# Reaction of singlet molecular oxygen, $O_2(^1\Delta_g)$, with the *Cinchona* tree alkaloids
# Effect of absolute configuration on the total rate constant

Else Lemp, Germán Günther, Rafael Castro, Manuel Curitol, Antonio L. Zanocco *

*Universidad de Chile, Facultad de Ciencias Químicas y Farmacéuticas, Departamento de Química Orgánica y Fisicoquímica, Olivos 1007, Casilla 233, Santiago, Chile*

**Abstract**

Detection of $O_2(^1\Delta_g)$ emission, $\lambda_{max} = 1270$ nm, following laser excitation and steady-state methods were employed to measure the total reaction rate constant, $k_T$, and the reactive reaction rate constant, $k_R$, for the reaction between singlet oxygen and the *Cinchona* tree alkaloids, cinchonidine, cinchonine, quinine and quinidine in several solvents. In most solvents, the $k_T$ values were close to $10^7$ $M^{-1}$ $s^{-1}$, indicating that these compounds are good singlet oxygen quenchers. The reactive rate constants are smaller than $10^4$ $M^{-1}$ $s^{-1}$, implying that quenching is essentially a physical process. The analysis of solvent effect on $k_T$ by using LSER equations indicates that singlet oxygen deactivation by these drugs is accelerated by solvents with large $\pi^*$ and $\beta$ values, being inhibited by hydrogen bond donor (HBD) solvents. Correlations employing theoretical solvent parameters, TLSER, give similar results. These data support the formation of an exciplex with charge transfer character, resulting from the singlet oxygen electrophilic attack on the quinuclidine moiety nitrogen. In most solvents, cinchonidine is more reactive than cinchonine and quinine is more reactive than quinidine although reactivity differences are small and only in a few solvents $k_T$ values of the *S*,*R*-isomer are about twice than those of *R*,*S*-isomer. The higher reactivity of *S*,*R*-isomers in these solvents is explained by the geometrically favorable intra-exciplex stabilizing interaction between the non-bonded pernitrone oxygen and the hydrogen of the hydroxyl substituent at C(9).

*Keywords:* Antimalarial drugs; *Cinchona* tree alkaloids; Singlet oxygen; Solvent effect; LSER; Time resolved kinetics

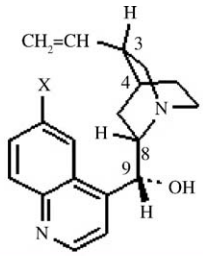
## 1. Introduction

Linear solvation energy relationship, LSER, and theoretical linear solvation energy relationship, TLSER, are very useful frameworks to analyze solvent effects on singlet oxygen reactions with several kinds of molecules. Treatments allow quantitative evaluation of solvent effects and are helpful tools for interpreting the mechanism of the process. These equations have also been employed to determine the main reaction center in polyfunctional compounds, to detect changes in the reaction mechanism with solvent properties and to evaluate the relative contribution of tautomers in equilibrium to the total reaction rate [1,2].

*Cinchona* alkaloids, the natural compounds extracted from barks of *Cinchona* tree have an ancient therapeutic heritage [3]. They have multiple biological activities, among them, the widely known antimalarial and antiarrhytmic properties. The most abundant constituents of *Cinchona* bark are the so-called *erythro* isomers: cinchonidine (**1**), quinine (**2**), cinchonine (**3**) and quinidine (**4**), which differ from each to other by the absence or presence of the methoxy substituent in the quinoline moiety and/or in the absolute configuration of two carbon atoms, C(8) and C(9), while the configuration of C(3) and C(4) is the same for all the alkaloids of this group.

* Corresponding author. Tel.: +56 2 6782878; fax: +56 2 6782809.
*E-mail address:* azanocco@ciq.uchile.cl (A.L. Zanocco).

Until relatively recently, quinine was the only remedy to treat malaria, the most awful disease for world civilization over the past three centuries [4], which caused the death of millions of peoples (over 1.1 million in 2001) [5]. Compounds (**1**)–(**4**) are antimalarials of comparable activity, unlike their C(9) epimers that are practically inactive. Since the beginning of the last century, synthetic antimalarials have also been employed to treat the disease. Most antimalarials have pharmacological activities beyond those for treating malaria, consequently a number of them have been used with several degrees of success to treat medical conditions other than malaria including lupus erythematosus, polymorphous light eruption, cutaneous lymphoma, and rheumatoid arthritis [6]. The majority of antimalarials derived from quinoline possessed undesirable photosensitizing properties that produce toxic side effects both in the skin and the eye [7–9]. Cutaneous and ocular effects, probably caused by light, include changes in the skin pigmentation, corneal opacity, cataract formation and other visual disturbances, including irreversible retinal damage that may lead to blindness [6]. The precise mechanisms for these reactions in humans remain unknown, although singlet molecular oxygen, $O_2(^1\Delta_g)$, and free radicals including superoxide/hydroperoxyl or peroxyl adduct, carbon- and nitrogen-centered radicals have been invoked as responsible of these phototoxic effects [10–14]. On the other hand, it is well known that singlet molecular oxygen ($^1\Delta_g$) reactions are important in biological systems, where it can play deleterious (damaging valuable biomolecules) and/or beneficial roles (photodynamic therapy of cancer) [15,16]. Furthermore, the relevance of the singlet oxygen-mediated photosensitizing effects of the antimalarials will be related to the efficiency with which the drug produces $O_2(^1\Delta_g)$ and/or to the reactivity of the molecule towards this active species of oxygen. Bimolecular rate constants for quenching of singlet oxygen by antimalaric drugs have been determined in $D_2O$ at pD = 7.4 by Motten et al. [14] and by Zanocco and coworkers in several solvents [17]. In view of the current interest in antimalarial drugs photoreactions due to their photosensitizing properties and the possible role of the singlet molecular oxygen to generate photooxidation products from the drugs that can also be photochemically active, we want to determine how reactive these compounds may be with singlet oxygen. If the structures of antimalarial drugs included in Fig. 1 are analyzed, several reactive centers can be visualized: the aromatic ring, the quinoline nitrogen and the bicyclic nitrogen that can interact with $O_2(^1\Delta_g)$. In addition, if the reactive center is the bicyclic nitrogen, the absolute configuration of the two carbon atoms, C(8) and C(9) could affect substrate reactivity towards singlet oxygen. In this study, we report on the LSER and TLSER analysis of the reactions of *Cinchona* tree alkaloids with singlet oxygen to determine if this framework provides information about the influence of carbon configuration in the vicinity of the reactive centre on substrate reactivity.




| Compound | Absolute Configuration | | X |
|---|---|---|---|
| | C(8) | C(9) | |
| Cinchonidine (1) | S | R | H |
| Quinine (2) | S | R | $OCH_3$ |
| Cinchonine (3) | R | S | H |
| Quinidine (4) | R | S | $OCH_3$ |

Fig. 1. Molecular structures of the principal *Cinchona* tree alkaloids.

## 2. Experimental

Cinchonidine hydrochloride and cinchonine hydrochloride (Sigma), 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine (TPP), 9,10-dimethylanthracene (DMA) and rubrene (Aldrich) were used without further purification. Rose Bengal (Fluka) was recrystallized from ethanol prior to use. All solvents (Merck) were of spectroscopic or HPLC grade. Quinine and quinidine (Sigma) were recrystallized from ethanol before used.

Free bases of cinchonidine and cinchonine were obtained by dissolving the corresponding salt in water, followed by addition of 10% NaOH until pH 12 and several extractions with chloroform or diethylether. The organic phase was dried over anhydrous sodium sulphate and the solvent removed. Both compounds were purified by at least three recrystallizations from ethanol. Purity of the free bases was assessed by its melting points, $^1$H NMR spectrum and GC-NPD chromatogram.

The measurements of total quenching rate constants, $k_T$, by time-resolved experiments were carried out observing the phosphorescence of $O_2(^1\Delta_g)$ at 1270 nm. TPP was irradiated by the 500 ps light pulse of a PTI model PL-202 dye laser (414 nm, ca. 200 μJ per pulse). When Rose Bengal was used as sensitizer, samples were excited with the second harmonic (532 nm, ca. 9 mJ per pulse) of 6 ns light pulse of a *Quantel Brilliant* Q-Switched Nd:YAG laser. The singlet oxygen emission was detected by using a liquid nitrogen cooled North Coast model EO-817P germanium photodiode detector equipped with a built-in preamplifier. The detector was coupled to a cuvette (1 cm optical path) in a right-angle geometry. An interference filter (1270 nm, Spectrogon US Inc.) and a cut-off filter (995 nm, Andover Corp.) were the only elements between the cuvette face and the diode cover plate. The output of the preamplifier was fed into the 1 MΩ input of a digitizing oscilloscope Hewlett Packard model 54540 A. Computerized experiment control, data acquisition

and analysis were performed by LabView based software developed in our laboratory [18].

The determination of the total rate constants by steady-state competitive techniques was done using TPP as sensitizer ($\lambda_{max}$ = 414 nm). Values of $k_T$ were determined by following the consumption of 9,10-dimethylanthracene upon drug addition [19]. The irradiation was performed with a visible Par lamp, 150 W, using a Schott cut-off filter at 400 nm. Values of $k_T$ measured employing the method of Carlsson et al. [20], observing the inhibition of rubrene autooxidation by addition of cinchonidine and cinchonine were obtained by irradiation of rubrene solutions in the absence and the presence of substrates with a visible halogen lamp, 50 W, using a Schott cut-off filter at 490 nm.

UV–vis absorption experiments were performed on a thermostated Unicam UV-4 spectrophotometer. A Hewlett Packard gas chromatograph equipped with a NPD detector and a Hewlett Packard Ultra-2 capillary column was employed in GLC experiments.

Quantum mechanical calculations were made using Gaussian 03W software. All structures were geometry optimized at the B3LYP/6-31G + d level. Atomic charges on reactive nitrogen were calculated by using the natural population analysis method at MP2/6-31G + d level.

Equation coefficients and statistical parameters were obtained by multilinear correlation analysis with STAT VIEW 5.0 (SAS Institute Inc.). Results were chosen on the basis of the *t*-statistic of the descriptors, correlation coefficients, standard deviations and the Fisher index of equation reliability. Only coefficients at the 0.95 significance level were considered. The number of solvents included in the correlation was as large as possible and at least three times the number of parameters used in the generalized equation.

## 3. Results and discussion

The values of total (physical and chemical) quenching rate constant, $k_T$, for the reaction of $O_2(^1\Delta_g)$ with the *Cinchona* tree alkaloids in several solvents were obtained from the experimentally measured first-order decay of $O_2(^1\Delta_g)$ in the absence ($\tau_0^{-1}$) and presence of the antimalarial compound ($\tau^{-1}$) according to:

$$\tau^{-1} = \tau_0^{-1} + k_T\,[\text{substrate}] \quad (1)$$

Fig. 2 shows a typical Stern-Volmer plot obtained for cinchonidine in acetonitrile. The triplet decay of the sensitizer (TPP or Rose Bengal) was not affected by the addition of the antimalarials even at concentrations higher than those used to quench the excited oxygen and linear plots of $\tau^{-1}$ versus drug concentrations were obtained in all the solvents employed. The intercept of these plots corresponds to the singlet oxygen lifetime in the solvent employed. In all the experiments, these values match very nearly with singlet oxygen lifetimes determined independently in a large set of experiments performed

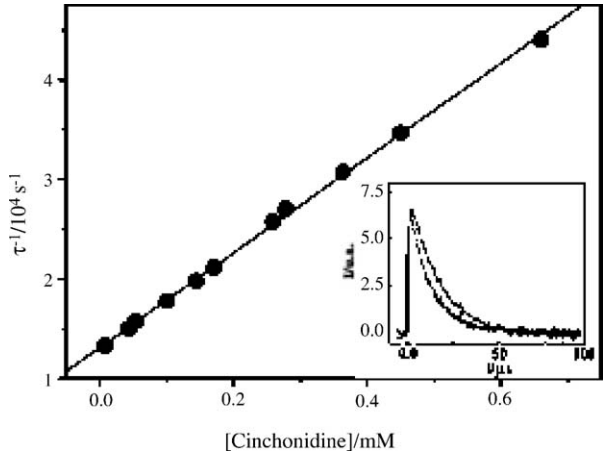



Fig. 2. Stern-Volmer plot for deactivation of singlet oxygen by cinchonidine in acetonitrile. Inset: Singlet oxygen phosphorescence decay at 1270 nm, following Nd:YAG laser excitation at 532 nm. Curve with decreasing lifetime represent an experiment with 0.18 mM of cinchonidine.

in our laboratory during past few years. The $k_T$ values calculated from the slope of these plots are given in Table 1. Possible rapid chemical changes of samples during illumination, or interference of the $O_2(^1\Delta_g)$ luminescence with the scattered laser light and the tail end of the sensitizer fluorescence [21] can be disregarded since the rate constant measured in some solvents by using competitive steady-state methods (data not included) afforded the same values than those obtained by time-resolved methods. As linear plots were obtained over a wide range of the drug concentrations in competitive steady-state experiments with DMA, the possible quenching of the sensitizer excited states by the antimalarials can be ignored.

Table 1 shows total rate constant data for the quenching of singlet oxygen by *Cinchona* tree alkaloids. Values are in the order of $10^7\ M^{-1}\ s^{-1}$ in most solvents, indicating that these compounds are good quenchers of singlet oxygen. A very low $k_T$ value ($5 \times 10^4\ M^{-1}\ s^{-1}$) was measured for both cinchonidine and cinchonine in trifluoroethanol. The values of $k_T$ for quinine were higher than those for quinidine in all solvents employed. Also, $k_T$ values for cinchonidine were slightly higher than those for cinchonine in most solvents, except for butanol. In addition, the $k_T$ values for all compounds were solvent-dependent. For example, the $k_T$ value for quinine increases in a factor 30 when *n*-hexane is changed to dioxane. These results can be associated to the presence of specific solute–solvent interactions. In this case, multilinear correlation of the logarithm of experimental rate constant with solvent microscopic properties, by using linear solvent free-energy relationships, is convenient in order to understand the effect of solvent on the singlet oxygen–antimalarial drug interaction. Then, we analyzed the quenching rate constant dependence on the microscopic solvent properties employing the equation of Kamlet and co-workers (Eq. (2)) [22,23]:

$$\log k = \log k_0 + s\pi^* + d\delta + a\alpha + b\beta + h\rho_H^2 \quad (2)$$

Table 1
Values of total rate constant, $k_T$, for reactions of *Cinchona* tree alkaloids with $O_2(^1\Delta_g)$ in different solvents obtained from time resolved methods

| Solvent | $k_T/10^7$ ($M^{-1}\,s^{-1}$) | | | |
|---|---|---|---|---|
| | Cinchonidine | Cinchonine | Quinine | Quinidine |
| *n*-Heptane | 0.58 ± 0.03 | 0.50 ± 0.04 | 0.86 ± 0.03 | 0.62 ± 0.03 |
| *n*-Hexane | 0.49 ± 0.03 | 0.43 ± 0.02 | 0.79 ± 0.03 | 0.58 ± 0.02 |
| Diethylether | 5.68 ± 0.28 | 4.67 ± 0.30 | 12.8 ± 0.36 | 10.7 ± 0.25 |
| 1-Hexanol | 0.65 ± 0.04 | 0.52 ± 0.04 | 1.04 ± 0.04 | 0.71 ± 0.03 |
| 1-Octanol | 0.62 ± 0.04 | 0.57 ± 0.04 | 1.96 ± 0.06 | 1.81 ± 0.09 |
| 1-Pentanol | 0.50 ± 0.03 | 0.47 ± 0.03 | 1.45 ± 0.03 | 1.08 ± 0.03 |
| 1-Butanol | 0.47 ± 0.03 | 0.51 ± 0.03 | 1.19 ± 0.06 | 0.77 ± 0.03 |
| 2-Propanol | 0.76 ± 0.04 | 0.69 ± 0.04 | 1.14 ± 0.07 | 0.92 ± 0.02 |
| 1-Propanol | 0.62 ± 0.04 | 0.54 ± 0.03 | 1.14 ± 0.05 | 0.78 ± 0.02 |
| Ethanol | 0.58 ± 0.03 | 0.50 ± 0.03 | 1.11 ± 0.05 | 0.98 ± 0.07 |
| Dioxane | 9.57 ± 0.55 | 9.36 ± 0.58 | 24.2 ± 0.98 | 13.2 ± 0.98 |
| Ethyl acetate | 6.87 ± 0.46 | 5.65 ± 0.46 | 8.30 ± 0.42 | 7.16 ± 0.24 |
| Chloroform | 2.09 ± 0.13 | 1.05 ± 0.13 | 2.33 ± 0.12 | 2.62 ± 0.11 |
| Tetrahydrofuran | 9.72 ± 0.11 | 8.68 ± 0.44 | 17.3 ± 1.03 | 14.8 ± 0.41 |
| Benzene | 6.64 ± 0.43 | 2.60 ± 0.14 | – | – |
| Methanol | 0.39 ± 0.02 | 0.22 ± 0.03 | 0.61 ± 0.08 | 0.42 ± 0.03 |
| Acetone | 7.09 ± 0.56 | 5.70 ± 0.37 | 12.0 ± 0.58 | 9.26 ± 0.39 |
| Anisole | 9.20 ± 0.45 | 5.21 ± 0.43 | 23.9 ± 0.51 | 10.9 ± 0.6 |
| Acetonitrile | 4.66 ± 0.30 | 4.38 ± 0.21 | 7.95 ± 0.32 | 5.60 ± 0.32 |
| Methylene chloride | 3.27 ± 0.18 | 2.59 ± 0.15 | 5.69 ± 0.34 | 3.88 ± 0.14 |
| Propylencarbonate | 8.36 ± 0.45 | 5.82 ± 0.29 | 10.9 ± 0.41 | 8.34 ± 0.54 |
| Dimethylacetamide | 13.8 ± 1.13 | 13.3 ± 0.62 | – | – |
| *N*,*N*-Dimethylformamide | 11.4 ± 0.92 | 11.1 ± 0.59 | 21.7 ± 0.94 | 17.0 ± 0.91 |
| Benzonitrile | 6.28 ± 0.50 | 5.17 ± 0.56 | 10.8 ± 0.48 | 10.1 ± 0.42 |
| Formamide | 0.87 ± 0.06 | 0.36 ± 0.02 | – | – |

where $\pi^*$ accounts for dipolarities and polarizabilities of solvents [22,24], $\delta$ parameter is a correction term for polarizability, $\alpha$ is related to the hydrogen bond donor solvent ability, $\beta$ indicates the solvent capacity as hydrogen bond acceptor, and $\rho_H^2$, the square of Hildebrand parameter, that accounts for the solvent cohesive energy density, model the cavity effects. Table 2 gives the coefficients of Eq. (2) obtained by multilinear correlation.

Correlation equations result from purely statistical criteria. The overall correlation equation quality is indicated by the sample size, *N*, the product correlation coefficient, *R*, the standard deviation, S.D., and the Fisher index of equation reliability, *F*. The consistency of each term is indicated by the standard error, ±, the 2-tail probability, *P* (2-tail), and the *t*-statistic. Good quality is indicated by higher *F* and *t*-stat values and smaller S.D. ones. Results show that not all the descriptors are significant. Descriptor coefficients accepted in the correlation equation were those that have a significance level ≥0.95. For this reason, parameters $\delta$ and $\rho_H$ were excluded from LSER equations. The coefficients included

Table 2
LSER correlation equations for the reaction of singlet oxygen with *Cinchona* tree alkaloids

| Compound | $\log k_0$ | $\alpha$ | $\beta$ | $\pi$ | *N* | *R* | S.D. | *F* |
|---|---|---|---|---|---|---|---|---|
| Cinchonidine | 6.997 | −1.509 | 0.756 | 0.821 | 25 | 0.970 | 0.148 | 110 |
| ± | 0.084 | 0.113 | 0.154 | 0.122 | | | | |
| *P* (2-tail) | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | |
| Cinchonine | 6.823 | −1.563 | 1.004 | 0.748 | 23 | 0.986 | 0.102 | 215 |
| ± | 0.059 | 0.088 | 0.115 | 0.094 | | | | |
| *P* (2-tail) | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | |
| Quinine | 7.091 | −1.448 | 0.930 | 0.851 | 18 | 0.971 | 0.135 | 78 |
| ± | 0.056 | 0.084 | 0.114 | 0.081 | | | | |
| *P* (2-tail) | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | |
| Quinidine | 6.985 | −1.570 | 1.018 | 0.852 | 20 | 0.966 | 0.172 | 62 |
| ± | 0.103 | 0.170 | 0.231 | 0.162 | | | | |
| *P* (2-tail) | <0.0001 | 0.0004 | <0.0001 | <0.0001 | | | | |
| TEA | 7.924 | −1.305 | 0.362 | 0.321 | 28 | 0.927 | 0.173 | 61 |
| ± | 0.111 | 0.134 | 0.184 | 0.151 | | | | |
| *P* (2-tail) | <0.0001 | <0.0001 | 0.0609 | 0.0444 | | | | |

in Table 2 show that all the compounds studied have the same behavior. The values of $k_T$ depend on the microscopic solvent parameters $\pi^*$, $\alpha$ and $\beta$, increasing in solvents with larger capacities to stabilize charges and dipoles, diminishing in solvents with high $\alpha$ values, and increasing in HBA solvents. Fig. 3 exhibits a plot of the logarithm of experimental $k_T$ value versus the logarithm of $k_T$ computed according to LSER equation for reaction of singlet oxygen with cinchonidine. A good lineal fit was found for the correlation between experimental and computed values ($R = 0.967$) with values of 0.015 and 0.997 for the intercept and the slope, respectively, that match closely with the expected values, which clearly indicates the significance of $\pi^*$, $\alpha$ and $\beta$ in the effect of the solvent on the reaction rate. The plot of residuals in the inset of Fig. 3 shows a random distribution of residuals in the range of $k_T$ values. Both results are strong evidence that the reaction mechanism is the same in all the solvents employed.

Solvent dependence for reactions of *Cinchona* tree alkaloids cinchonine with singlet oxygen is very similar to that reported for aliphatic amines of similar reactivity, such as triethylamine, TEA. The LSER data analysis for this compound is also included in Table 2 [25]. Tertiary aliphatic amines dramatically decrease their reactivity towards singlet oxygen when the media changes from aprotic polar solvents, such as acetonitrile or methylene chloride, to protic polar solvents, such as aliphatic alcohols [1]. This indicates that the role of the solvent is more complex than simple stabilization of the charge transfer intermediate through polar and/or dipolar interactions, and that other properties of the solvent (e.g. its acidic character) must be taken into account, solvents having a large $\pi^*$ and $\beta$ values stabilize the complex, while solvents with a high $\alpha$ value prevent its formation by impeding singlet oxygen access to the nitrogen lone pair. These results obtained for reactions of singlet oxygen with compounds (**1**)–(**4**) are evidently compatible with the formation of a charge transfer exciplex, the reactive center being the bicyclic nitrogen of the side chain in these molecules. The increase of $k_T$ in polar non-protic solvents is explained by stabilization of the charge transfer exciplex by dipolar interactions in these solvents. Stabilization increases when these solvents have a large capacity as electron pair donors due to the interaction with the positive charge developed on the reactive bicyclic amine nitrogen and HBD solvents inhibit the reaction by blocking sterically the reactive center through hydrogen bonding interactions with the lone pair of the reactive nitrogen.

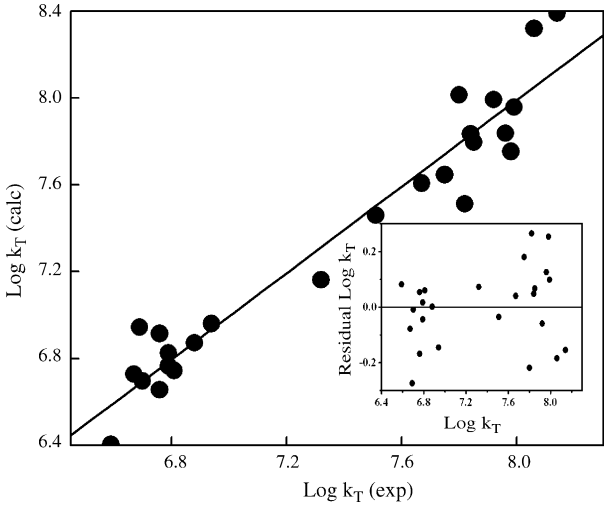



Fig. 3. Plot of $\log k_T$(exp) vs. $\log k_T$(calc) for the reaction of singlet oxygen with cinchonidine. Calculated $k_T$ values according to equation $\log k_T(\text{calc}) = 6.974 + 0.742\pi^* - 1.713\alpha + 1.005\beta$. Inset: Plot of the residuals of $\log k_T$ vs. $\log k_T$.

Furthermore, the $k_T$ dependence on the solvent was analyzed by using a theoretical set of parameters determined solely from computational methods [26–28]. The theoretical linear solvation relationship (TLSER) descriptors have been developed to give optimal correlation with the LSER descriptors [25,26]. The generalized TLSER equation proposed by Famini and coworkers Eq. (3), [26,29] can be used to analyze the dependence of reaction rates on solvent properties.

$$\log k = \log k_0 + a\pi_1 + b\varepsilon_b + cq_- + d\varepsilon_a + eq_+ + f\rho_H^2 \quad (3)$$

In Eq. (3), the theoretical bulk/steric term $V_{mc}$ of Famini equation was replaced by the square of Hildebrand parameter, $\rho_H^2$, the same parameter employed in the LSER treatment, because $V_{mc}$ gives a poor representation of the solvent cohesive energy density. The parameter $\pi_1$ corresponds to the polarizability index and accounts for the ease of moving or polarizing the electron cloud, and is obtained from the ratio between the polarizability volume and the molecular volume. The hydrogen bond acceptor (HBA) basicity involves covalent, $\varepsilon_b$, and electrostatic, $q_-$, terms. Similarly, the hydrogen bond donor (HBD) acidity includes covalent, $\varepsilon_a$, and electrostatic, $q_+$, terms [25,28]. Data from TLSER analysis of the solvent effect on $k_T$ are included in Table 3.

Such as in the LSER analysis, the quality of correlation equation was verified by the statigraphs from the correlation between calculated and experimental values, for which a good fitting was found, and by the random distribution of the residuals. However, the number of data points of TLSER correlation, in the reactions examined in this paper, is not coincident with the number of data points included in the LSER treatment, because for several solvents (e.g. propylene carbonate, ethylenglycol) theoretical parameters have not been reported and some data points are rejected by the statistical analysis being classified as outliers. In general, statistical parameters employed to account for the quality of the overall correlation equation improve considerably by neglecting outliers. Results obtained with Eq. (3) correlated closely with those obtained with LSER. TLSER treatment shows that solvents with higher $\pi_1$ values, which reflects the solvent's ability to stabilize dipole–induced dipole and induced dipole–induced dipole (dispersive) interactions, promote reaction of singlet oxygen with the *Cinchona* tree alkaloids. In addition, TLSER analysis indicates the singlet oxygen access to the reactive

Table 3
TLSER correlation equations for the reaction of singlet oxygen with *Cinchona* tree alkaloids

| Compound | $\log k_0$ | $\pi_1$ | $q_-$ | $q_+$ | $N$ | $R$ | S.D. | $F$ |
|---|---|---|---|---|---|---|---|---|
| Cinchonidine | 4.394 | 26.914 | 2.255 | −5.595 | 22 | 0.961 | 0.170 | 74 |
| ± | 0.517 | 4.111 | 0.348 | 0.577 | | | | |
| $P$ (2-tail) | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | |
| Cinchonine | 3.783 | 29.016 | 2.857 | −5.290 | 22 | 0.960 | 0.176 | 71 |
| ± | 0.495 | 4.076 | 0.318 | 0.600 | | | | |
| $P$ (2-tail) | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | |
| Quinine | 3.878 | 31.115 | 2.778 | −4.778 | 18 | 0.967 | 0.153 | 68 |
| ± | 0.457 | 3.577 | 0.360 | 0.564 | | | | |
| $P$ (2-tail) | 0.0003 | <0.0001 | <0.0001 | <0.0001 | | | | |
| Quinidine | 3.794 | 30.940 | 3.003 | −4.993 | 18 | 0.949 | 0.199 | 52 |
| ± | 0.572 | 4.846 | 0.420 | 0.740 | | | | |
| $P$ (2-tail) | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | |

centre on the quinuclidine moiety of the alkaloid, is sterically hindered by electrostatic interactions between HBD solvents and the lone pair on the nitrogen. Conversely, HBA solvents increase the reaction rate by means of stabilizing electrostatic interactions. This last effect would be understandable if a considerable positive charge, once the exciplex has been formed, is localized on the reactive nitrogen, resulting in formal electrostatic interactions with solvents possessing electron donor capacity.

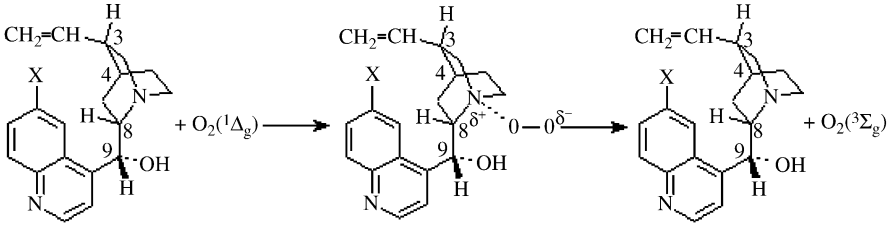
Furthermore, we perform experiments to measure the reactive rate constants, $k_R$, monitoring the alkaloid consumption employing a gas chromatograph equipped with NPD detection. In these experiments carried out employing Rose Bengal and TPP as sensitizers in acetonitrile and benzene as the solvents. For reaction of quinine, quinidine, cinchonidine and cinchonine with singlet oxygen no consumption of the alkaloids was observed after 48 h of irradiation. According to similar experiments carried out in our laboratory with analogous compounds containing tertiary amine groups, these results indicates that $k_R$ values are smaller than $10^4\ M^{-1}\ s^{-1}$. These data imply that quenching of singlet oxygen by the *Cinchona* tree alkaloids is essentially a physical process and can be described according to Scheme 1.

The data in Table 2 indicate that in all the solvents, but butanol, cinchonidine is more reactive than cinchonine and quinine is more reactive than quinidine, although reactivity differences are small and only in a few solvents $k_T$ values of the *S*,*R*-isomer are about twice than the *R*,*S*-isomer. This difference in reactivity could be explained in terms of charge density on the reactive nitrogen atom. Table 4 includes calculated charge density on the bicyclic nitrogen resulting from the NBO charge analysis on the MP2 minimized structure, a method currently employed to report charge analysis of organic molecules. Clearly, charge density on the bicyclic nitrogen shows very small variations and is not enough to explain the observed differences in reactivity.

Table 4
Charge density on the bicyclic nitrogen of gas-phase optimized structures (MP2-NBO) for the *Cinchona* tree alkaloids

| | |
|---|---|
| Quinine | −0.568 |
| Quinidine | −0.570 |
| Cinchonidine | −0.562 |
| Cinchonine | −0.558 |

After a careful examination of LSER coefficients and taking into account that the reaction mechanism is the same for compounds (**1**)–(**4**), we conclude that reactivity differences should be due to the absolute configuration these molecules. For all reactions, coefficients associated to the $\alpha$ parameter matched closely, indicating that steric effects imposed by HBD solvents due to hydrogen bond interactions with the reactive center are very similar for all *Cinchona* tree alkaloids. In addition, coefficients associated to the $\pi^*$ also follow a similar tendency although their values are larger than that reported for TEA, implying that in reactions of $O_2(^1\Delta_g)$ with *Cinchona* tree alkaloids containing a bicyclic bridge head reactive nitrogen, charge separation in the exciplex would be larger than the exciplex resulting from the interaction




Scheme 1.

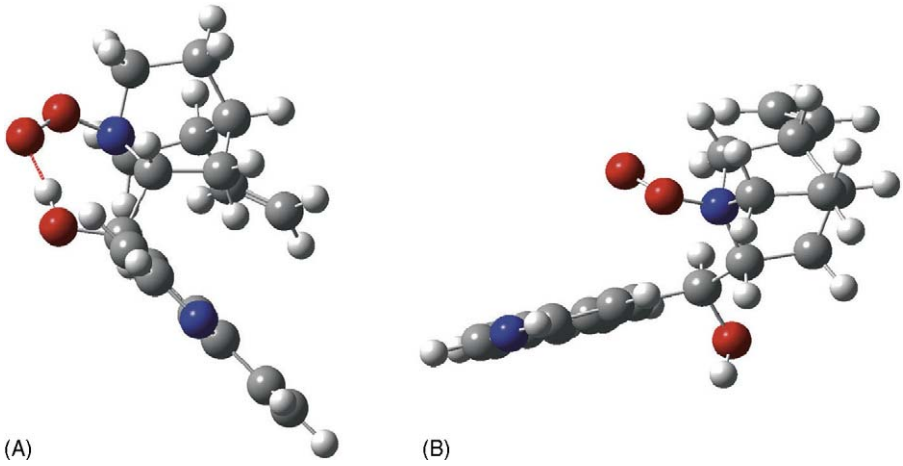



Fig. 4. DFT 631g + d gas-phase optimized structures of cinchonidine (A) and cinchonine (B) showing the most probable orientation of the electrophilic singlet oxygen attack on the nitrogen quinuclidine moiety.

of singlet oxygen with typical tertiary aliphatic amines. A similar result has been found previously for DABCO [29]. Consequently, a greater dependence on $\beta$ parameter for the most reactive S,R-isomers cinchonidine and quinine, resulting from the large positive charge developed on the quinuclidine nitrogen, once the exciplex has been formed, is expected. However, values of coefficients associated to the $\beta$ parameter (Table 2), do not follow this prediction. On the contrary, value of $\beta$ coefficient is markedly less important for reactions of singlet oxygen with cinchonidine and quinine than that observed for reactions of cinchonine and quinidine (coefficients of $\beta$ equal to 0.756 and 1.004 for cinchonidine and cinchonine, and 0.930 and 1.018 for quinine and quinidine, respectively). The dependence of $k_T$ on TLSER parameters follows a similar trend than that observed for the dependence on LSER parameters. These results can be explained on the basis of the isolated molecules structures. Fig. 4 shows the gas-phase DFT optimized structures of cinchonidine and cinchonine at the level 631g + d and the most probable orientation for singlet oxygen attack on the nitrogen of the quinuclidine moiety. Clearly, the structure of the cinchonidine-singlet oxygen exciplex of Fig. 4A, shows the feasibility of an intra-complex stabilizing interaction between the most negative non-bonded oxygen atom of the pernitrone and the hydroxyl substituent on the C(9) of the cinchonidine. Identical theoretical calculations were found for quinine–quinidine isomeric pair. This intramolecular interaction opens an additional stabilizing path, geometrically favorable, increasing the total reaction rate constant. Thus, the smaller $k_T$ dependence on $\beta$ solvatochromic parameter for reaction can be understood considering that the intramolecular interaction partially diminishes the stabilizing effect of HBA solvents once the exciplex has been generated. Fig. 4B shows that, in the event of electrophilic attack of singlet oxygen on cinchonine, it most probably proceeds on the quinuclidine nitrogen by the opposite side to which the hydroxyl substituent is attached at C(9), preventing eventual intra-exciplex stabilizing interactions between the terminal oxygen of the pernitrone and the hydrogen of the hydroxyl group at C(9).

Summing up, the *Cinchona* tree alkaloids cinchonidine, cinchonine, quinine and quinidine are good quenchers of singlet oxygen, physically reacting with this active species of oxygen through a charge transfer mechanism involving the electrophilic attack of the excited oxygen on the nitrogen quinuclidine moiety. The higher reactivity of the *S*,*R*-isomer relative to the corresponding *R*,*S*-isomer would be explained by the geometrically favorable intra-exciplex stabilizing interaction between the non-bonded oxygen of the pernitrone and the hydrogen of the hydroxyl substituent at C(9), that is sterically hindered for the reaction of $O_2(^1\Delta_g)$ with the *R*,*S*-isomers.

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