A kinetic study of the reaction between boldine and singlet oxygen [$O_2(^1\Delta_g)$]

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The sensitized photo-oxidation of boldine and its permethylated derivative, glaucine, has been studied in several solvents using steady-state kinetic methods. Values of k^B, the total rate constant for the reaction between boldine and $O_2(^1\Delta_g)$, vary from 3.44×10^7 M^{-1} s^{-1} in benzene to 150.00×10^7 M^{-1} s^{-1} in water. For glaucine, similar values were determined. Quenching is faster in solvents with high capacity to stabilize a charge or dipole (higher π^* parameter values). From these results, a charge-transfer quenching mechanism, to account for the interaction between boldine and $O_2(^1\Delta_g)$, is proposed. Rate constants for the chemical reaction are at least two orders of magnitude smaller than the total rate constants, which also show a large dependence on the π^* parameter. This last result can be explained in terms of a more polar transition state leading to products.

Introduction

In the last 10 years, several papers concerning the reactivity of a variety of naturally occurring molecules towards singlet molecular oxygen [$O_2(^1\Delta_g)$], have been reported. The great interest in investigating these reactions is due to the recognized participation of this active species of oxygen in pathological processes in living organisms.[1–10]

Boldo [*Peumus boldus* Molina], a shrub from Chile, grows along the Cordillera de la Costa from Aconcagua to Osorno [33 to 39° south]. Boldo leaves contain roughly 0.1% boldine, this being the principal alkaloid present. Boldo bark contains about eight-fold more boldine than boldo leaves and should be used as the starting material for this and other related alkaloid extraction.[11,12] Boldo as an extract, tincture or purified boldine has been used for a century in South America for hepatic disease therapy including lithiasis, diuresis, digestive disorders and cholagogue.[13–16]

Chemically, boldine corresponds to 1,10-dimethoxyaporphine-2,9-diol and was the first isolated alkaloid from *Peumus boldus*. More recently, the effect of boldine in preventing autoxidation of rat brain homogenates,[17] lysozyme inactivation induced by the thermolysis of 2,2-diamidino-2,2′-azopropane),[17] oxidation of fish oil,[18] and inhibition of rat liver microsomal lipid peroxidation[19] have been assessed. These studies reveal the ability of boldine to act as a scavenger of carbon- and oxygen-centred radicals. However, its reactivity towards other active species of oxygen, such as singlet molecular oxygen, has not been evaluated.

In this study, we report on the kinetic results obtained from a study of the sensitized photo-oxidation of boldine and its permethylated derivative, glaucine, using steady-state kinetic methods.[20,21] Furthermore, solvent effect correlation is employed to propose a mechanism for the reaction.

Experimental

The compounds boldine and glaucine (Sigma Chemical Co.), methylene blue, rubrene, 1,3-diphenylisobenzofuran, 9,10-dimethylanthracene and 9-anthrylmethanol (Aldrich Chemical Co), sodium azide, sodium acetate, sodium chloride, phosphate buffer and acetic acid (Merck) were used without further purification. Eosin Y (Hopkins and Williams) and Rose Bengal (Fluka) were recrystallized from dichloromethane and ethanol, respectively. All solvents used (Merck) were of spectroscopic quality.

UV–VIS absorption spectra and kinetic experiments were performed using a Unicam UV-4 spectrophotometer interfaced with a DTK personal computer. The cell holder was maintained at 25 ± 0.5 °C by circulating water from a Haake thermoregulated bath. Emission spectra and fluorescence measurements were performed in a Perkin-Elmer LS 50B luminescence spectrometer interfaced with a DTK personal computer.

Steady-state photolysis was performed in a double wall cell of 10 ml capacity, light-protected by black paint. A centred window allows irradiation with light of a determined wavelength by using a Shott cut-off filter. Water circulating around the cell allowed cell temperature to be maintained at 25 ± 0.5 °C.

Rubrene ($\lambda_{max} = 520$ nm), Rose Bengal ($\lambda_{max} = 557$ nm) and Eosin Y ($\lambda = 522$ nm) were employed as sensitizers. Illumination was performed with a visible, 150 W, Par lamp. The distance between the light source and cell was set for each experiment so that the initial substrate concentration diminished by *ca*. 40% in 20 min.

Boldine consumption rate constants were evaluated employing a Waters HPLC system equipped with a diode array detector and a Zorbax ODS 4.6 × 150 mm column. Elution of samples was performed in linear gradient mode using methyl alcohol and phosphate buffer 0.025 M as a mobile phase. 1,3-Diphenylisobenzofuran was employed to evaluate the steady-state concentration of $O_2(^1\Delta_g)$. Daily prepared in a dark room, 1,3-diphenylisobenzofuran solutions and an appropriate cut-off filter were used in these experiments. Autoxidation of this compound, measured using UV–VIS spectrophotometry, was lower than 1% under our experimental conditions.

Results and discussion

In polar solvents, the boldine photo-oxidation was performed employing Rose Bengal as the sensitizer. In these experiments the rate constant for the reaction of boldine with $O_2(^1\Delta_g)$ was determined using the competitive reaction method, by observing the decrease in the consumption rate of 9,10-dimethylanthracene (measured spectrophotometrically at 393 nm) by addition of variable quantities of boldine in the milimolar range. Eqns. (1)–(6) describe the most significant chemical process associated with the system.

Table 1 Rate constants for quenching of singlet oxygen by boldine in several solvents[a]

Solvent	ε^{27}	$k^B/10^7$ M^{-1} s^{-1}	π^*	δ	α	β
Benzene[b]	2.27	3.44	0.59	1.0	0.00	0.10
Chloroform[b]	4.81	2.90	0.58	0.5	0.44	0.00
Ethyl acetate	6.02	3.70	0.55	0.0	0.00	0.45
Isopropyl alcohol[c]	19.92	1.90	0.48	0.0	0.95	0.76
Acetone[b,c]	20.56	5.80	0.71	0.0	0.08	0.48
Ethyl alcohol[c]	24.55	2.44	0.54	0.0	0.83	0.77
Methyl alcohol[b]	32.66	1.80	0.60	0.0	0.93	0.62
Acetonitrile[b]	35.94	5.00	0.75	0.0	0.19	0.31
Dimethylformamide[b]	36.71	25.00	0.88	0.0	0.00	0.69
Water[d]	78.30	150.00	1.09	0.0	1.17	0.18

[a] Solvent parameters were taken from ref. 30. [b] Rubrene. [c] Rose Bengal–9,10-dimethylanthracene. [d] Eosin Y–anthracen-9-ylmethanol.

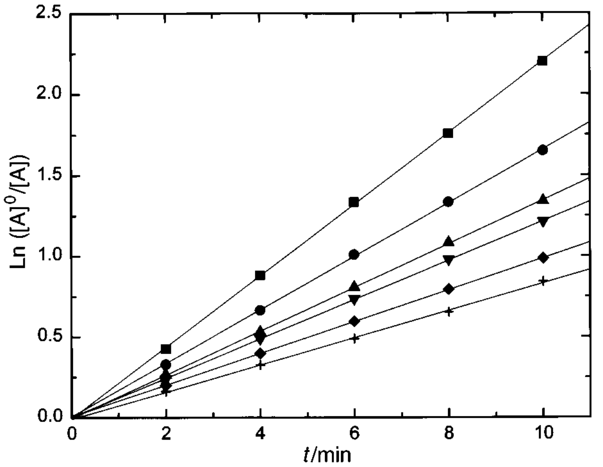


Fig. 1 Photobleaching of 9,10-dimethylanthracene by boldine. Solvent: acetonitrile; [Boldine]: ■ 0.0 mM; ● 0.75 mM; ▲ 1.49 mM; ▼ 2.24 mM; ◆ 3.00 mM; +4.50 mM.

$$\text{Sens} + h\nu \xrightarrow{I_a} \text{Sens}^* \tag{1}$$

$$\text{Sens} + O_2(^3\Sigma_g) \xrightarrow{k_\Delta} O_2(^1\Delta_g) + \text{Sens} \tag{2}$$

$$O_2(^1\Delta_g) \xrightarrow{k_d} O_2(^3\Sigma_g) \tag{3}$$

$$O_2(^1\Delta_g) + \xrightarrow{k^A} AO_2 \tag{4}$$

$$O_2(^1\Delta_g) + B \xrightarrow{k_r^B} \text{Products} \tag{5}$$

$$O_2(^1\Delta_g) + B \xrightarrow{k_q^B} O_2(^3\Sigma_g) + B \tag{6}$$

Reactions (3)–(6) describe the main molecular singlet oxygen deactivation paths, where k_d accounts for the rate constant of excited oxygen deactivation by solvent, k^A is the rate constant for the reaction between singlet oxygen and the anthracene derivative, k_r^B is the rate constant for the chemical reaction between boldine and $O_2(^1\Delta_g)$ and k_q^B is the rate constant for the physical quenching of singlet oxygen by boldine.

Applying the steady-state approach to the singlet oxygen concentration in reactions (1)–(6), expressions for the rate of anthracene derivative consumption in the absence and presence of boldine can be obtained. Furthermore, from the literature it is found that k_d takes values between (0.15–2.0) × 10^4 s^{-1}[22] and k^A is *ca.* 10^7 M^{-1} s^{-1},[23,24] then, if the anthracene derivative concentration is *ca.* 10^{-5} M, $k_d \gg k_r^A[A]$, pseudo-first-order conditions are fulfilled. Results obtained according to the above treatment are plotted in Fig. 1, when acetonitrile is used as the solvent. As is observed in this plot, boldine addition diminished

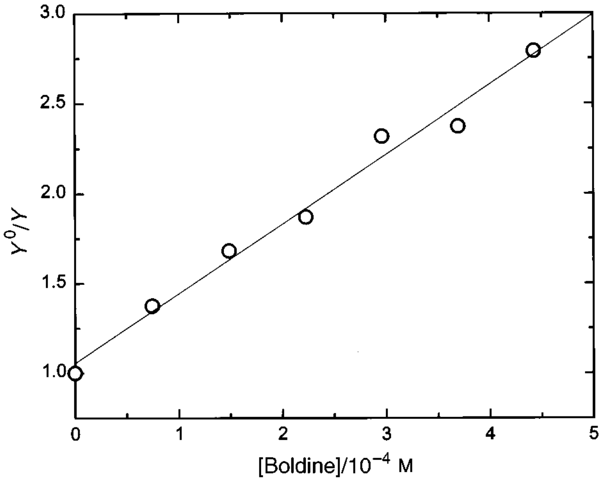


Fig. 2 Plot of $Y°/Y$ as a function of boldine concentration according to eqn. (7)

noticeably the consumption rate of 9,10-dimethylanthracene. From the ratio between the slope of the curve in the absence of boldine ($Y°$) and the slope at a given boldine concentration (Y) an expression to determine k^B is obtained [eqn. (7)], where [B] is

$$\frac{Y°}{Y} = 1 + k^B \tau_{O_2(^1\Delta_g)}[B] \tag{7}$$

the initial boldine concentration, k^B is the total rate constant for the reaction between $O_2(^1\Delta_g)$ and boldine, $k^B = k_r^B + k_q^B$ and $\tau_{O_2(^1\Delta_g)} = k_d^{-1}$. Typical results plotted according to eqn. (7) are presented in Fig. 2. Values of k^B obtained in several solvents by this procedure are summarized in Table 1. In non-polar solvents rubrene was used as the sensitizer. In these experiments values of k^B were determined by observing the inhibition of the rubrene autoxidation rate at 520 nm. In this case the kinetics do not correspond to first order and the rate constants were obtained from the initial rates of rubrene consumption[25] according to eqn. (8), where $[R]_0$ and [B] are the initial rubrene

$$k^B = \frac{k_d + k_{ox}[R]_0}{[B]}\left[\frac{(\partial[R]/\partial t)_i}{(\partial[R]/\partial t)_i^B} - 1\right] \tag{8}$$

and boldine concentrations, $(\partial[R]/\partial t)_i$ and $(\partial[R]/\partial t)_i^B$ are the initial consumption rates of rubrene in the absence and presence of boldine, respectively. Fig. 3 shows a decrease in rubrene concentration with irradiation time for experiments performed at different boldine concentrations.

Values of k^B obtained in different solvents by these methods are given in Table 1.

When buffered water was the solvent, Rose Bengal was not an appropriate sensitizer. Rose Bengal forms an interaction complex with boldine as was observed by a remarkable reduc-

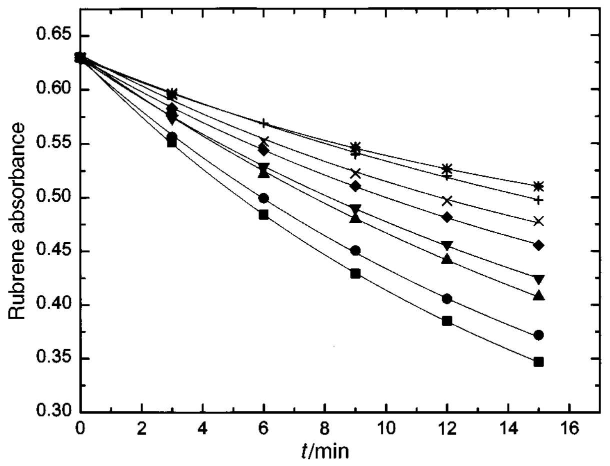


Fig. 3 Dependence of the autoxidation rate of rubrene with boldine concentration. [Boldine]: ■ 0.0 mM; ● 1.517 mM; ▲ 3.023 mM; ▼ 4.516 mM; ◆ 7.469 mM; × 8.928 mM; +11.810 mM; * 15.160 mM.

tion in the absorption maxima in the visible spectra of the dye. Therefore, in this solvent, eosin Y was employed as sensitizer, anthracen-9-ylmethanol was used instead of 9,10-dimethylanthracene and the consumption rate of the anthracene derivative was measured by the decrease in the fluorescence intensity. The k^B value measured in water is included in Table 1.

Regardless of the sensitizer employed to evaluate k^B, these methods are applicable only if boldine does not quench the sensitizer excited states, singlet or triplet under the experimental conditions employed.[26] We can disregard this possibility for the following reasons: (*i*) k^B values were found to be independent of 9,10-dimethylanthracene concentration; (*ii*) linear Stern–Volmer-type plots were obtained over a wide range of boldine concentrations; (*iii*) data obtained in double quenching experiments (using sodium azide) were compatible with those expected from a competition for $O_2(^1\Delta_g)$; (*iv*) the value of the ratio between the experimental rate constants for anthracene derivative bleaching in equivalent mixtures of acetonitrile–deuterium oxide and acetonitrile–water, determined in the absence of boldine, $(k_D/k_H)^\circ$, was very close to the value of the same parameter obtained in the presence of boldine, $(k_D/k_H)^B$, excluding secondary reactions that modify the steady-state singlet oxygen concentration; (*vi*) the boldine concentration employed was relatively low (<50 mM) and no significant quenching of the singlet excited state of the dye was detected.

The results summarized in Table 1 show that the rate constant is strongly solvent-dependent, increasing from 3.44×10^7 M^{-1} s^{-1} in benzene to 1.50×10^9 M^{-1} s^{-1} in water, the latter being close to the diffusion control limit. The k^B values are about one order of magnitude lower than those reported for other alkaloid compounds such as strychnine[27] and brucine,[28] showing that boldine is a powerful physical quencher of singlet oxygen. Values in chloroform, compare favourably with that previously reported by Larson and Marley.[28] Nevertheless, k^B dependence on the solvent is not simply related to macroscopic parameters such as relative permittivity ε_r, *e.g.* in chloroform ($\varepsilon_r = 4.81$), the k^B value is lower than that observed in benzene ($\varepsilon_r = 2.27$).

In order to obtain further details of the microscopic singlet oxygen–boldine interactions, solvent effect correlations employing a generalized solvatochromic equation[29,30] were performed.

Microscopic solvent parameters corresponding to the solvents in which values of k^B were measured are included in Table 1. Multiparametric correlation of the data, gives eqn. (9) that relates the dependence of k^B on the solvent parameters.

$$\ln k^B = 12.74 + 7.26\,(\pi^* + 0.043\delta) - 0.10\alpha + 0.22\beta \quad (9)$$

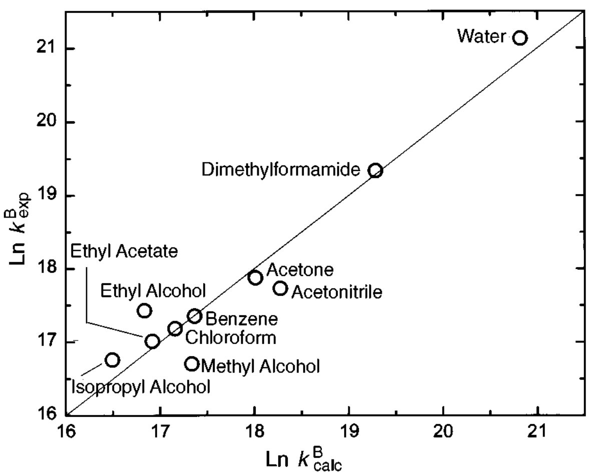


Fig. 4 Logarithmic plot of the experimental total rate constant k^B *vs.* theoretical rate constant $k_{calc}{}^B$ (calculated according to solvatochromic equation) for $O_2(^1\Delta_g)$ quenching by boldine

Table 2 Rate constants for quenching of singlet oxygen by glaucine and boldine in several solvents

Solvent	$k^G/10^7$ M^{-1} s^{-1}	$k^B/10^7$ M^{-1} s^{-1}
Isopropyl alcohol[a]	0.54	1.90
Ethyl alcohol[a]	0.84	2.44
Methyl alcohol[b]	1.80	1.80
Acetonitrile[b]	8.80	5.0
Dimethylformamide[b]	68.00	25.0

[a] Rose Bengal–9,10-dimethylanthracene. [b] Rubrene.

The independent coefficient that appears in eqn. (9) corresponds to the ln k^B value in cyclohexane, the reference solvent for the solvatochromic parameters employed.[30] Furthermore, eqn. (9) clearly shows that the dominant factor in determining the k^B value corresponds to the solvatochromic parameter π^*. If only π^* and δ parameters are included in the correlation, the independent coefficient for π^* is 7.13.

In Fig. 4, a plot of the logarithm of experimental k^B value *vs.* the logarithm of k^B computed according to eqn. (9) is shown. A good fit is found between experimental and computed values, a result that clearly indicates the significance of π^* in the effect of the solvent on the reaction rate. If the boldine structure is considered, it is possible to visualize three different reaction sites for the boldine–singlet oxygen interaction: (*i*) direct abstraction of one of the phenolic hydrogens;[31] (*ii*) charge transfer complex formation by interaction with the cyclic amine;[21,32] (*iii*) charge transfer complex formation by interaction with the aromatic system in a Diels–Alder type reaction leading to cyclic peroxides or hydroperoxides.[33]

The minor importance of the α parameter in eqn. (9) allows us to disregard the interaction with the tertiary amine group[31] in spite of k^B values that are in the same order as the rate constants reported for the reaction between cyclic amines and $O_2(^1\Delta_g)$.[32] Furthermore, k^B dependence on solvent polarity is appreciably larger than expected for a Diels–Alder type reaction.[34]

With the purpose of evaluating the relevance of the phenolic hydrogens in the boldine–singlet oxygen interaction, the rate constants, k^G, for the reaction between singlet oxygen and glaucine were measured in several solvents. Results obtained are given in Table 2.

The values of k^G are clearly dependent upon the solvent in a similar way to that observed for boldine. Moreover, the dependence of k^G on solvent parameters was also very similar to that observed for k^B, with a value of 12.3 for the polarity–polarizability coefficient. These results, combined with the similarity between the values of k^B and k^G, allows us to disregard the contribution of phenolic hydrogens in the transition

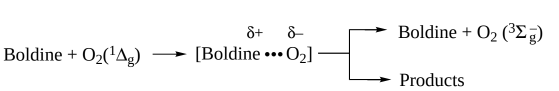
Table 3 Values of k_r^B and k_r^G in several solvents[a]

Solvent	$k_r^B/10^5$ M^{-1} s^{-1}	$k_r^G/10^5$ M^{-1} s^{-1}	π*
1-Propyl alcohol[b]	0.54	1.7	0.48
Ethyl alcohol[b]	1.8	—	0.54
Methyl alcohol[c]	3.0	4.8	0.60
Acetone[b,c]	4.2	—	0.71
Benzene[c]	6.0	—	0.59
Acetonitrile[c]	30.0	—	0.75
Dimethylformamide[c]	130.0	125.0	0.87

[a] Solvent parameters were taken from ref. 30. [b] Rose Bengal–9,10-dimethylanthracene. [c] Rubrene.

state formation for the reaction between boldine and singlet oxygen.

The similarity between k^B and k^G, their strong dependence on the π* parameter and low values associated with the coefficients of α and β, provide meaningful support to the postulate that in the reaction between boldine (or glaucine) and $O_2(^1\Delta_g)$ a charge transfer complex is formed resulting from the interaction between the aromatic rings and the excited oxygen. The complex can later either transfer the electron back, giving ground-state oxygen and boldine, or combine to yield products (Scheme 1).



Scheme 1

As was stated above, k^B includes both the contribution from the physical quenching of $O_2(^1\Delta_g)$, k_q^B and the one corresponding to the chemical reaction between boldine and singlet oxygen, k_r^B. To assess the contribution of the latter process to the total rate, k_r^B was measured in several solvents, observing boldine consumption by HPLC techniques. Under the conditions employed, the reaction follows pseudo-first-order kinetics. From the slope of the pseudo-first-order plots and using 1,3-diphenylisobenzofuran as actinometer, values of k_r^B were obtained. These results and those obtained for glaucine in similar experiments are given in Table 3.

The results summarized in Table 3 show that k_r^B and k_r^G are at least two orders of magnitude smaller than the total rate constant, nevertheless in solvents with larger π* values, *e.g.* dimethylformamide, the contribution of the chemical process to the total rate is *ca.* 5%. If the solvatochromic equation is applied to the data obtained for boldine, the dependence of k_r^B on π* parameters follows the same pattern as the one observed for k^B. However, the corresponding coefficient is about twice as great. This result can be explained in terms of a more polar transition state in the reaction path leading to the products.

Conclusions

Data obtained in this study reveal that boldine and its permethylated derivative, glaucine, are very effective quenchers for excited oxygen, particularly in polar solvents. This fact, in addition to the recognized ability of these natural compounds to act as scavengers for oxygen- or carbon-centred radicals, and their small systemic toxicity[35] bestow on boldine and their analogues remarkable characteristics which prevent photochemical and thermal oxidative damage in biological systems.

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