each pH reading. In general, 15 to 20 pH readings were taken for each titration. The pK_a was calculated by the nonlogarithmic method of Benet and Goyan.[32]

The partition coefficients (1-octanol/water) of the test compounds were determined by a similar titration method. A 10- to 40-mL aqueous aliquot of the test compound (1 mM) and 4 to 25 mL of 1-octanol was stirred vigorously and titrated with 0.1 N NaOH. From these data an apparent pK_a (pK_a') was calculated. The partition coefficient (P) of the free base was calculated from the pK_a and pK_a' according to the following formula:[33]

$$P = \frac{V_w}{V_0}(10^{pK_a'-pK_a} - 1)$$

where V_w = volume of aqueous solution and V_0 = octanol volume.

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QSAR Analysis. The coefficients for the independent parameters in the QSAR equations were arrived at by multiple regression using a standard matrix inversion technique.[34,35] A sequential F test was used to test for significant improvement when adding another term to the regression equation.[36]

Acknowledgment. We thank D. Charron, R. Heideger, G. H. Kronberg, K. Landry, and M. Wishengrad for excellent technical assistance and J. B. Keenaghan for the mass spectral data. We also thank Dr. D. O. Flanagan for the determination of pK_a values and partition coefficients and Dr. B. H. Takman for many valuable discussions.

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Methylangelicins: New Potential Agents for the Photochemotherapy of Psoriasis. Structure-Activity Study on the Dark and Photochemical Interactions with DNA

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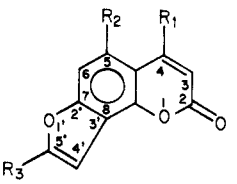
The interactions both in the ground and in the excited state between various methylangelicins, previously prepared with the aim to increase the low photobiological activity of the parent angelicin 1, and DNA have been studied. In general, the new methylangelicins show an increased capacity to photobind monofunctionally to DNA and a parallel increment of photobiological activity in comparison with the parent 1. This increase appears to be connected with various factors, such as the augmented affinity toward DNA for the dark complex formation and the electronic effect connected with the introduction into 1 of one or two methyl groups. The new compounds, on the basis of their photobiological activity and their lack of skin phototoxicity, appear as possible agents for the photochemistry of skin diseases characterized by cell hyperproliferation.

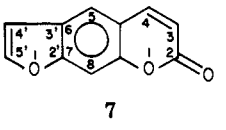
Photochemotherapy of psoriasis and other skin diseases characterized by hyperproliferation of the cutaneous cells is realized by oral or topical administration of psoralens to the patient and successive irradiation (UV-A) of the ill areas of the skin.[1-3] Psoralens, linear furocoumarins, are able to induce mono- and bifunctional photodamage to the DNA of the cutaneous cells in a selective way, thus inhibiting DNA functions and, as a consequence, the cell proliferation.[1]

The photodamages consist of the products of photocycloaddition between one molecule of psoralen and either one (monoadducts) or two pyrimidine bases of DNA (biadducts);[4,5] in the latter case, the pyrimidines belong to the two different complementary strands of DNA and their photocycloaddition with psoralens induces the formation of interstrand cross-linkages in the macromolecule.[5,6]

This therapeutical treatment, however, is accompanied by some undesired side effects, such as skin phototoxicity and risk of skin cancer.[7,8] In this connection, skin phototoxicity is strictly connected with the bifunctional lesions in DNA;[9] on the other hand, bifunctional lesions, in connection with the error-prone repair systems involved for their remotion, seem to be the main cause of the risk of skin cancer, while monoadducts, repaired through error-free systems, appear much less involved in this process.[10,11]

Chart I. Molecular Structures of Angelicin Derivatives 1-6 and Psoralen (7; Reported for Comparison)





7

	R_1	R_2	R_3
1	H	H	H
2	CH_3	H	H
3	H	CH_3	H
4	H	H	CH_3
5	CH_3	H	CH_3
6	H	CH_3	CH_3

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Also, the possible risk of cataract[12] and of hepatotoxicity[13] seems to be involved when psoralens are administered orally.

In spite of these undesired side effects, PUVA (psoralen + ultraviolet A) therapy is now considered one of the best and certainly the most effective clinical treatment for the cure of some skin diseases, such as psoriasis and mycosis fungoides.

In order to reduce or to eliminate some undesired side effects of psoralens, in recent times a new line of research has been developed with the aim of obtaining new DNA monofunctional reagents which, by maintaining the same selectivity of action as psoralens, should be able to inhibit cell division by inducing only monofunctional photolesions to DNA. A new series of DNA monofunctional reagents, as possible agents for the photochemotherapy of psoriasis, has been obtained by our team from unsubstituted agenlicin (1), introducing one or more methyl groups into its molecule;[14] this chemical modification has been made with the aim of increasing the low DNA photobinding capacity of 1 (Chart I).[14] In spite of its bifunctional character (from the photochemical point of view, two photorective sites, 3,4 and 4',5' double bonds, are present), this angular furocoumarin (1) is able to photoinduce only monofunctional adducts in DNA;[6,15] this behavior is strictly connected with the complex that 1 forms in the ground state with DNA undergoing intercalation between two base pairs of the macromolecule.[6] Intercalated 1, for geometrical reasons, can engage in the photocycloaddition with pyrimidine bases of DNA at only one of the two photoreactive sites, thus forming only monofunctional adducts.[6,15] In this connection also, its methyl derivatives may be expected to behave as pure monofunctional photoreagents toward the macromolecule.

In this paper the capacity of the new methylangelicins to form the dark complex with DNA and to photobind monofunctionally to the same macromolecule has been studied. Preliminary experiments concerning 4,5'-dimethylangelicin, made under slightly different experimental conditions, have been recently published.[16]

The influence on these properties into the molecule of angelicin has been evaluated and discussed. The extent of inhibition of DNA and RNA synthesis in Ehrlich ascites tumor cells irradiated in the presence of these compounds has been also determined.

Results

Noncovalent Binding with DNA. It is well known that furocoumarins in the ground state form a complex with DNA and that this step is a preliminary necessary event for the successive photoaddition.[17,18] From a qualitative point of view, the evidence of the formation of the complexes between the new methylangelicins and DNA has been obtained both by UV absorption spectra, where the complexed angelicins showed a red shift and lower absorption capacity, and by fluorescence spectra, where a strong quenching of fluorescence of the ligands was observed in the presence of DNA.

For a quantitative evaulation of the extent of complexation, the binding process between the methylangelicins and DNA has been followed by equilibrium dialysis experiments using tritiated furocoumarins.[17]

From the binding data the value of r (molecules of ligand bound per nucleotide) and c (ligand free in the system, moles/liter) have been calculated according to Peacocke and Skerret.[19]

These data are reported according to the classic Scatchard plot,[20] that is, plotting r/c against r (Figure 1).

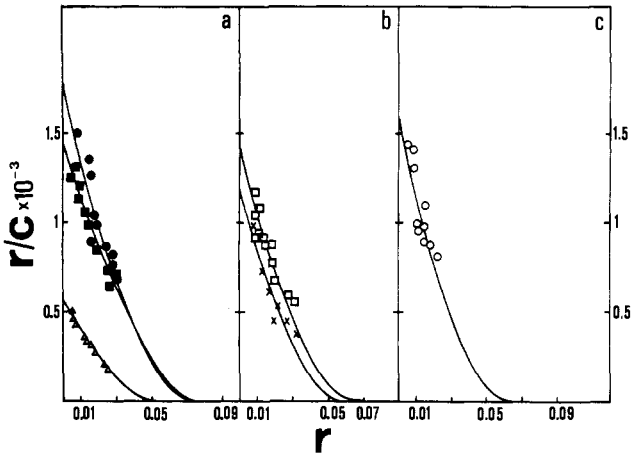

Figure 1. Scatchard plots for the binding in the dark of **5** (■–■), **6** (●–●), and **1** (Δ–Δ) as reference compound (part a), **2** (□–□) and **4** (x–x) (part b), and **3** (O–O) (part c) to calf thymus DNA. The curves were calculated by a computer according to the method of Mc Ghee and Von Hippel[21] on the basis of the experimental values of r and c reported in the figure.

Table I. Water Solubility of the Angelicin Derivatives, Binding Parameters of Their Complexes, and Rate Constants of Their Photoreactions with Calf Thymus DNA

	water solubility					
no.	μg/mL	mol/L	K values	n^a	$1/n^b$	rate constant, min^{-1}
1	20	10.7×10^{-5}	560	15.87	0.063	1.1×10^{-2}
2	4	2.0×10^{-5}	1400	13.16	0.076	1.6×10^{-2}
3	4.5	2.2×10^{-5}	1560	14.08	0.071	3.4×10^{-2}
4	12	6.0×10^{-5}	1200	14.92	0.067	2.1×10^{-2}
5	8	3.7×10^{-5}	1450	10.64	0.094	4.0×10^{-2}
6	3	1.4×10^{-5}	1750	11.76	0.085	2.7×10^{-2}

[a] According to McGhee and Von Hippel;[21] n is defined here as the number of nucleotides occluded by one bound molecule of angelicin derivative. [b] $1/n$ defines, according to Mc Ghee and Von Hippel, the frequency of binding sites, i.e., the number of ligands bound per nucleotide, and can be considered analogous to the "n" value obtained by the classic Scatchard method.[20]

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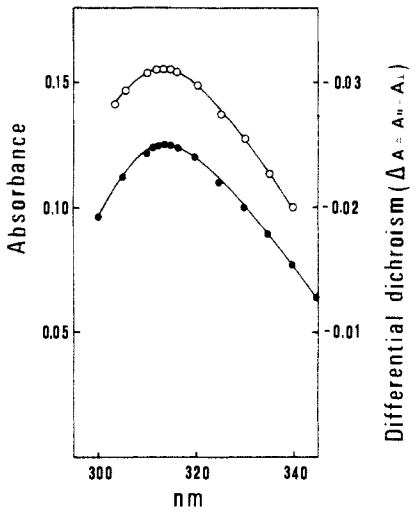


Figure 2. Differential dichroism (O–O) and UV absorption spectra (●–●) of compound **3** (2×10^{-4} M) in the presence of calf thymus DNA (3.78×10^{-3} M); optical cells having a 1-mm optical path were used. Velocity gradient of the laminar flow under our experimental conditions was 2900 s^{-1}.[17]

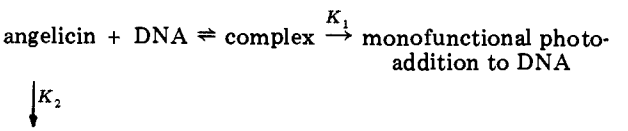
The isotherms reported have been calculated by a computer according to the method of Mc Ghee and von Hippel[21] on the basis of the experimental data of r and c. Also, the binding parameters K (the association constant to an isolated site), n (the number of nucleotides occluded by a bound angelicin), and $1/n$ (the frequency of the binding sites; in other words, the number of molecules of angelicin bound to every nucleotide) have been calculated by the same method.

We can observe in Figure 1 and in Table I that the introduction of one or two methyl groups into the molecule of **1** strongly increases the affinity toward DNA for the dark complex formation as shown by the markedly higher values of K and $1/n$ in comparison with those of unsubstituted **1** (see Table I). In the same table the water solubility of the various methylangelicins is also reported; these data show that the new methylangelicins show a hydrophobicity markedly higher with respect to that of unsubstituted angelicin.

Flow Dichroism Studies. In order to confirm whether these new angular furocoumarins also undergo intercalation between two base pairs of the macromolecule when complexed with native DNA, as in the case of the corresponding linear isomers, we have studied the flow linear dichroism (LD) of an aqueous solution of 5-methylangelicin (**3**) in the presence of DNA. By this technique the long and stiff molecule of DNA can be oriented in flow, and a peculiar negative dichroism of the macromolecule can be seen.[22] When a small planar ligand undergoes intercalation between two base pairs of the macromolecule, it assumes an ordered position similar to that of the purine and pyrimidine bases of the macromolecule; in such a way, a negative dichroism in the correspondence of the chromophore of the ligand (different from that of DNA) is observed when its transition moment is polarized in parallel with the planar chromophore as for purines and pyrimidines.[23]

Moreover, considering that all strong absorptions in the furocoumarins are π to π^* transitions which are polarized in the molecular plane,[24] the negative LD observed between

Scheme I



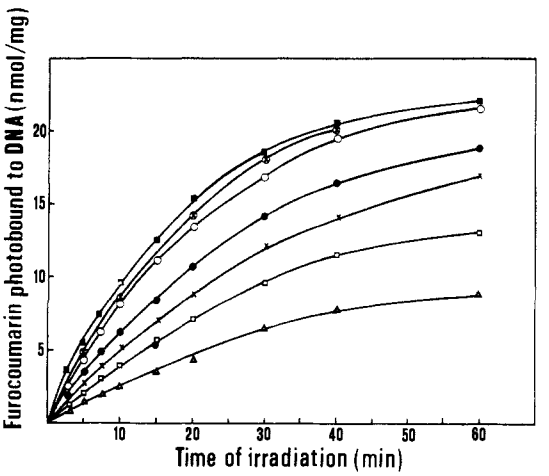


Figure 3. Photobinding capacities toward DNA shown by the new methylangelicins **5** (■–■), **3** (O–O), **6** (●–●), **4** (x–x), and **2** (□–□). **1** (Δ–Δ) and **7** (⊗–⊗), as reference compounds, are also reported.

305 and 330 nm (see Figure 2) is evidence that the complexed molecule of **3** assumes a position parallel to the plane of the DNA bases.[24] This orientation is consistent with the intercalation of this ligand between two base pairs of DNA.[17] Strictly analogous results have been obtained with other methylangelicins.

This fact assumes a marked importance because the capacity of these angular furocoumarins to behave as monofunctional reagents toward DNA, in spite of their bifunctional character from the photochemical point of view, is attributed just to their capacity to undergo intercalation and to assume an ordered position in the duplex DNA; they, in such a position, for geometrical reasons cannot form interstrand cross-linkages.

Photobinding Capacity toward DNA. The events occurring in the photoreactions between angelicins and DNA are reported in Scheme I. The factor that controls the photobinding to DNA is the amount of angelicin intercalated in duplex DNA. The complexed angelicin is in equilibrium with a certain amount of free angelicin dissolved in water, and the latter can undergo photomodification during the irradiation. In this way, a decrease of the free angelicin affects the extent of the complexed one.[18] This effect is, however, small, especially for the relatively short periods of irradiation (as long as 30 min), because of the low rate (K_2) of photoconversion of angelicins into their photooxidation products, as evidenced by irradiation of aqueous solutions of the compounds and by the successive evaluation of the unmodified angelicin by TLC.

The amounts of the various angelicins covalently photobound to DNA as a function of time of irradiation were determined by radiochemical measurements on DNA irradiated in the presence of tritiated angelicin (see Figure 3). These photocycloaddition reactions of the angelicins to DNA behave as pseudo-first-order reactions with respect

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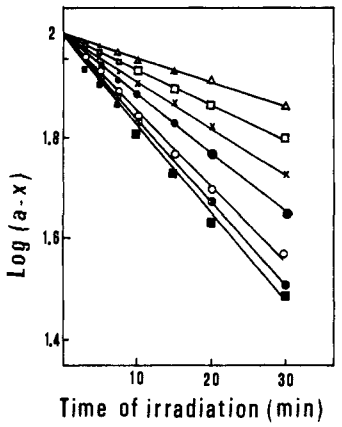

Figure 4. Plot of log $(a - x)$ against time of irrdiation; a = initial concentration of furocoumarin complexed with DNA; x = furocoumarin covalently photobound to DNA after various times of irradiation. 1 and 7 are reported as reference compounds. Symbols are the same in Figure 3.

to the complexed furocoumarins (see Figure 4). The rate constant values are reported in Table I.

We can distinguish three different levels of photoreactivity. The highest level includes 4,5′-dimethylangelicin (**5**) and 5-methylangelicin (**3**) and shows photobinding capacity toward DNA similar to that of psoralen (**7**). The intermediate level includes 5,5′-dimethylangelicin (**6**), 5′-methylangelicin (**4**), and 4-methylangelicin (**2**). Lastly, the lowest level consists of the parent, unsubstituted angelicin.

The introduction of one or two methyl groups in the angelicin molecule leads to a marked increase in the photobinding capacity toward DNA; in particular for the monomethyl derivatives, the role of the position where the methyl group is introduced for the increase of photoreactivity toward DNA has the following order of magnitude: 5 > 5′ > 4.

For dimethyl derivatives the increase of photoreactivity does not correspond to the simple addition of the effects of the two methyl groups evaluated separately; the order of effectiveness, in fact, is the following: 4,5′ > 5,5′.

Monofunctional Photocycloaddition to DNA: Heat Denaturation Studies. In order to verify the monofunctional cyclcoaddition to DNA of the various angelicins, the behavior of the samples of DNA irradiated in the presence of these compounds has been studied on hydroxylapatite column chromatography. By this technique the single-stranded (heat denatured) DNA is separated from double-stranded (native or renatured) DNA.

The elution profiles of DNA irradiated in the presence of **3** and for comparison in the presence of psoralen **7** before and after heat denaturation are reported in Figure 5. The lack of interstrand cross-linkages is shown by the identical behavior (complete denaturation) of the DNA sample irradiated in the presence of 5-methylangelicin with reference DNA; by contrast, the sample irradiated in the prsence of **7** underwent complete renaturation. Strictly similar results have been obtained with the other methylangelicins reported in this paper.

Skin Photosensitizing Activity. Some experimental evidence suggest that furocoumarins able to photoinduce monofunctional lesions in DNA, such as angelicin,[4,15] 3-(α,α-dimethylallyl)psoralen,[25] 3-carbethoxypsoralen,[26] 7-methylallosporalen, 4,7-dimethylallopsoralen,[27] or pyranocoumarins (xanthyletin), are not able to induce skin sensitization in guinea pig, while linear furocoumarins able to form interstrand cross-linkages provoke a marked skin phototoxicity. In this connection, a close correlation between the rate constant of cross-linkage formation in DNA and the skin photosensitizing activity of various linear furocoumarins has been observed.[9]

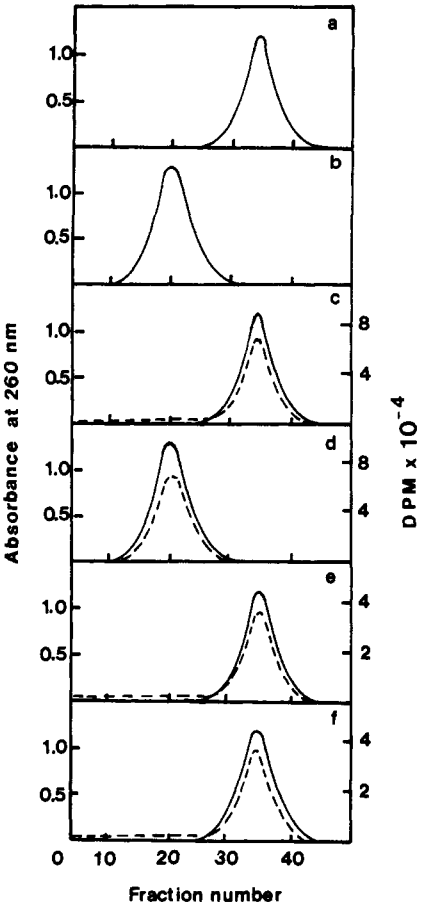

Figure 5. Hydroxylapatite column chromatography of calf thymus DNA (2.3×10^{-3} M) irradiated (120 min) in the presence of tritiated compound **3** and tritiated psoralen **7** (4.7×10^{-5} M). After the irradiation the various DNA samples were heat denatured, quenched in ice, and then chromatographed. Reference DNA: part a, native; part b, denatured. Irradiated DNA + ^{3}H-labeled compound **3**: part c, native; part d, heat denatured. Irradiated DNA plus ^{3}H-labeled compound **7**: part e, native; part f, heat denatured. Strictly analogous results have been obtained using compounds **2**, **4**, **5**, and **6**: (—) absorbance at 260 nm; (---) radioactivity.

In order to also have a further confirmation of the lack of skin phototoxicity in these new angelicins, they have been tested according to the classic test of Musajo et al.[4] All these compounds after the application of guinea pig skin and irradiation with UV-A light did not show any skin phototoxicity.

Inhibition of DNA and RNA Synthesis. While skin photosensitization has been for a long time a basic test for the evaluation of photobiological activity of linear furocoumarins,[4] in this case a more suitable substrate, such as Ehrlich ascites tumor cells, has been used in order to evaluate the photobiological activity of angelicins, as a consequence of their absence of any skin phototoxicity.

The inhibition of DNA and RNA synthesis in Ehrlich ascites tumor cells was determined after the irradiation

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Table II. Inhibition of DNA and RNA Synthesis in Ehrlich Ascites Tumor Cells by Irradiation (365 nm) in the Presence of Angelicins[a]

no.	D_{50}, quanta × 10^{-18} (confidence limits); $p = 0.05$ DNA	RNA
1	25 ± 1.1	23.3 ± 1.6
2	24 ± 3.1	18.1 ± 1.7
3	9.1 ± 1.2	8.3 ± 1.4
4	16.6 ± 1.4	17.4 ± 1.3
5	10.9 ± 1.54	13.5 ± 2.1
6	13.8 ± 1.2	6.6 ± 1.1
7	9.1 ± 0.8	14.0 ± 1.1

[a] The tumor cells were irradiated in the presence of 1.9×10^{-5} M of the compound studied and then incubated at 37 °C in the presence of [^{3}H]thymidine or of [^{3}H]uridine; the acid-insoluble radioactivity was determined. The results are expressed as D_{50}, i.e., the UV radiation dose that in the presence of the drug produces a 50% inhibition in respect of untreated cells. Incubation in the dark in the presence of the drugs or irradiation in their absence were both ineffective.

of the cells in the presence of the methylangelicins. The D_{50} values (amount of UV-A irradiation that in the presence of the angelicin under investigation causes a 50% inhibition of the synthesis of the macromolecule) are reported in Table II. These values are inversely correlated with the photobiological activity of the various angelicins. These data show that some compounds more effectively inhibit DNA synthesis in the cells, such as **3**, **5**, and **6**, while other derivatives show a gradually lower activity, such as compounds 4 and 2.

Discussion and Conclusions

The new compounds have shown a markedly increased capacity to photobind to DNA in comparison with the parent angelicin (1); all these compounds undergo photoaddition to the macromolecule only through monofunctional adducts.

Moreover, they do not show any skin phototoxicity on guinea pig skin, further supporting the fact that compounds able to photoinduce only monofunctional lesions to DNA do not appear to be able to provide skin photosensitization.

The introduction of one or two methyl groups into the molecule of angelicin strongly increases the capacity to form the complex with DNA in the ground state. This fact can be explained by considering that the introduction of one or more methyl groups into the molecule of angelicin produces a marked increment of hydrophobicity, with a consequent increase of the affinity toward the internal lipophilic part of DNA where intercalation occurs.

Considering that only the furocourmarin complexed with DNA can undergo covalent photoaddition to the macromolecule[18] (see Scheme I), in DNA photobinding experiments the higher capacity to form the dark complex increases the extent of molecules that could undergo photoaddition to DNA. This is one of the factors which contribute to the generally higher photobinding to DNA shown by the new methylangelicins.

However, if we examine in more detail these two apsects, i.e., dark binding and photobinding to DNA, by comparing the rate constants of the photoreaction with the binding parameters of the complexes, we see that some discrepancies can be observed in the above-mentioned general correlation. In fact, the variations among the various rate constants are more pronounced than those among the binding parameters of the complexes. Another factor therefore seems to be involved, that is, the electronic effect of the introduction of one or more methyl groups into the electronic arrangement of unsubstituted angelicin (1) both in the ground state and in the excited state. In other words, a possible modification of the photoreactivity of the angelicin molecule (1) should be connected with the number and the position of the methyl groups introduced; a study of the photophysical properties of various methylangelicins is now in progress in this line.

The new methylangelicins, on the one hand, show strong photobiological activity markedly increased in comparison with their parent compound, 1; on the other hand, they do not provoke any skin phototoxicity. These compounds moreover, show a much lower mutagenic activity than psoralens.[28,29] These data seem to support the application of the most active methylangelicins as possible agents for the photochemotherapy of skin diseases characterized by cell hyperproliferation as drugs alternative to psoralens.

Experimental Section

Furocoumarins. Compounds 1–6 were prepared by chemical synthesis in this institute,[14] psoralen (7) was extracted from fig leaves,[30] and the furocoumarins were labeled with tritiated water and catalyst by the Radiochemical Centre (Amersham, England); they were then purified by two runs on TLC (silica gel preparative plates, Merck, catalog no. 5717, developed with chloroform) and have, respectively, the following specific activity: 2.40, 4.78, 9.35, 5.88, 2.56, 13.9, and 5.23 Ci/mol.

DNA. Calf thymus DNA (cat. D 1501) was purchased from Sigma Chemical Co., St. Louis, MO. Hypochromicity of the sample, determined according to Marmur and Doty,[31] was higher than 40%. Hydroxylapatite Bio-Gel type was furnished by Bio-Rad Laboratories, Richmond, CA.

Equilibrium Dialysis Experiments. Cylindrical containers, 4-cm diameter, 1.6-cm depth, divided into two parts by a cellophane membrane (Visking Corp.) were used; in one part of the cells an aqueous solution of a labeled furocoumarin containing 0.02 M NaCl and 1 mM EDTA at a concentration a little under its water solubility was introduced; in the other part of the cells, aqueous DNA solutions in the presence of the same labeled angelicin, at the same ionic strength and having decreasing concentrations in the range between 3×10^{-3} and 3×10^{-4} M, were introduced. In these DNA solutions the initial concentration of the angelicin was constant and identical with that of the aqueous phase.

The cells were mechanically shaken for 12 h in a thermostat at 25 ± 0.05 °C in the dark; preliminary experiments showed that this period allowed the phases to reach equilibrium across the membrane.

After the shaking period, small volumes (0.2 mL) of the two phases were utilized for radiochemical measurements: in this way, the concentrations of the furocoumarin in the two phases were determined, and α, r, and c values were calculated.[20]

In order to evaluate the extent of furocoumarin complexed to DNA (100% of reactant at 0 times) for calculating the rate constants of the photoreactions, analogous experiments were made, however at lower ionic strength (2 mM NaCl); moreover, the concentrations of the furocoumarins and of DNA were varied so that in the DNA phase, once equilibrium was reached, the ratio of ligand to nulceotides (1:76) was strictly similar to that of the solutions used for the photobinding experiments.

Radiochemical Determinations. A liquid scintillation spectrometer (Packard Model 3375) was used. Small volumes (0.2 mL) of the solutions in which the concentration of furocoumarin had to be determined were added to 10 mL of dioxane

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base scintillator (PPO 5 g; POPOP, 0.075 g; naphthalene, 120 g; dioxane, up to 1000 mL of solution) and then counted. The apparatus efficiency for counting tritium was within the range of 28–35%.

Computation of the Interaction Parameters. The method of computation involved an iterative procedure apt to satisfy the following equation of Mc Ghee and Von Hippel:[21]

$$\frac{r}{c} = K(1 - nr)\left(\frac{1 - nr}{[1 - (n - 1)]r}\right)^{n-1}$$

given the experimentally determined values of r and c and the initial guess of K (the intrinsic binding constant to an isolated site) and of n (the number of nucleotides occluded by a bound furocoumarin molecule). The program, based on the least-squares method of the Taylor series expansion of the above-reported equation, was made to recycle until K and n changed by less than 1% and then, to give the final values of K and n, with a calculated binding isotherm at 5% saturation increments.

Irradiation Procedure. Aqueous solutions (2.3×10^{-3} M) of DNA containing 2×10^{-3} M NaCl at 1×10^{-3} M EDTA were added of the labeled furocoumarin to be examined (3×10^{-5} M). Measured volumes (2 mL) of the prepared solutions were introduced into calibrated glass tubes, and these were immersed into a thermostatically controlled bath and irradiated for ever-increasing periods of time by means of two HPW 125 Philips lamps (which emit almost exclusively at 365 nm);[32] the irradiation intensity, determined by using a chemical actinometer,[33] was 1.07×10^{16} quanta s^{-1} mL^{-1}. After irradiation, the macromolecule was precipitated by the addition of 2 M NaCl and 2 volumes of absolute ethanol, washed with 80% ethanol, and redissolved in the initial volume of water. The solutions so obtained were used for radiochemical determinations. Check experiments were carried out by precipitating DNA from a nonirradiated solution of the macromolecule in the presence of the labeled angelicin derivative. This DNA sample, after washing with ethanol and redissolution, did not show any radioactivity, indicating that the dark complex underwent complete dissociation as a consequence of the ethanolic precipitation of the macromolecule.

Evaluation of the Products of Photomodification of Angelicins. Two milliliters of an aqueous solution of the tritiated angelicin derivative (10 μg/mL) was maintained in the dark or irradiated for 30 and 60 min under the same conditions previously used for the photoreactions with DNA. The water was evaporated, and the residue was dissolved with hot ethanol and chromatographed on silica gel analytical plates (Merck, catalog no. 5715) developing with chloroform. The plate was cut into ten strips, and these were counted; in the case of the unirradiated sample the radioactivity was localized only in the area corresponding to the angelicin derivative (R_f between 0.7 and 0.8 for the various compounds), while in the case of the irradiated sample the radioactivity was present not only in the area corresponding to the unmodified angelicin but also in a second one where the products of its modification were present (R_f between 0 and 0.4). The extent of photomodification was calculated considering as 100% of the initially present compound the amount of angelicin derivative recovered from the plate where unirradiated solution was chromatographed. After 1 h of irradiation, the extent of photomodification was lower than 10% for the various compounds. Considering that in the system where the photoreactions occur about 65% of the angelicin derivatives are complexed with DNA and that the complexed angelicins cannot undergo photodegradation as occurs by their irradiation in aqueous solution,[18] the extent of this photochemical event in the above-mentioned system is small.

Evaluation of Cross-linkages. This evaluation was made directly on the irradiated DNA samples, without precipitation, according to ref 16.

Flow Dichroism Measurements. Linear flow dichroism of aqueous (NaCl, 2 mM) solutions of DNA in the presence of angular furocoumarin to be examined was determined by a Schimadzu QV 50 spectrophotometer equipped with the flow dichroism attachment.[17] This replaces the usual cell chamber and consists of a quartz cylindrical cell containing a quartz rotating cylinder and a calcite prism which polarizes the monochromatic light parallel (∥) and perpendicular (⊥) to the flow line.[22] The linear flow dichorism as well as the reduced dichroism were calculated according to Wada[34] and Wada and Kozawa.[22]

Water Solubilities of the Compounds. These have been carried out as described in ref 6.

DNA and RNA Synthesis. Ehrlich ascite tumor cells in Hank's solution (aliquots of 5 mL; 2×10^7 cells/mL) were irradiated in Petri dishes (5 cm in diameter) placed on ice by a Philips HPW 125 set at 20-cm distance (2.02×10^{16} quanta/s incident on the whole sample). Samples of 2×10^6 of the irradiated cells in 0.5 mL of the same medium containing 1 μCi of [^{3}H]thymidine or of [^{3}H]uridine were incubated for 15 min at 37 °C. The reaction was stopped by chilling in ice and adding 1 mL of 5 mM unlabeled nulceoside. The cells were collected by filtering on Whatman CF/c dishes (diameter 2.5 cm) and then washed three times with saline and treated with 10 mL of ice-cold 10% trichloroacetic acid. After 1 min the samples were filtered and washed six times with 10 mL of 10% trichloroacetic acid; the filters were dried and counted. Each UV-A radiation dose was studied at least in duplicate, while the controls were four samples of untreated cells. Results were calculated on the basis of the specific radioactivity incorporated into nucleic acids and expressed as the percentage of the incorporation observed in the untreated cells (2000 DPM/μg for DNA and 2400 for RNA). DNA and RNA contents were determined by the diphenilamine[35] and orcinol[36] reactions. The data were submitted to probit analysis and expressed as the D_{50}, i.e., the UV-A radiation dose that in the presence of the 1.9×10^{-5} M produces a 50% inhibition.

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