JOURNAL OF PHYSICAL ORGANIC CHEMISTRY
J. Phys. Org. Chem. 2004; **17:** 675–679
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/poc.755

Intensely fluorescent dipyrrinones

Stefan E. Boiadjiev and David A. Lightner*

Department of Chemistry, University of Nevada, Reno, Nevada 89557-0020, USA

Received 8 October 2003; revised 12 January 2004; accepted 14 January 2004

ABSTRACT: Intense fluorescence from a new, more highly conjugated (benzannelated) xanthoglow analog, 9,11-dimethyl-10-[2-(methoxycarbonyl)ethyl]-5*H*,7*H*-pyrrolo[2′,1′:6,1]pyrimidino[3,4-*a*]isoindole-5,7-dione (**1**), was determined accurately and compared with fluorescence from the parent xanthoglow, 1-ethyl-8-[2-(methoxycarbonyl) ethyl]-2,7,9-trimethyl-3*H*,5*H*-dipyrrolo[1,2-*c*:2′,1′-*f*]pyrimidine-3,5-dione (**2**). Benzoxanthoglow (**1**) gave a quantum yield for fluorescence (ϕ_F) of 0.78 in cyclohexane (λ_{exc} 412 nm, λ_{em} 487 nm), whereas xanthoglow methyl ester (**2**) gave $\phi_F = 0.80$ in cyclohexane (λ_{exc} 410 nm, λ_{em} 473 nm). In DMSO, **1** gave $\phi_F = 0.55$ (λ_{exc} 419 nm, λ_{em} 530 nm) and **2** gave $\phi_F = 0.65$ (λ_{exc} 419 nm, λ_{em} 508 nm), illustrating the large Stokes shifts and strong fluorescence properties of these easily synthesized yellow pigments. Copyright © 2004 John Wiley & Sons, Ltd.
Additional material for this paper is available in Wiley Interscience

KEYWORDS: fluorescence; xanthoglow; dipyrrinones

INTRODUCTION

Dipyrrinones (Fig. 1) are typically yellow chromophores, with a strongly allowed absorption ($\varepsilon \approx 30\,000$) near 400 nm.[1] In solution, the singlet excited state from absorption of an ~400 nm photon is rapidly relaxed by $4Z \rightarrow 4E$ double-bond isomerization. For example, the quantum yield for $Z \rightarrow E$ photoisomerization of xanthobilirubic acid (Fig. 1), $\phi_{Z \rightarrow E}$, is ~0.22 ($\phi_{E \rightarrow Z}$ ~0.40) in EPA (diethylether–isopentane–ethanol, 5:5:2 v/v/v) at 20 °C[1] and ~0.2 in pH 7.4 aqueous buffer containing human serum albumin (HSA).[2] Radiative decay by fluorescence is correspondingly very weak, with minute fluorescence quantum yields: $\phi_F < 1 \times 10^{-3}$ in EPA[1] and $\phi_F < 3 \times 10^{-3}$ in aqueous buffered HSA at 22 °C.[2] At very low temperatures (77 K) in glasses, $\phi_{Z \rightarrow E}$ decreases to $< 5 \times 10^{-4}$ and ϕ_F increases to ~0.33 in EPA. When the $Z \rightarrow E$ photoisomerization is prevented by bonding constraints that link the two nitrogens by one-carbon[3–6] (or longer[6]) belts, ϕ_F measured at room temperature rises considerably.

In this work, we examined the influence of extended conjugation, through benzannelation of the dipyrrinone, measuring ϕ_F for **1** and making comparisons with the parent xanthoglow (**2**).[5] Because most general references[7,8] that describe methods for determining quantum yields do not outline a step-by-step experimentally detailed procedure for measuring relative ϕ_F, we describe a systematic method for relative ϕ_F measurements that should be useful to those learning fluorescence spectroscopy.

**Correspondence to:* D. A. Lightner, Department of Chemistry, University of Nevada, Reno, Nevada 89557-0020, USA.
E-mail: lightner@scs.unr.edu
Contract/grant sponsor: National Institutes of Health; *Contract/grant number:* HD 17779.

RESULTS AND DISCUSSION

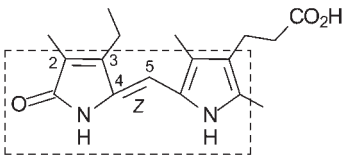
Synthesis

The parent, unbridged, methyl esters of xanthobilirubic acid (XBR, Fig. 1) and its 2,3-benzo analog, both known from earlier studies,[9,10] were dissolved in anhydrous methylene chloride solvent and reacted with 5 mol equiv. of 1,1′-carbonyldiimidazole in the presence of DBU base[5] to afford 90 and 93% isolated yields of **1** and **2**, respectively. Compounds **1** and **2**, solutions of which were intensely fluorescent to the naked eye, gave the expected ^{13}C and ^{1}H NMR spectra, with the latter showing the absence of the NH resonances and the former showing a new signal at 143–144 ppm for the imide carbonyl.

UV–visible absorption and fluorescence emission spectra

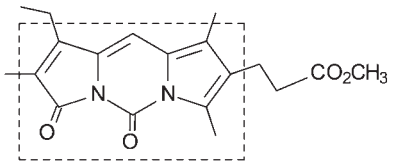
The UV–visible spectra of **1** and **2** (Table 1) reveal a long-wavelength maximum absorbance near 415–426 nm for **1** and 425–431 nm for **2** with a smaller associated ε, as has been noticed previously for planarized dipyrrinones.[3,4] In addition, a shorter wavelength UV band near 275–285 nm and a longer wavelength band lying close to 363–370 nm become evident in **1**. Only small wavelength shifts in the long-wavelength absorption

Xanthobilirubic Acid (XBR)



dipyrrinone

Methyl N,N'-Carbonylxanthobilirubinate (**2**)
(Xanthoglow methyl ester)



2,3-Benzo-N,N'-carbonylXBR methyl ester (**1**)
(2,3-Benzoxanthoglow methyl ester)

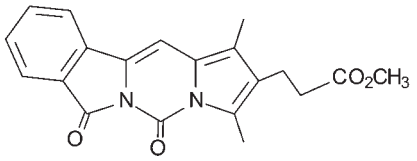


Figure 1. Structrues of (top) xanthobilirubic acid (XBR), with dipyrrinone chromophore in box; (middle) *N*,*N*′-carbonyl-bridged XBR, called 'xanthoglow', with *N*,*N*′-carbonyldipyrrinone chromophore (also called 3*H*, 5*H*,-dipyrrolo[1,2-*c*:2′,1′-*f*]pyrimidine-3,5-dione) in box; (bottom) benzannelated xanthoglow

attend the extended conjugation on going from **1** to **2**. In contrast, a more significant bathochromic shift is found from the second band on going from **2** to **1**, suggesting that the orientation of its transition dipole moment might be along the long axis of the chromophore. Most surprising, perhaps, is a new UV band near 363–370 nm found in **1** and absent from **2**. The origin of this band is as yet unclear.

The fluorescence spectra of **1** and **2** were measured exactly as described in the Supplementary Material (available in Wiley Interscience) for the standard reference compounds anthracene, 9,10-diphenylanthracene and perylene, keeping the resolution, scan rate and (especially) the slit widths constant. The last necessitated several adjustments of concentration to fulfill two requirements: sample absorbance <0.1 and emission intensity within the linear response range of the fluorimeter. The combined excitation, emission and UV–visible spectral curves are shown in Figs 2 and 3 for **1** and **2**, respectively. In these graphs, the fluorescence intensity (I) scaling is arbitrary from graph to graph, which means that direct comparison of I is not possible, except for overlaid spectra such as those from cyclohexane and DMSO, or $CHCl_3$ and CH_3OH solvents. A better visual comparison of the solvent dependency of the relative emission intensities is shown in Fig. 4.

Fluorescence quantum yields

In order to quantify the fluorescence of the new heterocyclic systems, a reliable protocol for reproducing literature quantum yield (ϕ_F) data of known standard compounds was first established (see Supplementary Material). In order to generate confidence in the methodology, only after the protocol produced satisfactory agreement between ϕ_F determined for at least two standards (with λ_{ex} and λ_{em} close to those expected for **1** and **2**) was obtained did we use one of them as a standard for the determination of ϕ_F of **1** and **2**. The equation used to interrelate ϕ of a standard (ϕ_{st}) with ϕ of an unknown sample (ϕ_F) is[11]

$$\phi_F = \frac{A_{st}}{A_s} \cdot \frac{I_s}{I_{st}} \cdot \frac{n_s^2}{n_{st}^2} \cdot \phi_{st} \qquad (1)$$

where A_{st} and A_s are absorbances at the excitation wavelength of the standard (st) and sample (s), respectively, I_{st} and I_s are integral fluorescence intensities and n_{st} and n_s are the refractive indices of the respective solvents. This relation requires knowledge of the absorbance (A_{st} and A_s) at the excitation wavelength for each compound. The absorbances were calculated from UV–visible spectra obtained independently in most cases from more concentrated solutions than those used for fluorescence measurements. The UV–visible spectra

Table 1. Solvent dependence of the UV–visible absorption spectral data of dipyrrinones **1** and **2**

	λ_{max} (ε)				
Compound	n-C_6H_{14}	C_6H_6	$CHCl_3$	CH_3OH	$(CH_3)_2SO$
1	439 (14 500)[sh]	444 (13 900)[sh]			
	415 (18 700)	423 (16 900)	426 (16 300)	424 (15 600)	425 (16 200)
	368 (12 000)	370 (9800)	368 (9800)	363 (9700)	365 (9500)
	350 (8400)				
	279 (9000)	277 (8700)	286 (10 900)	285 (12 400)	285 (11 600)
2	431 (19 700)	429 (18 600)	428 (18 500)	425 (18 400)	425 (18 400)
	411 (19 500)	421 (18 600)			
	272 (9600)		276 (11 000)	276 (11 400)	275 (11 100)

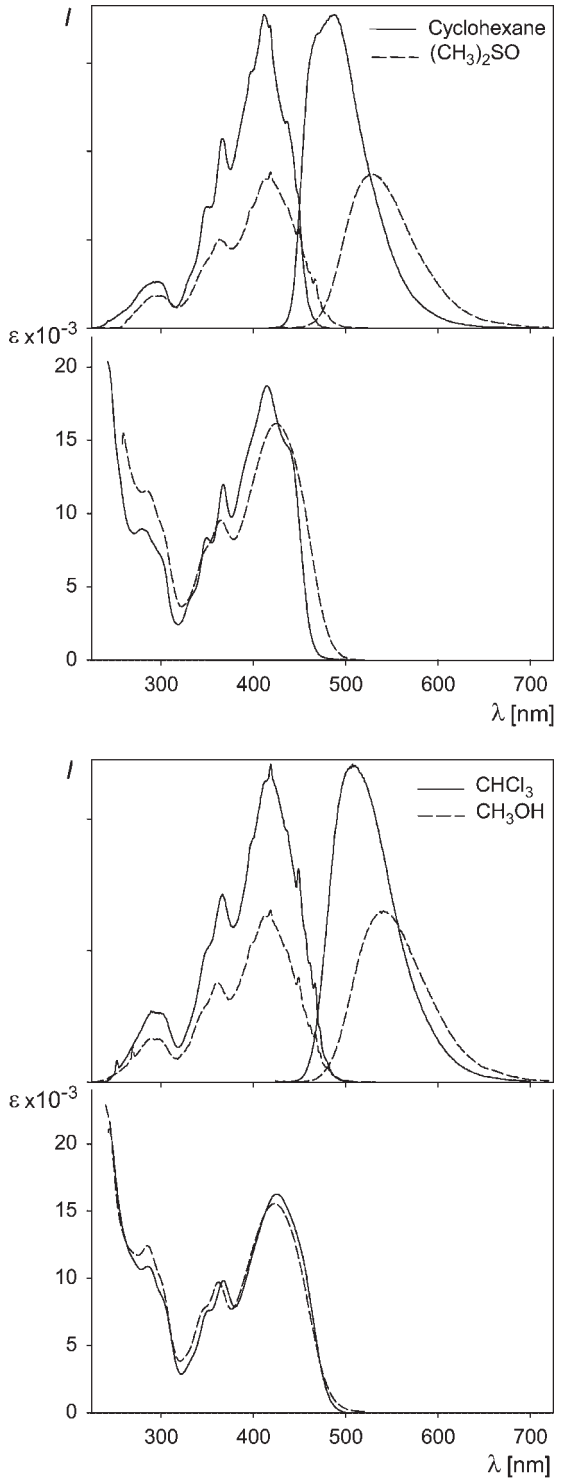


Figure 2. Fluorescence excitation and emission spectra of **1** (displayed in top half) and UV–visible spectra of **1** (displayed in bottom half of each graphic)

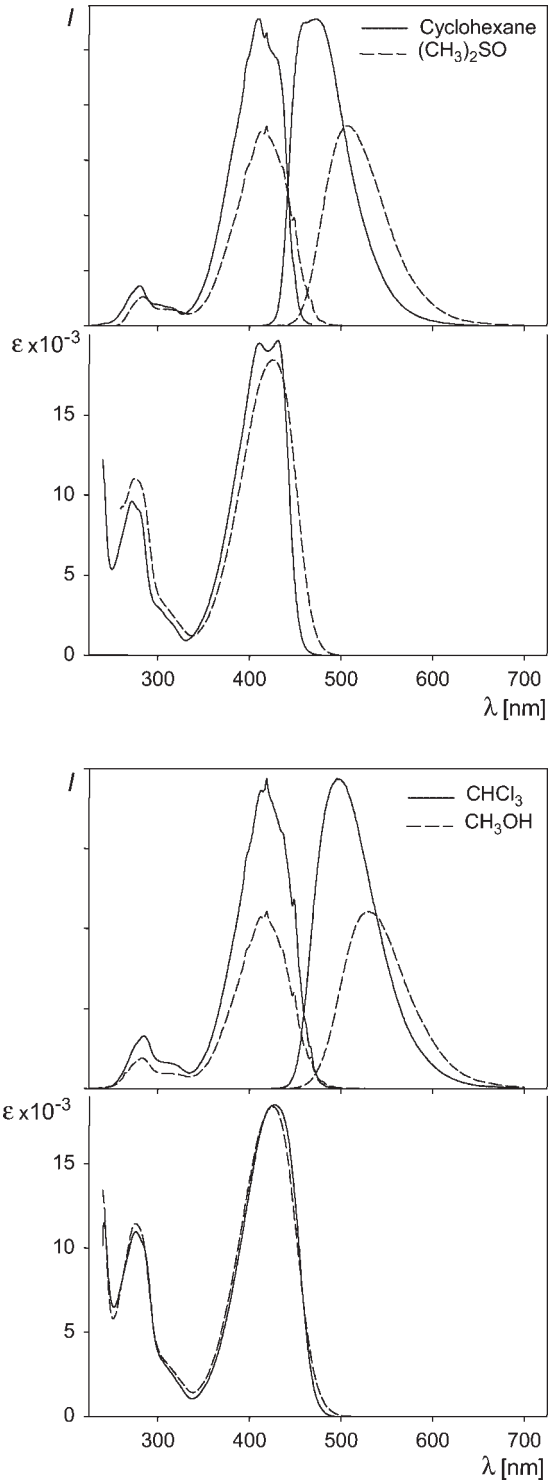


Figure 3. Fluorescence excitation and emission spectra of **2** (displayed in top half) and UV–visible spectra of **2** (displayed in bottom half of each graphic)

were corrected for instrument baseline drift and for concentration corresponding to that of the fluorescence measurement.

After collecting the emission spectra of **1** and **2** in five solvents (Fig. 4), each spectrum was printed together with the emission band of a fluorescence standard, 9,10-diphenylanthracene (DPA), using identical scaling. As an internal check for consistency in the ratio (I_s/I_{st}), we compared ϕ_F from the cut and weigh method vs ϕ_F from the integral values from the fluorimeter. For example, the results from one such pair of emission bands for **2** in $CHCl_3$ gave the weight of emission band of DPA in

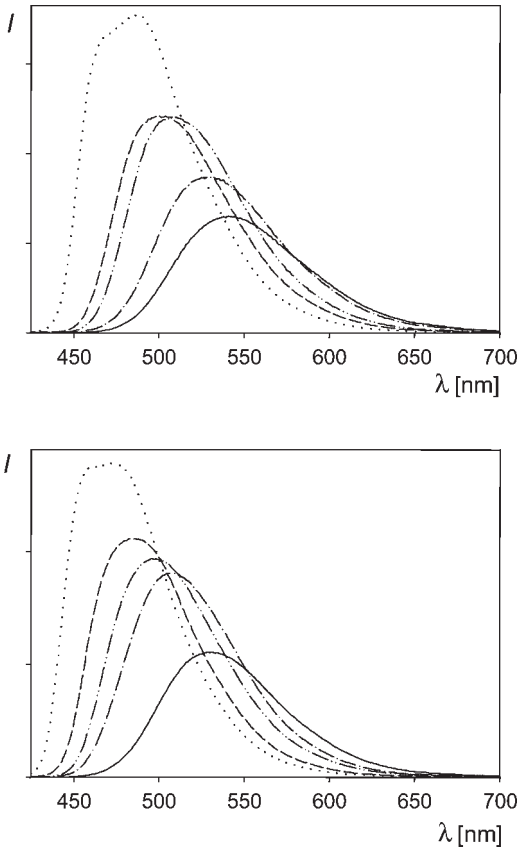


Figure 4. Solvent dependence of fluorescence emission from **1** (top) and **2** (bottom) in (• • •) cyclohexane, (-- -- --) benzene, (-- • • --) chloroform, (------) methanol and (-- • --) dimethyl sulfoxide

cyclohexane as 394.8 mg and that of **2** as 290.1 mg. From Eqn (1), and the available significant figures, then truncating at the final product, we found

$$\phi_F = (7.08_5 \times 10^{-2}/7.35_0 \times 10^{-2}) \times (290.1/394.8) \times (1.446^2/1.426^2) \times 0.90 = 0.66$$

Alternatively, using integral values from the fluorimeter:

$$\phi_F = (7.08_5 \times 10^{-2}/7.35_0 \times 10^{-2}) \times (1.57_2 \times 10^8/2.10_6 \times 10^8) \times (1.446^2/1.426^2) \times 0.90 = 0.67$$

The agreement between these two methods is excellent, and similarly excellent agreement was confirmed for three more samples in five different solvents. The disadvantage of the 'cut and weigh' method is obvious: the 'weight' of the emission band varies. Variations come from the different scaling factors along the *x*- and *y*-axes: in every sample and standard pair, the scaling must be adjusted to accommodate both band intensities. Thus, every pair (different solvent or sample) will give a different weight for the same standard (DPA). The numerical integral method avoids this complication, i.e. the intensity of DPA is always $2.10_6 \times 10^8$ (arbitrary units) and this value was used throughout the study. The various ϕ_F, λ_{exc} and λ_{em} values for **1** are summarized in Table 2. The fluorescence quantum yields of the parent xanthoglow methyl ester (**2**) were determined in the same manner and are shown in Table 2.

Clearly, the ϕ_F values of **1** and **2** are very large and approach unity in cyclohexane while tapering off in methanol. The latter solvent might have promoted self-association of these non-polar pigments; however, in the range of concentrations used, 10^{-5}–10^{-6} M, we found $\ll$2% deviation from Beer's law. The conjugation of **1** exhibits little effect on ϕ_F but a significant effect on λ_{em}, with the emission band showing a 10–22 nm bathochromic shift of **1** relative to **2**. Both **1** and **2** show large Stokes shifts, from 70 to 90 nm in non-polar solvents and from 110 to 120 nm in polar solvents.

CONCLUSION

Determinations of ϕ_F for **1** and **2** relative to three known standards reveal very high values of ϕ_F in a study that illustrates practical aspects, reproducibility and standardization of the method. The importance of **1** and **2** relates to the potential of those strongly blue–green-emitting yellow dyes in forming conjugates to proteins and nucleic acids, where they might serve as fluorophores in gene and protein profilings on DNA and protein chips.

EXPERIMENTAL

Fluorescence measurements were performed using a Jobin Yvon Fluorolog 3 Model FL3-22 instrument at

Table 2. Solvent dependence of the fluorescence quantum yields (ϕ_F) and excitation λ_{max} (λ_{exc}) and emission λ_{max} (λ_{em}) (nm) for **1** and **2**

	Cyclo-C_6H_{12}			C_6H_6			$CHCl_3$			CH_3OH			$(CH_3)_2SO$		
Compound	λ_{exc}	λ_{em}	ϕ_F	λ_{exc}	λ_{em}	ϕ_F	λ_{exc}	λ_{em}	ϕ_F	λ_{exc}	λ_{em}	ϕ_F	λ_{exc}	λ_{em}	ϕ_F
1	412	487	0.78[a]	419	500	0.68	419	508	0.67	419	541	0.36	419	530	0.55
2	410	473	0.80	419	484	0.72	419	497	0.67[b]	419	531	0.35[c]	419	508	0.65

[a] $\phi_F = 0.75$ using perylene standard ($\phi_{st} = 0.91$) in air-free cyclohexane.
[b] $\phi_F = 0.63$ using anthracene standard.
[c] $\phi_F = 0.34$ using anthracene standard.

295–297 K. UV–visible spectroscopy was carried out on a Perkin-Elmer Lambda 12 spectrophotometer, both in 1 cm quartz cells. All fluorescence spectra were acquired by using constant spectral parameters: a step resolution (increment) of 1 nm, both excitation and emission slits of 2 nm and an integration time of 0.5 s. The NMR spectra were acquired on a Varian Unity Plus spectrometer at 500 MHz proton frequency in $CDCl_3$ solvent and were referenced at 7.26 ppm (^{1}H) for the residual $CHCl_3$ signal and 77.00 ppm (^{13}C) for the $CDCl_3$ signal.

The solvents for optical spectroscopy were of HPLC grade and were distilled under a stream of argon just prior to use. Before the distillation, $CHCl_3$ was passed through a basic alumina column. Distillation of $(CH_3)_2SO$ solvent was carried out at 0.5 mmHg vacuum collecting the solvent at 0 °C and thawing it under Ar. The fluorescence standard compounds, *N*,*N*′-carbonyldiimidazole (CDI) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were obtained from Aldrich. Anthracene was recrystallized from ethanol, 9,10-diphenylanthracene (gold label, 99+%) was used as received and perylene was recrystallized from 1-methylnaphthalene as described.[12] The starting dipyrrinones, xanthobilirubic acid methyl ester (**4**)[9] and its benzo analog (**3**),[10] were synthesized as previously described.

N,N′-Carbonyldipyrrinones. General procedure. A mixture of 2 mmol of dipyrrinone **3** or **4**, 1.62 g (10 mmol) of 1,1′-carbonyldiimidazole, 1.50 ml (10 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene and 160 ml of anhydrous methylene chloride was heated under N_2 at reflux for 16 h. After cooling, the mixture was washed with 1% HCl (2 × 100 ml), then with water (3 × 100 ml), and the solution was dried over anhydrous $MgSO_4$. After filtration and evaporation of the solvent under vacuum, the residue was purified by radial chromatography on silica gel and recrystallized from ethyl acetate–hexane to afford >99.5% pure, bright-yellow tricyclic compounds.

9,11-Dimethyl-10-[2-(methoxycarbonyl)ethyl]-5H,7H-pyrrolo[2′,1′:6,1]pyrimidino[3.4-a]isoindole-5,7-dione (**1**). Obtained in 90% yield, m.p. 226–227 °C. ^{1}H NMR, δ 2.13 (3H, s), 2.44 (2H, t, $J = 7.7$ Hz), 2.63 (3H, s), 2.71 (2H, t, $J = 7.7$ Hz), 3.67 (3H, s), 6.73 (1H, s), 7.40 (1H, dd, $J = 7.3$, 7.8 Hz), 7.60–7.65 (2H, m), 7.87 (1H, d, $J = 7.8$ Hz) ppm; ^{13}C NMR, δ 8.97, 12.93, 19.43, 34.27, 51.68, 96.17, 119.48, 119.61, 125.21, 126.05, 126.28, 126.91, 127.01, 128.95, 130.76, 134.18, 134.30, 144.08, 164.23, 173.00 ppm. Anal: calcd for $C_{20}H_{18}N_2O_4$ (350.4), C 68.56; H 5.18, N 8.00; found, C 68.31, H 5.05, N 8.06%.

1-Ethyl-8-[2-(methoxycarbonyl)ethyl]-2,7,9-trimethyl-3H,5H-dipyrrolo[1,2-c:2′,1′-f]pyrimidine-3,5-dione (**2**). Obtained in 93% yield, m.p. 145–146 °C (lit.[5] m.p. 140–141 °C). ^{1}H NMR, δ 1.21 (3H, t, $J = 7.7$ Hz), 1.95 (3H, s), 2.12 (3H, s), 2.44 (2H, t, $J = 7.8$ Hz), 2.53 (2H, q, $J = 7.7$ Hz), 2.65 (3H, s), 2.74 (2H, t, $J = 7.8$ Hz), 3.67 (3H, s), 6.38 (1H, s) ppm; ^{13}C NMR, δ 8.36, 9.00, 12.90, 13.77, 17.87, 19.35, 34.28, 51.63, 96.86, 120.41, 125.78, 126.16, 126.22, 130.33, 131.45, 143.30, 146.42, 167.67, 172.97 ppm.

Acknowledgements

We thank the US National Institutes of Health (HD 17779) for generous support of this work and Professor S.-W. Tam-Chang for use of the spectrofluorimeter. Dr S. E. Boiadjiev is on leave from the Institute of Organic Chemistry, Bulgarian Academy of Sciences.

REFERENCES

1. Falk H. *The Chemistry of Linear Oligopyrroles and Bile Pigments*. Springer: Vienna, 1989.
2. Lamola AA, Braslavsky SE, Schaffner K, Lightner DA. *Photochem. Photobiol*. 1983; **37**: 263–270.
3. van Es JJGS, Koek JH, Erkelens C, Lugtenburg J. *Recl. Trav. Chem. Pays-Bas* 1986; **105**: 360–367.
4. Ma JS, Lightner DA. *Tetrahedron* 1991; **47**: 3719–3726.
5. Brower JO, Lightner DA. *J. Org. Chem*. 2002; **67**: 2713–2716.
6. Hwang KO, Lightner DA. *Tetrahedron* 1994; **50**: 1955–1966.
7. Demas JN, Crosby GA. *J. Phys. Chem*. 1971; **75**: 991–1024.
8. Berlman IB. *Handbook of Fluorescence Spectra of Aromatic Molecules*. Academic Press: New York, 1965.
9. Shrout DP, Lightner DA. *Synthesis* 1990; 1062–1065.
10. Boiadjiev SE, Lightner DA. *J. Heterocycl. Chem.* 2003; **40**: 181–185.
11. Eaton DF. In *The Handbook of Organic Photochemistry*, vol. 1, Scaiano J (ed). CRC Press: Boca Raton, FL, 1989; Chapt. 8.
12. Dewar MJS, Mole T. *J. Chem. Soc*. 1956; 1441–1443.