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Mechanism of the photochemical degradation of amlodipine

Elisa Fasani *, Angelo Albini, Silvia Gemme

Department of Organic Chemistry, University of Pavia, v. Taramelli 10, 27100 Pavia, Italy

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Abstract

A mechanistic investigation on the photodegradation of amlodipine, the corresponding besylate and a simple analogue lacking the β-aminoethoxy group has been carried out in water and in organic solvents. Irradiation leads to aromatization to the corresponding pyridines through an oxygen-independent process. The quantum yield for amlodipine base is $\Phi \cong 0.001$ under UV-A light, about one order of magnitude larger than that for the model bearing no amino group, supporting intramolecular assistance by that group. The value of Φ increases up to *ca.* 0.01 at shorter wavelength. The photolability of this drug according to ICH criteria is justified by the high absorptivity in the UV-A range ($\varepsilon_{\text{UV-A}}$), despite the low quantum yield. Some comments are added about the fact that product $\Phi \times \varepsilon_{\text{UV-A}}$ is more significative than Φ alone for the photolability (in solution) and about the lacking possibility to quench the photoreactivity where, as in the present case, this involves only short-lived intermediates.

Keywords: Photochemistry; Amlodipine; Mechanism; Quantum yield; Dihydropyridine; Photolability

1. Introduction

Photostability is a concern for drugs that absorb strongly in UV-A and in the visible. After the introduction of the ICH Guidelines (ICH, 1996) on this topic, several determinations and some comments have appeared in the literature (Tønnesen, 1996, 2004; Beijersbergen van Henegouwen, 1997; Albini and Fasani, 1998, 2003, 2004; Fasani and Albini, 2005; Fasani et al., 2006a,b). Isolated measurements have no predictive value, however, and it is important that reports on single drugs or groups of drugs are accompanied by mechanistic investigations, where a rationalization of the observed reactivity and of its dependence on changes in the structure and in the medium is offered. 1,4-Dihydropyridines antihypertensive drugs are a typical case. These absorb intensively in the UV-A (some derivatives also in the visible) and are known to be photolabile (Yeung et al., 1991; Marinkovic et al., 2000; Ragno et al., 2002, 2003). Furthermore, the efficiency of various methods for their protection from light has been tested (Skowronski et al., 1984; Thoma and Klimek, 1991; Béchard et al., 1992; Hasan, 1992; Jang et al., 2006). However, in front of a large number of photostability determinations under various conditions, there are only a few quantum yield measurements and the mechanism has been discussed in detail practically only for some 4-(2′-nitrophenyl) derivatives, in particular for nifedipine (Fasani et al., 2006a; Shim et al., 1988; Fujii and Berliner, 1999; Taiwo et al., 1999). These compounds, however, are clearly a special case. The efficient photoreaction in this case is due to the interaction of the nitro group with the easily accessible hydrogen in position 4 of the dihydropyridine ring, a mechanism that has no bearing on the photochemistry of derivatives with the nitro group in a different position (as demonstrated for some 4-(3′-nitrophenyl)-1,4-dihydropyridines) or lacking such group (Fasani et al., 2006b).

We were attracted by amlodipine, a term of the 1,4-dihydropyridine class of antihypertensive drugs used in the treatment of hypertension and angina. This acts as a calcium antagonist and inhibits the transmembrane influx of calcium ions into vascular smooth muscles and cardiac muscles, which in turn affects their contractile process and results in reduced blood pressure (Naidu et al., 2005). The structure characteristics that made this particular derivative worth studying were: (i) the fact that no nitro group was present, but rather a chloro atom in 2′, thus making it possible to test whether a different substituent in 2′ may exert any effect and (ii) the presence of an aliphatic amino group in the side chain. The latter moiety, an electron donor and a base, may on one hand introduce new paths in the

* Corresponding author. Tel.: +39 0382 987314; fax: +39 0382 987323.
E-mail address: elisa.fasani@unipv.it (E. Fasani).

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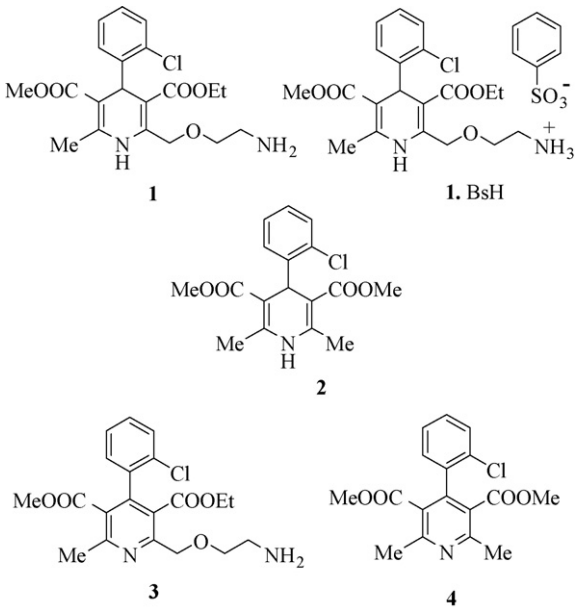

Fig. 1. Chemical structures of the studied compounds.

photochemical reaction and on the other widen the scope of solvents that could be tested, because the corresponding salts were more soluble in polar media than dihydropyridines generally are.

Therefore, we decided to explore in some detail the photoreaction in solution of amlodipine [3-ethyl-5-methyl-4-(2′-chlorophenyl)-2-(2-aminoethyloxymethyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate, **1**, see Fig. 1] and of the corresponding besylate (**1.BsH**), a salt that is one of the used pharmaceutical forms, as well as of a simple analogue lacking the amino group, viz. dimethyl 4-(2′-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate, **2**.

2. Materials and methods

2.1. Chemicals

The sample of amlodipine besylate (**1.BsH**) used was a generous gift by Pfizer Ltd., Sanwick, Kent (UK). The free base was prepared by treatment of the besylate with NaOH 5 M (pH 11) in CH_2Cl_2/H_2O under stirring, as indicated in a previous work (McDaid and Deasy, 1996). The corresponding pyridine **3** was prepared by oxidation of **1**, according to the literature (Beresford et al., 1989), and isolated as a difumarate salt, m.p. 135–138 °C (lit: 135–140 °C);

^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 0.83$ (t, 3H, C**H**$_3$CH$_2$COO, $J = 7$ Hz); 2.57 (s, 3H, C**H**$_3$-Py); 2.9 (t, 2H, –C**H**$_2$NH$_2$, $J = 5$ Hz); 3.49 (s, 3H); 3.58 (t, 2H, –OC**H**$_2$CH$_2$NH$_2$, $J = 5$ Hz); 3.94 (q, 2H, –COOC**H**$_2$CH$_3$, $J = 7$ Hz); 4.77 (AB quartet, 2H, Py-C**H**$_2$–O–, $^2J = 13$ Hz); 6.46 (s, 2H); 7.17-7.55 (m, 5H, aromatic protons).

^{13}C NMR (DMSO-d_6): $\delta = 13.2$ (CH_3); 22.8 (CH_3); 38.4 (CH_2); 52.3 (CH_3); 60.9 (CH_2); 67.7 (CH_2); 72.1 (CH_2); 126.1 (C); 126.6 (CH); 129.0 (CH); 130.3 (CH); 130.5 (CH); 131.7 (C); 134.1 (C); 135.0 (CH fumarate); 144.2 (C); 155.4 (C); 155.5 (C); 165.6 (C, carboxyl group); 166.5 (C, carboxyl group); 167.6 (2 C, fumarate carboxyl groups).

Samples of dihydropyridine **2** and pyridine **4** were prepared according to published procedures and showed spectroscopic characteristics as reported (Carabateas et al., 1984; Böcker and Guengerich, 1986; Salehi and Guo, 2004).

2.2. Photochemistry

Small-scale experiments were carried out on 3 mL samples of 2.5×10^{-4} M solutions of the dihydropyridines in MeCN or MeOH in quartz tubes after argon flushing when appropriate. These were irradiated by means of 15 W low-pressure mercury arcs (254 nm) or 15 W phosphor-coated lamps (center of emission, 366 nm; mid-height width of the emission range, 35 nm). The course of the reaction was monitored by TLC on silica gel (Fluka Silica gel/TLC-cards; F254, 0,2 mm thick) by eluting with cyclohexane/ethyl acetate 7:3 for compound **2** (R_f 0,15, fluorescent under UV_{366}), dichloromethane/methanol/ammonia 60/40/2 for compound **1** ($R_{f.}$ 0,4, fluorescent under UV_{366}) and by HPLC (Jasco PU1580, UV 1575 system) by using a C-18 reverse-phase Supelco Discovery 14518, 250 mm × 4.6 mm, 5 μm column and eluting with acetonitrile–water mixture (50:50, flow 1.3 mL/min, $\lambda_{an} = 250$ nm for compound **2**, r.t. 11,3 min) or phosphate buffer-triethylammine pH 3.14/MeCN mixture (65:35, flow 1 mL/min, $\lambda_{an} = 250$ nm for compounds **1**, r.t. 10,6 min, and **1.BsH**, r.t. 9,1 min). The phosphate buffer was prepared by dissolving 7.0 mL of triethylammine in 1 L of water adding H_3PO_4 to adjust the pH to 3.14).

Absorption spectra were registered on 5×10^{-5} M solutions in spectrophotometric cuvettes (1 cm optical path) on the range of 200–450 nm on a Jasco V-550 UV-Vis Spectrophotometer, using Spectra Manager as software UV, with scan rate 1 nm s^{-1}.

2.3. Quantum yields

The quantum yields of the reaction were measured in quartz tubes on 3 mL samples of 2.5×10^{-4} M solutions of the dihydropyridines in MeCN or MeOH after argon flushing when appropriate irradiated by means of a multilamps apparatus (Helios Italquartz Multirays apparatus). This was fitted with twelve 15 W phosphor-coated lamps (center of emission, 366 nm; mid-height width. 35 nm) or four 15 W low-pressure mercury arcs (254 nm), until a 10–25% conversion was reached (HPLC). The light flux was measured by ferrioxalate actinometry (Hatchard and Parker, 1956).

3. Results and discussion

The absorption spectrum of **1** (Fig. 2) exhibited a long-wavelength band with a maximum in the region 350–360 nm (in MeOH, $\varepsilon_{361} = 5740$ M^{-1} cm^{-1}) that tailed beyond 400 nm, as well as a more intensive band at shorter wavelength (in MeOH, $\varepsilon_{238} = 16850$ M^{-1} cm^{-1}). The spectrum of compound **2** was almost identical ($\varepsilon_{238} = 20400$, $\varepsilon_{359} = 7300$).

Irradiation of a 5×10^{-5} M solution of **1** in methanol at 366 nm (see Section 2 for detail) caused the decrease of both

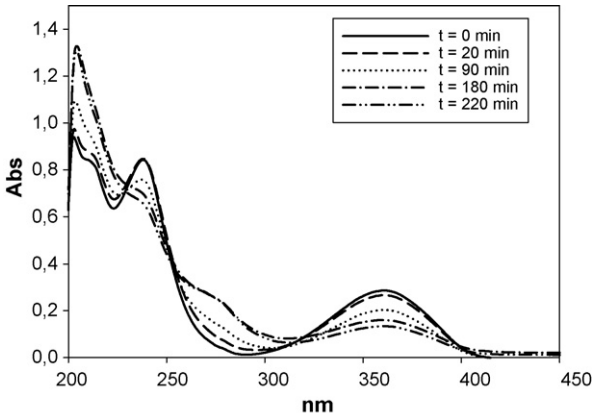

Fig. 2. UV spectra of a 5×10^{-5} M solution of compound **1** in methanol when irradiated with six 15 W phosphor-coated lamps (center of emission, 366 nm; mid-height width, 35 nm) after different irradiation intervals.

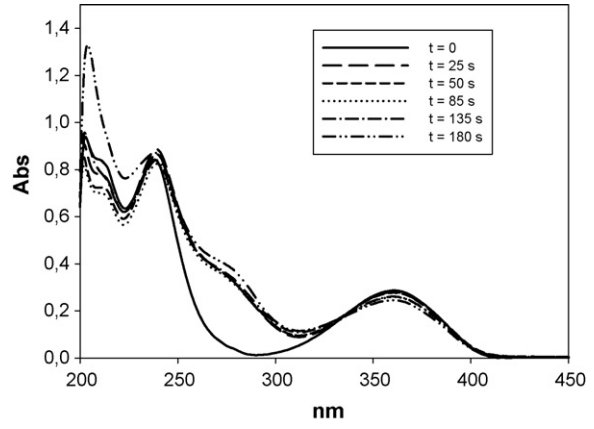

Fig. 3. UV spectra of a 5×10^{-5} M solution of compound **1** in methanol when irradiated with two 15 W low-pressure mercury arcs (254 nm) after different irradiation intervals.

bands at 360 and at 235 nm, while a new band peaking around 277 nm (characteristic of aromatized pyridine **3**) emerged. Isosbestic points were conserved up to >70% conversion (Fig. 2). This indicated that either a single product was generated, or a mixture with fixed composition. HPLC analysis showed the presence of a main peak with the same r.t. as an authentic sample of compound **3** (r.t. 6,4 min), confirming that this was by far the main product. Irradiation at 254 nm (Fig. 3) also led to the rearomatized product, though in a lower proportion. In both cases, experiments were carried out both in air-equilibrated and in argon flushed solutions, obtaining very similar results (see Table 1).

Dihydropyridine **2** showed a similar behaviour, both in air and in argon flushed solutions, with aromatized **4** (r.t. 10,2 min, identical with that of an authentic sample) as almost the only product in methanol at 366 nm, although the proportion decreased at 254 nm. The reaction was also tested in acetonitrile, again with closely similar results (Table 1).

Finally, besylate **1.BsH** was irradiated in water, both in the presence and in the absence of air, and was found to give again the aromatized pyridine **3**.

The quantum yield of reaction was measured under all of the above conditions. The results are gathered in Table 1. Considering first the simple derivative **2**, irradiation in the first absorption band produced a quite inefficient reaction, with quantum yield slightly below 1×10^{-4}, unaffected by the nature of the solvent and by the presence of oxygen. With these low Φ values it is difficult to discard the hypothesis that traces of non removed oxygen participate to the rearomatization process in the argon-flushed solution. However, the change in air-equilibrated solutions is so small that this seems unlikely. The simplest hypothesis is that the reaction involves hydrogen transfer from position 4 in the dihydropyridine ring, either via a singlet state or via a short-lived triplet. The transfer confronts a relevant barrier and is highly inefficient. If oxygen is not involved, the process might be due to the weakening of the C_4-H bond in the excited state, *i.e.* to the stability of the delocalized radical formed upon hydrogen loss (see Fig. 4). Irradiation in the high-energy band around 235 nm reasonably involves the same path, but apparently a vibrationally excited state is reached and the likehood of the reaction increases by about two orders of magnitude. In this case further reaction paths become also activated and rearomatization, though remaining a main process, is no more the only one. The photoreaction is little affected by the medium. However, the quantum yield for **1** at 366 nm is larger than that of **2** by a factor >10. This may be due to a participation of the amino group assisting the H transfer step (Fig. 4), as previously invoked for another dihyropyridine (nicardipine) bearing an amino group in a side chain (Fasani et al., 2006b). A possible mechanism is presented in Fig. 4, where it is shown that the excited state has an intramolecular charge transfer character (which justifies the

Table 1
Reaction quantum yields measured on 2.5×10^{-4} M solutions of the indicated compounds

Compound	Solvent (nm)	Air-equilibrated Product (%yield)	Φ	Argon-flushed Product (%yield)	Φ
1	MeOH, 254	**3** ((~30%)	8×10^{-3}	**3** ((~30%)	9×10^{-3}
	MeOH, 366	**3** (100%)	10^{-3}	**3** (100%)	7×10^{-4}
1.BsH	H_2O, 254	**3** ((~60%)	7×10^{-3}	**3** ((~50%)	6×10^{-3}
	H_2O, 366	**3** (100%)	2×10^{-4}	**3** (100%)	2×10^{-4}
2	MeCN, 254	**4** (24%)	6×10^{-3}	**4** (27%)	4×10^{-3}
	MeOH, 254	**4** (23%)	6×10^{-3}	**4** (31%)	3×10^{-3}
	MeCN, 366	**4** (66%)	7×10^{-5}	**4** (65%)	5×10^{-5}
	MeOH, 366	**4** (100%)	9×10^{-5}	**4** (99%)	7×10^{-5}

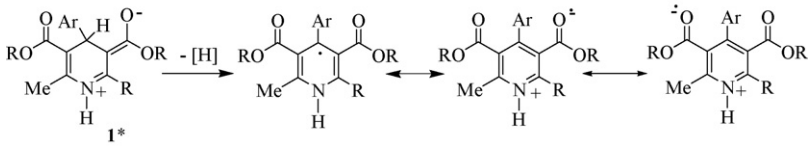

Fig. 4. Mechanism of degradation of **1** in the excited state.

strong absorption in the UV-A). Thus, hydrogen loss from position 4 is facilitated, since the resulting radical is stabilized by the contribution of (zwitterionic) aromatic formulas, as shown in the Figure. The tethered amino group ($R = OCH_2CH_2NH_2$) further increases this process, reasonably by acting as electron donor towards the pyridiniun moiety.

In fact, the difference is much reduced in the case of the besylate, where the amino group is protonated. On the contrary, there is no difference in the quantum yield at 254 nm between **1**, **1.BsH** and **2**, in accord with the hypothesis that a vibrationally excited state is involved.

To summarize, the quantum yield of reaction (Φ) is rather low, particularly in the environmentally relevant near UV, where it reaches a value around 0.001 in the case of amlodipine. However, the combination of this low value with the strong absorption of these molecules in the UV-A region (ε_{360} *ca.* 6000 M^{-1} cm^{-1}) is the cause of the conspicuous photodegradation observed in the tests, so that these molecules are classed as highly photolabile according to the ICH regulations. In fact, what is practically relevant for the photostability is the product $\varepsilon \times \Phi$, where ε is referred to wavelengths present in the environment, rather than Φ per se.

Furthermore, it is important to obtain some evidence on the mechanism of photodegradation or at least about the dependence of the photoreactions on conditions, in order to make rationalizing and, when possible, predicting the protective effect by various agents. In the present case the photochemistry, at least with respect to practically relevant UV-A light, is virtually independent on the experimental conditions and must involve very short-lived intermediates. Protection from photodecomposition is only possible by adding filters that absorb competitively. Indeed effective protection has been reported where light penetration is inhibited, that is in the solid state (Memariam and Mirjafari, 2005) and in dry emulsion (Jang et al., 2006), where loss by refraction is overwhelming.

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