Possible singlet oxygen generation from the photolysis of indigo dyes in methanol, DMSO, water, and ionic liquid, 1-butyl-3-methylimidazolium tetrafluoroborate

Naveen Gandra, a Aaron T. Frank, b Onica Le Gendre, b Nahed Sawwan, b David Aebisher, b Joel F. Liebman, c K. N. Houk, d Alexander Greer b, * and Ruomei Gao a, *

a Department of Chemistry, Jackson State University, Jackson, MS 39217, USA
b Department of Chemistry, Graduate Center and The City University of New York (CUNY), Brooklyn College, Brooklyn, NY 11210, USA
c Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, Baltimore, MD 21250, USA
d Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095, USA

Received 7 July 2006; revised 25 July 2006; accepted 8 August 2006
Available online 25 September 2006

Abstract—We suggest that singlet molecular oxygen \( [1O_2 (1D_g)] \) is formed upon irradiation of indigo \( 1 \) [in air or \( O_2 \)-saturated DMSO and DMSO (0.5% \( H_2SO_4 \))] and indigo carmine \( 2 \) [in air or \( O_2 \)-saturated \( CH_3OH, D_2O, \) and 1-butyl-3-methylimidazolium tetrafluoroborate (BmIm–BF4)]. The quantum yield for production of \( 1O_2 \) is estimated to be 0.6 for \( 1 \) and 0.3–0.5 for \( 2 \). The rates of reaction of \( 1O_2 \) with \( 1 \) and \( 2 \) were determined by monitoring the emission of \( 1O_2 \) at 1270 nm over time. Low molar absorptivities (at 532 nm) and rapid physical quenching caused by \( 1 \) and \( 2 \) limit their utility as \( 1O_2 \) photosensitizers in solution. Compounds \( 1 \) and \( 2 \) degrade slowly during the photolysis due to a self-sensitized (type I or II) photooxidation reaction. Oxidative cleavage of \( 1 \) by singlet oxygen and superoxide, and \( 2 \) by superoxide has been noted before (Kuramoto, N.; Kitao, T. J. Soc. Dyers Color 1979, 95, 257–261; Kettle, A. J.; Clark, B. M.; Winterbourn, C. C. J. Biol. Chem. 2004, 279, 18521–18525).

1. Introduction

Indigo (\( 1 \)) possesses a blue color, and has been of scientific interest given its historic provenance and esthetic hue. Indigo is a naturally derived dye that comes from a variety of plant species.1–5 Biosynthetic and laboratory preparations of \( 1 \) have been reported.3–6 Indigo \( 1 \) and indigo carmine \( 2 \) represent stable dyes and colorants, and are composed of a central carbon double bond with two imine nitrogens and two carbonyl groups affixed (Chart 1).7

Both \( 1 \) and \( 2 \) are quite photostable8 and this led us to raise the following question: How efficiently might they generate \( 1O_2 \) by photosensitization? The yield of \( 1O_2 \) formed by irradiation of triplet \( O_2 \) plus sensitizer varies depending on the structure of the photosensitizer, the nature of the photosensitizer’s excited state, and on the solvents used. Studies of indigo dye spectroscopy,8–12 excited state chemistry,13–24 and photofading25,26 date back to the 1950s. The mechanism of quenching of electronically excited states of thioindigo dyes (\( S \) replacing \( NH \)) by triplet molecular oxygen has been studied.27–35 Wyman and co-workers observed that dissolved oxygen quenched the photoisomerization of \( 3 \) without diminishing the intensity of the fluorescence.36 This suggested that the trans–cis isomerization of \( 3 \) may involve the excited triplet state. The triplet energy of \( 3 \) was determined to be 27–32 kcal/mol,16,17 which is greater than the 22.5 kcal/mol required to excite \( O_2 \) from its ground state to its first excited singlet state. The triplet state energies of \( 1 \) and \( 2 \) have not yet been reported.

\begin{align*}
\text{Chart 1. Structures of indigo and indigo carmine in their keto forms.}
\end{align*}
The photostability of indigo 1 has been attributed by Kobayashi and Rentzepis to the high internal conversion from excited singlet state \( S_1 \) to ground singlet state \( S_0 \) rather than trans to cis isomerization.\(^{14} \) The internal conversion process may be enhanced by excited state intramolecular proton transfer. However, Elsaesser et al. have concluded that the internal conversion is related to the vibrations of the \( \mathrm{N}–\mathrm{H} \) bonds, which are part of the intramolecular hydrogen bonds.\(^{13} \)

Despite the importance of indigo dyes, little information is available about whether they may serve as \( ^1\mathrm{O}_2 \) sensitizers.\(^{36} \) Recent work by Seixas de Melo and co-workers showed that the fluorescence efficiency \( \phi_F \) is favored by the leuco, but not keto forms, e.g., \( \phi_F \) of indigo is 0.0023 for the keto form, but 0.348 for the leuco form in DMF.\(^{37} \) The structures of the keto and leuco forms of indigo are shown below. The low fluorescence of indigo dyes in their keto form was explained by the small energy gap between \( S_0 \) and \( S_1 \). According to the golden rule for radiationless transitions, a small value in the \( S_1–S_0 \) energy gap favors a nonradiative decay pathway by increasing the vibrational overlap (Franck–Condon integrals) of the wave functions for the nonradiative modes of these two states.\(^{22,23} \)

The formation of \( ^1\mathrm{O}_2 \) is predominantly a triplet quenching process. The \( ^1\mathrm{O}_2 \) quantum yields (\( \phi_\Delta \)) were determined using Eq. \( 1.\)\(^{38} \) Eq. 1 represents a theoretical understanding of quantum yields of \( ^1\mathrm{O}_2 \) generation.

\[
\phi_\Delta = \phi_T P_{T,^1\text{O}_2} f_{T,^1\text{O}_2} \tag{1}
\]

Here \( \phi_T \) is the quantum yield of triplet formation for the photosensitizer, \( P_{T,^1\text{O}_2} \) the proportion of triplet quenched by ground state oxygen and \( f_{T,^1\text{O}_2} \) the fraction of triplet state quenched by ground state oxygen that leads to the formation of \( ^1\text{O}_2 \).

There is limited solubility of 1 in DMSO (\(~0.7 \text{ mM}\)) and in \( \text{CHCl}_3, \text{CH}_2\text{Cl}_2, \text{CH}_3\text{CN}, \) and \( \text{CH}_3\text{OH} (\sim0.1 \text{ mM}) \). However, because of its pair of sulfonate groups, indigo carmine 2 dissolves in water (\( \sim0.02 \text{ M} \)) and \( \text{MeOH} (\sim0.8 \text{ M}) \) to a higher extent. A number of reports discussing photochemistry in ionic liquids have appeared in the past several years.\(^{39–43} \) The ability of ionic liquids to retain other ionic compounds suggested the idea of using a photosensitizer bearing a charge, thereby making it preferentially soluble in the ionic liquid. Imidazole-tagged aryl ketones have been developed that can efficiently sensitize photochemical reactions in ionic liquids and are isolated simply by extraction of the ionic liquid solution with an appropriate organic solvent after use.\(^{44} \) The \([2+4]\) reaction between \( ^1\text{O}_2 \) and 1,4-dimethylnaphthalene has been investigated in several \( 1\)-methyl-3-butylimidazolium cation (\( \text{BmIm}^+ \)) ionic liquids along with the following counter anions: \( \text{PF}_6^–, \text{SbF}_6^– \), and \( \text{BF}_4^–. \)\(^{45} \)

Information regarding the quantum efficiency of \( ^1\text{O}_2 \) generation in ionic liquids is unavailable.

We report that absorption of visible light by 1 and 2 in the presence of \( \text{O}_2 \) leads to the production of \( ^1\text{O}_2 \). Singlet oxygen generation was measured for 1 in DMSO, and 2 in \( \text{CH}_3\text{OH}, \text{D}_2\text{O}, \) and \( \text{BmIm}–\text{BF}_4 \) using a time-resolved method. Oxidizing intermediates produced during the reaction led to the decomposition of 1 and 2.

2. Experimental

2.1. General aspects

Indigo 1, indigo carmine 2, isatin 4, rose bengal, methylene blue, and di-\( n\)-butyl sulfide were purchased commercially and used without further purification. The solvents used (DMSO, \( \text{D}_2\text{O}, \text{CH}_3\text{OH}, \text{and BmIm}–\text{BF}_4 \)) were of spectroscopic or equivalent grade and were used as received. Light at 532 nm was obtained from a time-resolved Nd:YAG laser set-up with pulse width 3–4 ns and maxima energy 50 mJ at 532 nm (Polaris II-20, New Wave Research Merchantek Products). A liquid \( \text{N}_2 \) cooled germanium photodetector (Applied Detector Corporation) was used for the determination of quantum yields and luminescence quenching rates of \( ^1\text{O}_2 \). A Rayonet 250 W tungsten lamp equipped with a 1 cm 1.5 M \( \text{NaNO}_3 \) solution cutoff filter was used for steady-state generation of \( ^1\text{O}_2 \). UV–visible spectra were recorded on a Varian 300 Bio (Cary) spectrophotometer. Mass spectrometry data was collected on an Agilent 1100 LC/MSD instrument using either electrospray ionization or atmospheric pressure photoionization methods. All experiments were carried out at room temperature and with air or \( \text{O}_2\)-saturated solutions. Shaking, stirring, and/or sonication were conducted to dissolve 2 and methylene blue in \( \text{BmIm}–\text{BF}_4 \).

2.2. \( ^1\text{O}_2 \) Quantum yield measurements

Absorptances of 1 and 2 ranged from 0.03 to 0.6 at the excitation wavelength 532 nm. The absorbance of 1 and 2, and rose bengal (known to possess a quantum yield of \( ^1\text{O}_2 \) generation 0.76 in DMSO, methanol, and heavy water\(^{46,47} \)) all matched to within 80%. The quantum yield values may change according to the reference used. The initial \( ^1\text{O}_2 \) intensity is extrapolated to \( t=0 \). The data points of the initial 3–4 ns are not used due to electronic interference signals from the detector. The intensity of the pulses at 532 nm were controlled between 40 and 50 mJ. The quantum yield for production of \( ^1\text{O}_2 \) is calculated according to Eq. 2. Unlike Eq. 1, Eq. 2 is practical to use along with the time-resolved method.

\[
\frac{\phi_{\Delta,\text{sample}}}{\phi_{\Delta,\text{reference}}} = \frac{S_{\text{sample}}}{S_{\text{reference}}} \tag{2}
\]

Here \( \phi_{\Delta,\text{sample}} \) and \( \phi_{\Delta,\text{reference}} \) are the quantum yields for samples and the reference. Rose bengal was used as a reference sensitizer. \( S_{\text{sample}} \) and \( S_{\text{reference}} \) represent the slopes obtained from the plot of initial intensity of \( ^1\text{O}_2 \) via the absorbance at excitation wavelength 532 nm for the sample and the reference, respectively.
2.3. Quenching rate constants of $^1$O$_2$

The phosphorescence of $^1$O$_2$ at 1270 nm was measured as previously described. The $^1$O$_2$ quenching rates were measured by monitoring the deactivation of $^1$O$_2$ by 1 and 2. Stern–Volmer analyses were conducted with rose bengal as the sensitizer in DMSO, methanol, and D$_2$O.

3. Results and discussion

3.1. Spectral properties of 1 and 2

Normalized UV–visible spectra for 1 and 2 are shown in Figure 1. The $\lambda_{\text{max}}$ and absorptivity at maxima of 1 and 2 and their 532 nm excitation wavelengths are collected in Table 1. The absorption of 1 and 2 consists of a band in the visible region between 500 and 700 nm with a small inflection or shoulder at shorter wavelengths (Fig. 1). The high absorptivity indicates that 1 and 2 can be excited upon the absorption of light in this range.

![Normalized absorption spectra for 1 and 2.](image1)

Figure 1. Normalized absorption spectra for 1 and 2. (1) DMSO solution of 1. (2) D$_2$O solution of 2. (3) BmIm–BF$_4$ solution of 2. (4) CH$_3$OH solution of 2.

<table>
<thead>
<tr>
<th>Sensitizer</th>
<th>Solvent</th>
<th>$\lambda_{\text{max}},$ nm</th>
<th>$\varepsilon_{\text{max}},$ M$^{-1}$ cm$^{-1}$</th>
<th>$\varepsilon_{532\text{max}},$ M$^{-1}$ cm$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DMSO</td>
<td>620</td>
<td>$2.26 \times 10^3$</td>
<td>$3.26 \times 10^2$</td>
</tr>
<tr>
<td>2</td>
<td>CH$_3$OH</td>
<td>598</td>
<td>$4.76 \times 10^3$</td>
<td>$1.59 \times 10^3$</td>
</tr>
<tr>
<td>3</td>
<td>D$_2$O</td>
<td>607</td>
<td>$7.92 \times 10^3$</td>
<td>$2.02 \times 10^3$</td>
</tr>
<tr>
<td>4</td>
<td>BmIm–BF$_4$</td>
<td>609</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Methylene blue</td>
<td>BmIm–BF$_4$</td>
<td>661</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

3.2. Possible $^1$O$_2$ quantum yields and quenching rates

The quantum yields for $^1$O$_2$ generation were determined by time-resolved laser measurements of its near-infrared luminescence (1270 nm) upon excitation at 532 nm with rose bengal $\phi_\Delta=0.76$ as the reference in DMSO, CH$_3$OH, and D$_2$O (Fig. 2). Ideally, the straight lines should go through zero in Figure 2, and we attribute the deviation to systematic errors. Thus, the quantum yields were determined from the slopes of the plots. Triplet–triplet annihilation appears to be negligible at absorbances ranging from 0.03 to 0.60 from excitation at 532 nm, as indicated by the $^1$O$_2$ intensity showing a linear correlation with the absorption of the complexes. We estimate that the $\phi_\Delta$ for 1 is 0.6, and for 2 is 0.3–0.6, where these $\phi_\Delta$’s were obtained in the common solvents noted above. No reference photosensitizer is available for ionic liquids. Thus, the relative $\phi_\Delta$ for 2 in BmIm–BF$_4$ was calculated assuming $\phi_\Delta=1.0$ for methylene blue. Our results show that $\phi_\Delta$ for 2 is about half that of methylene blue (Table 2).

![O$_2$ Emission intensity as a function of absorbance in DMSO with the excitation wavelength of 532 nm.](image2)

Figure 2. $^1$O$_2$ Emission intensity as a function of absorbance in DMSO with the excitation wavelength of 532 nm.

The total $^1$O$_2$ quenching rate constants ($k_T$) of 2 with $^1$O$_2$ were determined in D$_2$O and CH$_3$OH by Stern–Volmer analyses, since a large quenching rate would severely limit their potential as photosensitizers. The quenching rate constants range from $5 \times 10^7$ to $3 \times 10^9$ M$^{-1}$ s$^{-1}$ for 2 in CH$_3$OH and D$_2$O. This is similar to other photosensitizers, e.g., rose bengal ($2 \times 10^7$ M$^{-1}$ s$^{-1}$ in CH$_3$OH) and methylene blue ($2.3 \times 10^8$ M$^{-1}$ s$^{-1}$ in CH$_3$OH) and $4 \times 10^8$ M$^{-1}$ s$^{-1}$ in D$_2$O. The quenching rates of $^1$O$_2$ by 1 in DMSO and 2 in BmIm–BF$_4$ cannot be observed because of their limited solubility. The self-decay rate constants ($k_d$, s$^{-1}$) measured in the present work are comparable to published values of $7.1 \times 10^4$ s$^{-1}$ in DMSO, $8.9 \times 10^4$ s$^{-1}$ in CH$_3$OH, $1.8 \times 10^4$ s$^{-1}$ in D$_2$O, and $6.3 \times 10^4$ s$^{-1}$ in BmIm–BF$_4$.

Indigo 1 and indigo carmine 2 exhibit absorption bands in the range of 500–700 nm, and can be activated to higher energy levels by absorbing excitation light in this region. Photosensitization processes have been reviewed in detail by Wilkinson and Brummer. A brief summary is given below (Eqs. 3–10). A Type II mechanism involves the generation of $^1$O$_2$ via energy transfer between T$_1$ of a photosensitizer and ground state $^3$O$_2$ (Eq. 7). A Type I mechanism involves hydrogen-atom abstraction or electron-transfer between the excited photosensitizer and a substrate, and the subsequent generation of oxygen radicals. One example of the Type I reaction is an electron-transfer from an excited sensitizer to $^1$O$_2$, to form superoxide (Eq. 8).
Table 2. Summary of ¹⁰⁰₂ quantum yields (Φₐ), total quenching rate constants (kₑ, M⁻¹ s⁻¹) and self-decay rate constants in various solvents (kₛ, s⁻¹).

<table>
<thead>
<tr>
<th>Photosensitizer</th>
<th>Solvent</th>
<th>Φₐ±S.D.</th>
<th>Reference</th>
<th>kₑ, M⁻¹ s⁻¹</th>
<th>kₛ, s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DMSO</td>
<td>0.61±0.09</td>
<td>Rose bengal 0.76</td>
<td>—</td>
<td>(9.1±2.0)×10⁷</td>
</tr>
<tr>
<td>1</td>
<td>DMSO/0.5% H₂SO₄</td>
<td>0.62±0.05</td>
<td>Rose bengal 0.76</td>
<td>—</td>
<td>(6.0±1.0)×10⁷</td>
</tr>
<tr>
<td>2</td>
<td>CH₃OH</td>
<td>0.53±0.07</td>
<td>Rose bengal 0.76</td>
<td>(5.6±0.1)×10⁷</td>
<td>(8.1±0.5)×10⁷</td>
</tr>
<tr>
<td>2</td>
<td>D₂O</td>
<td>0.31±0.03</td>
<td>Rose bengal 0.76</td>
<td>(3.2±0.7)×10⁷</td>
<td>(1.9±0.3)×10⁷</td>
</tr>
<tr>
<td>2</td>
<td>BmIm–BF₄</td>
<td>0.53±0.11</td>
<td>Methylene blue</td>
<td>—</td>
<td>(6.1±0.9)×10⁷</td>
</tr>
</tbody>
</table>

* All experiments were measured at room temperature in air-saturated solutions with an excitation wavelength of 532 nm.
* There is no reference ¹⁰⁰₂ photosensitizer available for BmIm–BF₄.
* The ¹⁰⁰₂ quantum yield for indigo carmine 2 in BmIm–BF₄ was calculated assuming Φₐ=1.0 for methylene blue.

Due to the lack of reference photosensitizers in ionic liquids, the relative Φₐ values were determined by assuming Φₐ(methylene blue)=1.0 in BmIm–BF₄, yielding Φₐ(indigo carmine 2)=2.1. In contrast, the Φₐ ratio of methylene blue to indigo carmine 2 is 1:1 in CH₃OH. Furthermore, the Φₐ is 0.5 for methylene blue in CH₃OH has been reported previously. The change in relative ¹⁰⁰₂ quantum yields may result from a competition reaction for the triplet state. The enhancement of the lifetimes of triplet state and radical ions is observed for xanthone in 1-butyl-3-methylimidazolium hexafluorophosphate (BmIm–PF₆). This statement is speculative since influence of ionic liquids on the deactivation of triplet states is not established. Thus, it is difficult to assess whether the quantum yields for ¹⁰⁰₂ generation from rose bengal and indigo carmine 2 are significantly increased or decreased in different solvents.

3.3. Possible ¹⁰⁰₂ sensitization

Singlet oxygen may be formed under the conditions we have used in our experiments. Figure 3 contains the data of a representative 1270 nm signal decay produced by irradiation of 2 in CH₃OH. We attribute the 1270 nm signal to ¹⁰⁰₂, which is quenched by addition of Na₂O (0.20 mM) to the solution. The self-decay rate constants kₛ measured in various solvents are consistent with the literature values, where the kₛ ranges from 10⁴ to 10⁵ s⁻¹, which correspond to ¹⁰⁰₂ lifetimes between 10 and 100 μs (vide supra, Section 3.2). The 1270 nm signal also decreases with a decreasing concentration of oxygen in the solution. The generation of ¹⁰⁰₂ can be very sensitive to reaction conditions. The amount of ¹⁰⁰₂ produced by 1 and 2 is likely influenced by the concentrations of the sensitizer and oxygen, temperature, and irradiation time. Although the quenching rate constant of ¹⁰⁰₂ by 2 measured in this paper (5.6×10⁷ M⁻¹ s⁻¹ in methanol) is similar to that of rose bengal (2.0×10⁷ M⁻¹ s⁻¹ in methanol), higher concentrations of 2 will be required to obtain the same absorbance at the excitation wavelength because of a lower absorptivity [compare ε (2 at 532 nm)=1600 M⁻¹ cm⁻¹ with ε (rose bengal at 532 nm)=30,000 M⁻¹ cm⁻¹]. Therefore, low concentration, high excitation intensity, and long irradiation times should be employed when using 1 or 2 as ¹⁰⁰₂ photosensitizers.
3.4. Indigo and indigo carmine sensitized photooxidations

In 1979, Kuramoto and Kitao attributed the photofading of indigo 1 in solution to a self-sensitized photooxidation involving singlet oxygen.\(^\text{25}\) We observed a cleavage of 1 into isatin (4) after 8 h of visible light irradiation in an O\(_2\)-saturated solution of DMSO-\(d_6\), or in an O\(_2\)-saturated solution of CDCl\(_3\) in the presence of methylene blue. The LC–MS data is indicative of isatin 4 since we find a molecular ion peak \(m/z\) 148 (M+H)+, and under various ionization conditions find \(m/z\) 146 (M−H)−, \(m/z\) 165 (M+NH\(_3\))+, \(m/z\) 180 (M+CH\(_3\)OH)\(^+\), \(m/z\) 183 (M+Cl)− representing the addition of solvent molecules or ions. Isatin 4 was also identified by \(^1\)H NMR spectroscopy and the comparison to a commercial sample of isatin 4. Singlet oxygen may contribute to the oxidative degradation of 1 by a reaction with the central C=\(\equiv\)C double bond of 1. Alternatively, oxidation of 1 may take place via a Type I photooxidation pathway, similar to the reaction of 2 with superoxide reported recently.\(^\text{57}\) The mechanism by which active oxygen species initiates a process leading to the oxidative cleavage of the double bond of 1 and 2 will require further investigation. Greater yields of 4 are observed when a co-sensitizer, such as methylene blue is present.\(^\text{25}\)

A sulfide trapping experiment was conducted to study the indigo carmine 2—sensitized photochemical reaction. We detect dibutyl sulfoxide (51%) by GC–MS in a reaction of 2 (6×10\(^{-4}\) M) with dibutyl sulfoxide (0.15 M) in O\(_2\)-saturated CD\(_3\)OD after 48 h of visible light irradiation with a Rayonet lamp. An experiment with rose bengal (6×10\(^{-4}\) M) and butyl sulfoxide (0.15 M) in O\(_2\)-saturated CD\(_3\)OD led to 88% conversion to dibutyl sulfoxide after 1 h of visible light irradiation. These preliminary results may point to the intermediary of O\(_2\), which then oxidizes dibutyl sulfide. However, we have not explored this reaction in sufficient detail to confirm whether O\(_2\) is trapped and responsible for the oxidation of dibutyl sulfide.

4. Conclusion

Quantum yields for the possible generation of O\(_2\) by indigo 1 and indigo carmine 2 have been measured, which suggest that ISC competes with fluorescence and internal conversion for S\(_1\). We believe that 1 and 2 may serve as O\(_2\) photosensitizers in conventional polar solvents and an ionic liquid. However, low molar absorbivities and physical quenching of 1 and 2 limits their utility as O\(_2\) photosensitizers. High intensity of excitation light, longer irradiation times, and low concentrations of 1 or 2 would be needed when attempting to use them as O\(_2\) photosensitizers. A better understanding of the photooxidation mechanism of 1 and 2 under the conditions will require further study.

Acknowledgements

This work was supported by research grants to R.G. from the NSF-PREM program (DMR-0611539), A.T.F. from the NIH-MARC program (2T34 GM008078), and to A.G. from the National Institutes of Health (S06 GM076168-01) and PSC-CUNY. We thank Mark Kobrak (Brooklyn College) for useful comments on the manuscript, Cliff Soll (Hunter College Mass Spectrometry Facility) for conducting several mass measurements, and Bin Ye and Matthias Selke (California State University, Los Angeles) for carrying out several laser experiments. We thank a reviewer for comments on the manuscript.

References and notes
