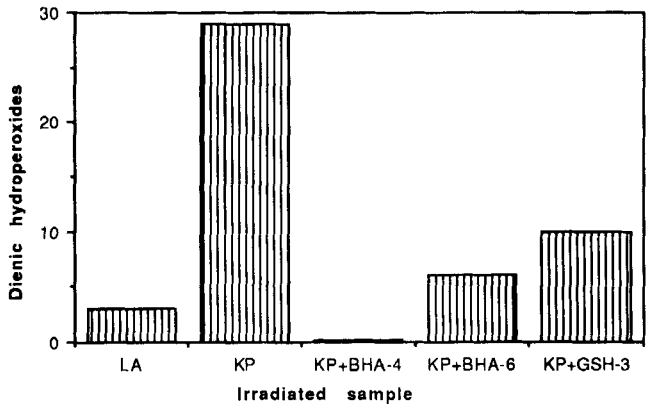

Figure 3. Peroxidation of LA photosensitized by KP in PBS. Solutions of 1 m*M* LA were irradiated alone and also in the presence of 10^{-5} *M* KP for 1 h. The extent of lipid peroxidation was followed spectrophotometrically by the increase of absorption at 233 nm. Parallel experiments were performed upon addition of the radical scavengers BHA (10^{-4} and 10^{-6} *M*) or GSH (10^{-3} *M*) to the mixtures of LA and KP.

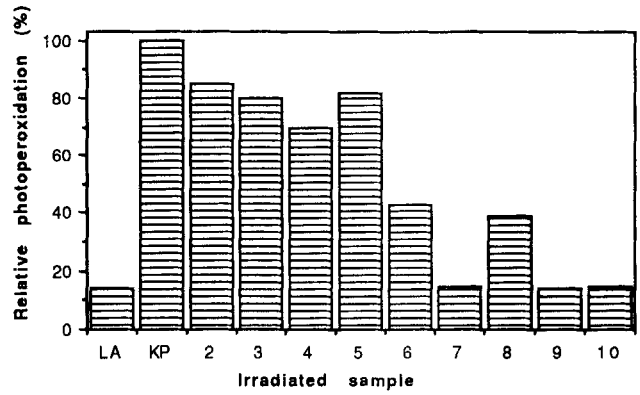

Figure 4. Photodynamic lipid peroxidation by the KP photoproducts. Solutions of 1 m*M* LA were irradiated alone and also in the presence of photoproducts **2–10** (10^{-5} *M*) and compared with the effect produced by KP. The formation of conjugated dienic hydroperoxides of LA was determined by measuring the optical absorption at 233 nm.

Photodynamic lipid peroxidation by KP

Having established the photochemical behavior of KP, its photosensitizing potential was evaluated through the ability to mediate photoperoxidation of LA. At a drug concentration of 10^{-5} *M*, a significant amount of lipid-derived dienic hydroperoxides was produced (Fig. 3), as evidenced by the new absorption band appearing at *ca* 233 nm.[2,14] This effect was inhibited by the radical scavengers GSH and BHA.[11] The latter was very efficient, even at concentrations as low as 10^{-6} *M*.

In order to know whether formation of lipid-derived dienic hydroperoxides might be photosensitized by the photoproducts of KP, analogous experiments were performed using 10^{-5} *M* concentrations of compounds **2–10**. The photoperoxidation of LA was efficiently promoted by the photoproducts containing the benzophenone chromophore (**2**, **3**, **4**, **5**, **6** and **8**) but not by the reduced ethyl derivative **9** or the hydrodimers **7** and **10** (Fig. 4).

DISCUSSION

Exposure of KP solutions in neutral aqueous media to sunlight resulted in rapid consumption of the drug, which was transformed into nine different photoproducts (compounds **2–10**, Fig. 1). Their formation is explained in Fig. 5 as the result of initial decarboxylation of the dissociated acid $\mathbf{1}^-$, which is the species actually existing at pH 7.2 (the pK_a of KP is *ca* 4.7). This process would be associated with the ejection of one electron, which would be efficiently scavenged by oxygen to give superoxide radical anion. Under these conditions, trapping of the benzylic radical $\mathbf{2}^{\cdot}$ would afford the oxygenated products **3**, **4** and **5**. Alternatively, dimerization of $\mathbf{2}^{\cdot}$ would give rise to **6**. This photoreactivity would be in agreement with the available literature data.[11,12] In the absence of oxygen, the benzylic radical/electron pair would collapse to the corresponding carbanion $\mathbf{2}^-$, whose protonation justifies the formation of **2**. This process is expected to be faster than hydrogen abstraction by $\mathbf{2}^{\cdot}$.[16] Similar photodecarboxylations occurring *via* benzylic carbanions have been previously reported.[17] The excited benzophenone chromophore of **2** might abstract hydrogen from suitable donors (including the ethyl side chain of a second molecule of **2**) to give radical $\mathbf{2H}^{\cdot}$. The formation of compounds **7**, **8** and **9** from the latter intermediates is rather obvious and does not deserve more detailed comments.

Irradiation of the nondissociated acid **1** in methanol gave KP hydrodimer **10** as the major photoproduct, which is in good agreement with the better hydrogen-donating ability of this solvent as compared with water. Moreover, the small amount of **10** found in aqueous solution might derive from some KP remaining in suspension (**1** in acidic form). In methanolic solution, the corresponding ion $\mathbf{1}^-$ afforded the decarboxylated hydrodimer **7**. In this case, loss of carbon dioxide must have occurred prior to dimerization, since hydrodimers do not absorb in the UVB/UVA region. A control experiment confirmed that irradiation of **10** did not produce detectable transformation of this compound (data not shown).

These results clearly show that the actually photodecarboxylating species is the dissociated acid $\mathbf{1}^-$. To ascertain whether the ethyl derivative **2** is formed from the benzylic anion $\mathbf{2}^-$ or, as previously proposed,[11] from the benzylic radical $\mathbf{2}^{\cdot}$, the sodium salt of KP was irradiated in CH_3OD.[16,18] Extensive deuterium incorporation into the benzylic position of **2** and **7** was in agreement with protonation of $\mathbf{2}^-$ as the almost exclusive route in methanol.

It has been previously reported that KP is able to induce polymerization of acrylamide in methanolic solution.[11] This has been taken as evidence for radical formation and has led to the proposal that photodecarboxylation of KP in aqueous medium occurs *via* intermediate radicals $\mathbf{2}^{\cdot}$. According to our results, the formation of radicals is indeed a major process in methanol, but these species have ketyl radical structure and lead to dimerization instead of decarboxylation. On the other hand, the behavior of the acid **1** differs from that of its conjugate base $\mathbf{1}^-$ and the observations in methanol cannot be directly extrapolated to the aqueous medium, owing to the poor hydrogen-donating properties of water.

The above facts underline the importance of the benzo-

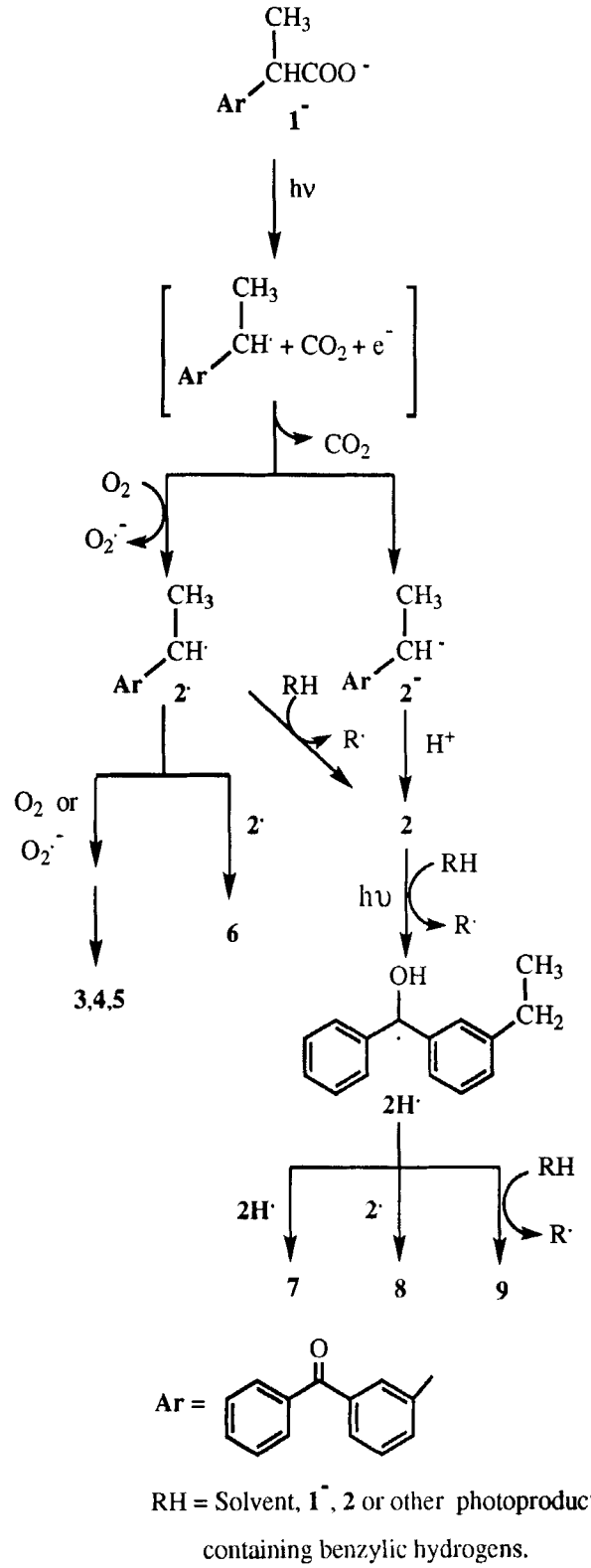

Figure 5. A comprehensive mechanistic scheme rationalizing the formation of all the different photoproducts obtained by irradiation of KP.

phenone chromophore in the photochemistry of KP. Its relevance to the photobiological properties of this drug was evidenced by means of the LA photoperoxidation test (Figs. 3 and 4). Irradiation of 10^{-3} *M* aqueous solutions of this model lipid in the presence of **1**$^-$ resulted in the formation of an appreciable amount of dienic hydroperoxides, as indicated by the appearance of a well-defined UV-absorption band at 233 nm.[14] This can be explained by abstraction of the double allylic hydrogen either by the radical **2**· or by excited benzophenone, which would be the initial step in a radical chain oxygenation of LA. The efficient inhibition of photoperoxidation by well-established radical scavengers, such as BHA or GSH (Fig. 4),[19] confirmed the involvement of a type I mechanism. Further support in favor of the key role of the benzophenone moiety[20,21] in this process was obtained by examining the ability of the KP photoproducts **2**–**10** to promote LA photoperoxidation (Fig. 4). The most relevant observation was the high photosensitizing potential of the major photoproduct **2**, in spite of the lack of the propionic acid side chain. A similar effect was detected in the benzophenone-containing photoproducts **3**, **4**, **5**, **6** and **8**. By contrast, the reduced ethyl derivative **9** and hydrodimers **7** and **10** were unable to produce significant amounts of dienic hydroperoxides, which was not surprising in view of the weak absorption properties of these compounds.

CONCLUSIONS

The photodecarboxylation of KP in neutral aqueous medium proceeds *via* benzylic radical and benzylic carbanion intermediates (**2**· and **2**$^-$). Protonation of **2**$^-$, as well as hydrogen abstraction by **2**·, justify the formation of an ethyl derivative (**2**) as a major photoproduct. The benzophenone chromophore of KP is able to mediate the photoperoxidation of LA through a type I mechanism. Because lipid peroxidation is indicative of cell membrane lysis, the above findings are highly relevant in explaining the photobiological properties of KP.

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REFERENCES

1. Kochevar, I. E. (1989) Phototoxicity of nonsteroidal anti-inflammatory drugs: coincidence or specific mechanism? *Arch. Dermatol.* **125**, 824–826.
2. Miranda, M. A. (1992) Phototoxicity of drugs. In *Alternative In Vitro Models to Animal Experimentation in Pharmaco-Toxicology* (Edited by J. V. Castell and M. J. Gomez-L.), chapter 10, pp. 239–270. Farmaindustria, Barcelona.
3. Diffey, B. and S. Brown (1983) A method for predicting the phototoxicity of non-steroidal anti-inflammatory drugs. *Br. J. Clin. Pharmacol.* **16**, 633–638.
4. Kochevar, I. E., K. W. Hoover and M. Gawienowski (1984) Benoxaprofen photosensitization of cell membrane disruption. *J. Invest. Dermatol.* **82**, 214–218.
5. Alomar, A. (1985) Ketoprofen photodermatitis. *Contact Dermatitis* **12**, 112–113.
6. Cusano, F., B. R. Rafenella and G. Errico (1987) Photo-contact dermatitis from ketoprofen. *Contact Dermatitis* **21**, 51.
7. Tosti, A., G. Gaddoni, F. Valeri and F. Bardazzi (1990) Contact allergy to ketoprofen. *Contact Dermatitis* **23**, 112–113.

8. Mozzanica, N. and P. D. Pigatto (1990) Contact and photocontact allergy to ketoprofen: clinical and experimental study. *Contact Dermatitis* **23**, 336–340.
9. Ljunggren, B. (1985) Propionic acid-derived non-steroidal antiinflammatory drugs are phototoxic *in vitro*. *Photodermatology* **2**, 3–9.
10. Przybilla, B., U. Schwab-Przybilla, T. Ruzicka and J. Ring (1987) Phototoxicity of non-steroidal anti-inflammatory drugs demonstrated *in vitro* by a photo-basophil–histamine-release test. *Photodermatology* **4**, 73–78.
11. Costanzo, L. L., G. De Guidi, G. Condorelli, A. Cambria and M. Fama (1989) Molecular mechanism of drug photosensitization—II. Photohemolysis sensitized by ketoprofen. *Photochem. Photobiol.* **50**, 359–365.
12. Pietta, P., E. Manera and P. Ceva (1987) High-performance liquid chromatographic determination of ketoprofen degradation products. *J. Chromatogr.* **390**, 454–457.
13. Berliner, E. and M. M. Chen (1958) Electronic effects of alkyl groups. XI. Rates of solvolysis of *m*-alkylbenzhydryl chlorides. *J. Am. Chem. Soc.* **80**, 343–347.
14. Recknagel, R. O. and E. A. Glende, Jr. (1984) Spectrophotometric detection of lipid conjugated dienes. In *Oxygen Radicals in Biological Systems. Methods in Enzymology*, Vol. 105 (Edited by L. Packer), pp. 331–337. Academic Press, New York.
15. Castell, J. V., M. J. Gomez-L., M. A. Miranda and L. A. Martinez (1994) A general procedure for isotopic (deuterium) labelling of non-steroidal antiinflammatory 2-arylpropionic acids. *J. Labelled Compd. & Radiopharm.* **34**, 93–100.
16. Epling G. A. and A. Lopes (1977) Fragmentation pathways in the photolysis of phenylacetic acid. *J. Am. Chem. Soc.* **99**, 2700–2704.
17. Budac, D. and P. Wan (1992) Photodecarboxylation: mechanism and synthetic utility. *J. Photochem. Photobiol. A Chem.* **67**, 135–166.
18. Vargas, F., C. Rivas, M. A. Miranda and F. Boscá (1991) Photochemistry of the non-steroidal anti-inflammatory drugs, propionic acid derived. *Pharmazie* **46**, 767–771.
19. Girotti, A. W. (1990) Photodynamic lipid peroxidation in biological systems. *Photochem. Photobiol.* **51**, 497–509.
20. Markovic, D. Z., T. Durant and L. K. Patterson (1990) Hydrogen abstraction from lipids by triplet states of derivatized benzophenone photosensitizers. *Photochem. Photobiol.* **51**, 389–394.
21. Markovic, D. Z. and L. K. Patterson (1993) Benzophenone-sensitized lipid peroxidation in linoleate micelles. *Photochem. Photobiol.* **58**, 329–334.