

Photophysical and Spectroscopic Studies of Indigo Derivatives in Their Keto and Leuco Forms

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A comprehensive spectroscopic and photophysical study of the keto and leuco forms of indigo and three other ring-substituted derivatives in solution was performed. The characterization involves absorption, fluorescence, and triplet–triplet absorption spectra, making it possible to obtain the quantum yields for fluorescence (ϕ_F), singlet–triplet intersystem crossing (ϕ_{ISC}), internal conversion (ϕ_{IC}), and lifetimes for fluorescence (τ_F) and triplet decay (τ_T). For the case of the keto forms, pulse radiolysis experiments have revealed the existence of a triplet acceptor (from energy transfer from different donors) for the indigo, purple, and indirubin compounds. It is shown that with the keto form the major deactivation pathway involves internal conversion from the lowest singlet excited state to the ground state whereas with the leuco form there is competition between internal conversion, triplet formation, and fluorescence deactivation processes. Furthermore, leuco forms present much higher Stokes shifts compared with keto ones, suggesting an excited-state geometry different from the ground-state geometry, possibly involving rotational photoisomerization.

Introduction

Indigo blue is one of the earliest and most popular dyestuffs known to man. Its use dates back to the Egyptian and Roman civilizations. Indigo (see Chart 1) is also one of the most stable organic dyes, which explains not only its wide use in antiquity and premodern times but also its longevity as a colorant. The stability of indigo is also the reason it was used in oil paintings by some of the great masters of the 17th and 18th centuries, such as Rubens.^{1,2} In *De bello Gallico*, Julius Caesar describes the warrior skin paintings of his Gaulish adversaries as being obtained from a blue juice; their faces were frightening and they believed that by this dyeing, the skin paintings would protect them and turn them into invulnerable warriors! Tyrian purple (see Chart 1) is another example of a ring-substituted derivative of indigo and is one of the most important and powerful ancient dyes. This was derived from the Mediterranean shell fish of the genus *Purpura*, whose exhaustive extraction led to the first historically reported ecological disaster.^{3,4} Surprisingly, in the very beginning of the 21st century, indigo is still a important modern dye. Making use of the concepts of green chemistry, the production of indigo by microorganisms is being actively investigated.^{5,6}

The indigo derivatives are generally known as vat dyes and are insoluble in water in their oxidized colored form, the keto species.^{3,4,7} A common characteristic of these vat dyes is the presence of one or more carbonyl groups that, when treated with a reducing agent in the presence of an alkali, form a water-soluble species known as the leuco form. This form is the water-soluble intermediate used in the dyeing process.^{8,9} The most well-known dye of this family, indigo blue, is still used to color the world-famous blue jeans.^{9,10}

The explanation of the blue of indigo and its derivatives is an intriguing and fascinating subject and was explored with great detail and ingenuity during the 1970s and 1980s.^{11–14} It has been found that the fundamental chromophore of the indigo dyes includes the central double bond (connecting the two rings), together with the nitrogen and carbonyl groups.¹³ Substitution at different ring positions of indigo is likely to promote significant shifts in both the long-wavelength visible and UV bands.^{15,16}

Despite the importance of indigo, few conclusive studies have been carried out to clarify and rationalize the photochemistry and photophysics of this molecule, particularly of its leuco form. In 1994, George Wyman nicely reviewed the state of the art for the spectroscopy of indigo and its derivatives known until then, showing that several mysteries regarding indigo's behavior were still left to be explained.¹⁷ Because of the complicated nature of the spectroscopy of indigo, Wyman reported that even though he had not been working with these compounds for more than 15 years, little or nothing had been done during that period.¹⁷ Because of misleading results for indigo's fluorescence,^{17,18} most of the work reported was for thioindigo derivatives.^{17,19–21} In contrast to the behavior of the heteroatom derivatives,^{19,21} the remarkable stability of indigo and its ring-substituted derivatives has been attributed to intramolecular hydrogen bonding between the two adjacent carbonyl and N–H groups (see Chart 2), which keeps the molecule in a trans planar configuration, preventing the photochemical cis–trans isomerization.^{18,22} The internal conversion from the first singlet excited state to the ground state has been shown to be much faster than the other S_1 deactivation processes (fluorescence, intersystem crossing, and photochemistry). This was attributed, by some authors, to fast intramolecular transfer of one or both protons from nitrogen to oxygen.¹⁸ However, other groups later concluded that this could not be the case.²³

Contrary to the behavior of the usual keto form of indigo²² and several of its derivatives,^{3,17,19–21,23} little attention has been

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CHART 1

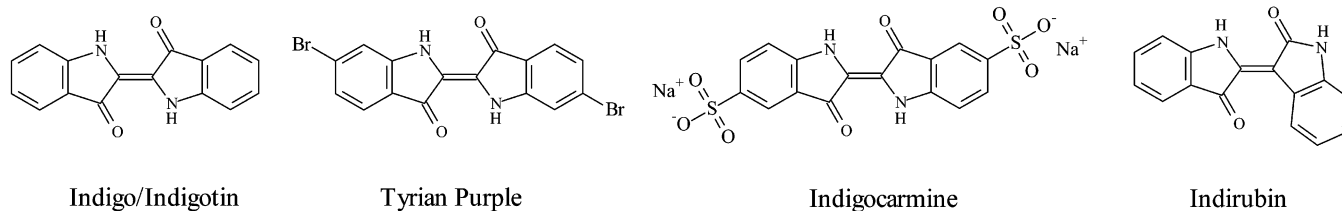
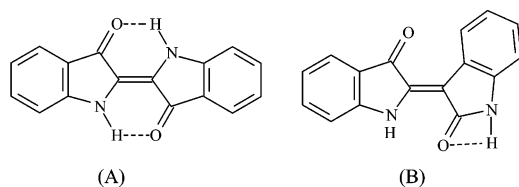


CHART 2



paid to the leuco forms^{24,25} and in particular to their photophysical properties. The present work aims to give more insight on the spectroscopic and photophysical properties of some indigo derivatives including indigo itself, purple, indigocarmine, and indigo's structural isomer indirubin (see Chart 1), in particular the behavior of the corresponding leuco forms. A better knowledge of the photophysics of the keto–leuco system will permit a better understanding of the factors that influence indigo photodegradation and also its lifetime as a painting material and dye.

Experimental Section

Indigo was prepared according to the procedure elsewhere described⁸ or purchased from Aldrich and used as received. Purple was obtained following a published procedure.^{26,27} Indirubin was synthesized following the procedure described in ref 28. The crystals were washed first with a solution of aqueous methanol (1:1.5) and then with methanol. Indigocarmine Merck was used as received.

The solvents used were of spectroscopic or equivalent grade. Ethanol and methanol were previously dried over CaO and then distilled. Dimethylformamide (DMF) was distilled and left over molecular sieves prior to and after use.

Indigo and its derivatives indirubin and purple are considered vat dyes, meaning that being water-insoluble (except indigocarmine), prior to the dyeing process they must be converted into a water-soluble form (leuco form), which is usually obtained by reducing the dye with a strong reducing agent (sodium dithionite in alkaline media) or alternatively through electrochemical reduction.²⁹

Preparation of the Leuco Species. To a solution of the dye in DMF (ethanol, methanol and benzene were also used as solvents), submitted to constant and gentle bubbling with Ar, 2–3 drops of a concentrated sodium dithionite/NaOH solution (0.15 g of sodium dithionite in 10 mL of a 1 M solution of sodium hydroxide) were added. The resulting solution was mixed, left bubbling for more 20 min, and then sealed.

Absorption and fluorescence spectra were recorded on Shimadzu UV-2100 and Jovin-Yvon Spex Fluorog 3-2.2 spectrophotometers, respectively. Fluorescence spectra were corrected for the wavelength response of the system. The fluorescence quantum yield for the keto forms of the compounds were determined using indigo ($\phi_F = 0.001$ in dichloroethane²²). For the leuco forms of the compounds the fluorescence quantum yields were determined using quaterthiophene ($\phi_F = 0.18$ in dioxane³⁰) and quinquethiophene ($\phi_F = 0.36$ in dioxane³⁰) as standards.

Fluorescence decays were measured using a home-built TCSPC apparatus with a D₂/N₂ filled IBH 5000 coaxial flash lamp as the excitation source, Jobin-Yvon monochromator, Philips XP2020Q photomultiplier, and Canberra instruments TAC and MCA.³¹ The fluorescence decays were analyzed using the method of modulating functions implemented by Striker.³² The fluorescence decays of the keto forms of the compounds were obtained with picosecond resolution in an apparatus described elsewhere.³³

Triplet–singlet difference absorption spectra and yields were obtained using an Applied Photophysics laser flash photolysis equipment pumped by a Nd:YAG laser (Spectra Physics) with excitation wavelengths of 355 nm (leuco forms) and 535 nm (keto forms) as described elsewhere.³⁴ First-order kinetics was observed for the decay of the lowest triplet state. The transient spectra (300–700 nm) were obtained by monitoring the optical density change at 5–10 nm intervals, averaging at least 10 decays at each wavelength. Also, depending on the wavelength region studied, two different photomultipliers were used: Hamamatsu IP28 and Hamamatsu R928.

In all cases the signal was assigned to a triplet state because (i) it decayed by first-order kinetics with microsecond lifetimes and (ii) in these experiments other possible transients, such as radical ions, were not produced on photolysis. Additionally for the keto forms these were found to be quenched by oxygen.

The triplet molar absorption coefficients for the leuco forms of the indigo derivatives in DMF were determined by the singlet depletion technique, according to the well-known relationship³⁵

$$\epsilon_T = \frac{(\epsilon_S)(\Delta OD_T)}{\Delta OD_S} \quad (1)$$

where both ΔOD_S and ΔOD_T are obtained from the triplet transient absorption spectra, and triplet formation quantum yields were derived from these and actinometry with benzophenone. The ϕ_T value was obtained by comparing the ΔOD at 525 nm of a benzene solution of benzophenone (the standard) and of an aqueous solution of the compound (optically matched at the laser excitation wavelength) using the following equation:³⁶

$$\phi_T^{\text{Indigo derivative}} = \frac{\epsilon_{TT}^{\text{Benzophenone}}}{\epsilon_{TT}^{\text{Indigo derivative}}} \frac{\Delta OD_{\text{max}}^{\text{Indigo derivative}}}{\Delta OD_{\text{max}}^{\text{Benzophenone}}} \phi_T^{\text{Benzophenone}} \quad (2)$$

with $\epsilon_{TT}^{\text{Benzophenone}} = 7200 \text{ M}^{-1} \text{ cm}^{-1}$ and $\phi_T^{\text{Benzophenone}} = 1.35$

Pulse radiolysis experiments were carried out at the Free Radical Research Facility, Daresbury, U.K., using 200 ns to 2 μs high-energy electron pulses from a 12 MeV linear accelerator (LINAC), which were passed through solutions in a 2.5 cm optical path length quartz cuvette attached to a flow system. All solutions were bubbled with argon for 30–40 min before experiments were taken. More details on the experimental setup and other experiments details can be found elsewhere.^{37,38}

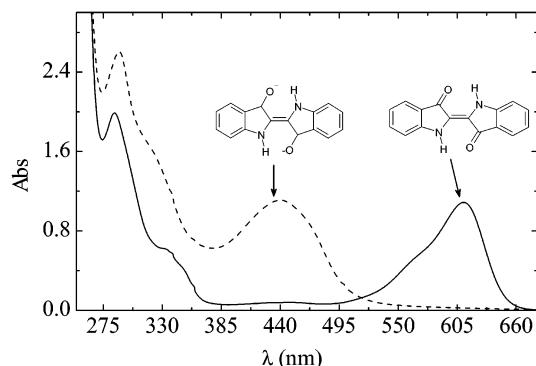


Figure 1. Absorption spectra of the keto (full line) and leuco (dashed line) forms of indigo obtained in DMF at $T = 293$ K.

TABLE 1: Absorption Maxima and Extinction Coefficients for the Keto and Leuco (in Parentheses) Forms of the Compounds Studied in DMF Solution at $T = 293$ K

compd	$\lambda_{\text{abs}}(\text{max})$ (nm)	ϵ_{max} ($\text{M}^{-1} \text{cm}^{-1}$)
indigo	610	22140
	(442)	(17400)
purple	601	12680
	(442)	(13050)
indigocarmin	618	8080
	(446)	(17250)
indirubin	546	9340
	(390)	(24770)

Results

1. Absorption Spectra. Keto Forms. The UV–vis absorption spectra of the four compounds (Chart 1) in dimethylformamide (DMF) display structurally common bands for indigo, purple, and indigocarmin consisting of a sharp band in the visible region with a slight inflection or shoulder at shorter wavelengths. In the case of indirubin the shoulder is absent. A representative spectrum is shown in Figure 1 for the leuco and keto forms of indigo. The wavelength maxima shifts are in the order indirubin (546 nm) < purple (601 nm) < indigo (610 nm) < indigocarmin (618 nm) (Table 1). The molar absorption coefficients, Table 1, with the exception of indigo ($22\,140 \text{ M}^{-1} \text{cm}^{-1}$) are low ($\sim 10\,000 \text{ M}^{-1} \text{cm}^{-1}$).

Leuco Forms. Adding some drops of an aqueous solution of sodium dithionite/sodium hydroxide to the DMF solution of the dyes leads to the formation of their leuco forms (Figure 1). The structures of these leuco forms have been clearly established by NMR spectrometry and deuterium substitution.^{24,25} The absorption spectra of the leuco forms of the compounds are blue-shifted ca. 150 nm relative to their keto counterparts (Table 1 and Figure 1). In contrast to what was observed for the keto forms, with the exception of indirubin, the absorption maxima for the three indigotin-based compounds are very similar, ~ 443 nm (Table 1). For the molar absorption coefficients (when compared to values for the keto species) for indigo, a lower value ($17\,400 \text{ M}^{-1} \text{cm}^{-1}$) is observed. For Tyrian purple there are no relevant changes ($13\,050 \text{ M}^{-1} \text{cm}^{-1}$), whereas for indigocarmin and indirubin the values doubled ($17\,250$ and $24\,770 \text{ M}^{-1} \text{cm}^{-1}$, respectively).

2. Photophysical Properties. Fluorescence Emission Spectra and Lifetimes. The fluorescence spectra of the compounds both for their keto and leuco forms are shown in Figure 2. For the four compounds studied, the emission wavelength maximum is in the region 640–661 nm. The very low values of the fluorescence quantum yields, ϕ_{F} , obtained for the keto forms of the indigo derivatives (Table 2), are in agreement with previous findings, where either very low values²² were observed

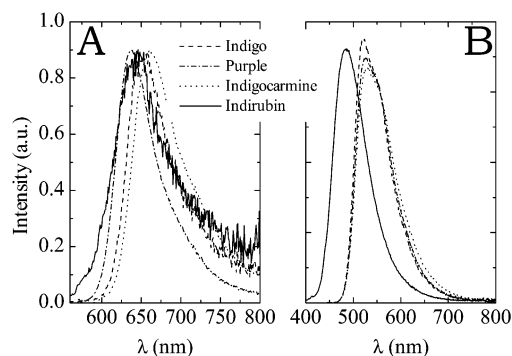


Figure 2. Fluorescence emission spectra in DMF for the keto (A) and leuco (B) forms of the compounds studied. $T = 293$ K.

TABLE 2: Spectroscopic and Photophysical Data for the Keto and Leuco (in Parentheses) forms of the studied compounds in DMF solution at $T = 293$ K

compd	$\lambda_{\text{fluo}}(\text{max})$ (nm)	ϕ_{F}	τ_{F} (ns)	k_{F}^a (ns^{-1})	k_{NR}^b (ns^{-1})
indigo	653	0.0023	0.14	0.0164	7.12
	(523)	(0.348)	(3.15)	(0.111)	(0.207)
purple	640	0.0071	0.323	0.022	3.07
	(526)	(0.225)	(3.77)	(0.060)	(0.206)
indigocarmin	661	0.0015	0.110	0.0136	9.08
	(529)	(0.292)	(3.50)	(0.083)	(0.202)
indirubin	645	0.00027	0.030	0.009	33.3
	(486)	(0.0145)	(1.78)	(0.008)	(0.554)

$$^a k_{\text{F}} = \phi_{\text{F}}/\tau_{\text{F}}. \quad ^b k_{\text{NR}} = (1 - \phi_{\text{F}})/\tau_{\text{F}}.$$

or no fluorescence^{17,18} was reported. The ϕ_{F} values for indigo, Tyrian purple, and indigocarmin are on the order of 10^{-3} , while that for indirubin is an order of magnitude lower. Also, the fluorescence lifetimes were found to be short, ranging from 0.110 to 0.323 ns for the indigotin type compounds, and even lower for indirubin (0.030 ns) (Table 2). This indicates that fluorescence is a nonefficient deactivation pathway for excited indigo derivatives in their keto forms. In fact, the calculated radiative rate constants are about 10^7 s^{-1} for indigotin-type compounds and 10^6 s^{-1} for indirubin. On the other hand, the radiationless rate constants display very high values, on the order of 10^9 s^{-1} for the indigotin type molecules and 10^{10} s^{-1} for indirubin. Therefore, the radiative constants for indigo, Tyrian purple, and indigocarmin are 2 orders of magnitude lower than the sum of the rate constants for the radiationless processes (k_{NR}). In the case of indirubin, the difference is now 4 orders of magnitude (Table 2)! The values of the radiationless rate constants are unusually high, which is consistent with molecules possessing excited-state proton transfer in contrast to what was suggested with some indigotin derivatives, where apparently this process is not viable.²³

For the leuco forms, the emission maxima are in the range 520–530 nm, i.e., a much narrower interval, with the exception of the leuco form of indirubin, which is found to be ca. 30 nm blue-shifted [$\lambda_{\text{max}}(\text{em}) = 486 \text{ nm}$] relative to the indigotin compounds (see Table 2). The fluorescence quantum yields for the leuco indigo derivatives are now ca. 2 orders of magnitude higher than those of the corresponding keto forms, with fluorescence lifetimes now found on a nanosecond time scale (Table 2 and Figure 3). Note that the radiative (k_{F}) and radiationless rate (k_{NR}) constants have approximately the same order of magnitude because of a decrease in the k_{NR} and increase in the k_{F} values, turning fluorescence emission into an efficient and competitive process for the deactivation of the excited state. In this case, indirubin is no exception (although there is a significant difference between the k_{F} and k_{NR} values), follow-

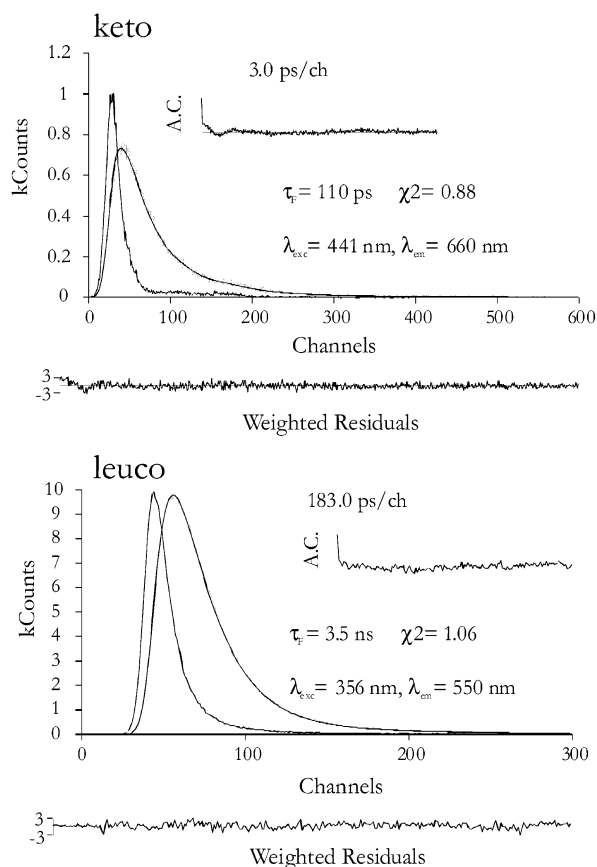


Figure 3. Fluorescence decays and pulse instrumental response obtained for the keto and leuco forms of indigocarmin in DMF at $T = 293 \text{ K}$. The experimental conditions and obtained decay times are shown as insets in the figure. Also shown are the weighted residuals, autocorrelation functions (A.C.) and the χ^2 values for a better judgment of the quality of the fits.

ing the same trends as the indigotin derivatives, even though the observed fluorescence quantum yields are lower and the lifetimes shorter.

For the keto forms of the indigo derivatives, the observed Stokes shifts (taken as the difference between the maxima of the absorption and emission spectra) are small, around 40 nm, with the exception of indirubin whose value is $\sim 100 \text{ nm}$ (see Tables 1 and 2). It is interesting to note that the leuco forms of the indigo derivatives present more pronounced Stokes shifts (Δ_{SS}) than the keto forms. With the exception of indirubin, which has an identical shift of 100 nm in the leuco and keto forms, the Stokes shifts for the leuco species are about 81–84 nm ($\sim 3500 \text{ cm}^{-1}$).

The small Stokes shifts and the mirror-image relationship (see Figures 1 and 2A) between the absorption and emission bands of the keto forms favor the hypothesis that identical conformational rigidity and structure are found in both the ground and singlet-excited states. This does not, however, occur with the leuco forms, where the much more pronounced Stokes shift suggests an excited-state species that is structurally different from the ground-state species. In addition, these show Franck–Condon (F.-C.) forbidden band shapes in the absorption spectra, which suggests, in terms of the F.-C. principle,³⁹ differences in the relative location of potential energy minima of ground and excited states. This once more indicates that there is a marked change in structure between the ground and first excited singlet state, where some considerable degree of conformational mobility (conformational isomerism) is possible (see Discussion).

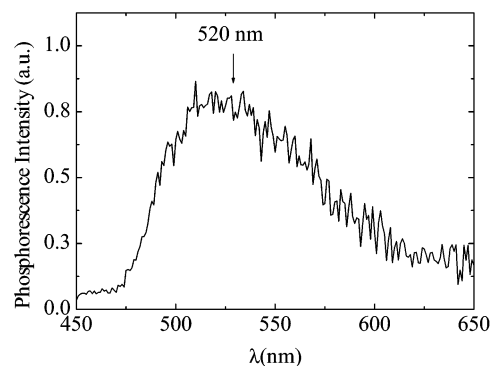


Figure 4. Phosphorescence spectra of the leuco form of indigocarmin in methanol at $T = 77 \text{ K}$ (a few drops of the sodium dithionite/NaOH aqueous solution were added to induce the formation of the leuco form).

Phosphorescence Emission Spectra. In the case of the water-soluble indigocarmin, it was possible to make a low-temperature luminescence study of the leuco form in methanol and thus to obtain information on the nature of the lowest triplet state. Figure 4 presents the phosphorescence spectrum of a methanol solution of indigocarmin. A very weak band is observed with a maximum at around 520 nm. A lifetime of 143 ms was obtained, and the excitation spectra collected with $\lambda_{\text{em}} = 520 \text{ nm}$ mirror the absorption spectra of the leuco form of indigocarmin. The proximity of the fluorescence (S_1 state) and phosphorescence (T_1 state) emission bands at ~ 529 and 520 nm, respectively, seems to explain the high efficiency of the $S_1 \rightsquigarrow T_1$ process in the leuco forms of indigocarmin when compared to the low efficiency of this process for the keto species. Note that the small blue shift of the phosphorescence relative to the fluorescence band results from the fact that the former is taken in a rigid matrix whereas the fluorescence spectrum was obtained at room temperature.

Triplet State Characterization by Laser Flash Photolysis and Pulse Radiolysis. Keto Forms. In general the triplet–triplet transient absorption spectra observed for the keto forms of the compounds studied are very weak or negligible, in agreement with negative results from previous studies on these systems. This is not, however, the case for diacyl and rigid trans and cis indigo analogues, where triplet transients could be obtained and characterized at low temperature under direct excitation,⁴⁰ or for thioindigo ring-substituted compounds, where it has been found that the triplet state plays a role in the cis \rightarrow trans isomerization of these compounds.⁴¹

In the present study, the observation of a transient absorption generated from direct excitation of the compound was only possible for purple in DMF solution (Figure 5). The spectrum is vibrationally resolved, and although weak, it was reproducible (Figure 5).

Attempts to obtain transient triplet–triplet absorption spectra under various conditions were made. Pulse radiolysis energy transfer experiments using biphenyl and naphthalene as triplet donors to indigo, purple, and indirubin were performed (Figure 6). In all these cases weak signals were obtained, and although quenching of the donors were observed, the absorptions were not intense enough to allow quantitative interpretation of the data. Moreover, in the pulse radiolysis experiments, benzene had to be used as a solvent, and under these conditions the solubility of the dyes is very low, reducing the sensitivity of the signals.

Triplet states can be selectively produced⁴² by energy transfer following pulse radiolysis of benzene (Bz) solutions of indigotin or indirubin in the presence of appropriate sensitizers (biphenyl,

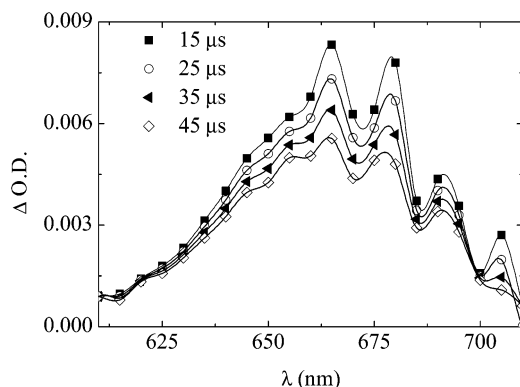


Figure 5. Transient triplet-triplet spectra obtained by direct excitation of purple in DMF at $T = 293$ K. Each data point is the result of an average of 15 shots.

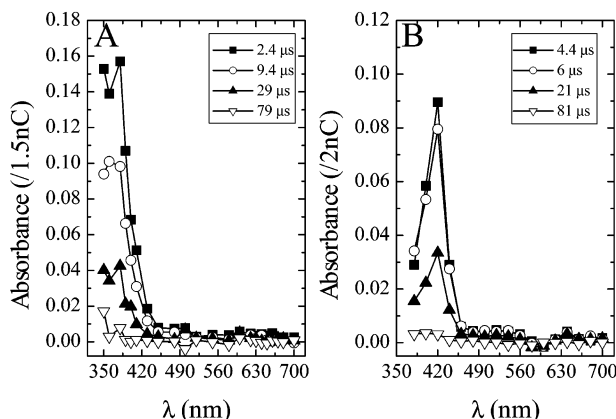
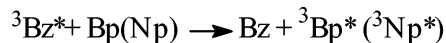
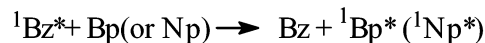
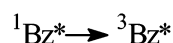
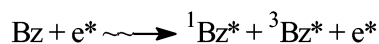


Figure 6. Spectral data observed following pulse radiolysis of argon saturated solutions of (A) purple (2.38×10^{-6} M) and biphenyl (10^{-2} M) in benzene and of (B) indigo (8×10^{-6} M) and naphthalene (10^{-2} M) in benzene at $T = 293$ K.

SCHEME 1



Bp, or naphthalene, Np, for example). A better understanding of the reactions occurring can be seen in Scheme 1. This is subject to the kinetically demanded concentration ratio $[\text{Bz}] \gg [\text{Bp}(\text{Np})] \gg [\text{indigotin}]$.⁴³

An argon saturated solution of purple was studied by pulse radiolysis in benzene solution in the presence of naphthalene (0.01 M) as the triplet sensitizer. Following pulse radiolysis, an initial absorption at 420 nm due to the naphthalene triplet was replaced by a new absorption at around 670 nm. The decay time for the naphthalene triplet (62 μs) was essentially identical to the grow-in of the new absorption at 640 nm (with a rise time of 61 μs), indicating that energy transfer from naphthalene to purple occurred. Under the same experimental conditions

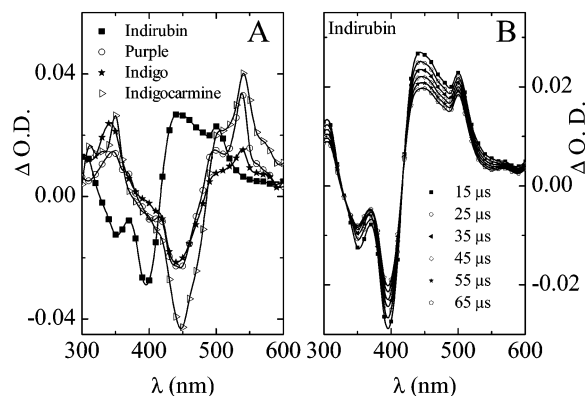


Figure 7. Transient triplet-triplet absorption spectra obtained for (A) the leuco forms of the four studied compounds and (B) for leuco-indirubin obtained with different delay times after laser flash at $T = 293$ K.

naphthalene itself was found to decay with a lifetime of 62 μs (decay collected at 420 nm). When biphenyl was used as a donor, the same weak signal at around 670 nm was found (Figure 6A), again indicating the formation of the transient triplet that is observed under the experimental conditions reported in Figure 5 (direct excitation of purple).

For indigo, similar experiments were performed using either biphenyl or naphthalene as donors (Figure 6B). In the case of biphenyl acting as the donor, a very weak signal between 450 and 700 nm together with a strong maximum at ~ 360 nm (biphenyl absorption) was observed in this situation. Although the biphenyl triplet lifetime decreases in the presence of indigo ($\tau_T = 16 \mu\text{s}$ in the presence vs $\tau_T = 56 \mu\text{s}$ in the absence), which is a clear sign that energy transfer from the biphenyl donor to indigo is occurring, the weakness of the signal precludes determination of the molar extinction coefficient for the generated transient. With naphthalene as the donor, similar signals were obtained in the 440–700 nm region (Figure 6B), although now the strong maximum is found at 420 nm (Np triplet maxima). Again, a decrease in the naphthalene triplet lifetime is obtained in the presence of indigo ($\tau_T = 24.5 \mu\text{s}$ in the presence vs $\tau_T = 62 \mu\text{s}$ in the absence), indicating once more that energy transfer from the naphthalene donor to indigo acceptor is occurring.

Leuco Forms. In contrast to what was observed for the keto compounds, for the leuco forms of the four compounds studied, intense and clear triplet signals were obtained. The triplet absorption spectra for the leuco forms of the compounds are presented in Figure 7. A strong depletion in the singlet absorption region followed by intense signals in the 450–600 nm visible region can be observed. The triplet maxima (~ 540 nm) and band shapes seem to be approximately identical for the compounds with the exception of indirubin, which possesses a much broader band with a blue-shifted maximum (~ 446 nm). Triplet intersystem crossing yields are usually parameters whose absolute value contains a degree of uncertainty that depends, among other things, on the method and techniques used.^{35,44} In the present case, these were determined by the singlet depletion technique,^{35,44} through eqs 1 and 2 (see Experimental Section). The criterion for the use of this technique implies absence of significant triplet absorption in the depletion region. In our case, since the depletion bands are strong and their maxima identical to those obtained in ground-state absorption spectra (see Table 1), this seems to indicate that little, or no triplet, is absorbing in the singlet depletion region. It is therefore possible to obtain the extinction coefficients for the triplet absorptions (eq 1), such that with the subsequent use of actinometry with benzophenone

TABLE 3: Transient Triplet–Triplet Parameters [Extinction Coefficients (ϵ_{TT}), Wavelength Maxima ($\lambda_{\max}^{T_1-T_n}$), Triplet Lifetimes (τ_T), and Intersystem Crossing Triplet Yields (ϕ_T)] and Internal Conversion Yield (ϕ_{IC}) Together with the Rate Constants Associated with These Processes and the Fluorescence Process (ϕ_F in Table 2) for the Leuco Forms of the Indigo Derivatives in DMF at $T = 293$ K

compd	ϵ_{TT} ($M^{-1}cm^{-1}$)	$\lambda_{\max}^{T_1-T_n}$ (nm)	τ_T (μs)	ϕ_T	ϕ_{IC}	k_F (ns^{-1})	k_{IC} (ns^{-1})	k_{ISC} (ns^{-1})
indigo	12 210	540	15	0.125	0.527	0.111	0.167	0.040
purple	18 900	538	20	0.190	0.585	0.060	0.155	0.050
indigocarmin	16 240	540	18	0.250	0.458	0.083	0.131	0.070
indirubin	23 130	446	80	0.171	0.815	0.008	0.458	0.096

(eq 2) it is possible to obtain the intersystem crossing triplet yields (see Table 3).

Discussion

The main experimental results obtained for the systems under study, both in their leuco and keto forms, are given in Tables 1–3. For the leuco forms, the complete photophysical characterization has been possible, and the complete set of results is summarized in Tables 2 and 3.

After determination of both the fluorescence quantum yields and the intersystem crossing triplet yields, it is now possible to establish the balance between all the yields and rate constants for the deactivation processes occurring with the leuco forms of the indigo derivatives (see Table 3). For the leuco species, the data in Table 3 show that with the exception of indirubin the fluorescence, intersystem crossing (k_{ISC}), and internal conversion (k_{IC}) rate constants are of the same order of magnitude, meaning that these processes compete for the excited-state deactivation. It is also observed that the level and kind of substitution in the fundamental indigotin moiety determine the balance between the different deactivation processes.¹¹ The introduction of bromine (purple) and sulfur (sulfoxide group in indigocarmin) increases the intersystem crossing triplet yield as a consequence of the classical heavy atom effect.³⁹ There is also a decrease in the fluorescence quantum yield and no significant change in the internal conversion quantum yield with this pattern of substitution, indicating that the increase in ϕ_T is made at the expense of the fluorescence channel (see Tables 2 and 3). A similar conclusion can be drawn from the rate constant data. The results obtained for these leuco species are in contrast with the general behavior found with the keto forms (Table 2), where the radiationless channels rule the deactivation processes.^{17,22}

The different behavior found for indirubin in its keto and leuco forms clearly shows that the structure influences the spectroscopic and photophysical properties of these compounds (Tables 2 and 3). Rotational photoisomerization could occur with indirubin, and this would explain the high Stokes shifts values observed. In fact, note that in the case of indigotin compounds, intramolecular hydrogen bonding between the amine hydrogen and the carbonyl oxygen (Chart 2A) possibly avoids rotation around the carbon–carbon central bond, whereas in the case of indirubin, intramolecular hydrogen bonding can be established in one individual indene ring (Chart 2B) liberating the molecule to freely rotate around the carbon–carbon central bond. The same line of argument is valid for the possibility of rotational isomerization to occur with the leuco form of the indigo derivatives (absence of intramolecular hydrogen bonding). In fact, it seems reasonable to associate the larger Δ_{SS} values in the leuco forms with some degree of rotation around the central C=C bond. This phenomenon has been observed for indigo derivatives with bulky groups on the nitrogen atom, such as *N,N'*-dimethylindigo⁴⁵ or *N,N'*-diacetylindigo⁴⁶ where this kind of substitution destroys the planarity of the indigo structure, in particular around the double bond, thus favoring

the excited-state cis–trans isomerization and photochromic properties of these derivatives.⁴⁵ This loss of planarity involves the two carbons of the central bond that adopt a slightly pyramidal bonding geometry and undergo a hybridization change from sp^2 toward sp^3 .⁴⁵

Concerning the explanation for the absolute prevalence of the radiationless deactivation pathway in the colored keto forms of the indigo type compounds, we comment on some of the arguments discussed in the literature, in particular on the data reported by Elsaesser et al.²³ In this work, on the basis of a pioneer picosecond infrared study, it was concluded that proton transfer did not take place in the S_1 state of the indigo derivative 4,4',7,7'-tetramethylindigo (TMI). TMI was chosen, in place of indigo, because of its higher solubility in organic nonpolar solvents and also because, as said by the authors, the four methyl groups prevent the formation of intermolecular H bridges. On the basis of the results obtained for TMI, it was stated that “the results give convincing proof that proton transfer does not occur in the first electronic state of indigo.”²³ This is the reference paper that has been used to exclude ESPT as being responsible for the high photostability of indigo.²² Moreover, in this paper for TMI in water, the reported value for the lifetime of S_1 is 30 ps, and for the fluorescence quantum yield it is 0.0014.²³ While in the case of the fluorescence quantum yield the values are in reasonable agreement with our data for indigo (Table 2), the same cannot be said for the fluorescence lifetime. If, as claimed, the introduction of the four methyl groups avoids intermolecular hydrogen bridge formation, the decrease from 140 ps (Table 2 for indigo) to 30 ps²³ could be considered to be due to a more efficient internal conversion in the case of the substituted indigo. Yet in our case, a value of 30 ps was obtained only for indirubin (see Table 2). This low lifetime,²³ which is consistent with that of indirubin, could point to a nonplanar structure for TMI and therefore suggests that some caution should be taken concerning the generalization of the absence of ESPT from TMI to the indigo molecule.

Another important seminal work on the photophysics of indigo, based on picosecond transient absorption studies, was published by Kobayashi et al.¹⁸ Their main conclusions are in agreement with our results, namely, that “the absence of fluorescence of indigo can be explained in terms of a very fast internal conversion from the lowest excited singlet state (S_1) to the ground state (S_0)”.¹⁸ Here, the word “absence” should instead be read as “very little” as attested by our data. In that work it was proposed that ESPT could enhance the internal conversion process.¹⁸ They observed in water (a bad solvent for the keto form of indigo) a value of 270 ps for the relaxation of indigo to the ground state, although the repopulation of its ground state was found to be characterized by a lifetime of 1.5 ns.¹⁸ To explain such observations, the authors have suggested the decay of the singlet state to an intermediate state having a lifetime 270 ps, which was followed by decay to the ground state within 1.5 ns.¹⁸ However, the nature of this intermediate was not clearly established.¹⁸

Without excluding ESPT, a possible explanation for the fast

internal conversion rate constants values lies in the small energy difference between the ground and lowest singlet excited state. In fact, as is well-known from the golden rule for radiationless transitions, a small value in the S_1-S_0 energy gap favors nonradiative vs radiative decay pathway by increasing the vibrational overlap (Franck-Condon integrals)³⁹ of the wave functions for the nonradiative modes of these two states, which in consequence gives rise to indigo's blue color and also to its magnificent photostability.

Conclusions

The photophysical properties of indigo and three of its selected derivatives have been studied in their oxidized (keto) and reduced (leuco) forms. For the first time, detailed singlet and triplet excited-state studies are presented for the leuco forms, including fluorescence (spectra, quantum yields, and lifetimes), triplet absorption spectra, intersystem crossing triplet yields, and triplet lifetimes. As a consequence of this, it has been possible to obtain for the leuco species all the excited-state rate constants, allowing a full photophysical characterization of these systems.

It was shown that completely different spectroscopic and photophysical parameters are presented by the two forms (leuco and keto). The comparison between the photophysical properties of the leuco and keto forms revealed that for all the keto forms studied the radiationless rate constants are lower by ca. 1 order of magnitude than those found for the leuco forms. As a consequence, fluorescence and triplet state formation are, in the case of the leuco forms, alternatives to the internal conversion and efficient deactivation channels for the singlet excited state. In the case of the keto forms, the very low energy difference between the ground and first excited singlet states (which will enable significant overlap of the vibrational levels of the two states) seems to be the main contributory factor to the high values observed for the radiationless rate constants. It cannot, however, be excluded that excited-state proton transfer could play a part in the observed photostability.

Furthermore, the high Stokes shift observed in the leuco systems, as well as for indigo's structural isomer indirubin, in comparison with the small values for the keto forms suggests a lack of rigidity, in the excited state, for the former structures when compared with the rigid structure of the keto form.

The differences observed between the two forms suggest that the well-known photostability of the keto forms of indigo may not be present with the reduced species.

Even if the enigma of *cis*-indigo, as described by George Wyman,¹⁷ is yet to be solved, we hope that our results will contribute to a better understanding of these dazzling molecules.

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