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Mass spectrometric study of the photooxidation of the ophthalmic drugs timolol and pindolol

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A mass spectrometric study of the photooxidation products of the ophthalmic drugs pindolol (1-[1*H*-indol-4-yloxy]-3-[isopropylamino]-2-propanol) and timolol (*S*-[–]-1-[*t*-butylamino]-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-2-propanol) in water has been performed by LC-MS. Based on these data and the assumption that photooxidation mainly occurs through singlet molecular oxygen attack, a possible reaction mechanism is proposed. The mechanistic pathways involve singlet oxygen attack to the pindolol indole ring and oxidation of the pindolol isopropyl or timolol *ter*butyl methyl groups.

1. Introduction

Timolol and pindolol are β -adrenergic receptor antagonists which are commonly used as ophthalmic medicaments for the treatment of open-angle glaucoma and ocular hypertension [1–3]. These drugs upon light exposure may undergo photo-damage generating toxic products, or simply degrade (lowering their therapeutic action) or generate electronic excited states which would be involved in unpredictable reactive and physical processes. In this context, the elucidation of photodegradation products constitutes a matter of enormous interest, in particular when the substrates belong to the group of topically administrable drugs.

Photo-damages *in vivo* may be caused by photodynamic action [4, 5] of colored sensitizer molecules that are present endogenously. Dye-sensitized photooxidations frequently occur through a singlet molecular oxygen [$O_2(^1\Delta_g)$] mechanism [6, 7] and they may affect ophthalmic medicaments. In a previous paper we reported a kinetic study on the $O_2(^1\Delta_g)$ -mediated timolol and pindolol degradation upon visible-light irradiation of solutions containing dye-sensitizers. We also found that both ophthalmic drugs can act themselves as potential $O_2(^1\Delta_g)$ generators for ulterior photooxidative damages [8]. We now identified the photooxidation products of timolol and pindolol and proposed a possible reaction mechanism. The study was done employing mass spectrometry, a powerful analytical tool in organic chemistry [9].

2. Investigations and results

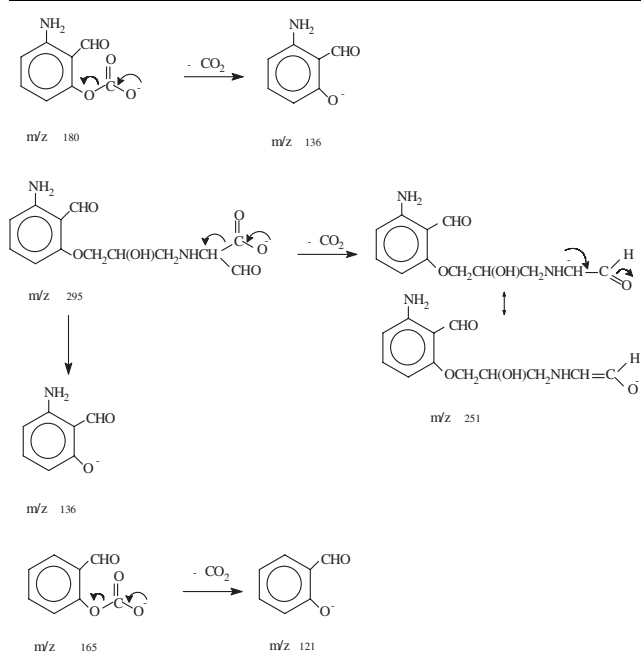
The Table shows relevant MS data obtained by electrospray ionisation (API) in the negative mode for pindolol and timolol and their photooxidation products, as well as the proposed structure of the products. A negligible ion production was found under the experimental conditions

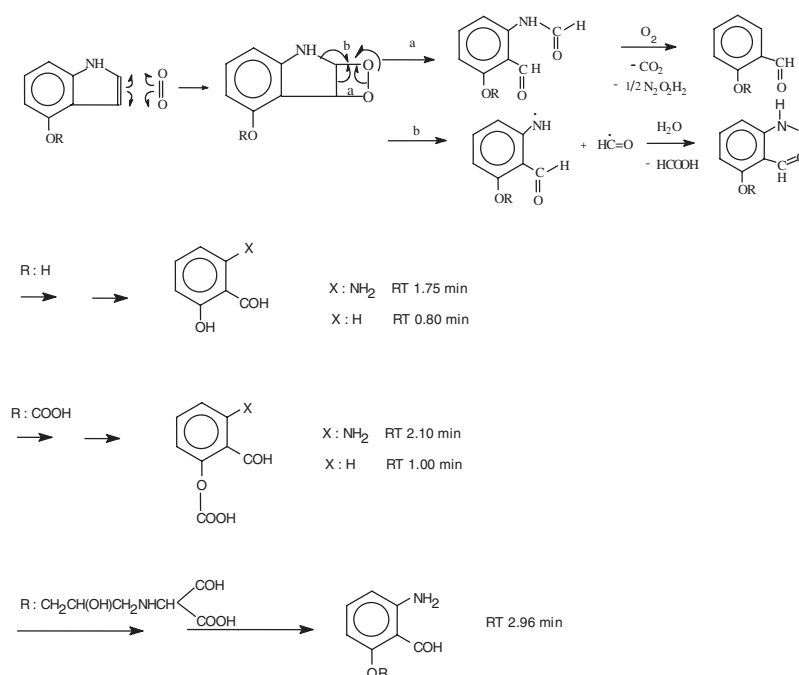
using the API source in the positive mode and the APCI source in any mode.

All the spectra are dominated by the pseudo molecular ions $(M-H)^-$. It should be noted that a) electrospray is a soft ionization technique with a decreased extension of the fragmentation, b) negative API allows the detection of compounds with acidic properties (carboxyl and hydroxyl containing compounds).

Scheme 1 shows the fragmentation pathways for some compounds of the Table (only observed for the mass spec-

Scheme 1 Fragmentation pathways for the mass spectra of the photooxidation products of pindolol



Scheme 2 Detected products and possible mechanism for their formation in the photooxidation of pindolol

tra of the photooxidation products of pindolol eluting at RT 1.00, 2.10 and 2.96 min), giving support to the proposed structures.

The available HPLC column was not the most appropriate with regard to the separation of photooxidation products for the early eluting compounds. Notwithstanding the use of a mass selective detector makes chromatographic separation less critical.

Structure assignment is not only based on the mass spectral data (molecular weight and fragment ions masses whenever low energy fragmentations occur) but partially also on the information obtained after consideration of singlet molecular oxygen attack supported by literature reports [10–13].

Scheme 2 shows mechanistic proposals for the formation of pindolol photolysis products.

3. Discussion

The proposals for products distribution upon RB-sensitized photooxidation (Table) involve the bond formation between oxygen and carbons or heteroatoms.

In particular the initial step of singlet molecular oxygen attack to the indole ring in the proposed mechanism for pindolol (Scheme 2) is similar to that reported by Wessels et al. [11] for the RB-sensitized photooxidation of tryptophan. The final photooxidation products for pindolol are salicylaldehyde, aminosalicylaldehyde and *o*-substituted derivatives. The breakage of the indolic group of tryptophan through reaction with singlet molecular oxygen leading to the formation of *N*-formyl kynurenine and L-kynurenine was also proposed by Langlois et al. [12].

The proposed pathways account for singlet molecular oxygen attack on the indole localized double bond. The radical nature of these reactions is indicated by half arrows notation.

Scheme 2 indicates the proposed singlet oxygen attack yielding the products of the Table for pindolol.

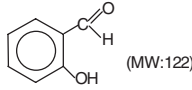
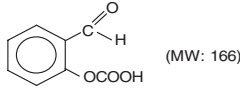
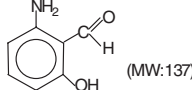
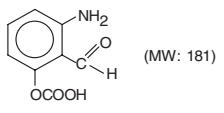
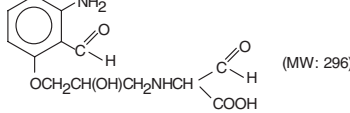
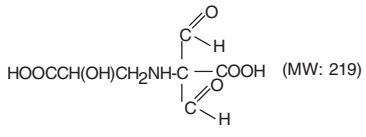
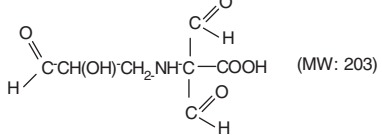
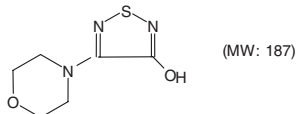
The detection of the oxidation product with RT 2.96 min does not exhibit breakage in the side chain but oxidation of the isopropyl moiety. The lack of detection of the analogue oxidation compound without the *ortho* amine group is likely due to the effect of the indicated hydrogen bonding (Scheme 2) on the carbonyl group action. This group has a relevant role on the homolytic rupture of the C–O bond of OR, a necessary step for the formation of phenols (RT 0.80 and 1.75 min).

In the case of timolol only one photoproduct retains the morpholine-thiadiazol structure (only one detected in the LC/MS conditions, Table), and the other two products are aliphatic amino acids. They are the result of the oxidation of the *tert*butyl moiety. As observed for pindolol, the oxidation of the *tert*butyl moiety would render only one carboxyl group which would inhibit further oxidation of the aldehyde groups.

Unfortunately, there are no previous investigations regarding the chemical interactions of singlet molecular oxygen with compounds containing morpholino or thiadiazol groups. However, Weigel and Henning [13] studied the photooxidation of several 2-morpholino cyclopropanols in the presence of the electron acceptors 9,10-dicyanoanthracene and triphenylpyrylium tetrafluoroborate. These authors ruled out the singlet oxygen photooxidation pathway because the substrates were completely recovered after photolysis ($\lambda > 395$ nm) in acetonitrile in the presence of methylene blue as photosensitizer for singlet oxygen. Thus, the morpholino ring seems to be non-reactive towards singlet molecular oxygen as also found here.

The high degree of oxidation observed in the identified products suggests that either important thermal reactions following the primary interaction between singlet oxygen and the ophthalmic drugs take place or that the reactions involve more than one singlet oxygen molecule per substrate molecule. Unfortunately, since the literature information on the singlet oxygen attack on such complex mole-

Table: Structure assignment from LC/MS data (API-Negative Mode) for the photooxidation products

Retention time (min)	Mass spectrum (m/z)	Proposed structure
Pindolol		
0.80	121	 (MW: 122)
1.00	165, 121	 (MW: 166)
1.75	136	 (MW: 137)
2.10	180, 136	 (MW: 181)
2.96	295, 251, 136	 (MW: 296)
Timolol		
1.70	218	 (MW: 219)
1.75	202	 (MW: 203)
11.03	186	 (MW: 187)

cules is very scarce, reasonable speculative mechanisms leading to all the products detected here cannot be proposed.

In conclusions the pharmaceutical topic antiglaucoma drugs timolol and pindolol, which act as β -adrenergic receptor antagonists, suffer singlet molecular oxygen mediated photodynamic action upon sensitized light irradiation.

Mass spectrometric data and literature information were used to make mechanistic proposals regarding the photooxidation of those drugs.

4. Experimental

4.1. Chemicals

Timolol, ((S)-[1-(1-butylamino-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-2-propanol), pindolol, (1-[1*H*-indol-4-yloxy]-3-isopropylamino-2-propanol), 4-hydroxyindole, 4-methylindole, 4-methoxyindole, sodium azide (NaN_3) and Rose Bengal (RB) were purchased from Sigma. Perinaphthene-2-sulfonic acid (1*H*-phenalen-1-one-2-sulfonic acid, PNS) was synthesized as described in the literature [14]. The solvent employed was triply distilled H_2O .

4.2. Photolysis

The irradiation device has been described elsewhere [15]. Singlet molecular oxygen was generated by irradiation of the solutions containing the sensitizer plus the individual ophthalmic medicament at wavelengths greater than 300 nm. Cut-off filters ensure that the light was only absorbed by the sensitizer. Light from a 150 W quartz-halogen lamp was passed through a water filter and focused into the reaction vessel (either the spectrophotometric cuvette or the hermetically sealed oxygen electrode reaction cell) containing the continuously stirred air-saturated solutions, both determined at identical substrate concentration (5×10^{-4} M).

4.3. Identification of photooxidation products

In order to identify the reaction products, steady-state photolysis experiments with air-saturated Rose Bengal solutions ($\text{Abs}_{530\text{ nm}} = 1.5$, pH 6) were irradiated at $\lambda_{\text{exc}} > 450$ nm with the set-up described above. The samples were analyzed with an Agilent 1100 LC-MSD modular system. The configuration was: binary pump, autosampler, thermostated column compartment, diode array detector and mass selective detector using API (electrospray) and APCI (atmospheric pressure chemical ionisation) interfaces. An Agilent RP-C18 amino acid column was used at 25 °C. The mobile phase was methanol: water (1:1). The flow rate was 1 ml/min and the injection volume 50 μl . The MSD parameters were: API and APCI interfaces, positive and negative modes, mass range 50–600 amu (0.5 amu mass resolution).

Two samples were run for each compound with different irradiation times. The first one for a relatively low conversion (approximately 15–20%) and the second one for a higher conversion (approximately 60%). The same compounds were identified in both runs finding higher amounts of products for the longer irradiation times.

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