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Note

Reversed-phase high-performance liquid chromatography, a tool for the study of bichromophoric systems including polymethylenic linking bridges

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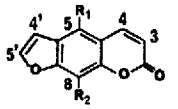
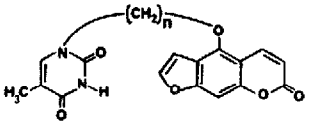
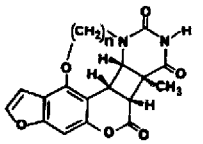
The photochemical and photobiological properties of furocoumarins such as 5-methoxypsoralen **1** (5-MOP) and 8-methoxypsoralen **2** (8-MOP) have been extensively studied, notably with respect to their use in the phototherapy of skin diseases.



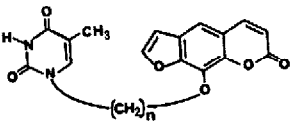
1: $R_1=OCH_3$, $R_2=H$, 5 MOP
2: $R_1=H$, $R_2=OCH_3$, 8 MOP



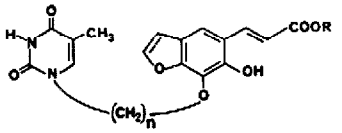
3: n = 2,3,4,5,6,12



5: n = 2,3,4,5,6,12



4: n = 3,4,5,6,12



6: $R=C_2H_5$; n = 3,4,5,6,12
7: R=H; n = 3–6,12

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The biological effects are generally related to the photochemical reactions which occur with DNA. After intercalation of the psoralen ring between adjacent complementary bases, the 3, 4 or/and 4', 5' double bond of the drug can add to the 5, 6 olefinic bond of a pyrimidine, usually thymine[1,2]. The intermolecular psoralen-thymine photoreactions cannot be studied in solution due to the highly favoured psoralen photodimerization[3]. With this aim, we have prepared compounds **3** and **4** in which the two chromophores are linked by a flexible polymethylene chain. They represent two series of models related to the important drugs 5-MOP and 8-MOP. In dilute solution, the polymethylene link allows intramolecular ring–ring stacking interactions and competitive photoaddition of the 5,6 double bond of thymine onto the 3, 4 or 4', 5' psoralen double bond. The study of models of various chain lengths $n = 2$, 3, 4, 5, 12 provides a control of the geometric strains imposed by the chain. We report here an interesting application of reversed-phase liquid chromatography (RPLC) for the characterization of the photoproducts as a function of the chain length.

EXPERIMENTAL

Detailed procedures for the preparation and irradiation at 365 nm of the models have been previously reported[4,5].

Analytical conditions

The apparatus was a Waters Associates chromatographic system equipped with a Model 660 programmer, two M-6000 pumps and a dual wavelength detector (254 or 280 and 365 nm). The irradiated solutions were analyzed on a C_{18} reversed-phase column (packed with RP-18 Prolabo phase with a Touzart and Matignon apparatus) using a linear gradient of two solvents water and methanol (from 50 to 95% of methanol in 10 min then 95% methanol, 2 ml/min). Water (pH 6) and buffered water (pH 5, 0.02 *M* ammonium acetate–acetic acid) were respectively used in the 5- and 8-series. The absorption ratios at the two wavelengths of detection were measured to characterize each compound.

RESULTS AND DISCUSSION

The photoreactivity of models **3** ($n = 2$–5, 12) in the 5-series was first studied. The compounds were irradiated at *very low concentration* ($2 \cdot 10^{-5} M$)[a] to avoid the photodimerization. From each model, a very major photoproduct was formed (as monitored by HPLC analysis). The photoproducts with $n = 2$, 4 and 12 were isolated and characterized by ^{1}H NMR, mass spectrometry and X-ray crystallography[4]. All three products possess a *cis* cyclobutane ring resulting from regio- and stereoselective intramolecular photoaddition of the thymine double bond onto the 3,4 pyrone ring of psoralen. The HPLC characteristics of those adducts **5b**, **5d**, **5f** were examined: their capacity factors, k', were plotted *versus* the capacity factors of the corresponding starting compounds **3b**, **3d**, **3l** (Fig. 1). A remarkably good linear correlation was observed ($r = 0.998$). The photoproducts **5c**, **5e**, **5f** resulting from the corresponding

[a] The use of HPLC is critical for this study at the limits of detection (150–200 μl of solution injected).

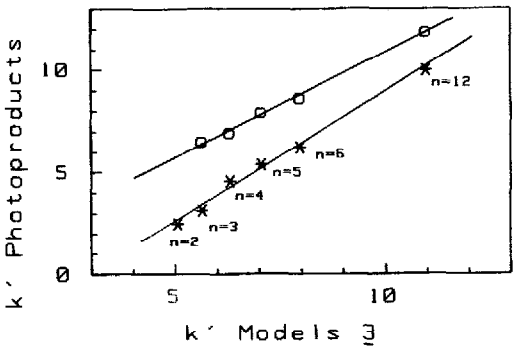

Fig. 1. Correlation between the RPLC capacity factors, k', of models **3** and the capacity factors of their photoproducts: intramolecular photoadducts **5** (*) and photodimers (○).

compounds **3c**, **3e**, **3f** were then examined. They were not isolated but their capacity factors were treated as previously, *i.e.*, they were plotted on the same graph as a function of the capacity factors of the starting compounds (Fig. 1). All points are remarkably localized on the same straight line ($r = 0.996$ for the six points).

The models **3** were then irradiated at a *more usual concentration* (10^{-3} *M*). From each model ($n = 3$–12)[a] a major photoproduct was formed. The photoproducts with $n = 4$ and 12 were identified by ^{1}H NMR spectroscopy as dimers resulting from a [2 + 2] photoaddition between the 3, 4 double bond of two psoralen rings[4]. As previously, the capacity factors of the photoproducts were plotted *versus* the capacity factors of the corresponding starting models **3**. All the five points obtained are again located on a straight line ($r = 0.998$).

Fig. 1 shows the remarkable relationships between the capacity factors of the two series of photoproducts, the intramolecular adducts **5** and the dimers, and the capacity factors of the parent compounds **3**.

The photoreactivity of models **4** ($n = 3$–6, 12) in the 8-series was studied at low concentration ($3 \cdot 10^{-5}$ *M*) in ethanol. From each model, a very major photoproduct was formed. The structure **6** resulting from a photolysis of the psoralen ring was assigned to the photoproducts with $n = 4$ and 12 (ref. 5). The capacity factors of all the observed photoproducts ($n = 3$–6 and 12) were plotted *versus* the capacity factors of the corresponding starting models **4** (Fig. 2). The localization of the five points on a straight line ($r = 0.999$) and the equality of the absorption ratios at the two wavelengths of detection led us to assign to each photoproduct the structure **6**. A similar photochemical study was realized in water and again HPLC allowed us to assign the same structure **7** to the major photoproduct observed after irradiation of each model **4** ($n = 3$–6 and 12, Fig. 2, $r = 0.999$)[5].

In each family of compounds possessing analogous structures, no good linear correlation was observed between log k' (capacity factor) and the number of methylenes in the linking chain. This is not unexpected as the HPLC analysis were not performed using isocratic elution. For practical reasons a linear gradient of solvents had to be used. However, for all the six families of compounds studied, remarkable good linear correlations were observed between the capacity factors of the com-

[a] No dimerization is observed for $n = 2$.

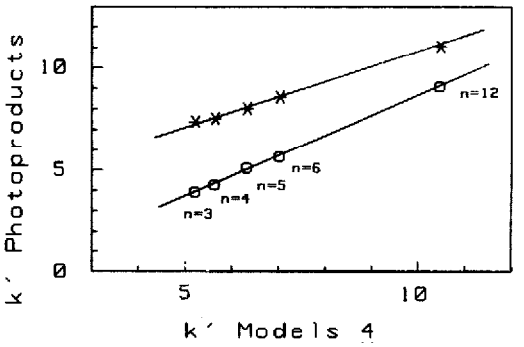

Fig. 2. Correlation between the RPLC capacity factors, k', of models **4** and the capacity factors of their photoproducts **6** (*) and **7** (○).

pounds *versus* the number of methylenes of their constitutive linking chain.

In conclusion, in each series of compounds, the capacity factors of the photoproducts are linearly related to the capacity factors of the irradiated compounds. Consequently, RPLC appears to be a powerful tool for the characterization and the study of bifunctional systems in which a polymethylene bridge of variable length separates the two functions. It should be especially useful for photochemical studies of bichromophoric systems which are currently the object of extensive investigations (for examples, see refs 6–12).

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