Journal of Photochemistry and Photobiology A: Chemistry 222 (2011) 360–365

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

Oxoisoaporphines: Regioselective deuterium labelling involving the metastable hydrogenated photoproduct anions

Christian Aliaga[a], Masiel Cerón-Neculpán[a], Claudio Saitz[a], Carolina Jullian[a], Eduardo Sobarzo-Sánchez[b], Julio R. De la Fuente[a,*]

[a] Departamento de Química Orgánica y Fisicoquímica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Casilla 223, Santiago 1, Chile
[b] Departamento Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Santiago de Compostela, Santiago de Compostela, Spain

ARTICLE INFO

Article history:
Received 19 January 2011
Received in revised form 22 June 2011
Accepted 17 July 2011
Available online 23 July 2011

Keywords:
Oxoisoaporphines
Isotopic labelling
Isotopic exchange
Heterocycle photoreduction
Azabenzoanthracenones
Benzoperimidinones

ABSTRACT

During the ^{1}H NMR study of the photoreduction of aza-oxoisoaporphines, aza-OIAs, (7H-benzo[*e*]perimidin-7-ones) by amines we found an unexpected H/D isotopic exchange in the aromatic positions 4 and 6 of these molecules. This surprising exchange motivated us to probe it with previously studied oxoisoaporphines, OIAs, (1-aza-benzo[de]anthracen-7-ones) and 2,3-dihydro-oxoisoaporphines, 2,3-dh-OIAs, (2,3-dihydro-7H-dibenzo[de,h]quinolin-7-ones). All of these compounds photoreduce efficiently in the presence of aliphatic tertiary amines through a stepwise mechanism of electron-proton-electron transfer. This photoreaction generates an AH^- anion hydrogenated either at the N-atom, for 2,3-dh-OIAs, or at the O-atom for aza-OIA and OIAs. These long-lived metastable photoproducts revert thermally to the initial oxoisoaporphines nearly quantitatively. In the presence of D_2O, regioselective exchange of the aromatic protons at positions 4 and 6 takes place to an extent greater than 90% under very mild conditions. This facile isotopic exchange reaction might be advantageously used to introduce deuterium, and likely tritium at these positions of aromatic oxoisoaporphines.

1. Introduction.

Stable isotopically labeled compounds are essential tools for mechanistic and metabolic pathways studies as well as for use as internal standards in mass spectrometry. There are numerous useful methods to incorporate deuterium into aromatic molecules, many of them by employing transition metal complexes in catalytic reactions [1–3]. These methods usually involve large amounts of catalyst, strong acidic or basic media and gaseous deuterium/hydrogen at high temperatures and pressures [4–6]. Some photoinduced H/D exchanges, involving radical intermediaries, have been reported for tryptophan and tyrosine [7,8]. Electrophilic aromatic substitution in dialkoxybenzenes, in strongly acidic media, has been reported [9], as well as excited-state proton transfer from the solvent in phenolic compounds and benzo- and methyl-phenones [10–13].

Some years ago we undertook a detailed study of the photoreduction by amines of synthetic 2,3-dihydro-oxoisoaporphines, 2,3-dh-OIAs, and aromatic oxoisoaporphine, OIAs [14–17]. For these compounds, the photoreduction mechanism involves a radical ion pair complex, $[A^{\bullet-}\cdots R_2N^{\bullet+}\text{–}CH_2R']$, formed by single electron transfer from the amine to the excited triplet state of the oxoisoaporphine derivatives, followed by a proton transfer from the amine radical cation to one of the substrate's heteroatoms, generating the non-ionic hydrogenated radical of the oxoisoaporphine derivatives, $AH^{\bullet}$, and the imine radical respectively. This latter, more reductive, imine radical [18–20] donates a second electron to $AH^{\bullet}$ giving the respective anion AH^- and the iminium cation of the amine, Eqs. (1)–(3), in a formal hydride transfer. The metastable hydrogenated photoproduct anion AH^- reverts slowly to the initial substrate in the dark. This reaction is faster if O_2 is admitted to the reaction cell. On the other hand, the iminium cation, Eq. (4), leads to amine oxidation products such as aldehydes, secondary amines and 1-diethylaminobutadiene when TEA was used [21]. This electron–proton–electron stepwise mechanism takes place with an amine α-H transfer to the N-atom of 2,3-dh-OIAs [14,15], while for OIAs the α-H transfer occurs over both the N1 and the O carbonyl atoms, with the generation of AN1H$^{\bullet}$ and AOH$^{\bullet}$ radicals, which are in equilibrium [16]. These photoreduction mechanisms were supported by spectral and kinetic characterizations of all the transient intermediaries by using laser flash photolysis and pulse radiolysis.

$$^3A^* + R_2N\text{–}CH_2R' \rightarrow [A^{\bullet-}\cdots R_2N^{\bullet+}\text{–}CH_2R'] \quad (1)$$

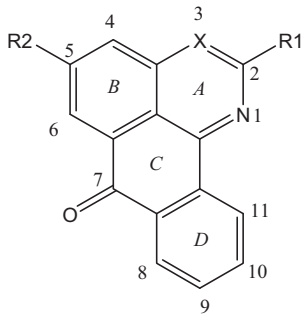
$$[A^{\bullet-}\cdots R_2N^{\bullet+}\text{–}CH_2R'] \rightarrow AH^{\bullet} + R_2N\text{–}C^{\bullet}HR' \quad (2)$$

$$AH^{\bullet} + R_2N\text{–}C^{\bullet}HR' \rightarrow AH^- + R_2N^+{=}CHR' \quad (3)$$

$$R_2N^+{=}CHR' \rightarrow \text{Amine oxidation products} \quad (4)$$

* Corresponding author. Fax: +56 2 978 2868.
E-mail address: jrfuente@ciq.uchile.cl (J.R. De la Fuente).

1010-6030/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotochem.2011.07.011



A1: X = N, R1 = H, R2 = H
A2: X = N, R1 = CH_3, R2 = H
A3: X = CH, R1 = H, R2 = H
A4: X = CH, R1 = H, R2 = -OCH_3

Chart 1. Structures of aza-oxoisoaporphines, aza-OIAs: 7H-benzo[*e*]perimidin-7-one, A1 and 2-methyl-7H-benzo[*e*]perimidin-7-one, A2, and oxoisoaporphines, OIAs: 1-aza-benzo[de]anthracen-7-one, A3 and 5-methoxy-1-aza-benzo[de]anthracen-7-one, A4. Atom, numbers used for ^{1}H NMR assignment and calculations.

Our more recent studies were focused in the photoreduction by amines of derivatives containing two N atoms in their structure (7H-benzo[*e*]perimidin-7-ones) named here as aza-oxoisoaporphines, aza-OIAs, see Chart 1, for which we expected a similar photoreduction behavior. During the study of the photoreduction of these compounds, we found an unexpected H/D isotopic exchange of aromatic protons at positions 4 and 6. This motivated us to probe whether the isotopic exchange occurs in the previously studied systems, and to find an explanation for this unusual H/D exchange taking place under very mild conditions.

2. Experimental

2.1. Materials

Acetonitrile Merck, HPLC grade, and acetonitrile-d_3 99% D, Aldrich, spectroscopic grade, were used as received. Triethylamine, TEA, and tripropylamine, TPA, both from Sigma Aldrich were distilled trap-to-trap and stored at −18 °C in vacuum sealed tubes. Before each experiment a new tube was opened to assure the freshness of the amine. Triethyl-d_{15}-amine, 99% D from Sigma Aldrich, was used as received in the ^{1}H NMR experiments.

2.2. NMR spectral measurements

Experiments were monitored with a Bruker Avance DRX-300, 300 MHz spectrometer. Reactions were carried out by direct photoreduction of Ar-purged solutions containing a weighed amount of the aza-oxoisoaporphines, typically 1 mg/ml in CD_3CN. Solutions were prepared directly in NMR tubes sealed with a septa and Ar-purged for almost 20 min. After the purge, an appropriate amount of normal or perdeuterated amine was added to give >8 fold molar excess over the aza-oxoisoaporphines. During photoreduction several ^{1}H NMR spectra were recorded in order to test for maximum concentrations of the metastable species. COSY spectra were taken when the ^{1}H NMR spectra were constant. Isotopic exchange experiments were performed by adding D_2O (10 μl) after or prior photolyses as described in the text. It was not possible to obtain a ^{13}C NMR spectrum of the irradiated samples due to the low concentration of the metastable photoproducts.

2.3. Synthesis of aza-oxoisoaporphines

Synthesis of 7*H*-benzo[*e*]perimidin-7-one, A1, was by cyclization of 1-aminoanthraquinone with *N*,*N*-dimethylformamide dimethyl acetal. The methyl substituted derivative, 2-methyl-7*H*-benzo[*e*]perimidin-7-one, A2, was obtained by reaction of 1-aminoanthraquinone with *N*,*N*-dimethylacetamide-dimethylacetal and phosphorus oxychloride. The formed intermediate was cycled in situ by addition of ammonium acetate in hot ethanol [22–24].

2.4. Mass spectra experiments

Measurements for aza-oxoisoaporphines were obtained in a Thermo Finnigan model MAT 95XP mass spectrometer, by using direct inlet of the recovered samples with a temperature ramp of 40 °C min^{-1} within 23–360° C during 8 min and electron impact ionization, EI, 70 eV and mass scanned between 200 and 500 Dalton.

The recovered oxoisoaporphines were dissolved in acetonitrile with formic acid 0.1% v/v, and the measurements were done in electrospray ion trap ESI-IT Esquire 4000 mass spectrometer (Bruker Daltonics, Inc., MA, USA), by using direct injection of the samples in a Cole-Palmer pump (IL, USA) with a flow-rate of 3.0 μL/min; nebulization at 325 °C, 10.0 psi and a gas flow of 5.0 L/min. Mass spectra were scanned between 20 and 2200 Dalton in positive polarity.

2.5. Quantum mechanical calculations

We used HyperChem-8.0 by HyperCube, Inc. The radical and anionic structures were built and minimized by using semi-empirical PM3 with UHF and multiplicity = 2 for the radicals and RHF and multiplicity = 1 for the anions. In this manner formation enthalpies (kcal mol^{-1}) and charge densities were obtained for each one of the calculated species.

3. Results and discussion

As we expected, the photoreduction of aza-OIAs by amines was completely analogous to the photoreduction of previously studied systems [14–17], showing the appearance of absorption bands attributed to the hydrogenated anion (AH^-). These bands revert to those of the precursor aza-OIAs when the photolyzed samples were stored in the dark, or if air O_2 was admitted to the samples, Fig. 1. Isosbestic points observed during the photolysis and the recovery reactions suggest the formation of single photoproducts, which we probed further with NMR experiments. These photoproducts were identified as the hydrogenated anions $A1H^-$ and $A2H^-$ for A1 and A2, respectively. Details concerning to the photoreduction reactions, including kinetic and time resolved characterization of the radical intermediaries will be published elsewhere.

3.1. NMR results

Direct photoreduction of Ar purged solutions of A1 and A2 with an excess of amine in CD_3CN allowed us to characterize the ^{1}H NMR spectra of the metastable photoproducts ($A1H^-$, $A2H^-$), shown in Table 1. The largest differences in chemical shifts, Δδ, Table 1, between the photoproducts and the precursors occur at protons in positions 2, 4, 5 and 6. This agrees with an increase in charge density, mainly in the rings *A B* of the respective photoproducts, Chart 1, which supports the formation of AH^- anions as photoproducts. Similar high field shifts were reported for the photoreduction of 2,3-dh-OIAs [14,15] and 3-phenyl-quinoxalin-2-ones [25,26] where the protonation occurs over the substrate's N heteroatom.

As in similar photoreduction systems [15,25] all the experiments (with triethylamine, TEA, perdeuterated triethylamine, TEA_{d15}, and tripropylamine, TPA) involved the rapid appearance of broad high-field-shifted signals, typical for negatively charged photoproducts. Considering the photoreduction mechanism, it is expected that there was a transfer of an additional proton from the amine to the aza-OIAs to form a labile O–H or N–H bond able to exchange H^+ by D^+ in the presence of D_2O, Eqs. (1)–(3).

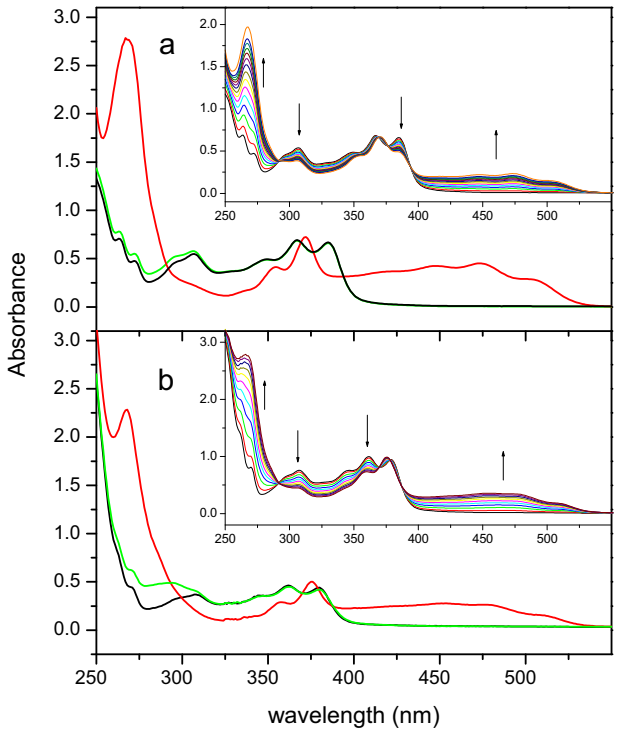


Fig. 1. (a) Spectra showing the reverse reaction in the presence of TEA of: (a) 2-methyl-3-aza-oxoisoaporphine, A2; and (b) 3-aza-oxoisoaporphine, A1. Black, $t = 0$; red, at the end of photolysis; and green after air admission recovery. Inset arrows show the increasing and decreasing absorption bands during photolysis. (b) Spectra showing the reverse reaction in the presence of TEA of: (a) 2-methyl-3-aza-oxoisoaporphine, A2; and (b) 3-aza-oxoisoaporphine, A1. $t = 0$ (—); at the end of photolysis (· – · – ·); and green after air admission recovery (· · · · ·). Inset arrows show the increasing and decreasing absorption bands during photolysis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

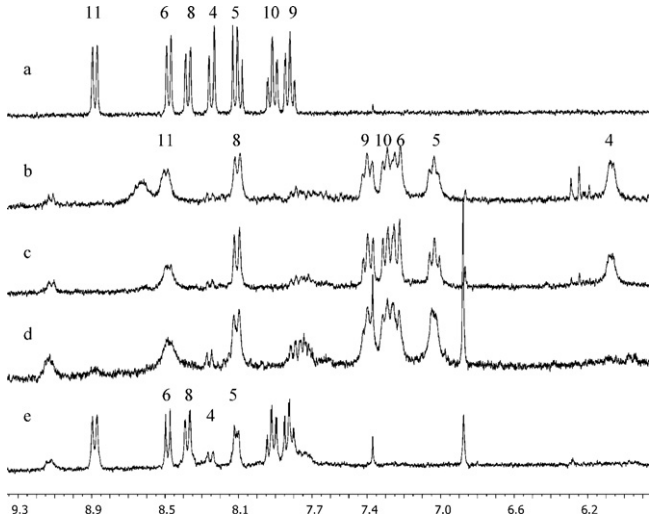


Fig. 2. ^{1}H NMR spectra of 2-methyl-3-aza-oxoisoaporphine A2, photoreduced by TEA: (a) unreacted sample, $t = 0$; (b) at the photolysis end, $t = 10$ min; (c) D_2O added to the photoreaction mixture; (d) sample containing D_2O after 48 h in darkness; and (e) sample after recovery by air admission.

In the photolysis of A2, Fig. 2, this additional signal clearly appears at 8.62 ppm, integrating for one proton, Fig. 2b, immediately disappearing after D_2O addition to the photoreaction mixture, Fig. 2c. Experiments performed with $TEA_{d\text{-}15}$ (not shown) show an integration of only 0.3 for the labile proton signal at 8.62 ppm. This demonstrates that this H or D was transferred from the amine to A2, providing a confirmation of the photoreduction mechanism reported previously for other derivatives [14–17].

Noteworthy, after storage in the dark (48 h) of the mixture of photoproducts, containing D_2O, the A2H$^-$ doublet at 6.07 ppm corresponding to H-4, almost completely disappears. In addition, the triplet at 7.04 ppm (A2H$^-$, H-5) becomes a broad signal, Fig. 2d. When air is admitted to this sample, Fig. 2e, the recovery of all A2 signals was observed with a notable decrease in the integration value of A2 H-4 and in less degree in the H-6 signals, as compared with the invariant H-11 signal. These observed changes are due to H/D exchange in the aromatic positions 4 and 6 of the A2H$^-$ photoproduct. Therefore, there are two isotopic exchange processes; a fast in the labile H transferred from the amine, and a slow one occurring on the aromatic carbons of the molecules of the photoproducts.

The photoreduction of A1 by TEA and the effects of D_2O addition to the photoreaction mixture, shown in Fig. 3, were similar to those described for A2. After D_2O addition all the A1H$^-$ photoproduct signals remain the same, Fig. 3c, not showing any labile hydrogen signal. However, after 24 h in the dark, in the presence of D_2O, the A1H$^-$ signals assigned to H-4 and H-6 disappeared and the triplet assigned to H-5 became a singlet, Fig. 3d, clearly indicating the exchange of H to D at aromatic positions 4 and 6 of the photoproduct molecule.

The isotopic exchange in these positions was confirmed, after admission of air, Fig. 3e, in the recovered A1 through: the integration of substrate signals H-6 and H-4 which decreased to ≈0.2 and to almost 0, respectively, as compared with the invariant H-11 sig-

Table 1
^{1}H NMR Proton assignment of 7H-benzo[*e*]perimidin-7-one, A1, and 2-methyl-7H-benzo[*e*]perimidin-7-one, A2, and their respective metastable hydrogenated photoproducts AH$^-$. Assignation was made based on photoproduct, ^{1}H RMN, COSY.

H position[b]	Chemical shift and multiplicity [δ ppm,[a] (multiplicity)]					
	A1	A1H$^-$	Δδ[c] A1	A2	A2H$^-$	Δδ[c] A2
2	9.50 (s)	7.31 (s)	−2.19	–	–	–
4	8.38 (d)	6.09 (d)	**−2.29**	8.25 (d)	6.07 (d)	**−2.18**
5	8.19 (t)	7.01 (t)	−1.18	8.11 (t)	7.04 (t)	−1.07
6	8.58 (d)	7.24 (d)	**−1.34**	8.48 (d)	7.23 (d)	**−1.25**
8	8.40 (d)	8.10 (d)	−0.30	8.38 (d)	8.10 (d)	−0.28
9	7.82 (t)	7.40 (t)	−0.42	7.82 (t)	7.40 (t)	−0.42
10	7.92 (t)	7.31 (t)	−0.61	7.92 (t)	7.29 (t)	−0.63
11	8.90 (d)	8.39 (d)	−0.51	8.88 (d)	8.50 (d)	−0.38
CH_3	–	–	–	2.94 (s)	–	–
Labile H	–	–	–	–	8.62 (s, broad)	–

Bold indicates the larger changes in properties associated with the atom positions.
[a] In ppm relative to TMS.
[b] Numbers are those of Chart 1.
[c] (δ product AH$^-$ − δ A).

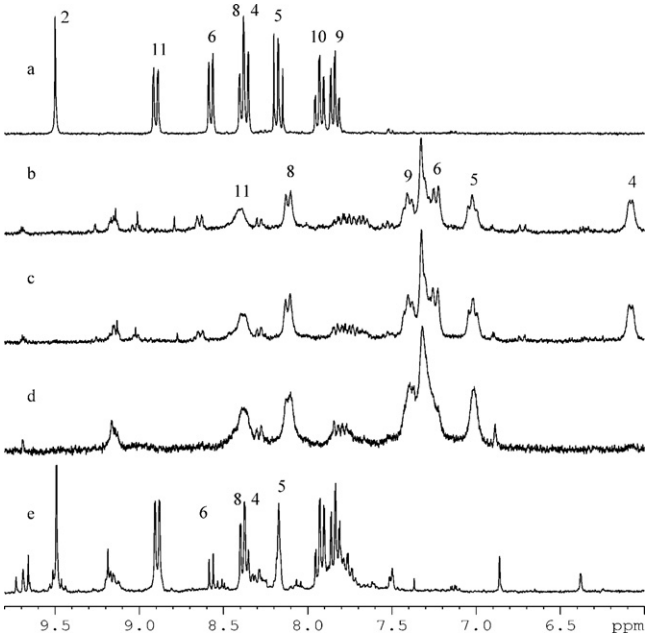


Fig. 3. ^{1}H NMR spectra for photoreduction of 3-aza-oxoisoaporphine A1 and effects after D_2O addition. (a) Before photolysis $t = 0$; (b) at the end of photolysis, $t = 30$ min; (c) photoreduction mixture after 10 μl D_2O addition; (d) after 24 h storage in darkness; and (e) after recovery by air admission into the NMR tube.

nal, and the loss of the triplet character of the H-5 signal which became a singlet.

By considering the similarities in the chemical shifts of both metastable photoproducts, see Table 1, we conclude that the proton transfer from the amine should take place also in A1 photoreduction, although we cannot detect the labile proton in the A1H$^-$ photoproduct.

Photolysis experiments, for both compounds A1 and A2, in the absence of D_2O showed the almost complete recovery of the signals after the admission of air. But, in a separate experiment, wherein D_2O was present (added prior to photolysis) and a fast recovery was induced by admission of O_2, no H/D exchange was observed in the aromatic positions. This clearly indicates that the isotopic exchange took place over the metastable photoproducts, during the storage in the dark, and not in the photolyses or in the recovering reactions.

3.2. Aza-oxoisoaporphine mass spectra

After photolysis, D_2O addition and storage in the dark for 170 h, the isotopic exchange was confirmed in recovered aza-OIAs samples by mass spectra. For 3-aza-oxoisoaporphine, A1, we detected the following molecular ions (M^-) with: $m/z = 232$ (6%); 233 (30%) and 234 (64%), corresponding to the normal, mono and di-deuterated A, respectively. For the recovered normal, mono and di-deuterated A2, we found (M^-) with $m/z = 246$ (10%); 247 (68%) and 248 (22%), respectively. These data show that almost 90% of both aza-OIAs exchanged deuterium at position 4, as indicated by the ^{1}H NMR experiments.

This unexpected H/D exchange observed for aza-OIAs encouraged us to test the isotopic exchange with the previously studied derivatives oxoisoaporphines, OIAs [16], and 2,3-dihydro-oxoisoaporphines, 2,3-dh-OIAs [14,15,21].

3.3. Oxoisoaporphines and 2,3-dihydro-oxoisoaporphines results

We photolyzed samples containing the respective oxoisoaporphines and excess TEA, and after photolysis was complete, D_2O was added. The samples were stored protected from light, for six days

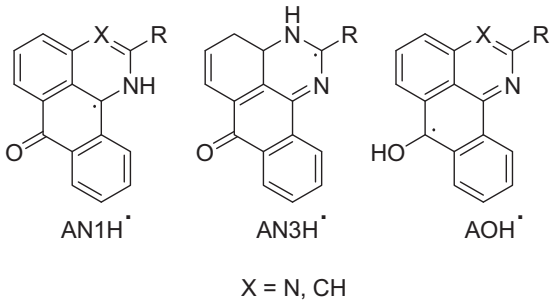


Chart 2. Structures for aza-OIAs possible hydrogenated radicals, AH•, A1 (R = H) and A2 (R = CH_3). The only explicitly shown H is the α-H transferred from the amine.

to allow for the H/D exchange. After this storage in the dark, air O_2 was admitted into the cells, and the solvent was evaporated. Each sample was redissolved in $CDCl_3$ for a ^{1}H NMR spectrum. As we expected, for both OIAs A3 and A4 an almost complete H/D exchange occurred in positions 4 and 6 as seen in the NMR spectra (not showed). In contrast, no exchange was observed for the 2,3-dihydro derivatives.

3.3.1. Oxoisoaporphines mass spectra

Mass spectra analysis of the recovered A3 and A4 samples showed molecular ions, corresponding to the $(M+H)^+$ species with: $m/z = 232$ (10%); 233 (19%) and 234 (71%); and $m/z = 262$ (2%); 263 (5%) and 264 (93%) for the normal, mono and di-deuterated A3 and A4 respectively. Details of the mass analysis are provided in Section 2.4.

In conclusion, we have a regioselective H/D exchange for both families of aromatic derivatives, OIAs and aza-OIAs, which do not take place for the 2,3-dihydro-oxoisoaporphines. This behavior has to be related to the protonation site of the respective metastable photoproducts AH$^-$. Hence, we calculated the formation enthalpies, of the AH• radicals and AH$^-$ anions, protonated either in the N1, N3 or carbonyl O atoms for aza-OIAs and in the N1 or O atoms for OIAs, as shown in Chart 2.

3.4. Semi-empirical quantum mechanic calculations

The radical species and the corresponding anions were geometrically optimized by using the PM3 method and their formation enthalpies were evaluated disregarding any solvent effect. From these calculations, we also obtained the charge densities of the photoproduct anions, which were used to evaluate the electronic density changes relatives to the substrate molecules. These results are summarized in Table 2.

For the aza-OIAs A1 and A2, as can be expected, the most stable AH• radicals are those protonated at the O-atom with the respective anions following the same trend. In contrast, for the OIAs, the most stable radicals are those protonated on the N1 atom, but the respective AOH$^-$ anions were the most stable anionic photoproducts [16].

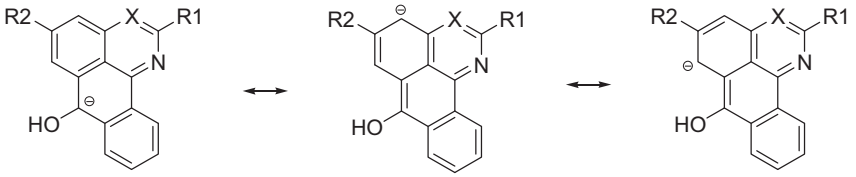
Therefore, for the aromatic OIAs and aza-OIAs a preferential protonation in the carbonyl O-atom generating the respective AOH$^-$ anions should be expected. Moreover, these photoproducts explain the isotopic exchange at positions 4 and 6 by considering simple resonance structures, Chart 3. Resonance locates negative charges at positions 4 and 6 allowing a D$^+$ electrophilic attack in these positions. For the anions protonated on the N-atom, AN1H$^-$ and AN3H$^-$, resonance locates the charges at positions 5, 9, and the bridgehead C-atoms.

These empirical resonance structures for aza-OIAs and OIAs, agree with PM3 charge density calculations, showing the largest increases in charge at the same positions for both types of anions,

Table 2
PM3 calculated formation enthalpies, ΔH_f (kcal mol^{-1}), for aza-OIAs and OIAs hydrogenated radicals, AH•, and photoproducts anion, AH$^-$. Difference in charge density, Δ(CD), between the AH$^-$ photoproducts and precursors.

	Aza-oxoisoaporphines						Oxoisoaporphines			
	A1			A2			A3		A4	
Specie ΔH°_f (kcal mol^{-1})	A1N1H•	A1N3H•	A1OH•	A2N1H•	A2N3H•	A2OH•	A3N1H•	A3OH•	A4N1H•	A4OH•
	40.90	40.26	**37.67**	31.16	30.40	**28.15**	**45.45**	47.64	**7.84**	10.22
	A1N1H$^-$	A1N3H$^-$	A1OH$^-$	A2N1H$^-$	A2N3H$^-$	A2OH$^-$	A3N1H$^-$	A3OH$^-$	A4N1H$^-$	A4OH$^-$
	−5.89	−7.82	**−9.18**	−14.96	−16.99	**−16.35**	−6.92	**−9.54**	−45.33	**−48.83**
Atom numbers	Δ(CD) AH$^-$ – A									
N1	0.235	0.112	−0.178	0.233	0.119	−0.182	0.213	−0.183	0.226	−0.162
C2	−0.078	−0.225	0.049	−0.084	−0.233	0.040	−0.083	0.059	−0.085	0.054
N3/C3	−0.058	0.168	−0.186	−0.045	0.181	−0.164	−0.054	−0.181	−0.054	−0.179
C4	−0.014	−0.067	**−0.238**	−0.012	−0.068	**−0.217**	−0.026	**−0.236**	0.011	**−0.223**
C5	**−0.128**	**−0.111**	0.094	**−0.129**	**−0.109**	0.069	**−0.125**	0.081	**−0.128**	0.095
C6	0.017	0.005	**−0.258**	0.020	0.006	**−0.208**	0.017	**−0.226**	0.020	**−0.286**
C7	0.015	0.017	−0.468	0.014	0.017	−0.483	−0.761	−0.506	0.009	−0.492
C8	0.058	0.038	−0.074	0.058	0.038	−0.118	0.056	−0.126	0.056	−0.083
C9	**−0.154**	**−0.132**	−0.004	**−0.154**	**−0.131**	0.024	**−0.148**	0.030	**−0.147**	0.005
C10	0.022	0.000	−0.124	0.023	0.001	−0.146	0.017	−0.151	0.015	−0.128
C11	−0.131	−0.092	0.050	−0.130	−0.09	0.071	−0.118	0.073	−0.117	0.052

Bold indicates the larger changes in properties associated with the atom positions.



A1: X = N, R1 = R2 = H; A2: X = N, R1 = -CH_3, R2 = H
A3: X = CH, R1 = R2 = H; A4: X = CH, R1 = H, R2 = -OCH_3

Chart 3. Resonant structures for AOH$^-$ photoproducts anions of aza-OIAs and OIAs.

see Table 2. In contrast, for the 2,3-dh-OIAs, protonation at the N1 atom is far more favorable (≈10 kcal mol^{-1}) [14,15] than at the carbonyl O-atom giving the respective AN1H$^-$ as the photoreduction products which cannot locate charges at positions 4 or 6 and hence exchange H^+ by D^+ there.

4. Conclusions

Our current findings show that, for the aza-OIAs and OIAs the formation of the metastable photoproducts hydrogenated at the carbonyl O-atom, AOH$^-$, explain the observed H/D isotopic exchange through a simple aromatic electrophilic substitution of the photoproducts, during storage in the dark, in the presence of D_2O. These mild conditions contrast with the use of catalysts, strong acidic or basic media and gaseous deuterium/hydrogen at high temperatures and pressures [1–6]. The efficient H/D exchange we are reporting might be advantageously used to introduce regioselectively deuterium, and likely tritium, in positions 4 and 6, of the oxoisoaporphines and aza-oxoisoaporphines. On the other hand, results show that the aza-OIAs photoreduction mechanism follows essentially the same stepwise electron–proton–electron transfer photoreduction mechanisms proposed earlier for the previously studied oxoisoaporphine derivatives [14–17].

It is noteworthy that many derivatives of OIAs and aza-OIAs, synthesized as non-cardiotoxic alternatives of anthraquinone antitumor drugs have been studied, showing antineoplastic activity and cytotoxicity against different tumoral cell lines [22–24,27–29]. Therefore, the regioselective isotopic labelling reported here may prove useful for the preparation of these compounds for use as tracers in biomedical studies or as internal standards in mass spectroscopy.

From the present results, it is possible to propose a simple method for isotopic labelling, at positions 4 and 6, of these compounds by the following sequence: (a) photoreduction in the presence of tertiary aliphatic amines; (b) D_2O or T_2O addition; (c) storage in the dark for several days; and finally (d) separation of deuterated or tritiated products.

Acknowledgments

We thank FONDECYT grants Nos. 1070623 and 1100121 for the financial support, to CEPEDEQ of Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, for the use of mass and NMR facilities. C.A. thanks CONICYT No. 24091001 and Ph.D. fellowships. E. S-S. thanks to Isidro Parga Pondal fellowship from Xunta de Galicia, Spain. We thank Prof. Gordon L. Hug for the critical reading of this manuscript.

References

[1] J. Atzrodt, V. Derdau, T. Fey, J. Zimmermann, The renaissance of H/D exchange, Angew. Chem. Int. Ed. 46 (2007) 7744–7765.
[2] C.M. Yung, M.B. Skaddan, R.G. Bergman, Stoichiometric and catalytic H/D incorporation by cationic iridium complexes: a common monohydrido-iridium intermediate, J. Am. Chem. Soc. 126 (2004) 13033–13043.
[3] M.B. Skaddan, C.M. Yung, R.G. Bergman, Stoichiometric and catalytic deuterium and tritium labeling of "unactivated" organic substrates with cationic Ir(III) complexes, Org. Lett. 6 (2004) 11–13.
[4] V. Derdau, J. Atzrodt, J. Zimmermann, C. Kroll, F. Bruckner, Hydrogen–deuterium exchange reactions of aromatic compounds and heterocycles by NaBD4-activated rhodium, platinum and palladium catalysts, Chem. Eur. J. 15 (2009) 10397–10404.
[5] V. Derdau, J. Atzrodt, W. Holla, H/D-exchange reactions with hydride-activated catalysts, J. Labelled Compd. Radiopharm. 50 (2007) 295–299.
[6] A. Martins, M. Lautens, A simple, cost-effective method for the regioselective deuteration of anilines, Org. Lett. 10 (2008) 4351–4353.

[7] R.E. London, S.A. Gabel, Photoactivated H/D exchange in tyrosine: involvement of a radical anion intermediate, J. Am. Chem. Soc. 128 (2006) 2268–2275.
[8] I. Saito, H. Sugiyama, A. Yamamoto, S. Muramatsu, T. Matsuura, Photoinduced reactions. 158. Photochemical hydrogen–deuterium exchange reaction of tryptophan. The role of nonradiative decay of singlet tryptophan, J. Am. Chem. Soc. 106 (1984) 4286–4287.
[9] R. Mosi, G.Z. Zhang, P. Wan, Enhanced basicity of the 2-position of 1,3-dialkoxybenzenes in S-1 – acid-catalyzed photochemical proton deuteron exchange, J. Org. Chem. 60 (1995) 411–417.
[10] C. Sabot, K.A. Kumar, C. Antheaume, C. Mioskowski, Triazabicyclodecene: an effective isotope exchange catalyst in $CDCl_3$, J. Org. Chem. 72 (2007) 5001–5004.
[11] L.A. Huck, P. Wan, Photochemical deuterium exchange of the m-methyl group of 3-methylbenzophenone and 3-methylacetophenone in acidic D_2O, Org. Lett. 6 (2004) 1797–1799.
[12] Q. Chen, W.J. Walczak, M.D. Barkley, Excited-state proton transfer from solvent to aromatic carbons in tomaymycin, Am. Chem. Soc. 117 (1995) 556–557.
[13] M. Lukeman, P. Wan, A new type of excited-state intramolecular proton transfer: proton transfer from phenol OH to a carbon atom of an aromatic ring observed for 2-phenylphenol1, J. Am. Chem. Soc. 124 (2002) 9458–9464.
[14] J.R. De la Fuente, V. Neira, C. Saitz, C. Jullian, E. Sobarzo-Sánchez, Photoreduction of oxoisoaporphine dyes by amines: transient-absorption and semiempirical quantum-chemical studies, J. Phys. Chem. A 109 (2005) 5897–5904.
[15] J.R. De la Fuente, C. Jullian, C. Saitz, E. Sobarzo-Sánchez, V. Neira, C. González, et al., Photoreduction of oxoisoaporphines. Another example of a formal hydride-transfer mechanism, Photochem. Photobiol. Sci. 3 (2004) 194–199.
[16] J.R. De la Fuente, C. Aliaga, C. Poblete, G. Zapata, C. Jullian, C. Saitz, et al., Photoreduction of oxoisoaporphines by amines: laser flash photolysis, pulse radiolysis and TD-DFT studies, J. Phys. Chem. A 113 (2009) 7737–7747.
[17] J.R. De la Fuente, G. Kciuk, E. Sobarzo-Sánchez, K. Bobrowski, Transient phenomena in the pulse radiolysis of oxoisoaporphine derivatives in acetonitrile, J. Phys. Chem. A 112 (2008) 10168–10177.
[18] L.Y.C. Lee, X. Ci, C. Giannotti, D.G. Whitten, Photoinduced carbon–carbon bond cleavage via electron-transfer reactions: visible-light-mediated scission of tertiary amines, J. Am. Chem. Soc. 108 (1986) 175–177.
[19] H. Gan, X. Zhao, D.G. Whitten, Amine photoredox reactions: a photoinduced methylene shuttle initiated via two-electron oxidation of a tertiary amine by anthraquinone, J. Am. Chem. Soc. 113 (1991) 9409–9411.
[20] G. Pandey, S.R. Gadre, Sequential two electron photooxidation of t-amines: generation of a regiospecific iminium cation and its application in organic synthesis, Arkivoc (2003) 45–54.
[21] J.R. De la Fuente, C. Jullian, C. Saitz, V. Neira, O. Poblete, E. Sobarzo-Sánchez, Unexpected formation of 1-diethylaminobutadiene in photosensitized oxidation of triethylamine induced by 2,3-dihydro-oxoisoaporphine dyes. A ^{1}H-NMR and isotopic exchange study, J. Org. Chem. 70 (2005) 8712–8716.
[22] B. Stefanska, M. Dzieduszycka, M.M. Bontemps-Gracz, E. Borowski, S. Martelli, R. Supino, et al., 8,11-dihydroxy-6-[(aminoalkyl)amino]-7H-benzo[*e*]perimidin-7-ones with activity in multidrug-resistant cell lines: synthesis and antitumor evaluation, J. Med. Chem. 42 (1999) 3494–3501.
[23] X. Bu, J. Chen, L.W. Deady, C.L. Smith, B.C. Baguley, D. Greenhalgh, et al., Synthesis and cytotoxic activity of N-[(alkylamino)alkyl]carboxamide derivatives of 7-oxo-7H-benz[de]anthracene, 7-oxo-7H-naphtho[1,2,3-de]quinoline, and 7-oxo-7H-benzo[*e*]perimidine, Bioorg. Med. Chem. 13 (2005) 3657–3665.
[24] X. Bu, L.W. Deady, G.J. Finlay, B.C. Baguley, W.A. Denny, Synthesis and cytotoxic activity of 7-Oxo-7H-dibenz[f,ij]isoquinoline and 7-Oxo-7H-benzo[*e*]perimidine derivatives, J. Med. Chem. 44 (2001) 2004–2014.
[25] J.R. De la Fuente, A. Cañete, A.L. Zanocco, C. Saitz, C. Jullian, Formal hydride transfer mechanism for photoreduction of 3-phenylquinoxalin-2-ones by amines. Association of 3-phenylquinoxalin-2-one with aliphatic amines, J. Org. Chem. 65 (2000) 7949–7958.
[26] J.R. De la Fuente, A. Cañete, C. Saitz, C. Jullian, Photoreduction of 3-phenylquinoxalin-2-ones by amines: transient-absorption and semiempirical quantum-chemical studies, J. Phys. Chem. A 106 (2002) 7113–7120.
[27] M. Dzieduszycka, S. Martelli, M. Arciemiuk, M.M. Bontemps-Gracz, A. Kupiec, E. Borowski, Effect of modification of 6-[(Aminoalkyl)amino]-7H-benzo[*e*]-perimidin-7-ones on their cytotoxic activity toward sensitive and multidrug resistant tumor cell lines. Synthesis and biological evaluation, Bioorg. Med. Chem. 10 (2002) 1025–1035.
[28] B. Stefanska, M. Dzieduszycka, S. Martelli, J. Tarasiuk, M. Bontempsgracz, E. Borowski, 6-[(Aminoalkyl)amino]-substituted 7h-benzo[*E*]perimidin-7-ones as novel antineoplastic agents – synthesis and biological evaluation, J. Med. Chem. 36 (1993) 38–41.
[29] I.V. Borovlev, O.P. Demidov, A.V. Aksenov, A.F. Pozharskii, Heterocyclic analogs of pleiadiene: LXXIV. peri-cyclizations in the perimidine series. Synthesis of 1,3-diazapyrene derivatives, Russ. J. Org. Chem. 40 (2004) 895–901.