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SYNTHETIC MODELS RELATED TO DNA-INTERCALATING MOLECULES
A STUDY OF THE RING-RING STACKING INTERACTIONS BETWEEN 8-ALKOXYPSORALEN AND THYMINE.

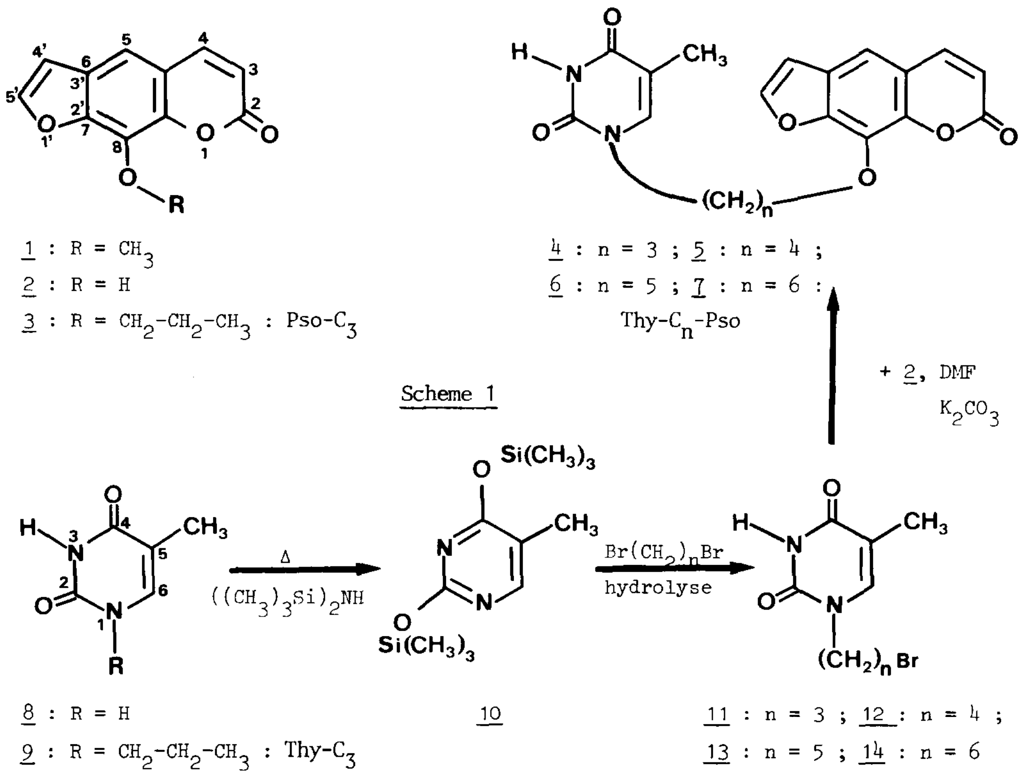
Jean-Luc DECOUT and Jean LHOMME*
Laboratoire de Chimie Organique Biologique, associé à l'ENSCL,
ERA CNRS 827 - Université de LILLE I - 59655 Villeneuve d'Ascq - France

Summary : As a contribution to the problem of the intercalation and photobinding of psoralens to DNA, model compounds were prepared in which the psoralen ring is linked to thymine by a polymethylene bridge. The ring-ring intramolecular interaction in the models was assessed by hypochromism measurement in the UV.

Furocoumarins of the psoralen type 1 possess a strong photodynamic activity which has been related to their ability to bind photochemically to nucleic acids[1]. The binding is the consequence of two successive events : 1/ intercalation of the drug between the base pairs of the nucleic acid 2/ photocyclization with the pyrimidine bases (notably thymine) involving cyclobutane formation between the pyrimidine 5-6 double bond and the 3-4 or/ and 4'-5' double bond of the psoralen molecule. In order to gain insight into these two processes, and following a methodology which we used previously for other intercalating drugs[2], we have prepared models 4 - 7 in which the psoralen moiety is linked to the pyrimidine base by a flexible chain. The polymethylene bridge (n = 3, 4, 5, 6) allows intramolecular ring-ring stacking between the two aromatic units with possible superposition of the double bonds involved in the photoreaction.

Model compounds 4 - 7 were prepared as shown in scheme 1. Alkylation of silylated thymine 10 with α,ω-dibromoalkanes[3] afforded after hydrolysis the corresponding ω-bromoalkylthymines 11 - 14 in 50-80 % yields. Reaction of these intermediates with 8-hydroxypsoralen 2 in refluxing acetone or acetonitrile in the presence of K_2CO_3 furnished the models 4 - 7. An improved yield (60-85 %) was obtained by reacting the products in DMF at 70°C. Reference compound 3 Pso-C_3 was obtained similarly by alkylation of 2 with 1-bromopropane. Thy-C_3 9 had been already prepared[3]. All new compounds were confirmed by IR, UV, NMR, mass and analytical data[4] (and by X-ray analysis[5] in the case of 5).

Intramolecular ring-ring stacking between thymine and furocoumarin in models 4 - 7 was assessed by hypochromism measurement in the UV. The utility of this technique to study intramolecular interactions between aromatic moieties has been amply demonstrated[6], since very low concentrations are employed under which conditions intermolecular contributions are avoided. Moreover, the interactions of psoralen with nucleotide bases as they occur in the complex with DNA, have been most frequently detected by this method. Figure 1 compares the



$\underline{1}$: R = CH_3

$\underline{2}$: R = H

$\underline{3}$: R = CH_2-CH_2-CH_3 : Pso-C_3

$\underline{4}$: n = 3 ; $\underline{5}$: n = 4 ;

$\underline{6}$: n = 5 ; $\underline{7}$: n = 6 :

Thy-C_n-Pso

Scheme 1

$\underline{8}$: R = H

$\underline{9}$: R = CH_2-CH_2-CH_3 : Thy-C_3

$\underline{10}$

$\underline{11}$: n = 3 ; $\underline{12}$: n = 4 ;

$\underline{13}$: n = 5 ; $\underline{14}$: n = 6

electronic absorption spectrum of the model Thy-C_4-Pso $\underline{5}$ with those of the two references Thy-C_3 $\underline{9}$, Pso-C_3 $\underline{3}$ and a 1 : 1 mixture of the two latter compounds. The spectra were recorded in a water-ethanol mixture (95 : 5)[7] at equimolar concentrations of the chromophores. The spectrum of the model is characterized by a decrease of the absorption intensity at all

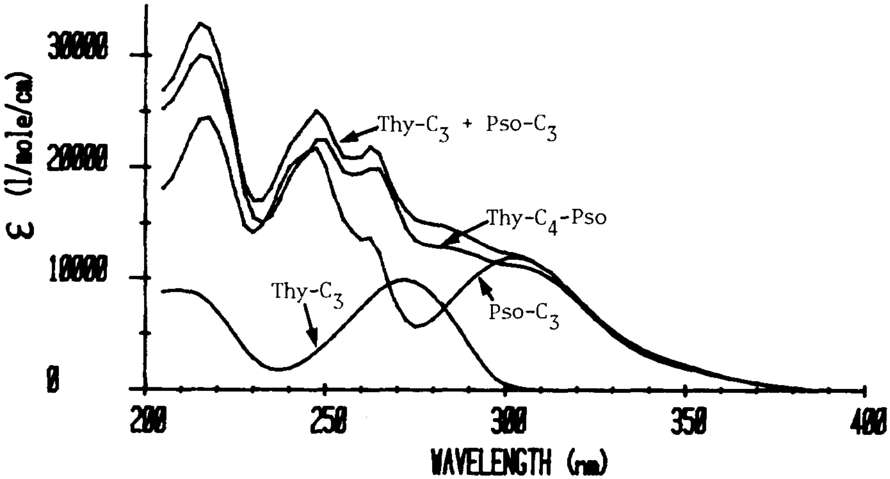


Figure 1 : comparative electronic absorption spectrum of Thy-C_4-Pso versus the addition spectrum of Thy-C_3 + Pso-C_3. Also indicated are Thy-C_3 and Pso-C_3. All spectra were measured in the same conditions : 5.10^{-5}M, 20°C, H_2O - EtOH (95 : 5).

wavelengths. This "hypochromic effect" is indeed quite comparable to that observed for the psoralen-DNA complex[8]. It can be quantitatively evaluated by the "percent of hypochromism" % H which is a measure of the interaction[6-9]. Table I indicates the % H values calculated for the four models between different wavelength limits. Inspection of the above table indicates that all systems show appreciable hypochromism, which is indicative of substantial intramolecular ring-ring stacking[10]. Interestingly, the forces responsible for this interaction are strongly solvent dependent. For example : the stacking almost disappears in organic solvent such as dichloromethane or ethanol (% H = 1.5 and 1.0, respectively, for Thy-C_4-Pso 5). It decreases rapidly with the percentage of ethanol in a water-ethanol mixture (% H = 2.0 for model 5 in a 50 : 50 mixture). Temperature is also an important factor. Model 5 exhibits a linear decrease of % H with increasing temperature (% H = 10 at 2°C and 4 at 80°C). This corresponds to the progressive opening (unfolding) of the system with temperature, a phenomenon which has already been observed in models studied previously. An interesting point which needs further investigation is raised by the comparison of % H for the four models 4 - 7 possessing chains of different lengths. The % H values, calculated for the whole spectrum (between 230 and 400 nm), is practically invariant with chain length. However, a more detailed examination of % H, calculated for limited parts of the spectrum (for example in the range 400 - 280 nm - mainly furocoumarin absorption) indicates a progressive variation with the number of methylene groups in the bridge. This suggests that the complexes adopt different preferred geometries according to the chain length.

TABLE I : Percent hypochromism values (% H) calculated for models 4 - 7 between different wavelength limits. Spectra measured in H_2O - EtOH (95 : 5), 20°C.
Relative precision on % H can be evaluated to ± 0.5 %.

Model Wavelength range (nm)	Thy-C_3-Pso 4	Thy-C_4-Pso 5	Thy-C_5-Pso 6	Thy-C_6-Pso 7
400-300	7.0	7.0	4.5	4.0
400-280	9.5	9.0	6.5	5.5
400-260	10.0	8.5	8.5	8.5
400-230	9.5	9.0	9.0	9.0

This point is now under investigation, as information concerning the relative orientation of the double bonds in the two reactive molecules should contitute the basis for a better understanding of the photocyclization in the models[11].

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4. 3 : mp 78.5-79°C, 4 : mp 220-221°C, 5 : mp 202-204°C, 6 : mp 182-183°C, 7 : mp 167 - 168°C. All spectra were in accordance with the structures indicated. For example, the NMR spectrum ($CDCl_3$) of 5 shows : psoralen protons :δ 7.65 (d, 1H, J= 10 Hz, C 4H), δ 7.5 (d, 1H, J=2 Hz, C 5'H), δ7.2 (s, 1H, C 5 H), δ6.7 (d, 1H, J=2 Hz, C 4'H), δ 6.2 (d, 1H, J=10Hz, C 3 H), thymine protons : δ8.7 (s, broad, 1 H, NH), δ7.1 (s, broad, 1H, C 6 H) δ1.9 (s, 3H, CH_3), bridge protons : δ4.4 (t, broad, 2H, J = 6 Hz), δ3.8 (t, broad, 2H, J=6 Hz), δ1.9 (m, broad, 4H) ; UV (EtOH) λ_{max}= 250 nm (ε=27000),λ_{max}=262 (21000).
   The purity of models 4 - 7 and references 3 and 9 was checked by TLC in different conditions of elution and by analytical HPLC.

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7. Pure water could not be used for the spectroscopic measurements due to the poor solubility of the model compounds. Even with 5 % ethanol in water, solutions of 4, 6 and 7 had to be studied in 10 cm cells at concentrations of about $5.\ 10^{-6}$M.

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9. The percentage of hypochromism % H corresponds to an integrated hypochromic effect. It is calculated according to the equation $\% H = \left| 1 - f\ Thy\text{-}C_n\text{-}Pso/(f\ Thy\text{-}C_3 + f\ Pso\text{-}C_3) \right| 100$ where f is the oscillator strength of the transition, i.e., a measure of the intensity of the absorption : $f = (4.32 \times 10^{-9}) \int \varepsilon(\lambda)/\lambda^2\, d\lambda$, where ε is the molecular extinction coefficient. The f values were obtained from optical densities measured every 2.5 nm by application of the Simpson's rule, as described in precedent publications[2].

10. No intermolecular contribution to % H can be detected as Beer's law is obeyed for the different compounds in our experimental conditions.
   The magnitude of % H is of the same order of magnitude as that observed for the comparable Thy-C_3-Thy model : % H = 10.5 (N.J. Leonard and R.L. Cundall, J. Am. Chem. Soc., 96, 5904 (1974)) and for Thy-C_3-Quinoleine[2b] : % H = 10.

11. Preliminary photochemical experiments indicate that the reaction is quite complex leading to labile photoproducts.

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