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Photochemical conversion of sanguinarine to oxysanguinarine

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Abstract

The photochemical properties of the alkanolamine form of sanguinarine, a benzophenanthridine plant alkaloid, were studied using absorption, fluorescence and high pressure liquid chromatography techniques. An excitation time dependent decrease in the fluorescence intensity of its emission maximum, along with changes in the emission and absorption spectral pattern was observed when the solution of alkanolamine form was excited under stirring. The rate of the reaction was dependent on the number of photons and the process was found to be irreversible. The irreversibility of the fluorescence excitation, emission and absorption spectra of the alkanolamine solution on excitation confirmed that this form of the alkaloid was undergoing a photochemical conversion in the excited singlet state. Further evidence for a photo-oxidation reaction was obtained from the experiments performed in the absence of molecular oxygen. On saturation of the solution with nitrogen gas, the photochemical conversion was not found to occur indicating an absolute requirement of molecular oxygen in the reaction. The photoproduct was isolated and identified as oxysanguinarine. On the basis of these observations, it is concluded that the sanguinarine alkanolamine form undergoes an irreversible photo-oxidation in the excited singlet state to produce oxysanguinarine. © 1997 Elsevier Science S.A.

Keywords: Sanguinarine alkanolamine form; Absorbance; Fluorescence; Photo-oxidation; Oxysanguinarine

1. Introduction

The importance of alkaloids in medicinal chemistry is extremely noteworthy. Fused aromatic ring structured alkaloids could form intercalation complexes with DNA and this property could most likely be the molecular basis for several of their biological properties [1]. In trying to understand the biological properties, it is very important to look at their photochemical and photophysical properties as well. Sanguinarine, a naturally occurring benzophenanthridine plant alkaloid has been the focus of recent attention from the standpoint of its diverse biological, DNA binding and photophysical properties [2–19]. This alkaloid exhibits promising antimicrobial, antitumor and antimicrotubule properties [2–7]. At the same time, the alkaloid is phototoxic and produces H_2O_2 [8,9]. Its ability to generate singlet oxygen has also been recently demonstrated [10]. Our laboratory has unequivocally demonstrated the pH dependent structural rearrangement of this alkaloid between the iminium (Fig. 1(a)) and alkanolamine forms (Fig. 1(b)) [11,12]. The stability of these two forms was later confirmed by studies of Jones et al. [13]. We have also demonstrated that the sanguinarine iminium form binds to DNA by a mechanism of intercalation with a high preference to GC base pairs [14–20], while the sanguinarine alkanolamine form does not bind to DNA [19]. The mode, mechanism and specificity of binding of these forms to DNA is now clearly understood [14–20]. In this paper we report that the alkanolamine form of sanguinarine undergoes an irreversible photoreaction on excitation, resulting in the formation of a stable photoproduct. The photoproduct has been isolated and characterized as oxysanguinarine.

2. Experimental details

2.1. Materials

Sanguinarine chloride was a product from Aldrich Chemical Co., Milwaukee, WI, USA. The purity of the sample was always confirmed by thin layer chromatography, melting point determination and NMR spectroscopy and was thereafter used without further purification. The alkaloid solution was prepared fresh each time and was always kept protected from light. A molar extinction coefficient (ϵ) of 30 700 M^{-1} cm^{-1} at 327 nm in 0.1 N HCl was utilized to determine the alkaloid concentration [13]. Chemically pure dihydrosanguinarine was provided by Dr. S.B. Mahato of this Institute

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and its purity was further checked by melting point determination and NMR spectroscopy. Adjustment of pH was achieved by simply adding HCl or NaOH to triply distilled water as reported previously [12].

2.2. Absorption spectra

UV–Vis absorption spectral measurements were performed on a Shimadzu UV-260 (Shimadzu Corporation, Japan) automatic recording spectrophotometer as described earlier [20].

2.3. Fluorescence spectra

A Hitachi Model F-4010 (Hitachi Ltd., Tokyo, Japan) was employed for fluorescence measurements. The temperature of the sample was controlled using an EYELA Uni Cool 55 water bath. Fluorescence spectra were recorded under constant stirring of the sample, and are uncorrected.

2.4. High pressure liquid chromatography

A Beckman System Gold 125 instrument equipped with a diode array detector system gold 165 and controlled by system gold chromatography software was used. Reverse phase columns of small pore size (Beckman, ODS Ultrasphere, 4.6×250 mm^2) and an isocratic system of 60% CH_3CN in 0.03 M KH_2PO_4, pH 5.5 was employed for high pressure liquid chromatography (HPLC) elution.

2.5. Preparation of the photoproduct

Sanguinarine chloride (5 mg) was dissolved in 100 ml of H_2O/NaOH solution of pH 11.0. The solution was kept stirred for about 1 h. This was then transferred into a photochemical apparatus (Applied Photophysics, USA) with a 400 W mercury lamp and filtered through a pyrex water jacket to screen out wavelengths less than 290 nm. The lamp was kept inside the sample chamber and was surrounded by the solution of the alkaloid. The reaction chamber was kept cool by circulating cold water and the sample was kept stirring by passing dry air during irradiation. Aliquots of sample were withdrawn at intervals of 1 h and the fluorescence spectrum was monitored to ascertain the progress of the reaction. The reaction was over in about 4 h and then the solution was concentrated to about 25 ml on a rotary evaporator. This solution was then extracted with chloroform (3×15 ml) in a separatory funnel. The chloroform extract was then washed with H_2O/HCl solution (pH 5.0) to remove any unconverted sanguinarine alkanolamine form. The chloroform extract was dried over anhydrous $MgSO_4$ and then evaporated to dryness to yield a pale yellow solid.

2.6. Preparation of oxysanguinarine

Sanguinarine undergoes oxidation in the presence of $K_3[Fe(CN)_6]$ [21]. To prepare oxysanguinarine, 1 mg of sanguinarine was dissolved in 10 ml of H_2O/HCl solution of pH 5.0. Freshly prepared saturated $K_3[Fe(CN)_6]$ solution (10 ml) was mixed with sanguinarine solution under stirring. The orange colour faded suddenly and a precipitate appeared. Stirring was continued for another 10 min. The turbid solution was extracted with chloroform (2×10 ml) and the extract was then washed with H_2O/HCl solution (pH 5.0) twice to remove any unconverted iminium ion form of sanguinarine. The chloroform extract was dried over anhydrous $MgSO_4$ and then evaporated to dryness to yield a pale yellow solid.

3. Results

The emission spectra of sanguinarine iminium ion (Fig. 1(a)) and alkanolamine form (Fig. 1(b)) are depicted in Fig. 2. The former is characterized by the 580 nm band while

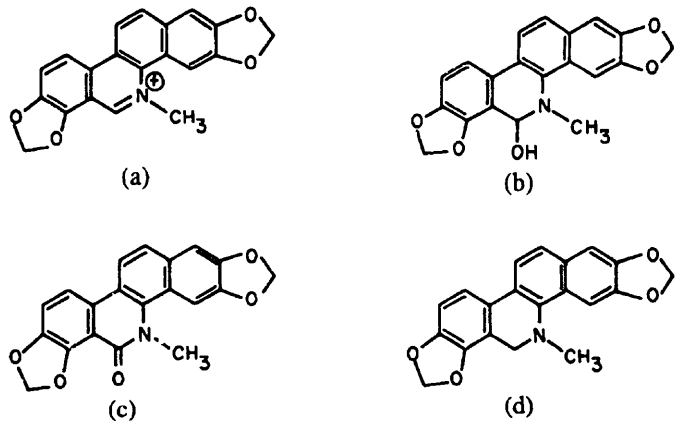


Fig. 1. Chemical structure of sanguinarine and its derivatives. (a) Sanguinarine iminium form, (b) sanguinarine alkanolamine form, (c) oxysanguinarine, and (d) dihydrosanguinarine.

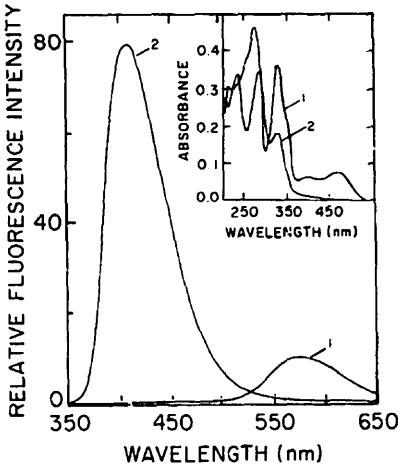


Fig. 2. Fluorescence spectrum of sanguinarine (2.5 μM) in aqueous buffer at (1) pH 5.0, and (2) pH 11.0. Excitation wavelength, 329 nm; scan speed, 120 nm min^{-1}; emission and excitation band pass, 5 nm each. Inset: absorption spectrum of sanguinarine (11.9 μM) in aqueous buffer at (1) pH 5.0, and (2) pH 11.0.

the latter has a maximum at 418 nm. At any particular concentration, the fluorescence intensity of the alkanolamine form is several fold higher than that of the iminium form. The pH dependent equilibrium between these two forms has a characteristic isoemissive point at 528 nm. The absorption spectra of these two forms are shown in the inset of Fig. 2.

In Fig. 3, the excitation time dependent excitation and emission spectra of alkanolamine form (structure b) are shown. The excitation and emission spectra underwent a continuous decrease in intensity and after about 120 min stable spectral patterns were obtained. These spectra have gross features identical to the initial spectra, except that the intensity has been reduced by about twenty times. In the emission spectrum, however, the wavelength maximum has also been red shifted by about 27 nm from the initial 418 nm. This emission spectrum does not undergo any further changes even after the excitation was stopped. The non-reversibility of the spectral changes has also been observed for the intermediate conditions. To verify this aspect further, a time scan of the emission spectrum was performed on continuous excitation but with the excitation shutter of the instrument being on and off alternatively. This data, shown in Fig. 4, indicates that there is no reversal of intensity during the shutter off period and the observed changes are clearly irreversible.

3.1. Effect of photons

The fluorescence intensity at the emission maximum of the sanguinarine alkanolamine form is gradually diminished (up to a limiting value) with increasing the time of excitation, because this form giving rise to the fluorescence is gradually converted to a less fluorescence product. The rate of photoreaction is increased with increasing light intensity, i.e. with increasing the number of photons available to the reaction.

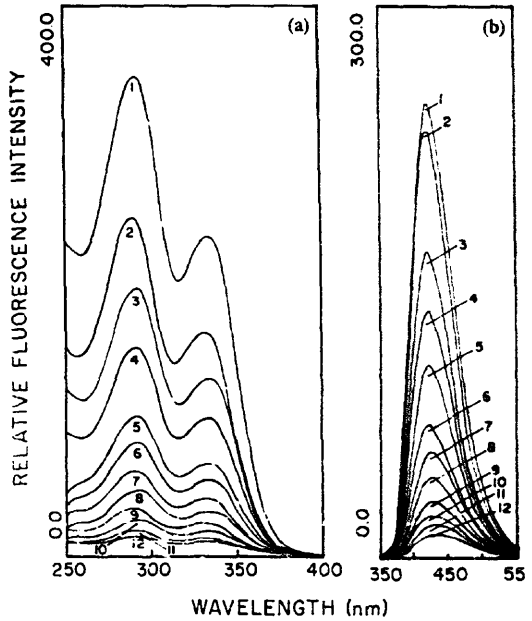


Fig. 3. Excitation time dependent excitation (a) and emission (b) spectra of sanguinarine alkanolamine form (7.2 μM, in aqueous buffer of pH 11.0) under stirring. The excitation time for curves 1–12 were 0, 1, 4, 7, 10, 15, 28, 38, 53, 73, 93, 110 and 120 min, respectively. Emission wavelength for the excitation spectrum was fixed at 418 nm and excitation wavelength for the emission spectrum was fixed at 329 nm. Emission and excitation band pass were 5 nm each.

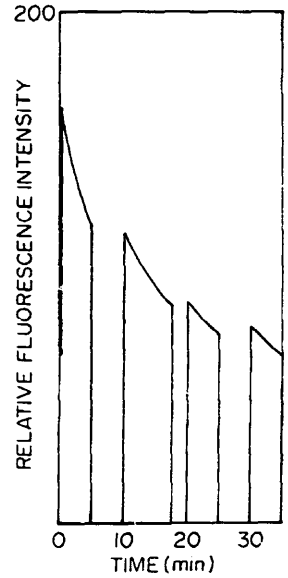


Fig. 4. Time scan of relative fluorescence intensity of sanguinarine alkanolamine form (3.8 μM, in aqueous buffer of pH 11.0) at 418 nm when excited at 329 nm under stirring with 5 min excitation and 5 min shutter off alternatively. Other experimental conditions were the same as in Fig. 3.

This aspect of the photoreaction was verified by performing a series of time scan experiments on the alkanolamine form by employing different excitation band pass keeping the emission band pass fixed at 5 nm. It has been observed that changing the excitation band pass from 1.5 to 20 nm produced a 15-fold enhancement in the rate constant (data not shown). Further, experiments performed conversely, with constant excitation band pass and varying emission band pass causing only marginal changes in the rate constant, again confirming the effect of photons on the rate of the reaction.

3.2. Absorption spectra

The absorbance changes corresponding to the fluorescence changes in Fig. 3 have also been monitored. Excitation was done in the spectrofluorimeter and the sample was transferred immediately to the UV cuvettes. In Fig. 5, the absorbance spectral changes are depicted. On progressive excitation, the absorbance spectral intensity gradually decreased concomitant with the fluorescence changes. A stable spectrum was achieved after 120 min. This final spectrum has a broad absorption maximum around 280 nm followed by a broader shoulder in the 310–340 nm region. This spectrum did not show any changes on further excitation or on keeping for a prolonged time.

3.3. Comparison of the fluorescence and absorption spectra of the photoproduct and oxysanguinarine

The photoproduct was prepared on a large scale as described in Section 2. The fluorescence spectrum of the photoproduct was compared with that of oxysanguinarine

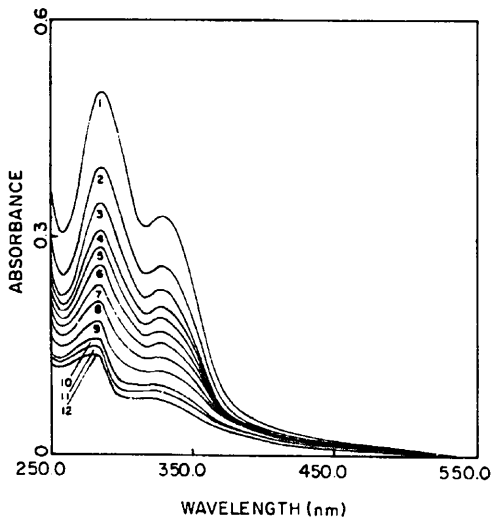


Fig. 5. Absorption spectrum of sanguinarine alkanolamine form (20.0 μM, in aqueous buffer of pH 11.0) after excitation in fluorimeter for different times. Excitation times for curves 1–12 were 0, 5, 10, 15, 20, 25, 35, 45, 60, 75, 95, 110 and 118 min, respectively. Other experimental conditions were the same as in Fig. 3.

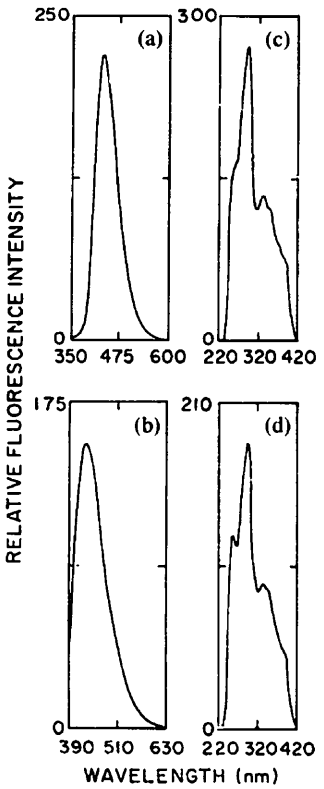


Fig. 6. Emission ((a) and (b)) and excitation ((c) and (d)) spectra of photoproduct (12.0 μM) and oxysanguinarine (8.0 μM) in chloroform. Excitation wavelength for the emission spectrum was fixed at 333 nm and emission wavelength for the excitation spectrum was fixed at 430 nm using an excitation and emission band pass of 5 nm each.

(structure (c) in Fig. 1) prepared chemically. In Fig. 6, the excitation and emission spectra of the two compounds are depicted. It can be observed that there is close similarity between the excitation spectra of both compounds, in both peak intensities and wavelength maxima. Again, the emission spectra of both compounds showed a single peak with a maximum around 430 nm. We have measured the fluorescence and absorption spectra of dihydrosanguinarine (structure (d) in Fig. 1) and compared it with the photoproduct (data not shown). It was observed that the spectrum of dihydrosanguinarine was vastly different from that of the photoproduct, ruling out the possibility that the photoproduct is dihydrosanguinarine.

3.4. Effect of dissolved oxygen

The role of molecular oxygen on the excitation dependent photoproduct formation was investigated by performing the time scan of solutions saturated with nitrogen gas. The rate constant values calculated in these cases were reduced by 90% when compared to those performed in the presence of oxygen (data not shown).

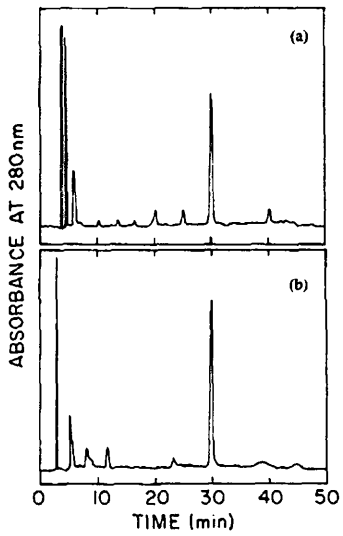


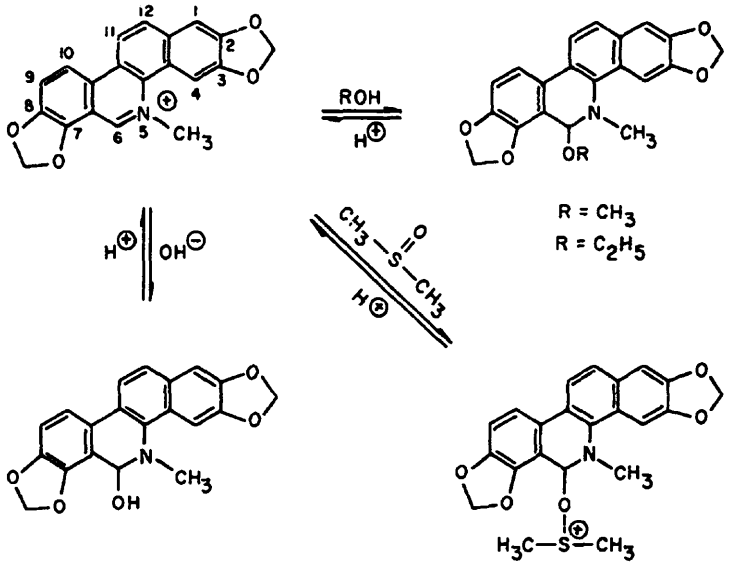
Fig. 7. HPLC elution profiles of (a) photoproduct, and (b) oxysanguinarine. Elution time of both compounds was 30.05 min.

3.5. HPLC analysis of the photoproduct and comparison with oxysanguinarine

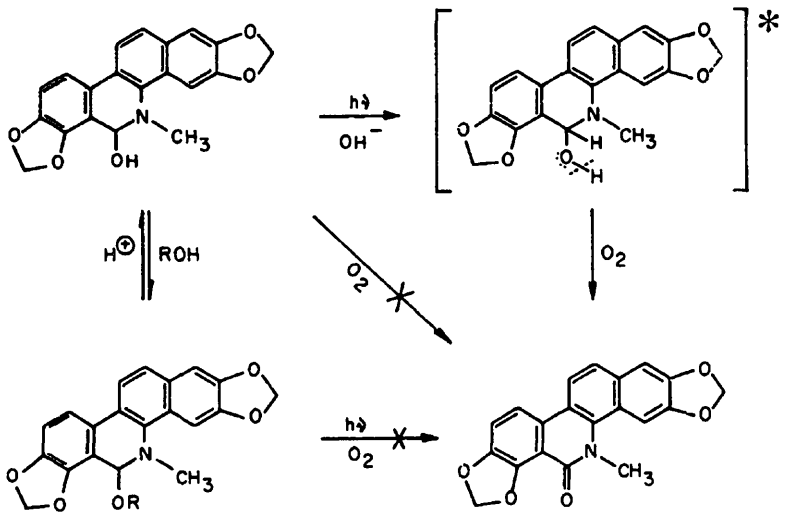
In Fig. 7, the HPLC elution pattern of the photoproduct is compared with that of oxysanguinarine. It is observed that both the photoproduct and oxysanguinarine showed identical elution times. Furthermore, a coinjection of a mixture of the photoproduct and oxysanguinarine (data not shown) gave a single peak confirming that the two compounds are identical.

4. Discussion

Sanguinarine exhibits a pH dependent structural transition between the iminium (structure (a) in Fig. 1) and the alkanolamine form (structure (b) in Fig. 1) which is represented by Scheme 1. This structural transition was evidenced by changes in absorption and fluorescence spectral properties in aqueous and organic media under various environmental conditions [11,12]. We have observed the steady state fluorescence property of the sanguinarine iminium form in methanol (–OMe), ethanol (–OEt) or dimethyl sulfoxide (DMSO) [12]. Thus, the iminium ion form exhibits steady state fluorescence properties and also the steady absorption spectral characteristics, indicating that this form does not undergo any changes on photoirradiation. On the other hand, the alkalonamine form of sanguinarine shows an excitation time dependent decrease in its fluorescence intensity, ultimately resulting in a product having lesser fluorescent properties. This establishes that the photoreaction property is exhibited only in the alkanolamine form and furthermore a stable end product of photoreaction is formed as a result of continuous excitation. Studies in the presence of methanol, ethanol and dimethyl sulfoxide indicated that the photoreaction was totally abolished in these solvents. Clearly, when –OMe, –OEt or DMSO replaces the –OH group at the C_6 position of sanguinarine (Scheme 1), the photoreaction is not possible. This evidence strongly suggests that the photoproduct could most likely be a compound with substitution at the C_6 position of sanguinarine. The absorption spectra of the excited samples of sanguinarine alkanolamine form also indicated a decrease in intensity. Moreover, the absorption and fluorescence spectra of the stable end product of photoirradiation do not undergo any changes for a long time when the excitation is stopped.



Scheme 1.



Scheme 2.

Experiments performed in the absence of molecular oxygen from the sample of sanguinarine alkanolamine form show that there is no gradual diminishing of emission intensity with different times of excitation. Thus, saturating with nitrogen gas abolishes the photoreaction almost completely, indicating clearly the absolute requirement of molecular oxygen in the reaction process. The isolation of the product and comparison of its spectral characteristics to two known derivatives of sanguinarine, namely oxysanguinarine (Fig. 1(c)) and dihydrosanguinarine (Fig. 1(d)) enabled us to identify the photoproduct to be oxysanguinarine. The absorption and fluorescence spectra of the photoproduct were closely similar to structure (c) in Fig. 1, confirming the photo-oxidation of the sanguinarine alkanolamine form in the excited singlet state. Further confirmation that the photoproduct is oxysanguinarine comes from the HPLC analysis. The photoproduct and oxysanguinarine have identical elution time and furthermore they coelute at the same time in a mixed injection proving unequivocally the photo-oxidation of the sanguinarine alkanolamine form to produce oxysanguinarine. A probable mechanism of the photo-oxidation reaction is illustrated in Scheme 2 although the actual mechanism could be more complex. In conclusion, the results presented here show that the sanguinarine alkanolamine form undergoes an irreversible photo-oxidation in the excited singlet state to produce oxysanguinarine.

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