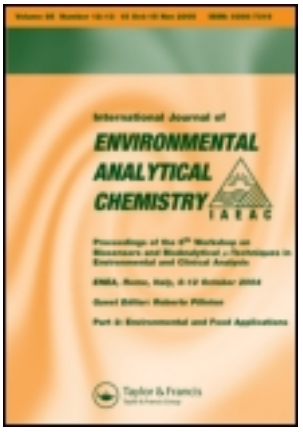

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Photochemical behaviour of propranolol in environmental waters: the hydroxylated photoproducts

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Photochemical behaviour of propranolol in environmental waters: the hydroxylated photoproducts

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The UV irradiation of propranolol, one of the beta-blockers currently used in cardiac medicine, was studied. During irradiation, the samples were analysed by LC/MS/MS, using a Waters Symmetry C_{18} (3.5 μm 150 × 2.1 mm) column. The main photoproducts were identified from mono-, di- and tri-hydroxylation of the propranolol naphthalenic skeleton. Hydroxyl group positions were predicted by means of theoretical calculations. Partial charge calculations of the propranolol atoms predicted the formation of four mono-hydroxylated compounds, two of them being the most probable. From these two compounds, three di-hydroxylated compounds were predicted. Then the calculations from the two most probable di-hydroxylated compounds were used to propose three tri-hydroxylated propranolol compounds. It was found that the same hydroxylated photoproducts are formed in pure water and in treatment plant waste water.

Keywords: propranolol; water treatment plant; liquid chromatography; mass spectrometry; photodegradation; hydroxylated photoproducts

1. Introduction

Beta-blockers are high prescription drugs used in human medicine to treat cardiac illness or disorders such as hypertension and arrhythmia [1–3]. Like other pharmaceuticals, β-blockers are partially metabolised by the human body and are then released in urine and faeces as a mixture of unchanged molecule and its metabolites. This mixture can then reach waste treatment plants (WTP) and surface waters. Currently, unchanged molecules have been detected in the aquatic compartment by several studies [4–9].

Moreover, β-blockers have been shown to be ecotoxic substances because they can affect cardiac rhythm in fish or reduce its spermatozoid mobility or viability [10–12]. It is therefore vital to assess their fate in environmental waters as they can undergo metabolisation by microorganisms or abiotic degradation by hydrolysis or photolysis.

In a previous paper, we showed that β-blockers were photolabile compounds [13]. In this preliminary study, photodegradation of several β-blockers was compared at two concentration levels (10 μg L^{-1} and 10 mg L^{-1}) and in two different matrices (pure and waste waters). We found that the concentration level does not influence either β-blocker degradation kinetics or photoproduct formation. Moreover, among the molecules studied

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in this previous paper, it was observed that an environmental matrix sped up their degradation without modifying photoproducts. This suggested that their characterisation from experiments in pure water should correlate with what occurs in the environment. However, no structures were proposed for photoproducts.

Among β-blockers, propranolol is a highly prescribed drug (12 tons year^{-1} in France [14], and 3 tons year^{-1} in Germany [15]). It is one of the β-blockers showing a low stability. Its half-life has been measured in different conditions:

2 h in waste water and 8 h in pure water at 10 μg L^{-1}, under UV light from a mercury lamp [13];
12 h at 0.3 μg L^{-1} and 21 h at 1 mg L^{-1}, under UV light from a xenon lamp [16];
0.6 h at 0.3 μg L^{-1}at a latitude of 52°N in June to 6.3 h at 0.1 mg L^{-1} at a latitude of 40°N in December under solar radiation [16];
6.0 h in August and 8.3 in May at 100 μg L^{-1} in a Japanese river [17].

From quantum yield measurements, its half-life was predicted to be from 2 h in summer (latitude, 20°N or 50°N) to 16 h in winter at a latitude of 50°N [18]. It is therefore vital to focus on propranolol degradation products.

Propranolol photodegradation leads to a very complex mixture, as shown on Figure 1. Some studies have already been published on propranolol photodegradation; however, most of them are not relevant from an environmental point of view as they were carried out on solid pharmaceutical tablets [19] or in buffered medium [20].

Nevertheless, in an environmental context, Liu *et al*. [16] proposed structures with loss of aromaticity for three propranolol photoproducts which appear when a propranolol solution is placed in a small borosilicate glass reaction vessel (4.6 cm i.d. × 3.2 cm depth) and submitted to a 1.1-kW xenon arc lamp. However, it is also important to characterise other compounds which can be observed with lower irradiation conditions (Figure 1). Indeed, according to Matsuura *et al*. [21] UV irradiation of aromatic compounds can lead to the addition of hydroxyl radicals, without loss of aromaticity. Moreover, during

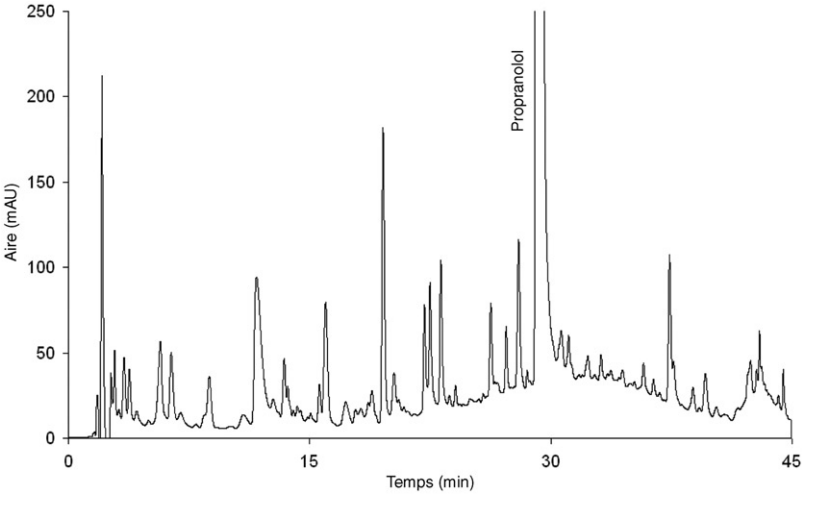

Figure 1. DAD chromatogram of a 10-mg L^{-1} propranolol solution in pure water when irradiated for 21 h. The solution was 100-fold concentrated by lyophilisation.

previous testings [13], we observed β-blocker photoproduct masses which could tally with the addition of 1, 2 or 3 hydroxyl radicals.

The aim of this study was therefore to propose propranolol hydroxylated photoproduct structures, never before reported in the literature. Because of the very close physicochemical properties of such compounds, isolating each photoproduct quantitatively for NMR analysis would prove to be very difficult. The characterisation proposed in this study was therefore based on mass spectrometry. Theoretical calculations of the partial charges carried out by each individual propranolol atom were used to predict the most probable isomers.

2. Experimental

2.1 *Chemicals*

Propranolol hydrochloride (99%) (Pr) was provided by Sigma-Aldrich (Saint Quentin Fallavier, France). HPLC-grade methanol (MeOH) was provided by SDS-Carlo-Erba (Peypin, France). LC/MS-grade acetonitrile (AcN), ammonium formate (99%) and formic acid (FOA) were obtained from Fisher Scientific (Val de Rueil, France). Ultra-pure water was generated by an Elgastat UHQ PS from Elga LabWater (High Wycombe, Bucks, UK). Propranolol stock solution was prepared in MeOH at 1000 mg L^{-1}. Then a 50 mg L^{-1} standard working (WS) propranolol solution was prepared in MeOH. These solutions were stored at 4°C, in the dark. Low-concentration (10 µg L^{-1}) experiments were conducted by spiking 1 L of pure or WTP water with 200 µL of WS to estimate the matrix's influence on propranolol degradation kinetics. The propranolol photoproducts were characterised separately from 10 mg-L^{-1} solutions prepared by dissolving 11.4 mg of propranolol hydrochloride in 1 L of water.

2.2 *Sampling*

Environmental matrix samples were collected from Pierre Benite WTP (downstream from Lyon, France). These effluent samples were filtered up to 0.45 µm on membrane filters provided by Millipore (Molsheim, France) and stored at 4°C. They were used as the photodegradation matrix within 1 week.

2.3 *Photodegradation under UV light*

Irradiation was carried out in a 3-L Pyrex glass immersion photochemical reactor, cutting out wavelengths shorter than 280 nm. The photoreactor was charged with 1 L of aqueous solution prepared as quoted above. A high-pressure vapour mercury lamp (HPK 125 W, Cathodeon, Cambridge, United Kingdom), emitting in the 250 to 600 nm range with a maximum emission at 365.5 nm, was placed in a water-jacked Pyrex tube (diameter, 3 cm) centred in the reactor. The progress of the reaction was followed by successive samplings (1 mL). The solution was verified in the dark by covering flasks with aluminium foil in order to confirm that propranolol did not degrade by hydrolysis.

For the preliminary general survey, 100 mL of a 21-h degradation mixture was lyophilised to dryness and dissolved in 1 mL of water/AcN (75/25, v/v).

2.4 *Liquid chromatography-tandem mass spectrometry*

2.4.1 *Apparatus*

The HPLC device consisted of the Agilent 1100 series pump, autosampler and diode-array detector (Massy, France). The HPLC device was coupled to a Sciex API 300 triple quadrupole mass spectrometer from MDS Sciex (Toronto, ON, Canada) equipped with a TurboIonspray Source (TIS) operating in positive ion mode. Instrument verification, data analysis and processing were performed using the associated Analyst 1.4.1 software.

The nebuliser (zero air) and the curtain gas flow (nitrogen) were set at 10 arbitrary units. The TIS source operated at 500°C, with the auxiliary gas flow (zero air) set at $8\,L\,min^{-1}$. The TIS voltage was set at 5000 V, and the orifice and ring voltage were set at 16 V and 140 V.

Chromatographic analysis was performed on a Symmetry C_{18} (3.5 µm 150 × 2.1 mm id), operating at $0.2\,mL\,min^{-1}$. A gradient elution was performed in two solvents: solvent A was formate buffer (10 mM ammonium formate acidified to pH 3.8 by FOA addition) and solvent B was AcN.

2.4.2 *Preliminary general survey*

A linear gradient from 95% to 0% solvent A over 40 min was used. The chromatographic column was then washed by 100% solvent B over 5 min, then returned to the initial conditions over 7 min prior to the next injection. The detection was successively performed using an Agilent diode array detector operating at 220 nm, followed by a full scan mass spectrometric analysis, scanning a mass range from 100 to 800 amu with a high (conditions for interface fragmentation) and a low declustering potential.

2.4.3 *Detection of hydroxylated photoproducts*

To achieve a better separation of hydroxylated photoproducts, the chromatographic gradient was adjusted. The initial composition of the mobile phase was 95% solvent A. An isocratic elution was used for 5 min followed by a linear gradient over 2 min to 90% solvent A. A linear gradient was then used to 30% solvent A over 23 min. The chromatographic column was then washed by 100% solvent B over 5 min then returned to the initial conditions over 7 min prior to the next injection. The mass spectrometer operating in single-ion monitoring mode (SIM) was used for detection. The molecular ion $[M+H]^+$ of propranolol and masses corresponding to multiple additions of 16 (hydroxyl formation) were followed, to analyse $[M+H]^+$ at 260 amu, $[M+H+16]^+$ (mono-hydroxylated photoproducts) at 276 amu, $[M+H+32]^+$ (di-hydroxylated photoproducts) at 292 amu, and $[M+H+48]^+$ (tri-hydroxylated photoproducts) at 308 amu.

2.5 *Computer simulations*

The experiment was completed by computational studies using density functional theory (DFT) within the Kohn-Sham approach. Kohn-Sham DFT is becoming an important first principles computational method to predict chemical properties accurately and optimise molecule geometry of reactants or products. For this purpose, the Amsterdam Density Functional (ADF 2006) software [22–23] was used. These calculations were made using a triple zeta basis set augmented by a polarisation function (TZP) and a small frozen core

approximation was used for 1s orbital shells. Geometry optimisations were carried out with no symmetry restriction. The exchange-correlation functional adopted was the PBE [24], which is considered one of the most accurate functionals belonging to the generalised-gradient-approximation (GGA) family [25].

3. Results and discussion

When attempting to conduct an environmentally relevant experiment at the laboratory scale, a problem arises. Indeed, high concentrations are necessary for photoproduct characterisation, whereas propranolol was detected in the $\mu g\,L^{-1}$ range in WTP [26]. Moreover, environmental matrices are likely to interfere with both degradation kinetics and photoproduct formation, because other substances such as nitrogen species or humic acids can modify propranolol's photochemical behaviour. Particular attention was therefore given to estimating the influence of these two factors on propranolol degradation kinetics so that the experiments would be environmentally relevant.

3.1 *Photodegradation kinetics*

Photochemical degradation kinetics were therefore studied at two concentration levels ($10\,\mu g\,L^{-1}$ and $10\,mg\,L^{-1}$) and in two different matrices (pure water and WTP water). Whatever the concentration or the matrix used in this study, propranolol showed pseudo-first-order degradation kinetics as the evolution of $\ln(c/c_0)$ varied linearly with degradation time, as shown on Figure 2 (where c represents the propranolol concentration after t hours of irradiation, and c_0 its initial concentration). The resulting half-lives were calculated from the equation $t_{1/2} = \ln 2/k$. As could have been theoretically predicted from the kinetic order of the reaction, the concentration level did not influence propranolol's half-life. However, the matrix showed a strong influence on propranolol's photodegradation rate. Whatever concentration was used, propranolol's half-life was 8 h in pure water and 2.5 h in waste water. These results show that the concentration level did not influence propranolol's disappearance kinetics.

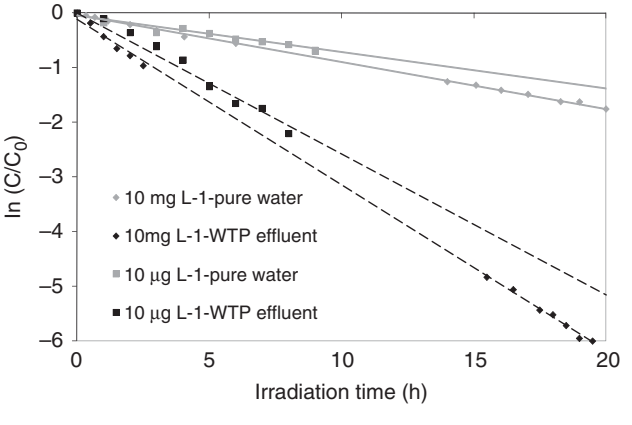

Figure 2. Influence of the concentration ($10\,mg\,L^{-1}$ or $10\,\mu g\,L^{-1}$) and the matrix (pure water or waste water) on propranolol degradation kinetics.

As has been reported in the literature for other β-blockers [13], this study shows that the photoproducts can be accurately identified from 10 mg L^{-1} pure water degradation solution experiments.

3.2 *Preliminary testing for propranolol photoproduct characterisation*

To obtain preliminary information on photoproduct masses, a 10-mg L^{-1} propranolol solution was irradiated for 21 h in order to achieve a high conversion of propranolol. The degradation mixture was concentrated 100-fold by lyophilisation in order to reach better analytical sensitivities and analysed in the LC/DAD/MS/MS device operating in 'full scan' mode. The resulting DAD chromatogram (Figure 1) reveals the presence of a large number of photoproducts, as has been previously observed in the literature [16]. The MS analyses of the peaks show that most of the photoproducts are heavier than propranolol. These results are quite surprising because usually, photochemical processes lead to intramolecular bond linkage and the formation of smaller molecules. These photoproducts should be generated by radical additions. As has previously been observed for other β-blockers, several photoproducts had masses which matched 16-, 32- or 48-amu mass shifts on 260 amu propranolol molecular ions. This could tally with the addition of one (276 amu), two (292 amu) or three (308 amu) hydroxyl radicals. As these phenomena had never been observed previously on propranolol photodegradation, we particularly focused on the identification and separation of these so-called hydroxylated photoproducts. With this objective in mind, LC/MS experiments were conducted on each aliquot from the degradation mixture. The resulting chromatograms are shown on Figure 3 for two degradation times and two matrices. Compared to the LC/DAD chromatogram, the resulting LC/MS chromatograms in single-ion monitoring mode (SIM) were highly simplified, leading to the isolation of a particular kind of photoproduct of interest.

3.3 *Photoproduct formation in pure or environmental waters*

To ensure that the hydroxylated photoproducts identified from degradation in pure water were also formed when propranolol was degraded in environmental water, their appearance and disappearance were compared from experiments in both matrices. For example, chromatogram acquired in pure or WTP water after 2 or 18 h of propranolol degradation are presented on Figure 3. The chromatograms in Figure 3a were obtained by selecting the 276-amu ion mass on the MS detector, while the chromatograms presented in Figures 3b and 3c were obtained by the selection of masses of 292 or 308 amu, respectively, corresponding to mono-hydroxylated, di-hydroxylated and tri-hydroxylated compounds. No tetra-hydroxylated photoproduct was observed. From these chromatograms, it can be concluded that the same hydroxylated photoproducts are formed in both matrices given that the peaks presenting the same retention time are observed during LC/SIM-MS experiments. At the same degradation time, one can observe that chromatographic peak intensities are very different in each matrix. This is explained by an increase of photoproduct appearance/disappearance kinetics in WTP water, as discussed previously [13].

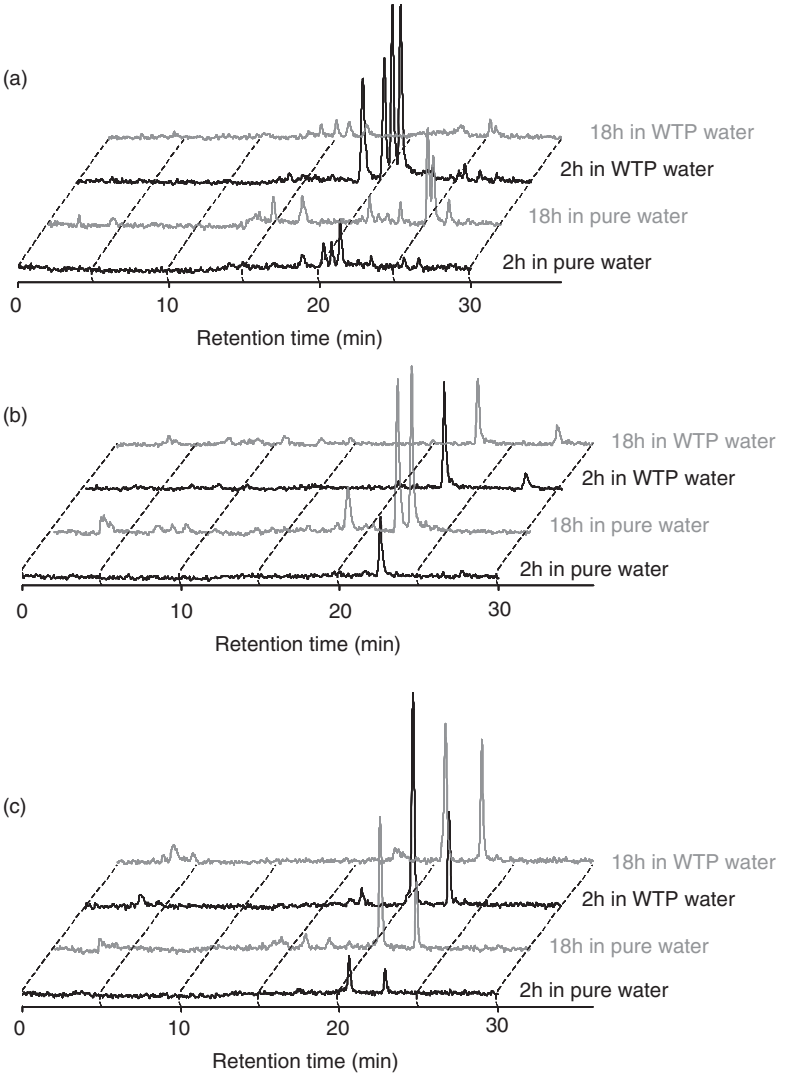

Figure 3. Chromatograms of propranolol degradation mixtures in pure water or WTP effluent after 2-h or 18-h UV irradiation: (a) single-ion monitoring at 276 amu (mono-hydroxylated photoproduct), (b) single-ion monitoring at 292 amu (di-hydroxylated photoproduct), (c) single-ion monitoring at 308 amu (tri-hydroxylated photoproduct).

3.4 *Analytical study of hydroxylated photoproducts*

Photochemical hydroxylations have been widely studied in the past. Matsuura *et al.* [21], for example, published a review on the photochemical hydroxylation of aromatic compounds. This article shows that, in the presence of oxygen, direct hydroxylation can involve replacement of one hydrogen atom by one hydroxyl group both from the photochemical generation of hydroxyl radical in aqueous solution and from atomic oxygen at the excited ^{3}P state. Both hydroxyl radicals and O (^{3}P) have been shown to be electrophilic species.

To determine the position of the addition (aromatic or aliphatic moieties), propranolol mass fragmentation spectra (Figure 4) were first compared to the photoproduct masses observed during full-scan analysis with a low and high declustering potential and/or product ion scan analysis.

It is particularly interesting to observe that propranolol mass fragmentation leads to the formation of an intense product ion at $m/z = 116$ amu, which is characteristic of the side chain. It was then considered that if this product ion existed on a target photoproduct mass fragmentation, the chemical modification would have taken place on the aromatic moiety. Otherwise, if a photoproduct does not present a 116-amu product ion, it would come from a modification of the side chain.

During high declustering potential full-scan and product ion scan analysis, the presence of a 116-amu product ion was systematically observed at hydroxylated photoproduct retention times. So, it can be concluded that hydroxylation takes place only in the aromatic moiety. Hydroxylation of the aliphatic moiety was never observed.

Moreover, as shown on Figure 5, the more the photoproduct is hydroxylated, the more its appearance kinetics is slow. It therefore seems that propranolol follows successive hydroxylations leading to tri-hydroxylated photoproducts. The $(n+1)$-hydroxylated photoproduct should thus result from the photochemical degradation of the n-hydroxylated compound.

To properly characterise (hydroxylation position) these compounds, it would be necessary to isolate each hydroxylated compound from the degradation mixture and to perform its NMR analysis. However, because of the complexity of the degradation mixture (Figure 1) and of the very close polarity of several photoproducts, it seemed very difficult to separate one compound from the mixture. Moreover, LC-NMR was not possible due to the small amount of each compound. Therefore, in order to determine the most probable positions attacked by a hydroxyl radical, theoretical calculations may be performed (e.g. work by Carrier *et al*. [27]).

3.5 *Proposal for the most probable propranolol hydroxylated photoproducts*

The partial charge of all individual atoms was calculated from both Mulliken's and Hirschfeld's formulations. The resulting data are shown in Table 1 for the Mulliken calculations, which were always confirmed by the Hirshfeld partial charge approach [28]. In addition, both hydroxyl radicals and atomic oxygen O (^{3}P) were identified as electrophilic species [29]. Thus, whatever the hydroxylation mechanisms, hydrogen atom replacement by a hydroxyl group should preferentially attack atoms or groups of atoms with a negative partial charge. The larger the partial charge on a position, the more this position is likely to be hydroxylated. For this prediction, it has been considered that n-hydroxylated compounds resulted from n successive reactions, i.e. that di-hydroxylated molecules were photoproducts of mono-hydroxylated molecules and that tri-hydroxylated compounds resulted from the subsequent hydroxylation of a di-hydroxylated molecule. It is then necessary to determine the most probable mono-hydroxylated propranolol photoproducts.

3.5.1 *Mono-hydroxylated photoproducts*

Partial charges of all propranolol individual atoms are shown in Table 1a. It is noteworthy that the largest negative charges are carried out by heteroatoms, which would mean that

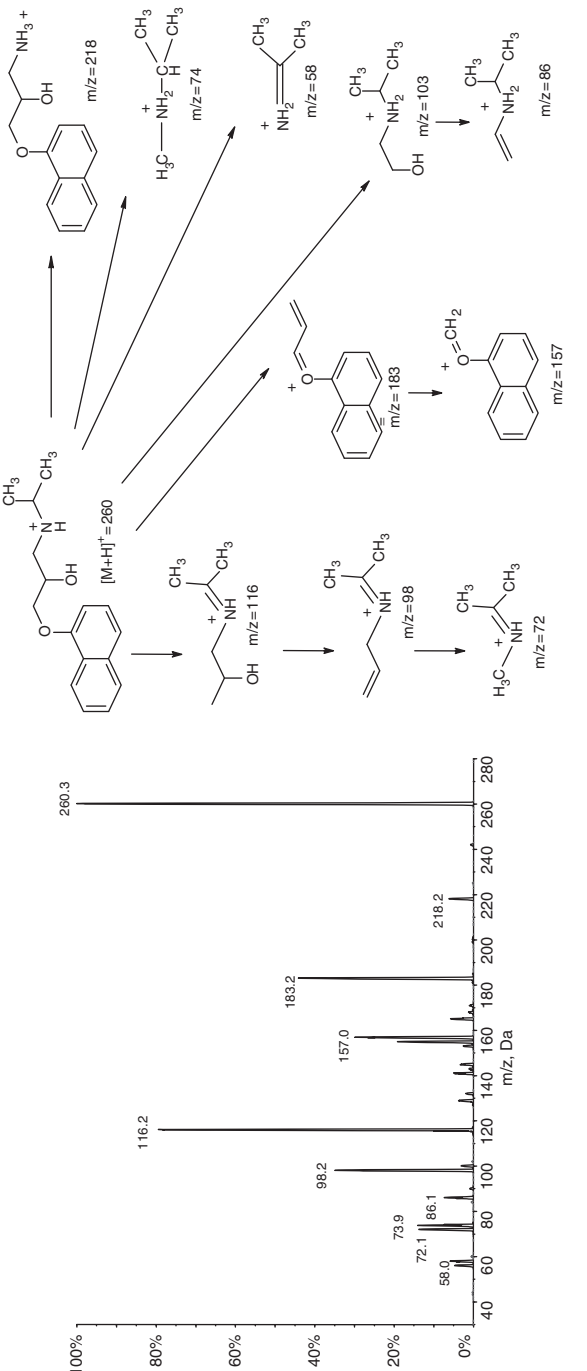

Figure 4. Product ion scan of propranolol and fragmentation pathway in positive mode.

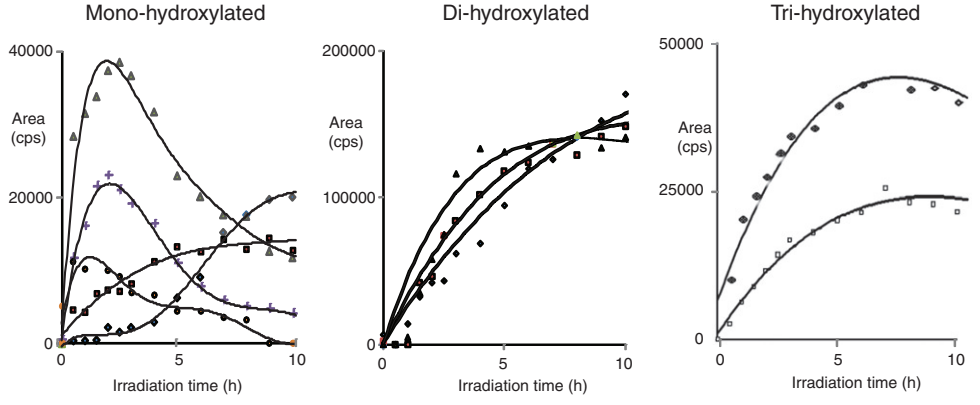

Figure 5. Appearance/disappearance kinetics of hydroxylated photoproducts resulting from the degradation under high-pressure vapour mercury lamp exposure of a 10-μg L^{-1} propranolol solution in waste water.

heteroatoms are the most likely to be hydroxylated. However, both O–O and N–O bonds are very weak and the resulting compounds should be easily hydrolysed or photolysed. Consequently, it was considered that these hypothetic degradation products should be degraded faster than their formation and could not be detected during off-line analysis. Moreover, no hydroxylated compounds were observed in the aliphatic moiety by mass spectrometry. It seems more realistic to focus on nucleophilic carbons.

Carbon atom number 11 (Figure 6 and Table 1a) carries out the largest negative charge, leading to the *ortho*-hydroxylation of the naphthalene moiety (the *ortho* position is compared to the side chain attachment). As several position isomers were observed, interpretation of theoretical data must be continued. With this model, carbon atoms number 1 and 13 carry out the same partial charge, because the difference between the two is not significant. However, these theoretical calculations consider a single molecule in a fixed state and do not take into account free rotation around single bonds. It should be noted that because of this free rotation the two carbon atoms, numbers 1 and 2, are equivalent and the partial charge calculated for both methyl groups should be averaged. With this consideration, atom carbon number 13 is most likely to undergo hydroxylation than the methyl number 1. These results are in accordance with the literature: it has already been observed that *ortho* and *para* additions were major products of the photochemical hydroxylation of phenol [30]. By pursuing the interpretation in the same way, the lost negative carbon atoms remaining are carbon numbers 12 and 17, leading to the formation of two other mono-hydroxylated compounds. In conclusion, four compounds, *o*-HO-Pr, *p*-HO-Pr, *m*-HO-Pr and *m'*-HO-Pr (Figure 6), can be proposed.

As *ortho* and *para* additions should lead to the major photoproducts, similar calculations have been performed on these two compounds in order to identify di-hydroxylated photoproducts.

3.5.2 *Di-hydroxylated photoproducts*

The partial charge for all individual atoms of *ortho* and *para* hydroxylated photoproducts are shown on Table 1b. As was observed during the interpretation of mono-hydroxylated

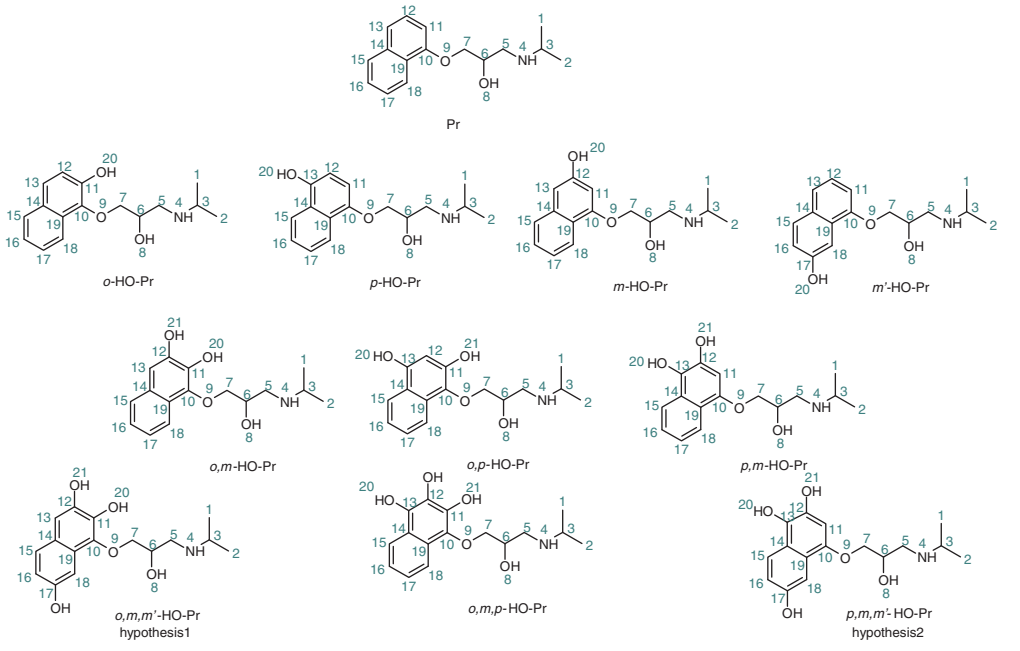

Figure 6. Main most probable mono-hydroxylated, di-hydroxylated photoproducts and tri-hydroxylated.

photoproducts, heteroatoms present the largest negative charge, because they are more electronegative. However, they were not considered a possible location for hydroxylation, as discussed above.

For both mono-hydroxylated photoproducts, carbon atom number 12 is the most nucleophilic atom. Thus in both cases, hydroxylation should occur on the meta position, leading to the structures *o*,*m*-HO-Pr and *p*,*m*-HO-Pr (Figure 6). By pursuing the interpretation, the carbon atom number 11 is the most likely to be hydroxylated on *p*-HO-Pr and the carbon atom number 13 on *o*-HO-Pr, leading to an identical photoproduct called *o*,*p*-HO-Pr (Figure 6).

3.5.3 *Tri-hydroxylated photoproducts*

With a similar discussion, photoproducts *o*,*m*,*p*-HO-Pr (Figure 6) can be identified. This photoproduct should be the main degradation product of the two main predicted di-hydroxylated photoproducts. However, the chromatograms in Figure 3c show that two trihydroxylated compounds are formed. By pursuing the interpretation, two structures can be proposed for the other trihydroxylated photoproduct, as shown on Figure 6. With these theoretical calculations, it is not possible to conclude which one appears because the carbon atom number 17 presents a close partial charge in both di-hydroxylated compounds. Another possibility would be that both hypotheses (*o*,*m*,*m*′-HO–Pr and

Table 1. Partial charges of atoms calculated from both Mulliken and Hirshfeld models, using ADF software: (a) prediction of mono-hydroxylated photoproducts; (b) prediction of di-hydroxylated photoproducts; (c) prediction of tri-hydroxylated photoproducts.

(a)	Propranolol	
Function labelling	Function	Mulliken partial charge
1	CH_3	−0.0511
2	CH_3	−0.0022
3	CH	0.1462
4	NH	−0.2573
5	CH_2	0.1878
6	CH	0.2742
7	CH_2	0.2939
8	OH	−0.3106
9	O	−0.531
10	C	0.4204
11	CH	−0.0971
12	CH	−0.0455
13	CH	−0.0504
14	C	0.1048
15	CH	−0.0237
16	CH	−0.027
17	CH	−0.0343
18	CH	−0.0048
19	C	0.0075

(b)	*o*-hydroxylated propranolol		*p*-hydroxylated propranolol	
Function labelling	Function	Mulliken partial charge	Function	Mulliken partial charge
1	CH_3	−0.0533	CH_3	−0.0522
2	CH_3	−0.0033	CH_3	−0.0022
3	CH	0.145	CH	0.1467
4	NH	−0.2511	NH	−0.257
5	CH_2	0.1825	CH_2	0.1875
6	CH	0.2843	CH	0.2755
7	CH_2	0.3021	CH_2	−0.0463
8	OH	−0.3145	OH	−0.312
9	O	−0.5374	O	−0.5366
10	C	0.3478	C	0.4073
11	C	0.3512	CH	−0.1099
12	CH	−0.1064	CH	−0.1346
13	CH	−0.0539	C	0.4213
14	C	0.0972	C	0.0207
15	CH	−0.0271	CH	−0.0081
16	CH	−0.0327	CH	−0.0297
17	CH	−0.0331	CH	−0.0304
18	CH	0.0021	CH	−0.0021
19	C	0.0068	C	0.0209
20	OH	−0.3061	OH	−0.2938

(*continued*)

Table 1. Continued.

(c)	*o,m*-hydroxylated propranolol		*p,m*-hydroxylated propranolol	
Function labelling	Function	Mulliken partial charge	Function	Mulliken partial charge
1	CH_3	−0.003	CH_3	−0.0026
2	CH_3	−0.0536	CH_3	−0.0528
3	CH	0.1445	CH	0.1469
4	NH	−0.2515	NH	−0.2576
5	CH_2	0.1825	CH_2	0.1936
6	CH	0.2834	CH	0.271
7	CH_2	0.3045	CH_2	0.2951
8	OH	−0.3139	OH	−0.3094
9	O	−0.5355	O	−0.5319
10	C	0.3436	C	0.4148
11	C	0.2941	CH	−0.1538
12	C	0.3711	C	0.3999
13	CH	−0.1591	C	0.2671
14	C	0.0893	C	0.0194
15	CH	−0.0366	CH	−0.0273
16	CH	−0.0309	CH	−0.0237
17	CH	−0.0417	CH	–0.04
18	CH	0.0054	CH	0.0021
19	C	−0.0063	C	0.0038
20	OH	−0.3104	OH	−0.3468
21	OH	−0.3345	OH	−0.2809

p,m,m′-HO–Pr) should be right and the compounds should be coeluting within the LC experiment used.

4. Conclusion

This study focused on hydroxylated propranolol compounds which have never been reported before. Previous experiments have been conducted in particular conditions. Indeed, laser flash photolysis leads to 6-hydroxy-1,4-naphtoquinone as the sole stable photoproduct [20], while irradiation of pharmaceutical tablets leads to 1-naphtol, N-acetylpropranolol and N-formylpropranolol [19].

Liu *et al*. [16] worked in environmental conditions, but they used stronger irradiation conditions that those reported herein. Thus, hydroxylated propranolol compounds detected in our study are probably precursors of the degraded compounds observed by Liu *et al*. involving naphthalenic ring oxidation aperture. Different mechanisms can lead to the hydroxylated compounds evidenced in this study. In waste water, inorganic ions such as nitrite or nitrate favour production of free hydroxyl radicals, which are likely to oxidise organic pollutants [31]. In pure water, where the formation kinetics are slower than in waste water, hydroxylate products may stem from a nucleophilic attack by water to propranolol molecules in the excited state.

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