Synthetic Models related to Deoxyribonucleic Acid Base–Psoralen Interactions. Syntheses and Studies of Bichromophore Systems containing Psoralen Moieties [1]

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The syntheses of new bispsoralen derivatives (II_n) and (III_m) are reported. The photophysical properties of these potential deoxyribonucleic acid (DNA) bisintercalating drugs are studied and compared with compounds (I) and (IV) where the psoralen moiety is linked with a DNA base. The singlet and triplet deactivation processes underline the role of intercalation in the photobiological activity of psoralen with DNA. Some attempts at complexation of compounds (II_n) and (III_m) with DNA are also reported.

The photosensitization of skin by psoralen has been actively investigated in recent years.[2] The photobiological activity of these drugs is generally recognized to be due to covalent bonding of the furocoumarins with deoxyribonucleic acid (DNA) bases under u.v.A light involving the formation, in the dark, of a molecular complex (intercalation type). This dark complex between psoralen and DNA bases has been studied mainly by u.v. absorption spectroscopy where characteristic bathochromic and hypsochromic effects are revealed.[3] Although Rapoport [4] provided information about the geometry of this complex by structural assignment of some photoproducts obtained from the irradiation of a psoralen derivative and native DNA, synthetic models appeared to be very convenient for this purpose.[2,4–6] Among these models, the bichromophore (I) in which a psoralen moiety and a DNA base (thymine) are linked together by a flexible polyoxyethylene chain, allowed, for the first time, the direct observation of specific overlap between furocoumarin and thymine in the crystalline state.[7] On the other hand, bichromophoric compounds are also useful for the location of intercalating sites of the drugs in DNA. In the bis-9-aminoacridine series, it has been shown that both acridine rings can intercalate if the linkage is just long enough to span one base pair.[8] Moreover, to probe packaging of nucleic acids, bismonoazidomethanium derivatives having interhelical DNA–DNA cross-linking have also been synthesized.[9]

In this paper we report synthetic and spectroscopic studies of the novel bichromophoric compounds (II_n) and (III_m) which contain two psoralen rings capable of displaying bis-intercalating properties in DNA. Bichromophores (II_n) containing flexible polyoxyethylene chains [10] of differing lengths (4—13 atoms) are compared with their polymethylene homologues (III_m). Moreover, the bichromophore (IV) in which psoralen is linked to a purine DNA base (adenine) is studied for comparison with (II_n), (III_m), and (I).

Results and Discussion

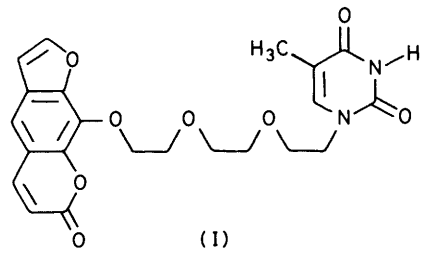
Syntheses and Characterisation of Models.—*O*-Alkylation of 8-hydroxypsoralen has been shown to be efficient using iodoalkanes in refluxing acetone with K_2CO_3 as the base.[11] Some bichromophores were prepared from stoicheiometric amounts of 8-hydroxypsoralen and 1,5-di-iodo-3-oxapentane [(II_1)] and 1,8-di-iodo-3,6-dioxaoctane [(II_2)], and the monochromophore R_1† was formed from 1-iodo-3,6,9-trioxadecane; however, this method failed for (II_3) largely because of the thermal instability of 1,11-di-iodo-3,6,9-trioxaundecane.[12] Under the same experimental conditions tetraethylene glycol ditosylate, which is stable, did not prove reactive enough to yield (II_3). This compound was, however, obtained in a one-pot synthesis by the addition of a stoicheiometric amount of sodium iodide to a tetraethylene glycol ditosylate–8-hydroxypsoralen mixture in refluxing acetone in the presence of K_2CO_3. Sodium iodide probably allowed iodide–tosylate exchange before alkylation.

The same procedure applied to a mixture of ethylene glycol ditosylate, sodium iodide, and K_2CO_3 in refluxing acetone yielded, instead of (III_1), 1-(psoralen-8-yloxy)propan-2-one as the major product indicating the probable transient formation of iodoacetone. The bichromophores ($III_{1,4,7,10}$) were synthesized from the commercial α,ω-dibromoalkanes, 8-hydroxypsoralen, and K_2CO_3 in dimethylformamide (DMF) at 80 °C under nitrogen. The unsymmetrical bichromophore (IV) was prepared by reaction in DMF of the adenine anion on 1-(psoralen-8-yloxy)-8-iodo-3,6-dioxaoctane previously obtained by the action of a seven-fold excess of 1,8-di-iodo-3,6-dioxaoctane on 8-hydroxypsoralen according to the procedure used for (II_1) and (II_2). The yields of pure products (II_n), (III_m), R_1, and (IV) are listed in Table 1 (the reaction yields were not optimized).

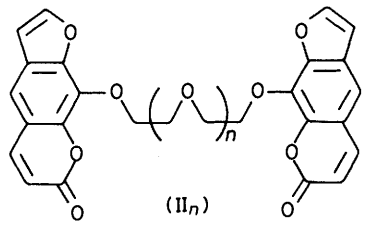
Compounds (II_n), (III_m), (IV), and R_1 were purified by column chromatography (silica gel) and recrystallized (except for R_1 which is an oil). Their purity was checked by t.l.c. and h.p.l.c. (reverse phase). After purification they were stored in the dark under an inert atmosphere (nitrogen or argon).

The molecular structures of compounds (II_n), (III_m), and (IV) were established principally by their ^1H n.m.r., u.v., and mass spectra. The pattern of the furocoumarin protons in the ^1H n.m.r. spectra of compounds (II_n) and (III_m) is the same and similar to that in the spectrum of 8-methoxypsoralen (8-MOP).[13] The aromatic region of compound (IV) is easily analysed as the sum of the furocoumarin and adenine patterns observed in the ^1H n.m.r. spectra of 8-MOP and 9-ethyladenine. The signals corresponding to the polyoxyethylene protons are characteristic of the chain length and their assignment was made by comparison with the bis-

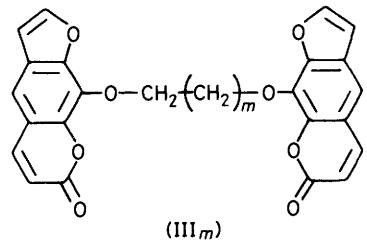
† 1-(Psoralen-8-yloxy)-3,6,9-trioxadecane.



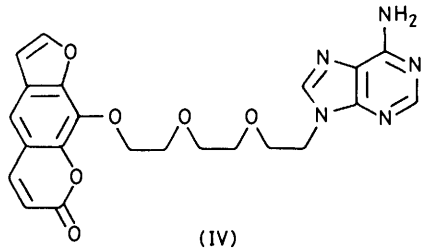
(I)



(II_n)
$n = 0,1,2,3$



(III_m)
$m = 1,4,7,10$



(IV)

anthryloxypolyoxa-alkane series.[14] No ground-state interactions in bichromophoric compounds are revealed by ^1H n.m.r. analysis at room temperature. The mass spectra always displayed an intense peak at m/z 202 corresponding to [hydroxypsoralen]$^{+\cdot}$ and a signal associated with the loss of 201 (oxypsoralen); moreover, with compounds (II_n), the characteristic peak at m/z 229 (psoralen $O-CH_2-CH_2)^+$ and the fragmentation of the polyoxyethylene chain into CH_2-CH_2-O portions were observed. Details of mass spectra and elemental analyses are in Supplementary Publication No. SUP 23726 (4 pp.).*

Spectroscopic Studies.—Owing to the poor solubility of bispsoralens (II_n) and (III_m) in aqueous solution, the photophysical studies were performed in spectroscopic grade alcohol.

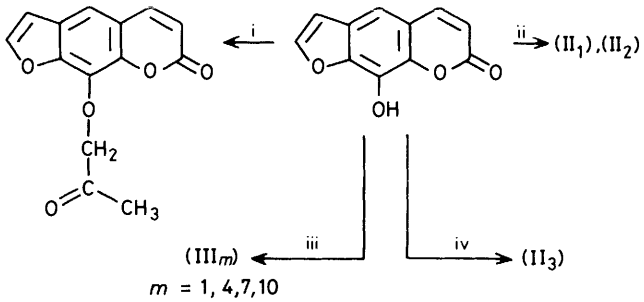
Electronic absorption spectra in fluid solution (ground-state interactions). U.v. absorption spectroscopy is a powerful technique for studying molecular interactions in the ground state. These interactions might induce new electronic transitions (charge transfer, *etc.*) or some modification of the intensity and/or the location in the absorption bands. This technique has already been used for certain bichromophoric systems, to measure at high dilution interactions between the chromophores (intramolecular process),[15] and also in complexation studies of psoralen with DNA.[3]

The electronic absorption spectra of compounds (II_0) and (II_3) in ethanol are reported in Figure 1 and compared with the reference compound 8-MOP.† Only a weak perturbation brought about by the presence of the second psoralen moiety is observed; this effect, similar for all compounds (II_n) and (III_m), decreases as the chain lengthens.

For compounds (I) and (IV), the spectra were also slightly modified in ethanol compared with the isolated single chromophores (Figure 2). However, in water, net hypochromism is revealed indicating a preferred folded conformation [16] in this solvent (Figure 3).

Emission spectroscopy and lifetime measurements. The biological activity of psoralen is associated with the electronic excited states (singlet and/or triplet [2]), which induce photochemical reaction; fluorescence and phosphorescence spectroscopy should provide information on the behaviour of these states and also reveal their ability to form excited complexes (excimers and exciplexes [17]). Here we report the emission spectra (fluorescence and phosphorescence) at low temperature (15 K) of all the compounds above (Figures 4a—k) and the emission spectrum at room temperature (Figure 4l) in alcoholic solvents of compound (III_1) which is representative of the series.

The lifetimes and quantum yields of fluorescence and also the triplet lifetimes (measured by triplet–triplet absorption spectroscopy) are compiled in Table 2.



Scheme 1. *Reagents and conditions:* i, $TsO(CH_2)_2OTs$, K_2CO_3, NaI, acetone, reflux; ii, $ICH_2(CH_2OCH_2)_nCH_2I$ (n = 1 or 2), K_2CO_3, acetone, reflux; iii, K_2CO_3, dimethylformamide, 80 °C, $BrCH_2-(CH_2)_mBr$; iv, $TsOCH_2(CH_2OCH_2)_3CH_2OTs$, K_2CO_3, NaI, acetone, reflux

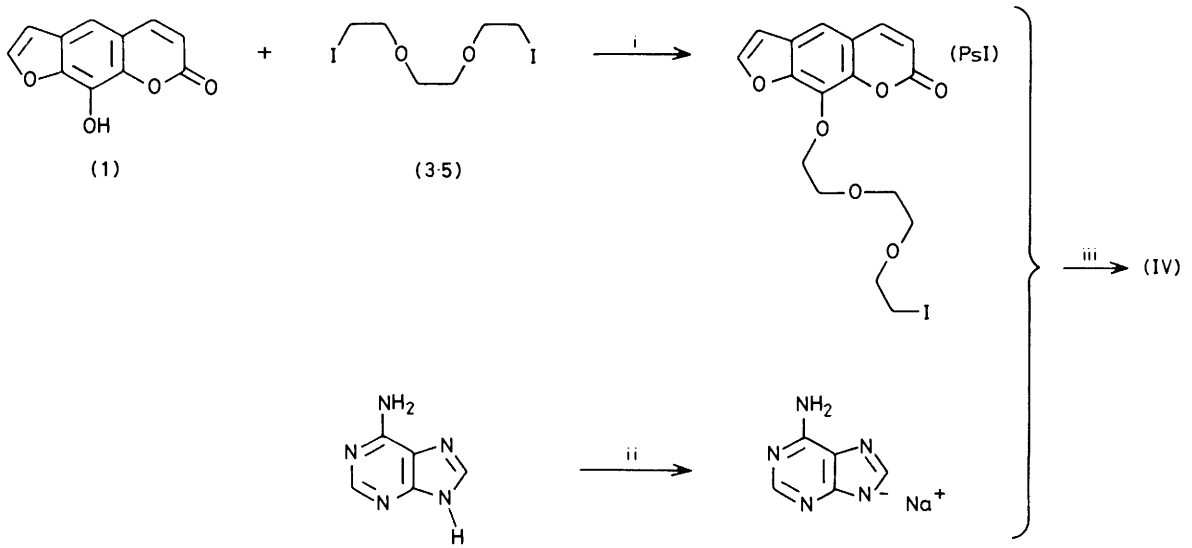
Table 1. Yields and m.p.s of novel bichromophoric systems and reference compounds

Compounds	Yields (%)	M.p. (°C)
(II_1)	17	174—176
(II_2)	8	124—125
(II_3)	18	74—76
(III_1)	24	226—227
(III_4)	38	141—142
(III_7)	31	145—146
(III_{10})	28	101—102
R_1	58	Oil
(IV)	39 *	150—151

* From PsI.

* For details of Supplementary Publications see Instructions for Authors, *J. Chem. Soc., Perkin Trans. 2*, 1983, Issue 1, p. xvii.

† The u.v. absorption spectra of 8-MOP and R_1 are identical.



Scheme 2. *Reagents and conditions:* i, K_2CO_3, acetone, reflux; ii, NaH, dimethylformamide, room temperature; iii, N_2, dimethylformamide, 80 °C

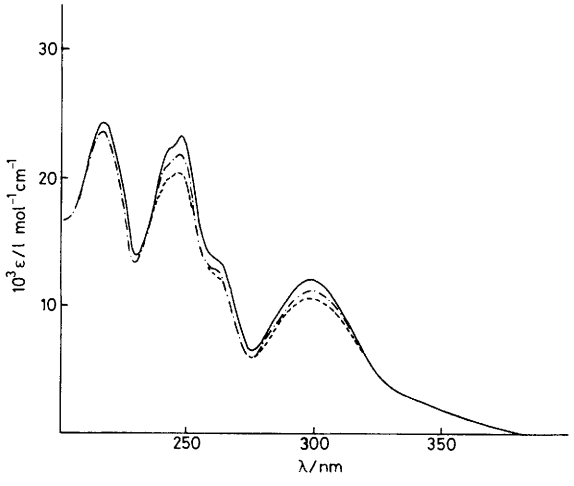


Figure 1. Comparison of the u.v. absorption spectra in ethanol at room temperature of 8-MOP (——) (ε), (II_0) (– – – – –) (ε/2), and II_3 (—·—·—) (ε/2). $c \leqslant 10^{-5}$M

Table 2. Lifetimes (τ/ns) and quantum yields (φ_F) of fluorescence and triplet lifetimes (τ/μs) of studied compounds at room temperature, in alcoholic solvents

Compounds	Singlet excited state * φ_F (10^{-3})	τ (ns) ‡	Triplet excited state † Concn. (10^{-4}M)	τ (μs) ‡
8-MOP	1.6	0.5	4.3	1.3
R_1	2.8	1	5.3	1.3
(II_0) ≡ (III_1)	3.5	0.9	1.9	0.1
(II_1)	4.0	0.6	2.0	0.24
(II_2)	3.2	0.5	2.0	0.20
(II_3)	2.3	0.5	2.1	0.17
(III_4)	3.0	0.7	1.7	0.16
(III_7)	3.0	0.6	2.0	0.15
(III_{10})	2.6	1	2.1	0.15
(I)	2.2	0.7	4.8	1.6
(IV)	2.2	0.7	4.4	0.76

* Solvent methanol, $c \leqslant 5 \times 10^{-5}$M, $\lambda_{exc.}$ 350 nm, room temperature. † Solvent ethanol, Nd laser $\lambda_{exc.}$ 353 nm, $\lambda_{abs.}$ 370 nm, room temperature. ‡ Single exponential decay was assumed.

At room temperature, the emission spectra of the compounds under study are structureless and similar in shape with a maximum at 495 nm; this luminescence might be attributable to a fluorescence process as previously reported by Hammond for 8-MOP in water.[13] This emission, characteristic of the psoralen group with low quantum yields and short lifetimes [2] (at the limits of the instrumental response), is weakly dependent on the nature of the chain and the chromophores (Table 2).

At room temperature, the triplet lifetimes have been determined according to Bensasson's method for psoralen derivatives.[18] Since the psoralen triplet lifetimes were shown to be sensitive to the furocoumarin concentration,[18] the triplet measurements were performed on closely related optical density solutions at 353 nm. For the symmetrical bichromophores (II_n) and (III_m) the triplet lifetime values, although lower than these observed for the reference compounds 8-MOP and R_1, are not very sensitive to the chain length, except for (III_1) where a net decrease is noticed ($\leqslant$ 100 ns). No effect from the thymine moiety was observed on the psoralen triplet lifetimes in compound (I), indicating that the intramolecular quenching process between psoralen and thymine is not the major deactivating pathway. On the other hand, the purine base in compound (IV) appeared to be a more efficient quencher of the furocoumarin triplet state. These results are different from previously published data obtained in water between psoralen and DNA bases for an intermolecular process.[19]

The emission spectra recorded at 15 K in rigid matrices (Figures 4a—k) (ethanol) revealed, in the phosphorescence region,[20] some intramolecular interactions between the chromophores in the symmetrical compounds (II_n) and (III_m). This effect appears to be sensitive to the chain length and emphasizes, in this solvent, the strong affinity of psoralen for itself. This interaction is not observed for psoralen and the DNA bases thymine and adenine.

Complexation studies with DNA. A u.v. spectroscopic method was used for the determination of complexation constants.[3] Measurements are based on the hypochromism which appears in the u.v. spectrum of furocoumarin in the presence of DNA. In this part, the complexation equilibrium constants

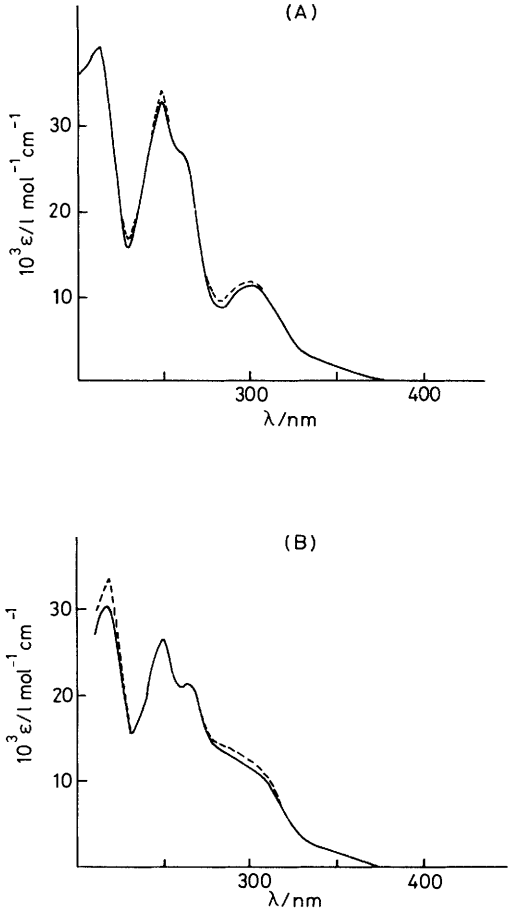


Figure 2. Comparison of the u.v. absorption spectra at room temperature of (IV) (——) and 8-MOP + 9-ethyladenine (– – – – –) in ethanol (A); and (I) (——) and 8-MOP + 1-ethylthymine (– – – – –) in ethanol (B)

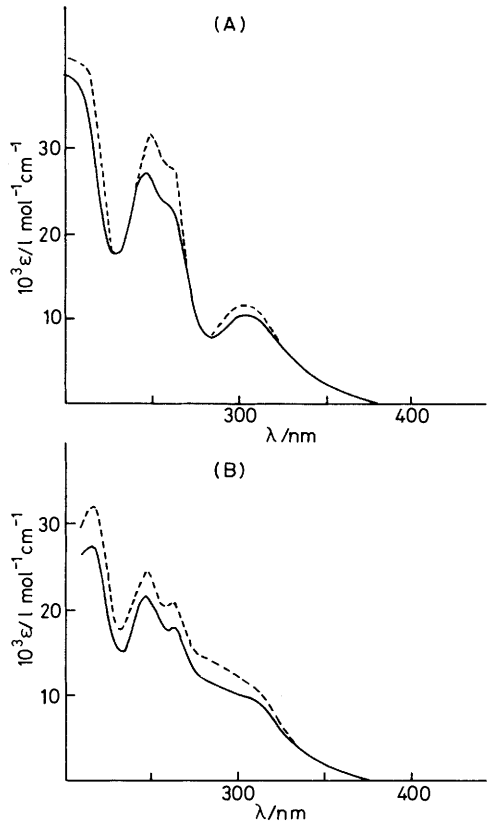


Figure 3. Comparison of the u.v. absorption spectra at room temperature of (IV) (——) and 8-MOP + 9-ethyladenine (– – – – –) in water (A); and (I) (——) and 8-MOP + 1-ethylthymine (– – – – –) in water (B)

were measured using dilute solutions of bichromophores (II_n) and (III_m) containing various concentrations of DNA. In each solution, the concentration of base pairs of DNA is far higher than the concentration of complexing compounds. The parent solutions of DNA are prepared by dissolving, at 35—38 °C, DNA fibres in aqueous NaCl solution (10^{-2}M) and the stability of the double strand structure of DNA is checked from the hyperchromicity which appears at 260 nm upon HCl denaturation (42%). The complexation study was carried out in aqueous solution containing 5% ethanol by weight because of the poor solubility of the compounds in water. The presence of ethanol might induce some weak denaturation of DNA and subsequently a small increase in the absorbance at 260 nm. However, since measurement of hypochromism of psoralens is carried out at 302 nm, the small ethanol-denaturation of DNA cannot affect the total absorbance at this wavelength. Hypochromism was only observed with compound (II_3). From the absorbance at 302 nm of the solutions containing 4.3×10^{-6}M (II_3) (*i.e.* 8.6×10^{-6}M of single chromophore) and, respectively, 1.1 or 0.9 g l^{-1} DNA (*i.e.* 1.8×10^{-3}M-base pairs), an intrinsic association constant K was graphically determined using relationship (1) [3b] where ε_{SF} is the molar

$$D = \varepsilon_{SF}[F] - (D - D_F^\circ)/K[S] \qquad (1)$$

extinction coefficient of complex SF between psoralen F and base pairs S, D_F° is the absorbance at 302 nm of psoralen (concentration [F]) in the absence of DNA, and D is the absorbance at 302 nm of psoralen (concentration [F]) in the presence of DNA (concentration of base pairs [S]).

A K value of 90 ± 10 l mol^{-1} was obtained for (II_3). As compound (II_3) contains two psoralen chromophores, the association constant between one psoralen chromophore and the base pairs is K' 350 ± 50 l mol^{-1}; under the same conditions, the association constant K between DNA and 8-MOP is 800 l mol^{-1}.[21]

It must be stressed again that the compound (II_3) with the longer polyoxyethylene chain is the only compound which is complexed by DNA. The other molecules of type (II) studied probably do not have adequate flexibility to allow efficient complexation.

Compound (III_{10}) which contains a chain of 13 members as does compound (II_3) is not complexed by DNA; it is assumed that the polymethylene chain, being less flexible and more hydrophobic than the polyoxyethylene chain, prevents the intercalation of psoralen in the DNA helix.

Conclusions.—The fluorescence emission studies suggest that a geometrical intercalation complex between psoralen and DNA bases must be involved in order to allow intermolecular photocycloaddition from the short singlet-excited state of furocoumarins; such an interaction, probably hydrophobic in nature, is revealed in the u.v. absorption spectra of compounds (I) and (IV). The bichromophores studied reveal in fluid solution (ethanol) more efficient self-quenching of psoralen in its triplet state than its deactivation by the DNA bases adenine and thymine; this is also observed at low temperature (15 K) in rigid matrices. These results underline the role of the intercalation process even in the triplet state. The poor intercalation

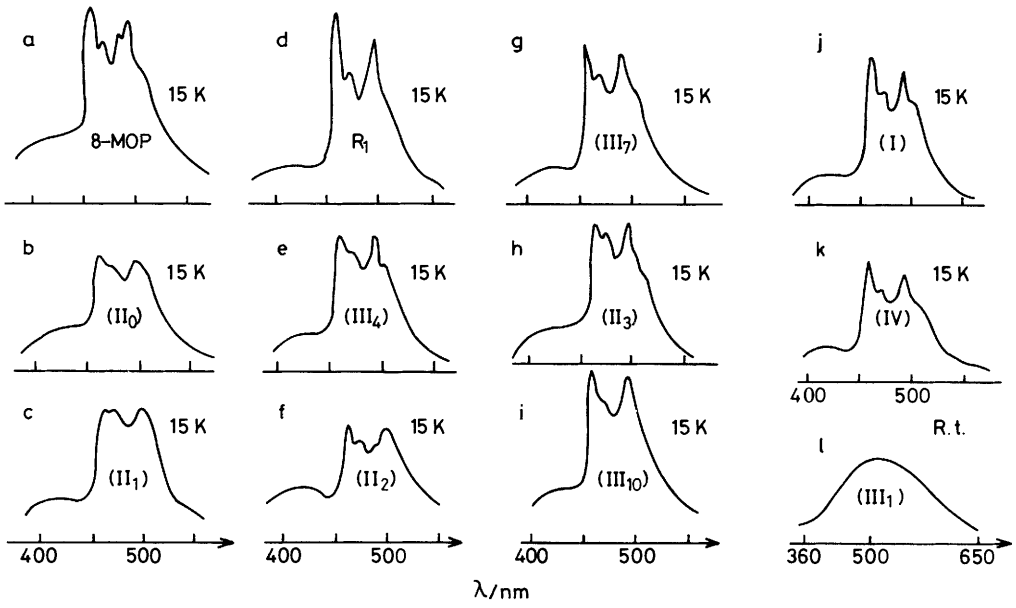


Figure 4. Emission spectra at 15 K of 8-MOP, R_1, (I), (IV), (II_n), and (III_m) in ethanol (a—k). Emission spectra of (III_1) in methanol at room temperature (l)

properties of the bispsoralen (II_n) and (III_m) might be due to unfavourable conformational effects preventing their insertion in DNA.

Experimental

Physical Methods.—M.p.s were determined with a Kofler block and are uncorrected. A microbalance (Mettler ME 30; sensitivity 10^{-6} g) was used for spectroscopic measurements. I.r. spectra were recorded on a Perkin-Elmer model 412 instrument with KBr pellets or on films between NaCl plates. Electronic absorption spectra were recorded on a Cary 219 spectrophotometer. ^{1}H N.m.r. spectra were obtained using Perkin-Elmer R12 and R-24B (60 MHz) instruments with Me_4Si as internal standard. Mass spectra were recorded using a VG Micromass 70/70 instrument. The purity of all new compounds was checked by t.l.c. (silica gel) and reverse-phase h.p.l.c. [S_5 octadecylsilane, SiC_{18} Spherisorb (eluant methanol)]. Satisfactory elemental analyses were obtained.

At room temperature, fluorescence spectra were recorded with a Hitachi–Perkin-Elmer MPF 44 fluorimeter corrected for emission and excitation. The sample vessels were degassed on a high-vacuum line with several freeze–pump–thaw cycles and sealed. The fluorescence quantum yields were determined according to the usual method [22] as described in preceding papers.[23] The fluorescence-lifetime measurements were performed with a single-photon-counting apparatus (Applied Photophysics) as described before.[10] Low-temperature phosphorescence spectra (15 K) were performed by Dr. P. Garrigues with a homemade spectrofluorimeter previously described.[24] The triplet lifetime measurements were determined at room temperature by Dr. R. Bonneau with a laser photolysis apparatus described elsewhere; [25] the excitation wavelength was 353 nm and analytical beam 370 nm.

Materials.—8-Methoxypsoralen (8-MOP) was purchased from the Sigma Chemical Co. 8-Hydroxypsoralen was quantitatively prepared from 8-MOP as already described.[11] The polyethylene glycol ditosylates were obtained according to the method of Dale [26] and the di-iodo derivatives were prepared by halogen exchange from the chlorine compounds.[12] Commercial α,ω-dibromoalkanes (Aldrich or Fluka) were used as received. For spectroscopic measurements ethanol (Prolabo; u.v. spectroscopic grade) was used without further purification. Water was distilled twice in quartz vessels before use (pH 6.9). No fluorescent impurities were detected in the emission spectral range of the psoralen chromophore. Acetone (Aldrich) was distilled over P_2O_5 and DMF (Aldrich) on CaH_2 just before use. Potassium carbonate and sodium iodide were dried overnight in an oven (170 °C). For complexation studies, aqueous solutions of calf thymus DNA (sodium salt) from the Sigma Chemical Co. were used.

1,2-Bis(psoralen-8-yloxy)ethane (II_0).—(a) *From 1,2-dibromoethane in DMF.* To a stirred suspension of K_2CO_3 (5 g, 3.6×10^{-2} mol) in dry DMF (100 ml) was added 8-hydroxypsoralen (1 g, 0.5×10^{-2} mol) and 1,2-dibromoethane (0.47 g, 0.25×10^{-2} mol). The mixture was heated for four days at 80 °C under nitrogen. After cooling, the solution was added dropwise to dilute HCl solution (10%) and extracted with chloroform. After removing the solvent the solid residue was washed with acetone. Crystallization in benzene gave pure (II_0) as fine crystals (0.25 g, 24%), m.p. 226—227 °C, $\nu_{max.}$ (KBr) 3 150, 3 120, 3 060, 2 950, 1 720, 1 625, 1 590, 1 470, 1 440, 1 405, 1 330, 1 295, 1 220, 1 150, 1 100, 1 095, 1 030, 990, 875, 830, and 755 cm^{-1}; δ ([$^2H_6$]DMSO) 4.85 (4 H, s), 6.40 (2 H, d, *J* 10 Hz, 3-H), 7.05 (2 H, d, *J* 2 Hz, 4′-H), 7.60 (2 H, s, 5-H), 8.00 (2 H, d, *J* 2 Hz, 5′-H), and 8.10 (2 H, d, *J* 10 Hz, 4-H); $\lambda_{max.}$ (ethanol) 300 (log ε 4.31), 263 (4.38), 248 (4.60), and 218 nm (4.65); *m/z* 430 (M^+, 40%), 229 (100), and 202 (44).

(b) *From ethylene glycol ditosylate in acetone.* By the procedure used for preparing (II_3), from 8-hydroxypsoralen (1 g), dry acetone (100 ml), K_2CO_3 (5 g), the ditosylate (0.925 g), and NaI (0.375 g), we obtained 1-(psoralen-8-yloxy)propan-2-one (0.2 g, 16%), m.p. 151—152 °C (yellow crystals from acetone–ligroin), $\nu_{max.}$ (KBr) 3 170, 3 130, 3 110, 2 940, 1 725, 1 620, 1 595, 1 475, 1 460, 1 410, 1 380, 1 340, 1 305, 1 230, 1 190, 1 160, 1 120, 1 050, 1 000, 885, 775, 760, 565, and 510 cm^{-1}; δ ($CDCl_3$) 2.45 (3 H, s), 5.08 (2 H, s), 6.38 (1 H, d, *J* 10 Hz, 3-H), 6.82 (1 H, d, *J* 2 Hz), 7.40 (1 H, s, 5-H), 7.68 (1 H, d, *J* 2 Hz, 5′-H), and 7.80 (1 H, d, *J* 10 Hz, 4-H); *m/z* 258 (M^+, 47%) and 215 (100).

1,5-*Bis*(*psoralen*-8-*yloxy*)-3-*oxapentane* (II_1).—To a stirred suspension of K_2CO_3 (10 g, 7.5 × 10^{-2} mol) in dry acetone (150 ml), 8-hydroxypsoralen (1 g, 0.5 × 10^{-2} mol) and 1,5-di-iodo-3-oxapentane (0.815 g, 0.25 × 10^{-2} mol) were added. The mixture was refluxed (17 h) and after cooling, filtered. After removal of the solvent, the crude product was chromatographed twice on silica gel (ligroin–diethyl ether 1 : 1), giving (II_1) as a pale yellow solid (0.2 g, 17%), m.p. 174—176 °C, $\nu_{max.}$ (KBr) 3 140, 3 110, 3 040, 2 920, 2 860, 1 720, 1 620, 1 585, 1 455, 1 440, 1 400, 1 330, 1 290, 1 210, 1 145, 1 090, 1 025, and 750 cm^{-1}; δ ($CDCl_3$) 4.1 and 4.7 (8 H, AA′BB′) [the protons belonging to the psoralen ring display an identical pattern with those in (II_0)]; $\lambda_{max.}$ (ethanol) 300 (log ε 4.31), 263 (4.39), 248 (4.61), and 218 nm (4.67); *m/z* 474 (M^+, 13%), 272 (11), 229 (100), and 202 (60).

1,8-*Bis*(*psoralen*-8-*yloxy*)-3,6-*dioxaoctane* (II_2).—By the procedure for (II_1), from 8-hydroxypsoralen (1 g, 0.5 × 10^{-2} mol), dry acetone (100 ml), K_2CO_3 (10^{-2} mol), and 1,8-di-iodo-3,6-dioxaoctane (1.1 g, 0.3 × 10^{-2} mol) we obtained, after chromatography on silica gel (acetone–ligroin 3 : 10), pure (II_2) as crystals (0.1 g, 8%), m.p. 124—125 °C, $\nu_{max.}$ (KBr) 3 135, 3 120, 3 080, 2 960, 2 890, 1 720, 1 620, 1 590, 1 450, 1 410, 1 340, 1 300, 1 170, 1 130, 1 105, 1 030, 1 000, 870, and 760 cm^{-1}; δ ($CDCl_3$) 3.60 (4 H, s), and 3.9 and 4.8 (8 H, AA′MM′) [psoralen protons are identical with those in (II_0)]; $\lambda_{max.}$ (ethanol) 300 (log ε 4.36), 263 (4.41), 248 (4.65), and 218 nm (4.70); *m/z* 518 (M^+, 9%), 317 (17), 273 (5), 229 (100), and 202 (36).

1,11-*Bis*(*psoralen*-8-*yloxy*)-3,6,9-*trioxaundecane* (II_3).—To a stirred suspension of K_2CO_3 (5 g, 3.6 × 10^{-2} mol) in dry acetone (100 ml), 8-hydroxypsoralen (1 g, 0.5 × 10^{-2} mol), tetraethylene glycol ditosylate (1.25 g, 0.25 × 10^{-2} mol) and NaI (0.375 g, 0.25 × 10^{-2} mol) were added. The mixture was refluxed for 4.5 h and the usual work-up and chromatography on silica gel (acetone–ligroin 1 : 4) gave (II_3) (0.25 g, 18%) as a yellow oil which solidified, m.p. 74—76 °C, $\nu_{max.}$ (KBr) 3 145, 3 110, 2 900, 1 720, 1 620, 1 585, 1 400, 1 330, 1 150, 1 090, 1 030, 990, and 760 cm^{-1}; δ ($CDCl_3$) 3.73 (8 H, s) and 4.0 and 4.70 (8 H, AA′MM′) [see (II_0) for psoralen ring protons]; $\lambda_{max.}$ (ethanol) 300 (log ε 4.36), 263 (4.43), 248 (4.65), and 218 nm (4.69); *m/z* 562 (M^+, 1.1%), 361 (8.5), 317 (4.3), 273 (4.3), 229 (100), and 202 (31).

1-(*Psoralen*-8-*yloxy*)-8-*iodo*-3,6-*dioxaoctane* (*PsI*).—By the procedure for (II_2), from K_2CO_3 (30 g, 0.2 mol), 8-hydroxypsoralen (2 g, 10^{-2} mol), 1,8-di-iodo-3,6-dioxaoctane (13 g, 3.5 × 10^{-2} mol), and dry acetone (100 ml), we obtained PsI as a yellow oil (1.3 g, 29%), $\nu_{max.}$ (NaCl) 3 140, 3 110, 2 860, 1 720, 1 620, 1 585, 1 470, 1 440, 1 400, 1 330, 1 150, 1 090, 1 030, 985, 865, and 760 cm^{-1}; δ ($CDCl_3$) 3.25 (2 H, m, CH_2I), 3.5—4.1 (8 H, m, chain), and 4.75 (2 H, m, CH_2O psoralen) [see (II_0) for psoralen ring protons]; *m/z* 444 (M^+, 15%), 229 (11), 216 (10), 202 (51), 201 (40), and 155 (100).

1-(*Psoralen*-8-*yloxy*)-3,6,9-*trioxadecane* (R_1).—As for PsI, from 8-hydroxypsoralen (1 g, 0.5 × 10^{-2} mol), K_2CO_3 (15 g), 1-iodo-3,6,9-trioxadecane (2.74 g, 0.1 mol), and dry acetone (100 ml), we obtained R_1 (1 g, 58%) as a yellow oil, $\nu_{max.}$ (NaCl) 3 160, 3 120, 3 075, 2 880, 1 720, 1 620, 1 580, 1 470, 1 445, 1 400, 1 330, 1 150, 1 090, 1 030, 990, 870, and 750 cm^{-1}; δ (CCl_4) 3.23 (3 H, s), 3.35—3.67 (8 H, m, chain), 3.83 and 4.53 (4 H, AAMM′, OCH_2CH_2O psoralen), 6.13 (1 H, d, *J* 10 Hz, 3-H), 6.66 (1 H, d, *J* 2 Hz, 4′-H), 7.17 (1 H, s, 5-H), 7.56 (1 H, d, *J* 10 Hz, 4-H), and 7.53 (1 H, d, *J* 2 Hz, 5′-H); $\lambda_{max.}$ (ethanol) 300 (log ε 4.08), 263 (4.12), 248 (4.36), and 218 nm (4.39); *m/z* 348 (M^+, 21%), 290 (2), 273 (1), 229 (19), 202 (42), 147 (40), 103 (27), and 59 (100).

1,5-*Bis*(*psoralen*-8-*yloxy*)*pentane* (III_4).—By the procedure for (II_0), 8-hydroxypsoralen (1 g, 0.5 × 10^{-2} mol), K_2CO_3 (5 g, 3.6 × 10^{-2} mol), and 1,5-dibromopentane (0.575 g, 0.25 × 10^{-2} mol) in dry DMF (100 ml) gave, after the usual work-up, a crude product which was purified by chromatography on silica (twice; CH_2Cl_2) and finally crystallized from benzene. We obtained (III_4) (0.45 g, 38%) as crystals, m.p. 141—142 °C, $\nu_{max.}$ (KBr) 3 140, 3 110, 2 950, 1 720, 1 620, 1 585, 1 465, 1 440, 1 400, 1 330, 1 295, 1 150, 1 090, 1 030, 990, 870, 820, and 750 cm^{-1}; δ ($CDCl_3$) 1.85—2.40 (6 H) and 4.60 (4 H, t) [for psoralen ring protons see (II_0)]; $\lambda_{max.}$ (ethanol) 300 (log ε 4.37), 263 (4.44), 248 (4.66), and 218 nm (4.70); *m/z* 472 (M^+, 6%), 271 (25), and 202 (100).

1,8-*Bis*(*psoralen*-8-*yloxy*)*octane* (III_7).—As described above, 8-hydroxypsoralen (1 g, 0.5 × 10^{-2} mol), K_2CO_3 (5 g, 3.6 × 10^{-2} mol), and 1,8-dibromo-octane (0.682 g, 0.25 × 10^{-2} mol) in dry DMF gave a crude product which was purified by chromatography on silica (twice; CH_2Cl_2–ligroin–acetone 10 : 10 : 4). Compound (III_7) (0.4 g, 31%) was obtained as a pale yellow product, m.p. 145—146 °C, $\nu_{max.}$ (KBr) 3 140, 3 120, 2 820, 2 750, 1 720, 1 620, 1 580, 1 465, 1 445, 1 400, 1 335, 1 290, 1 220, 1 180, 1 160, 1 090, 1 030, 1 010, 880, 830, and 760 cm^{-1}; δ ($CDCl_3$) 1.35—2.33 (12 H) and 4.60 (4 H, t) [for psoralen ring protons see (II_0)]; $\lambda_{max.}$ (ethanol) 300 (log ε 4.37), 263 (4.43), 248 (4.65), and 218 nm (4.69); *m/z* 514 (M^+, 2%), 313 (12), and 202 (100).

1,11-*Bis*(*psoralen*-8-*yloxy*)*undecane* (III_{10}).—According to the usual procedure but using 1,11-dibromoundecane (0.785 g, 0.25 × 10^{-2} mol) we obtained, after purification on silica gel (ligroin–CH_2Cl_2–acetone 4 : 1 : 1), compound (III_{10}) (0.4 g, 28%) as a pale yellow solid, m.p. 101—102 °C; $\nu_{max.}$ (KBr) 3 140, 3 120, 2 920, 2 850, 1 720, 1 620, 1 580, 1 460, 1 440, 1 400, 1 330, 1 290, 1 150, 1 090, 1 030, 860, and 750 cm^{-1}; δ ($CDCl_3$) 1.3—2.3 (18 H), and 4.60 (4 H, t) [for psoralen ring protons see (II_0)]; $\lambda_{max.}$ (ethanol) 300 (log ε 4.37), 263 (4.44), 248 (4.65), and 218 (4.69); *m/z* 556 (1%), 355 (6), and 202 (100).

1-(*Psoralen*-8-*yloxy*)-8-(*adenin*-9-*yl*)-3,6-*dioxaoctane* (IV).—To a stirred suspension of adenine (0.19 g, 0.14 × 10^{-2} mol) in dry DMF (100 ml) NaH (0.033 g, 0.14 × 10^{-2} mol) was added. The mixture was stirred for 3 h at room temperature. To the sodium adenide salt, PsI (0.62 g, 0.14 × 10^{-2} mol) in DMF (20 ml), was added dropwise. The resulting mixture was stirred for 17 h at room temperature. The solvent was removed by vacuum leaving a brown oily residue which solidified. The solid was dissolved in CH_2Cl_2 and purified by column chromatography on silica (acetone–methanol 90 : 10) to give (IV) (0.25 g, 39%) as a powder, m.p. 150—151 °C, $\nu_{max.}$ (KBr) 3 420, 3 120, 2 880, 1 710, 1 670, 1 600, 1 470, 1 440, 1 410, 1 340, 1 330, 1 300, 1 250, 1 220, 1 160, 1 090, 1 030, and 870 cm^{-1}; δ ([$^2H_6$]DMSO) 3.5–4.2 (8 H, complex), 4.2—4.8 (4 H, complex), 6.55 (1 H, d, *J* 10 Hz, 3-H), 7.20 (1 H, d, *J* 2 Hz, 4′-H), 7.20 (s, NH_2), 7.70 (1 H, s, 5-H), and 8.10—8.35 (4 H, m, 2 H [adenine], 4- + 5-H); $\lambda_{max.}$ (ethanol) 300 (log ε 4.05), 263 (4.42), 250 (4.51), and 213 nm (4.59); *m/z* 451 (M^+, 8%), 272 (24), 250 (49), 206 (67), 202 (55), 178 (60), 162 (100), and 135 (55).

9-*Ethyladenine*.—As described above for compound (IV), adenine (1.35 g, 10^{-2} mol), bromoethane (1.09 g, 10^{-2} mol), and NaH (0.24 g, 10^{-2} mol) in dry DMF (200 ml) gave, after purification on silica (acetone–methanol 85 : 15) and crystallization from acetone–methanol (85 : 15), 9-ethyladenine (0.31 g, 18%) as a solid, m.p. 193 °C (lit.,[27] 193 °C); $\nu_{max.}$ (KBr) 3 400—3 100s, 2 700, 1 680, 1 610, 1 575, 1 485, 1 420, 1 385, 1 350, 1 330, 1 310, 1 250, 1 215, 1 100, 1 075, 1 025, 960, 800.

710, 695, 600, 545, 535, 510, and 480 cm^{-1}; δ ([$^2H_6$]DMSO) 1.57 (3 H, t), 4.30 (2 H, q), 7.30 (NH_2), and 8.27 (2 H).

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References

1 Presented at the IXth IUPAC Symposium on Photochemistry, Pau, 1982.
2 (*a*) P. S. Song and K. J. Tapley, *Photochem. Photobiol.*, 1979, **29**, 1177; (*b*) B. J. Parsons, *ibid.*, 1980, **32**, 813; (*c*) F. Dall'Acqua, S. Marciani Magno, I. Zambon, and G. Rodighiero, *ibid.*, 1979, **29**, 489.
3 (*a*) G. C. Goyal and L. I. Grossweiner, *Photochem. Photobiol.*, 1979, **29**, 847; (*b*) R. Arnaud, A. Deflandre, G. Lang, and J. Lemaire, *J. Chim. Phys.*, 1980, **77**, 501.
4 K. Straub, D. Kanne, J. E. Hearst, and H. Rapoport, *J. Am. Chem. Soc.*, 1981, **103**, 2347.
5 J. L. Décout and J. Lhomme, *Tetrahedron Lett.*, 1981, **22**, 1247.
6 A. Castellan and J.-P. Desvergne, *Photochem. Photobiol.*, 1981, **34**, 183.
7 J.-P. Bideau, G. Bravic, C. Courseille, A. Castellan, and J.-P. Desvergne, *Eur. J. Med. Chem. Chim. Ther.*, 1982, **17**, 95.
8 L. P. G. Wakelin, M. Romanos, T. K. Chen, D. Glaubigel, E. S. Canellakis, and M. J. Waring, *Biochemistry*, 1978, **17**, 5057 and references therein.
9 M. A. Mitchell and P. B. Dervan, *J. Am. Chem. Soc.*, 1982, **104**, 4265 and references therein.
10 J.-P. Desvergne, A. Castellan, and R. Lesclaux, *Chem. Phys. Lett.*, 1980, **71**, 228.
11 A. Schönberg and A. Sina, *J. Am. Chem. Soc.*, 1950, **72**, 4826.
12 H.-M. Li, B. Post, and H. Morawetz, *Makromol. Chem.*, 1972, **154**, 89.
13 H. R. Bhattacharjee, E. L. Menger, and G. S. Hammond, *J. Am. Chem. Soc.*, 1980, **102**, 1977.
14 J.-P. Desvergne and H. Bouas-Laurent, *Isr. J. Chem.*, 1979, **18**, 220.
15 D. T. Browne, J. Eisenger, and N. J. Leonard, *J. Am. Chem. Soc.*, 1968, **90**, 7302.
16 M. M. Warshaw and I. Tinoco, Jr., *J. Mol. Biol.*, 1966, **20**, 29.
17 J. B. Birks, 'Photophysics of Aromatic Molecules,' Wiley–Interscience, London, 1970.
18 J. C. Ronfard-Haret, D. Averbeck, R. V. Bensasson, E. Bisagni, and E. J. Land, *Photochem. Photobiol.*, 1982, **35**, 479 and references therein.
19 R. V. Bensasson, E. J. Land, and C. Salet, *Photochem. Photobiol.*, 1978, **27**, 273.
20 (*a*) W. W. Mantulin and P. S. Song, *J. Am. Chem. Soc.*, 1973, **95**, 5122; (*b*) P. S. Song, W. W. Mantulin, D. McInturff, I. C. Felkner, and L. Harter, *Photochem. Photobiol.*, 1975, **21**, 317.
21 R. Arnaud, A. Deflandre, G. Lang, and J. Lemaire, *J. Chim. Phys.*, 1981, **78**, 597.
22 C. A. Parker in 'Photoluminescence of Solutions,' Elsevier, New York, 1968, 262.
23 (*a*) A. Castellan, J.-M. Lacoste, and H. Bouas-Laurent, *J. Chem. Soc., Perkin Trans. 2*, 1979, 411; (*b*) A. Castellan, J.-P. Desvergne, and H. Bouas-Laurent, *Nouv. J. Chim.*, 1979, **3**, 231.
24 P. Garrigues, M. Lamotte, M. Ewald, and J. Joussot-Dubien, *C.R. Acad. Sci., Serie II*, 1981, **293**, 567.
25 R. Bonneau, *J. Am. Chem. Soc.*, 1980, **102**, 3816.
26 J. Dale and P. O. Kristiansen, *Acta Chem. Scand.*, 1972, **26**, 1471.
27 K. Yamauchi, M. Hayashi, and M. Kinoshita, *J. Org. Chem.*, 1975, **40**, 385.

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