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Photoproducts and proposed degradation pathway in the riboflavin-sensitised photooxidation of isoproterenol

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Products of the aerobic visible-light-promoted riboflavin-sensitised photooxidation of the sympathomimetic drug isoproterenol were identified by means of HPLC and spectrophotometric techniques. The oxidative process, mediated by superoxide radical anion, generates *N*-isopropylaminochrome as a main photoproduct with a quantum yield of 0.15. In parallel, the photodecomposition of riboflavin is prevented in the presence of isoproterenol. A reaction scheme for the photooxidation pathway of isoproterenol is proposed in analogy to former reports for related compounds.

1. Introduction

The sensitised photoprocess of pharmaceutical and bioactive drugs, especially under visible-light irradiation, has received particular attention in the last decades because the photoreaction can give rise to products with different, no or even undesirable activity (Straight and Spikes 1985; Tonnesen and Moore 1991; Albini and Fasani 1998). The mechanism governing the photoprocesses is strongly dependent on the characteristics of the sensitiser. (Chacon et al. 1988; Posadaz et al. 2000; Pajares et al. 2001).
An important family of pharmaceutical drugs, very sensitive to photoreactions, are catecholamines (Ctchs) (Jahnke and Frenkel 1978; Mol et al. 1979; Kruk 1985). It has been reported that Ctchs can undergo photooxidation upon direct absorption of UV light, forming aminochromes and melanins (Mol et al. 1979; Kruk 1985). One of the Ctchs employed in medicine is isoproterenol (Iso, Scheme), a synthetic sympathomimetic amine structurally related to epinephrine, that produces pronounced stimulation of both beta-1 and beta-2 receptors (Litter 1988). Under direct UV photoirradiation Iso generates *N*-isopropylaminochrome (IPA) as an unique product (Mol et al. 1979; Kruk 1985). Most common Ctchs, including Iso, are transparent to visible light; however, they may be deliberately or accidentally exposed to environmental illumination, in the presence of different dyes, pigments or colored substances during manufacturing, handling, storage or even after medicinal administration.
A particularly interesting visible-light-absorber sensitiser is riboflavin (Rf), which is a natural compound present in most living organisms (Heelis 1982). Rf can potentially act as a natural endogenous photosensitiser towards natural or externally added compounds (Edwards and Silva 2001). The usual mechanism of action of this sensitiser is rather complex. Rf photodecomposes upon aerobic UV or visible illumination, in most of the cases with the concurrent involvement of ROS such as singlet molecular oxygen $[O_2(^1\Delta_g)]$ and superoxide radical anion $(O_2^{\bullet -})$ (Heelis 1982; Chacon et al. 1988; Krishna et al. 1991; Pajares et al. 2001; Ahmad et al. 2004).
In a previous work we reported kinetic and mechanistic results on the Rf-sensitised photooxidation of Iso (Massad et al. 2004). The paper shows that although both ROS, namely $O_2(^1\Delta_g)$ and $(O_2^{\bullet -})$, are formed during Rf-sensitised photolysis of the mixture Rf-Iso, only the species $O_2^{\bullet -}$ effectively oxidises isoproterenol.
In the present contribution, and as complementary work, we focused on the reaction products and reaction mechanism in the Rf-photosensitised oxidation of Iso.

2. Investigations, results and discussion

Usually, the chemical nature of the compound eluting in a specific chromatographic band can be assessed by collecting the band of interest and subjecting it to analysis by an ancillary technique (e.g. nuclear magnetic resonance (NMR) or mass spectroscopy (MS)). However, if a chemical standard of the suspected compound is available, the matching of their capacity factors k' (eq. 1), can be used to assign the chemical identity of the eluting band (Snyder et al. 1997).

$$k' = \frac{(t_r - t_0)}{t_0} \qquad (1)$$

In the above equation, t_r is the retention time of the analyte and t_0 is the retention time of the injection peak. Analysis of these parameters is performed on standards and unknown mixtures run under two chromatographic modes – reversed phase and normal phase for instance – to determine the capacity factors.
Moreover, if the k' values of the standard and the unknown match after a change in mobile phase selectivity, this result is strong evidence for the chemical similarities of the eluting compounds, a criterion that has been applied in the

present case for the identification of photoproducts, in the Rf-sensitised photooxidation of Iso.

An aqueous solution of Rf (0.03 mM) plus Iso (1 mM) was photolysed with light of 445 nm, within the absorption band of Rf in the visible region (Fig. 1). The difference spectrum taken to photolysed *vs.* non-photolysed solutions was almost identical to the spectrum of IPA, the single photoproduct obtained through direct photolysis of Iso. This concordance is shown in the inset of Fig. 1.

High performance liquid chromatography (HPLC) analysis on the photolysed Rf-Iso aqueous solution showed the presence of two peaks, belonging to the photoproduct and to remaining Rf respectively. The first peak exhibits the same retention time as IPA, obtained by direct UV photolysis of Iso (Table, Fig. 2). Using the injection peack as t_0, the respective capacity factors, of IPA and the photoproduct obtained in the Rf-sensitised photolysis were 0.81 for both derivatives with MeOH-water (15 : 85, v/v) as a mobile phase and 0.27 and 0.28 respectively in MeCN-water (10 : 90, v/v) as a mobile phase, in the HPLC analysis. These results, collected in the Table, strongly suggest that the identity of both photoproducts is the same.

Rf-sensitised photolysis of Iso produces N-isopropylnoraminochrome as a main product with a quantum yield 0.15. This value indicates that the Rf-sensitized photooxidation of Iso is a relatively efficient process, as compared with the value of 0.009 reported for the photodegradation of Iso to produce *N*-isopropylnoraminochrome through direct photolysis at 254 nm (Mol et al. 1978).

Adrenochrome, a photoproduct structurally related to IPA, has been described in the methylene blue-sensitised photooxidation of adrenaline, through a process also mediated by $O_2^{\bullet -}$ (Jahnke and Frenkel 1978). In a similar fashion we propose the following reaction sequence (Scheme) for the Rf-sensitised photoprocess. Iso semiquinone is produced by $O_2^{\bullet -}$ attack. After disproportionation the ortho-quinone is produced. This compound undergoes cyclization forming *leuco*aminochrome, which is finally oxidized to aminochrome The overall reaction leads to *N*-isopropylnoraminochrome after consecutive reactions involving trihydroxy-*N*-methylindole and dihydroxy-*N*-mehtylindole.

Neither spectroscopic nor chromatographic evidence for the disappearance of the sensitiser Rf has been observed even after prolonged photoirradiation. Nevertheless, the photodegradation of Rf occurs at a considerably rate in the absence of Iso. As described in our previous work (Massad et al. 2004), the species $Rf^{\bullet -}$, which is formed from electron transfer from Iso to the electronically excited pigment, reacts with dissolved oxygen to produce $O_2^{\bullet -}$, regenerating ground state Rf This is an important pathway in living organisms, since it constitutes a source of Rf recovery from the semireduced Rf species (Kanofsky 1991). The second possible pathway of Rf photodegradation is via $O_2(^1\Delta_g)$ (Chacon et al. 1988). However, this mechanism can be disregarded, because, based on the known rate constants for the interaction with $O_2(^1\Delta_g)$, the oxidative species is predominantly physically quenched by Iso, without generation of photooxidation products, as extensively discussed previously (Massad et al. 2004).

Table: Retention times (R_t) and capacity factors (k') for *N*-isopropylaminochrome obtained through direct (A) and Rf-sensitised (B) photolysis of isoproterenol in two mobile phase systems

	R_t (min) [MeOH-water 15 : 85 v/v	K'	R_t (min) MeCN-water 10 : 90 v/v]	K'
A	3.96	0.81	3.16	0.27
B	3.90	0.81	3.19	0.28
C	10.63	3.94	4.19	1.68

Row C shows values for the eluting band corresponding to Rf, employed in the Rf-sensitised runs

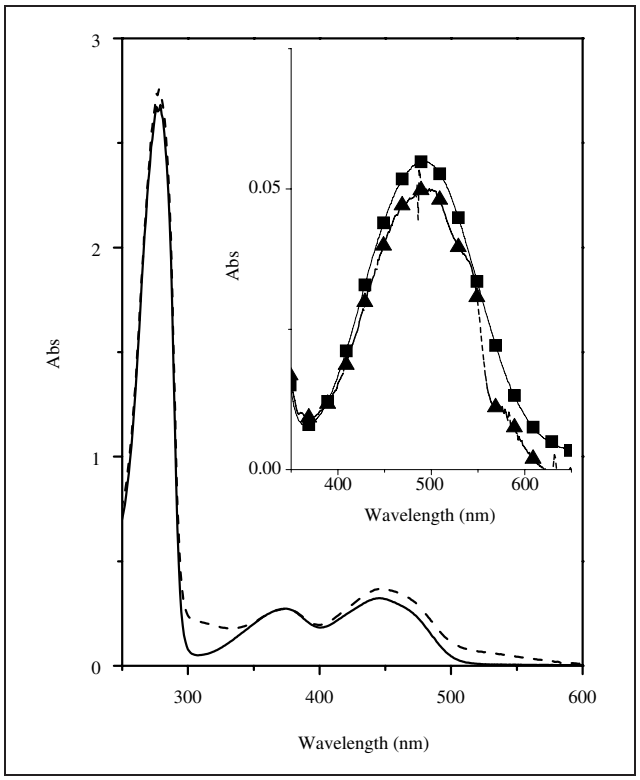


Fig. 1: Changes in the UV-Vis absorption spectra of Rf (0.03 mM) + Iso (1 mM) vs. water, upon photoirradiation at 445 nm under air-saturated conditions. (——) non photoirradiated; (- - - - -) t = 30 min photoirradiation. The two bands of the non irradiated solution, in the visible spectral region, correspond to Rf. Inset: comparison between the spectra of the products of direct (■) and Rf-sensitised (▲) photoirradiation of Isoproterenol

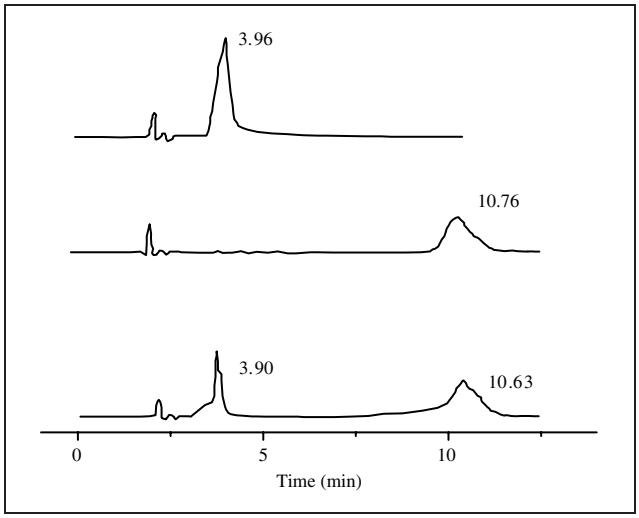
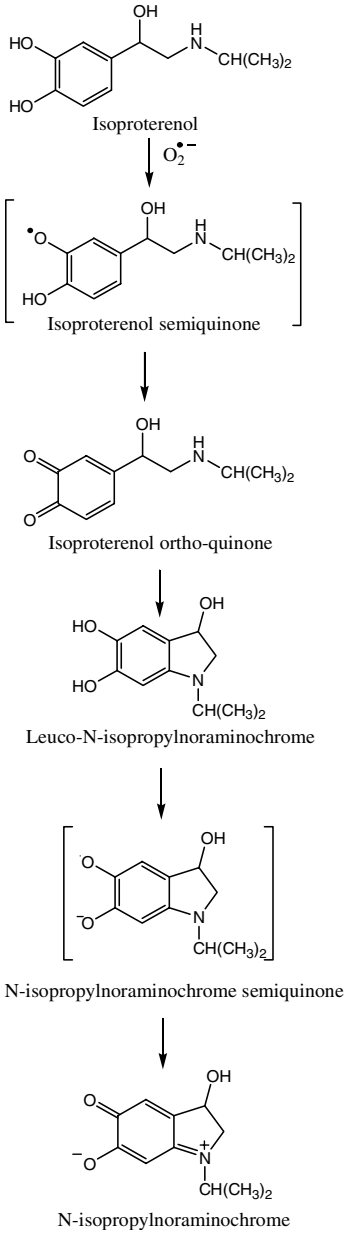


Fig. 2: Top: HPLC chromatogram of IPA obtained through direct photolysis of Iso. Medium: HPLC chromatogram of Rf. Bottom: HPLC chromatogram of Rf + Iso after 90 min of photoirradiation at 445 nm. Eluent MeOH-water 15 : 85 v/v, pH = 5 and 1 mM triethylamine. $\lambda_{detection}$ 494 nm

Scheme



As a conclusion we can say that the visible-light-irradiation of aqueous solutions of Rf in the presence of Iso produces IPA as a main product, through a relatively clean photoreaction, a fact demonstrated through HPLC and spectroscopic evidences. The species $O_2^{\bullet -}$ is involved in the photoprocess.

3. Experimental

3.1. Chemicals

Riboflavin 98% and 1-[3',4'-dihydroxyphenyl]-2-isopropyl-aminoethanol 98% (Iso) were purchased from Sigma Chem. Co. (St. Louis, MO, USA). Triethylamine 99% was purchased from Sintorgan (Bs. As., Argentina). All chemicals were used as received. An IPA standard was obtained by direct photolysis of Iso as described in the literature (Mol et al. 1979). Water was triply distilled. The non aqueous solvents were methanol (MeOH) and acetonitrile (MeCN), both HPLC quality, provided by Sintorgan (Bs. As., Argentina).

3.2. Methods

Steady state photolysis of aqueous solutions was carried out in a Photon Technologies International (PTI) unit, composed by a high pass monochromator and a 150 W Xe lamp. The irradiation of the sample was done in fluorescence quartz cell of 1 cm pathlenght placed at the output of the monochromator.

The quantum yield of formation *N*-isopropylnoraminochrome, photosensitized by Rf (irradiation wavelength 445 nm) in water was determined by relative actinometry. The photodecomposition of Rf, in aqueous pH 8 solution, with a reported quantum yield of 7.8×10^{-3}, was employed as a reference (Holzer et al. 2005). The rate of the process was determined by monitoring the decrease of the 445 nm-absorption band of the flavin, whereas the rate of generation of *N*-isopropylnoraminochrome was evaluated by means of the 495 nm-absorption band. A Hewlett Packard 8452A diode array spectrophotometer was employed.

The formation of *N*-isopropylnoraminochrome was determined by HPLC analysis, employing a Varian 5000 coupled to a UV-Vis detector (Varian 2550). A stainless steel analytical column (Varian SP-C8-IP-5 5 μm 4×150 mm) was used for the chromatographic analysis. The mobile phase consisted of H_2O : MeOH (85 : 15) or H_2O : MeCN (90 : 10), in both cases pH = 5 and 1 mM triethylamine. The flow rate was adjusted to 0.5 ml/min. The detection wavelength was 492 nm.

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References

Ahmad I, Fasihullah Q, Noor A, Ansari IA, Ali QNM (2004) Photolysis of riboflavin in aqueous solution: a kinetic study. Int J Pharm 280: 199–208.

Albini A, Fasani E (1998) Drugs: Photochemistry and Photostability. The Royal Society of Chemistry, Cambridge.

Chacon JN, McLearie J, Sinclair RS (1988) Singlet oxygen yields and radical contributions in the dye-sensitised photo-oxidation in methanol of esters of polyunsatured fatty acids (oleic, linoleic, linolenic and arachidonic). Photochem Photobiol 47: 647–656.

Edwards AM, Silva E (2001) Effect of visible light on selected enzymes, vitamins and amino acids. J Photochem Photobiol B: Biol 63: 126–131.

Heelis PF (1982) The photophysical and photochemical properties of flavins (isoalloxazines). Chem Soc Rev 11: 15–39.

Holzer W, Shirdel J, Zirak P, Penzkofer A, Hegemann P, Deutzmann R, Hochmuth E (2005) Photo-induced degradation of some flavins in aqueous solution. Chem Phys 308: 69–78.

Jahnke LS, Frenkel AW (1978) Photooxidation of epinephrine sensitized by methylene blue-evidence for the involvement of singlet oxygen and of superoxide. Photochem Photobiol 28: 517–523.

Kanofsky JR (1991) Singlet oxygen production from the reactions of superoxide ion in aprotic solvents: implications for hydrophobic biochemistry. Free Rad Res Comms 12–13: 87–92.

Krishna CM, Uppuluri S, Riesz P, Zigler JS, Balasubramanian D (1991) A study of the photodynamic efficiencies of some eye lens constituents. Photochem Photobiol 54: 51–58.

Kruk I (1985) The identification by electron spin resonance spectroscopy of singlet oxygen formed in the photooxidation of catecholamines. Z Phys Chemie (Leipzig) 266: 1239–1242.

Litter M (1988) Farmacología experimental y clínica, 7th Ed. edn. El Ateneo, Buenos Aires.

Massad WA, Bertolotti S, García NA (2004) Kinetics and Mechanism of the Vitamin B_2-sensitized Photooxidation of Isoproterenol. Photochem Photobiol 79: 428–433.

Mol NJD, Beijersbergen Van Henegouwen GMJ, Gerritsma KW (1979) Photochemical decomposition of catecholamines-II. The extent of aminochrome formation from adrenaline, isoprenaline and noradrenaline induced by ultraviolet light. Photochem Photobiol 29: 479–482.

Pajares A, Gianotti J, Stettler G, Bertolotti S, Criado S, Posadaz A, Amat Guerri F, Garcia NA (2001) Modelling the natural photodegradation of water contaminants – A kinetic study on the light-induced aerobic interactions between riboflavin and 4-hydroxypyridine. J Photochem Photobiol A: Chem 139: 199–204.

Posadaz A, Sánchez E, Gutiérrez MI, Calderón M, Bertolotti S, Biasutti MA, García NA (2000) Riboflavin and rose bengal sensitized photooxidation of sulfathiazole and succinylsulfathiazole. Kinetic study and microbiologiacal implications. Dyes Pigm 45: 219–228.

Snyder LR, Kirkland JJ, Glajch JL (1997) Practical HPLC method development. J. Wiley & Sons, Inc., New York.

Straight RC, Spikes JD (1985) Photosensitised oxidation of biomolecules. In: Frimer AA (ed) Singlet Oxygen. CRC Press, Boca Raton, pp 91–143.

Tonnesen HH, Moore DE (1991) Photochemical stability of biologically active compounds. III. Mefloquine as a photosensitizer. Int J Pharm 70: 95–101.