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Investigations on the Mechanism of Photodynamic Action of Different Psoralens with DNA

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Abstract. Investigations on the photodynamic action of psoralens with DNA were performed, using experimental techniques of fluorescence lifetime and NMR-CIDNP, as well as SCF-MO and CNDO molecular orbital calculations. It has been shown that the formation of a biradical through the triplet state is the decisive step for psoralen dimer formation, as well as for cyclobutane addition with thymine, while singlet oxygen production is responsible for enzyme inactivation (e.g., lysozyme and trypsin). The molecular orbital calculations, in agreement with experimental results, indicate that the differences in biological effectivity of different psoralens are based on variations in triplet formation probability.

Key words: Psoralen –DNA – Enzymes – Photodynamic action

Introduction

Psoralens, mainly 8-methoxypsoralen (8-MOP), are used as photosensitizers in photochemotherapy of skin diseases such as psoriasis and vitiligo (Parrish 1981). The combination of psoralens and UV-light controls excess cell division in the skin by its ability to damage DNA. The lesions in DNA lead to inhibition of DNA synthesis, skin pigmentation and erythema production. On the other hand, psoralens show mutagenic and carcinogenic effects (Song and Tapley 1979). The danger of initiating skin cancer in humans is, of course, of great concern for dermatologists (Grekin and Epstein 1981).

Psoralens intercalate in a dark reaction between pyrimidine base pairs in duplex DNA (Parsons 1980; Song and Tapley 1979). After light absorption, covalent bonds can be formed between pyrimidine bases and the 3,4- or

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4′,5′-double bond of the psoralen molecule (monoadducts). Under further irradiation a conversion of certain monoadducts into interstrand crosslinks is possible. The kinetics of these photoreactions are described by Dall'Aqua et al. (1979). Differently substituted psoralens show varying biological effectivities. For example, methylsubstituted psoralens are known as very active; psoralen, as well as the methoxypsoralens are active.

This paper presents theoretical and experimental investigations with differently substituted psoralens for a better understanding of the molecular basis of the photodynamic action of psoralens and to elucidate the structure – biological activity variations caused by different substitutents. Furthermore, a better knowledge about the excited electron states of psoralens could be of interest to estimate undesired reactions for the photochemotherapy.

We performed theoretical calculations of electron densities and mobile bind orders using SCF-π-molecular orbital and CNDO-programs, fluorescence lifetimes measurements of psoralens and NMR-CIDNP-spectroscopy. The method of *c*hemically *i*nduced *d*ynamic *n*uclear *p*olarisation allows the detection of radical intermediate substances (Closs 1974). Moreover, a comparison of the photodynamic and biological activities of different psoralens is given. The paper concludes with a proposed overall reaction scheme.

Experiments concerning the photodynamic sensitization by 8-MOP via the singlet oxygen mechanism were published earlier by Poppe and Grossweiner (1975), Singh and Vadasz (1978), De Mol and van Henegouwen (1979) and Muller-Runkel and Grossweiner (1981). Experimental and theoretical investigations on the molecular structure of poly dA-dT-complexes will be published separately (Römer and Anders 1983, in press).

Materials and Methods

Materials

The psoralens used (Fig. 1) are: 8-methoxypsoralen (Sigma); 4′,5′-dihydrosporalen and psoralen were synthesized after Hornig and Reisner (1948) and 8-hydroxypsoralen after Schönberg and Sina (1950). Bergapten, Bergaptol and 4,5′,8-trimethylpsoralen were kindly placed at our disposal from G. Rodighiero, University of Padua, and Angelicin from Chandra University of Frankfurt. Thymine (Serva) and poly dA · dT (PL Biochemistry) were used as received. DMSO, D_2O-DMSO mixture (20% DMSO), dimethylformamide, acetonitrile, and methanol (Sigma) were used as solvent.

SCF-π-MO and CNDO/II-Calculations

The calculations were done on a CDC-Cyber 76–12. The programs for calculating the molecular orbitals were obtained from the "Quantum Chemistry Program Exchange", Indiana University, Bloomington, IN, USA, (QCPE No. 71.2; SCF-PPP; Bloor et al. 1964, and QCPE No. 174; CNDO: Del Bene and

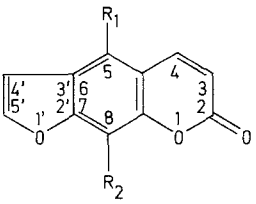

Fig. 1. Nomenclature for molecular orbital calculations and NMR-spectroscopy

R_1 = H,

R_2 = CH_3: 8-Methylpsoralen
R_2 = OCH_3 : 8-Methoxypsoralen, 8-MOP (Xanthotoxin)
R_2 = OH: 8-Hydroxypsoralen, 8-OH-Psoralen (Xanthotozol)
R_2 = H: Psoralen

R_2 = H,

R_2 = CH_3: 5-Methylpsoralen
R_1 = OCH_3: 5-Methoxypsoralen, 5-MOP (Bergapten)
R_1 = OH: 5-Hydroxypsoralen, 5-OH-Psoralen (Bergaptol)

Jaffe 1968). Atomic coordinates were taken from the data of 8-MOP from Stemple and Watson (1972) and were extended to the derivatives considering the usual atomic distances. The SCF-molecular orbitals were calculated with optimized parameters after Sarnow and Niemann (1975). For the ionization potentials of the different C-atoms with their substituents, the following values are taken: $I_{C\text{-}H}$ = 11,42 eV and $I_{C\text{-}CH_3}$ = 11,1 eV and for the substituents, which contribute to the π-system, the following values: I_{OCH_3} = 34 eV and I_{CH} = 34,31 eV.

CIDNP-Spectroscopy

Solutions of different psoralens were irradiated with UV-light in a Fourier-NMR-spectrometer WH 90 (Bruker Physik). The pulse length was 3 μs, the acquisition time 3.407 s (no pulse delay) resulting in measuring times from 1–10 min and 20–200 scans were used. A 200 W HBO-lamp (whole spectrum) served as a light source and was coupled into the spectrometer via a fiber optic technique. The different irradiation times showed no effect on the intensity of the CIDNP signals. The doses were not measured. The temperature was varied from 190 to 370 K.

Fluorescence Apparatus

The excitation part of the fluorescence apparatus consisted of a pulsed lamp (Optitron; pulse half-bandwidth: 1 ns, risetime: 0,5 ns, repetition rate: 200 Hz to 10 kHz) coupled with a monochromator for the selection of the excitation

wavelength, or a nitrogen-laser (Lambda Physik; pulse width; 6 ns, $\lambda = 337$ nm, repetition rate: 100 Hz (Poppe 1977). The solutions were kept in quartz cuvettes; the fluorescence was observed rectangular to the excitation light. The analysis part consisted of a monochromator, multiplier (Valvo, SEV XP1210), Boxcar averager (PAR, model 163) and a sampling head (Tektronix).

The detector system has a risetime $\tau < 1$ ns. Even if the excitation pulse has a risetime of $\tau > 1$ ns, we can observe time structures $t < 1$ ns, because the measured signal can be described as a convolution consisting of the excitation pulse and the fluorescence decay function. This problem can be described generally with the convolution integral

$$I(t) = \int_0^t F(t) \cdot L(t - t')\, dt' ,$$

[$I(t)$: measured signal, $F(t)$: fluorescence decay function, $L(t)$: excitation pulse, t: time in ns]. This integral was deconvoluted after Demas and Adamson (1971) or after Ware et al. (1973). In the latter case, the convolution integral was solved with a least square fit-method. The fit function consisted of a sum of exponential functions. We used several sets of decay frequency parameters depending on the fluorescence decay. After this deconvolution procedure, we analysed the decay curves with a kinetic program. In the first part of this calculation, the number of components, k, was computed from the deconvoluted function. A regression analysis determined the lifetimes of the k^{th} component. In the second part, the program fitted the a_i coefficients of the k^{th} component decay function $f(t)$,

$$f(t) = \sum_{i=1}^{k} a_i \cdot e^{-t/\tau_i} .$$

Finally the part of the different components were calculated in percent P_i (T: time range of the deconvoluted function).

$$P_i = \frac{a_i \int_0^t e^{-t/\tau_i} \cdot dt}{\int_0^t \left(\sum_{i=1}^{k} a_i \cdot e^{-t/\tau_i} \right) dt} = 100 .$$

Determination of Enzyme Activity

(*a*) *Lysozyme:* The activity was determined measuring the decrease of the extinction coefficient in a cell suspension of *Micrococcus lysodeikticus*. A HBO 200 W/2 (Osram) served as the irradiation source (Poppe 1977). Test solution: 30 mg *M. lysodeikticus* with 0.05 M phosphate buffer in 100 ml, pH 7.4.

(b) Trypsin: test solution: 46.7 mM *Tris*-buffer, pH 8; 0.9 ml BAEE (α-N-benzoyle-argininethylester) 19 mM $CaCl_2$ in 100 ml.

(c) Oxidation of I^- (I^- to I_3^-); solution: 20 µM psoralen and *KI* in D_2O, C_I = 0.15 M.

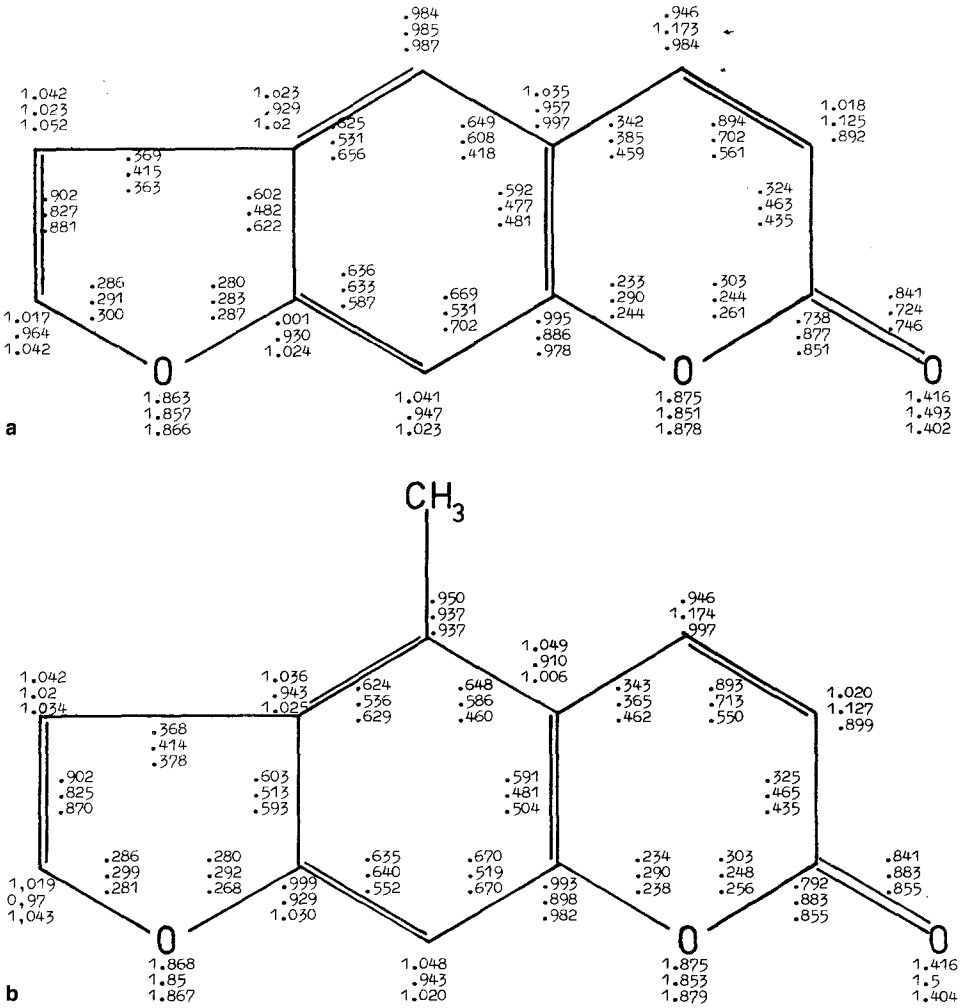

Fig. 2a–g. Electron densities and binding orders (figures inside) of different psoralens; ground state S_0, first excited singlet state S_1 and triplet state T_1 (from above). **a** psoralen, **b** 5-Methylsporalen, **c** 8-Methylsporalen, **d** 8-Methoxypsoralen, **e** 5-Methoxypsoralen, **f** 8-Hydroxypsoralen, **g** 5-Hydroxypsoralen

Results and Discussion

1. SCF-π-Molecular Orbital Calculations

In Fig. 2 the results of the SCF-π-molecular orbital calculations of the electron densities and the mobile bind orders are shown for two biologically inactive psoralens (8-hydroxypsoralen and 5-hydroxypsoralen), three active ones (psoralen, 8-methoxypsoralen and 5-methoxypsoralen), as well as for the very active methyl-substituted psoralens (8-methylpsoralen and 5-methylpsoralen).

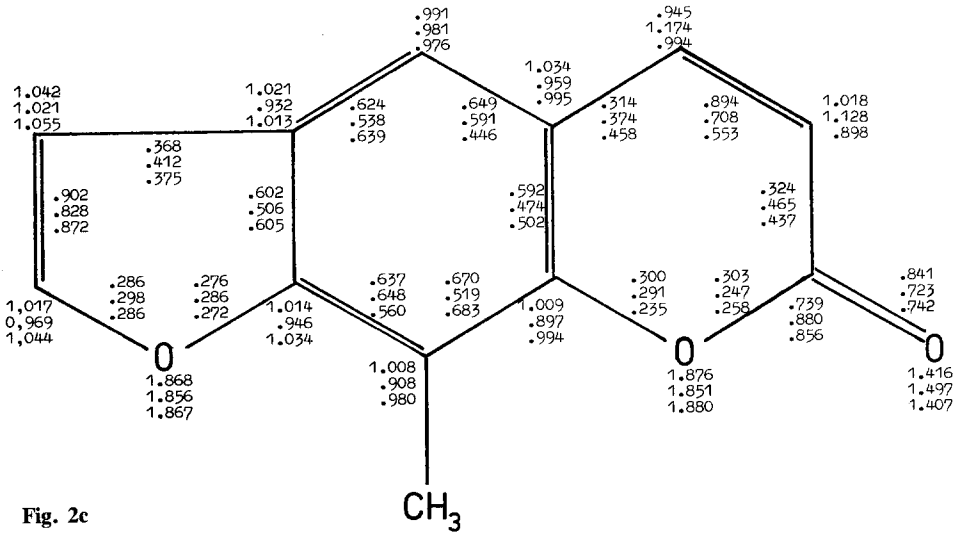

Fig. 2c

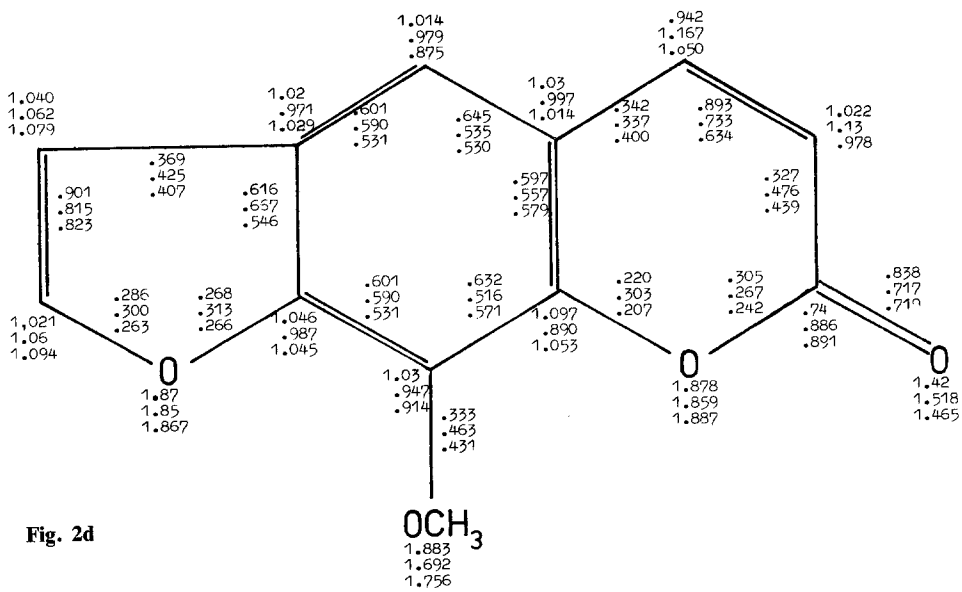

Fig. 2d

The calculations were made for the ground state, the first excited singlet state and the triplet state.

The atomic centres and bondings in the molecule are of interest in which strong shifts upon electronic excitation occur. This was found for the bond orders of the C_3-C_4 double bond and the electron densities at these C-atoms. This confirms the view that the C_3-C_4 double bond is the most reactive site,

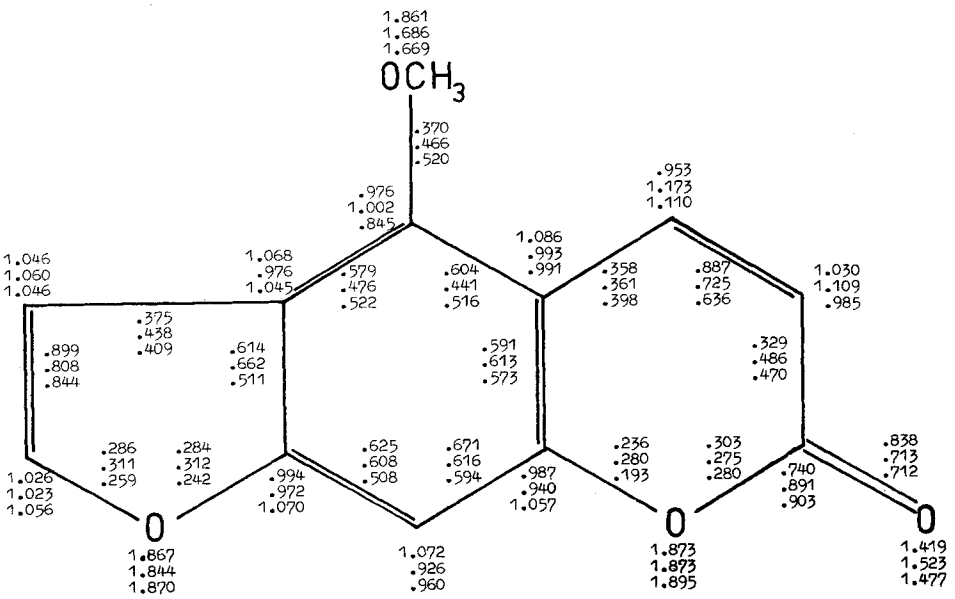

Fig. 2e

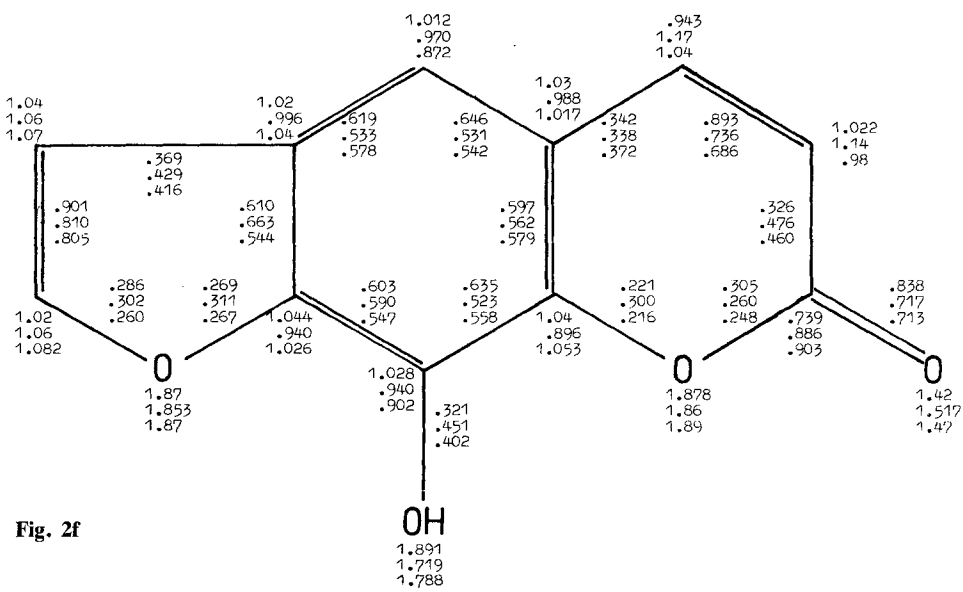

Fig. 2f

whereas the 4′−5′ bond only shows small shifts. The strongest shifts of the bond orders of the 3,4-double bond were found for all investigated psoralens in the triplet state.

Table 1 gives a comparison of the results. A decrease of the bond order in the excited singlet state occurs for psoralens of about $\Delta_S = 0{,}192$ e and in the triplet

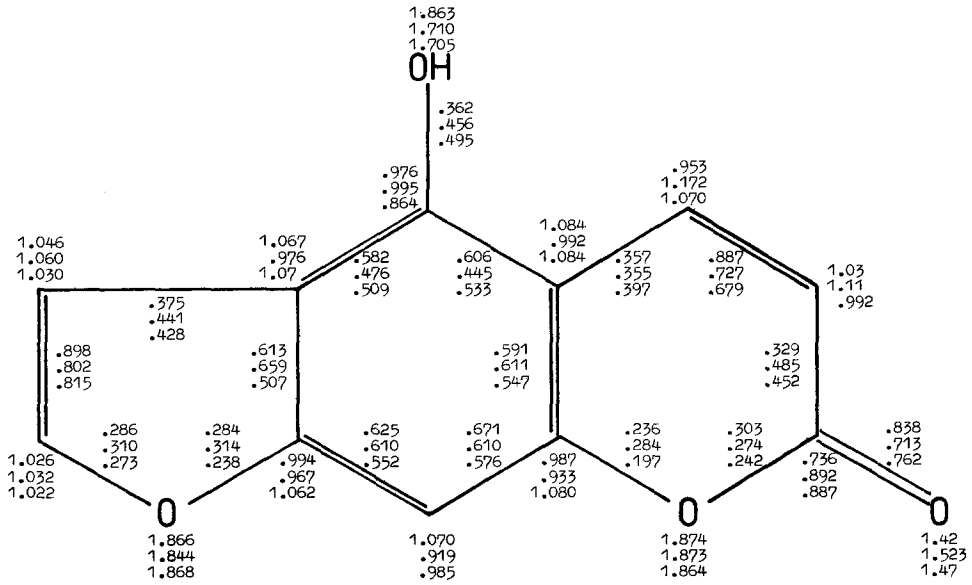

Fig. 2g

state of about $\Delta_T = 0.333$ e, for example. A comparison of the 3,4-double bond of the different substituents demonstrates that this effect is still relatively strong for 8- and 5-methoxy-psoralen ($\Delta_T = 0.25$ e), less strong for the hydroxy-substituted ones ($\Delta_T = 0.2$ e), and it is highest for the methyl-substituted compounds ($\Delta_T = 0.343$ e). Therefore, if the 3,4 bond order increases, the biological activity of the psoralens increases as well.

Summerizing the results, the calculations demonstrate that the 3,4-double bond represents the reactive centre of the excited psoralen molecule. Furthermore, the C-atom in paraposition to the substituent shows strongly increased electron density in the triplet state. The reactivity is a function of the corresponding substituent, which is caused by the variation in triplet fomation probability.

A kinetic study of the photoreactions between psoralen and DNA from Dall-Aqua et al. (1979) also points out a preference for the reaction from the 3,4-double bond of psoralen. The yield for 3,4-cycloadduct formation with DNA is considerably higher than for 4′,5′ cycloadducts (rate constant for 3,4-cycloadducts; 7.35×10^{-2} m · s^{-1}; for 4′,5′-cycloadducts: $2.17-2.55 \times 10^{-2}$ m · s^{-1}).

2. *Fluorescence Spectroscopy*

The fluorescence lifetimes of different psoralens are 1.0–6.0 ns. The results are summarized in Table 2 and compared to the spectral data for absorption and fluorescence maxima, as well as for triplet-singlet transitions, the phosphorescence lifetimes and $\lambda_{P\,0-0}$. The kinetics of the experimental decay curves of

Table 1. Centres and binding orders of psoralens after electronic excitation (see Fig. 1)

Dye	Binding 3–4		Binding 4′–5′		C_2		C_5	C_8		Substituted at C_5		Substituted at C_8	
	ΔS	ΔT	ΔS	ΔT	ΔS	ΔT	ΔT	ΔS	ΔT	ΔS	ΔT	ΔS	ΔT
Psoralen	0.192	0.333	0.075	0.021	− 0.194	− 0.11	–	0.094	–	–	–	–	–
8-MOP	0.16	0.259	0.086	0.078	− 0.146	− 0.15	0.14	–	0.116	–	–	0.179	0.127
5-MOP	0.162	0.251	0.091	0.055	− 0.151	− 0.16	0.13	0.141	0.112	0.175	0.19	–	–
8-OH-Psoralen	0.157	0.207	0.091	0.096	− 0.147	− 0.16	0.14	0.078	0.126	–	–	0.172	0.103
5-OH-Psoralen	0.16	0.208	0.096	0.083	− 0.15	− 0.15	0.11	0.151	0.085	0.15	0.16	–	–
8-Methyl-Psoralen	0.186	0.341	0.075	0.03	− 0.14	− 0.12	–	0.1	–	–	–	–	–
5-Methyl-Psoralen	0.18	0.343	0.077	0.03	− 0.11	− 0.14	–	0.1	–	–	–	–	–

Table 2. Spectroscopic properties of several psoralens a) own data: H_2O, 300K; b) own data: MeOH, 300K; c) own data: MeOH, 77K; d) Mantulin (1973): EtOH. 77K; e) Song et al. (1975): EtOH, 300K; f) steric not comparable; g) own calculations, SCF; h) own calculations, CNDO; i) Moore et at. (1971), SCF

τ: lifetime, *F*: fluorescence, *P*: phosphorescence, f: oscillator strength, ϕ: quantum yield

Furocoumarin	Absorption $\pi-\pi$ [nm]	$\pi-\pi$ calculated [nm]	λ_F max/[nm]	τ_F/[ns] (a)	λ_{P0-0} [nm]	τ_{P0-0} [s]
Psoralen	a) *295*, 335 d) 313	i) 329 f = 0.12 g) 295 f = 0.079 h) 306 f = 0.27	a) 440 c) 418 d) 409 ϕ = 0.019	1.4 ± 0,2	c) 455 d) 456	d) 0.66
4',5'-Dihydropsoralen	a) 295, *335* b) 295, *335*	h) 330 f = 0.15	a) 410	3.81 ± 0,15	c) 475	c) 1,22
4,5',8-Trime Hylpsoralen	a) *300*, 340 d) 337	More than 30 centres	a) 470 d) 416	1.02 ± 0,1	c) *475*	c) 1.25
5-Methoxypsoralen (Xanthotoxin)	a) *304*, 340 d) 345	g) 324 f = 0.019 h) 332 f = 0.036 i) 331 f = 0.04	a) 495 d) 440 ϕ = 0.013	1.9 ± 0,2	c) 452 d) 456	c) 0.74 d) 0.77
5-Methoxypsoralen (Bergapten)	a) *313*, 340 d) 335	g) 321 f = 0.727 h) 322 f = 0.22	a) 460 dc) 427 ϕ = 0.013 e) 471	3.1 ± 0,5	d) 472	d) 1.21
8-Hydroxypsoralen (Xanthotoxol)	a) 315, 350	g) 321 f = 0.017 h) 333 f = 0.027	a) 460 (low)	3.66 ± 0,3	e) 468	c) 0.62
5-Hydroxypsoralen (Bergaptol)	a) 315, 350 d) 345	g) 321 f = 0.727 h) 322 f = 0.22	d) 442 ϕ = 0.024	5.28 ± 0,32	d) 466	d) 1.34
Isopsoralen (Angelicin)	a) *300*, 330 d) 330	f)	a) 442 d) 401	6.07 ± 0,34	d) 452	d) 0.77

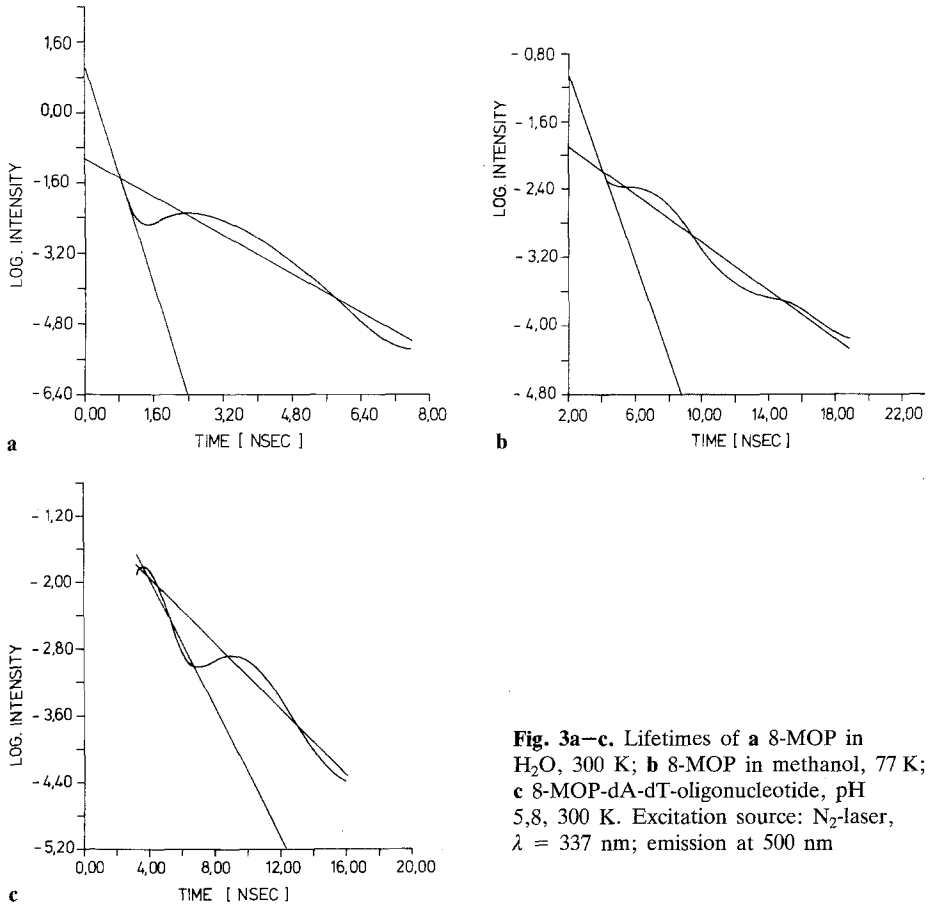

Fig. 3a–c. Lifetimes of **a** 8-MOP in H_2O, 300 K; **b** 8-MOP in methanol, 77 K; **c** 8-MOP-dA-dT-oligonucleotide, pH 5,8, 300 K. Excitation source: N_2-laser, λ = 337 nm; emission at 500 nm

Table 3. Lifetimes (τ) of 8-MOP. $P_{1/2}$ represents the portion of the different components in percent (see "Materials and Methods")

	1. component		2. component	
	ns	P_1 [%]	ns	P_2 [%]
8-MOP in A-T-oligonucleotide	2.58	11.4	5.04	88.6
8-MOP in water	0.38	98.8	1.69	1.2
8-MOP in methanol 77 K	1.81	28.5	7.08	71.5

8-MOP were analysed with the deconvolution method after Ware et al. (1973). It was possible to analyse two components in the spectra (Fig. 3 and Table 3). The *P*-values represent the portion of the different components in percent. A decay mechanism with more than one component is an indication that an electronic

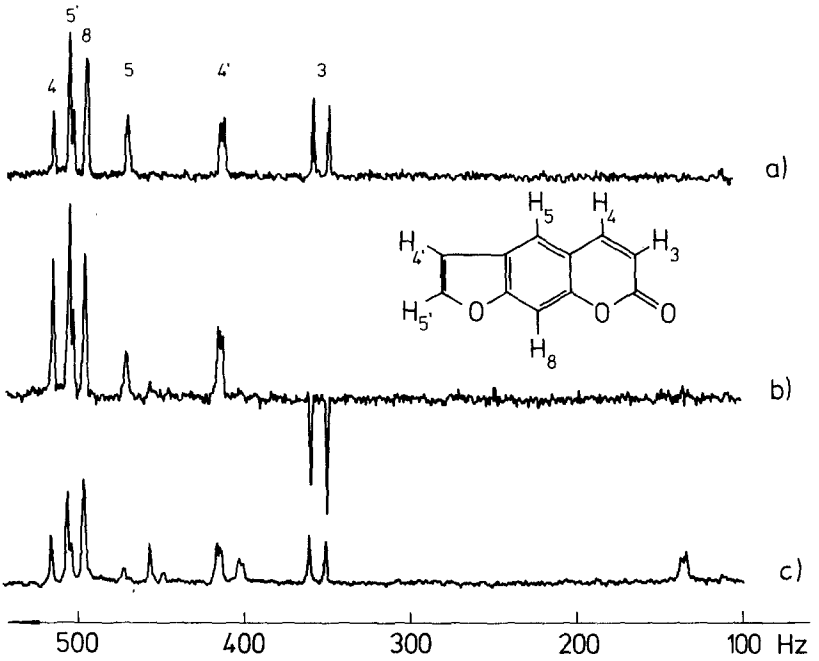

Fig. 4a–c. Spectrum of psoralen obtained at 900 MHz upon irradiation in DMSO. The high field emission is assigned to the proton at C_3, the low field enhanced absorption to the proton at C_4. The chemical shift scale is in Hz from DMSO; **a** dark spectrum 100 scans, **b** 100 scans + hν, **c** dark spectrum after 15 min of irradiation in the spectrometer

excited molecule relaxes in several competing processes to the ground state. These processes strongly depend on the interaction of the molecules with their surrounding molecules. The long component of 8-MOP in water is only 1.2%. At 77 K the lifetime increased due to the vibrational relaxations. A similar effect is observed, when the psoralen molecule is intercalated in the oligonucleotide.

3. CIDNP-Spectroscopy

In DMSO-solutions of 4′,5′-dihydropsoralen, psoralen, 8-methoxypsoralen and coumarin, CIDNP effects could be observed. In agreement with the theoretical calculations and the kinetic study from Dall'Aqua et al. (1979), only resonance signals of the protons at the 3,4-double bond are polarized as shown in Figs. 4 and 5 for the compounds psoralen and 4′,5′-dihydropsoralen. In all cases the proton H_3 shows emission or at least diminished absorption and H_4 enhanced absorption: 8-MOP shows identical but slightly weaker CIDNP-effects than psoralen. Irradiation of the exo-head-to-head photodimer of 4′,5′-dihydropsoralen under the same conditions again exhibits a polarized spectrum (Fig. 6). The chemical shifts of the polarized signals correspond with the protons H_3 and

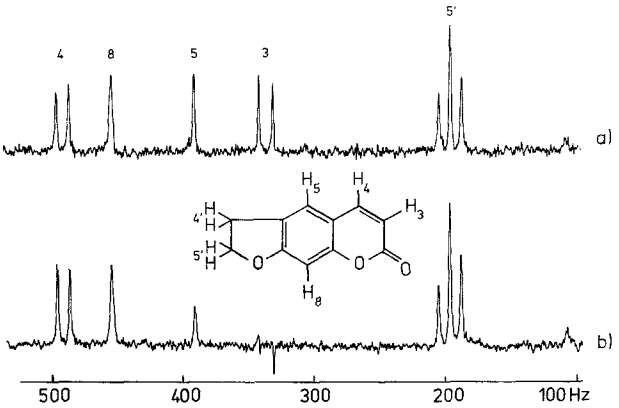

Fig. 5a and b. Spectrum of 4′,5′-dihydropsoralen in DMSO; **a** dark spectrum, 100 scans, **b** 100 scans + hν, conditions: see Fig. 1

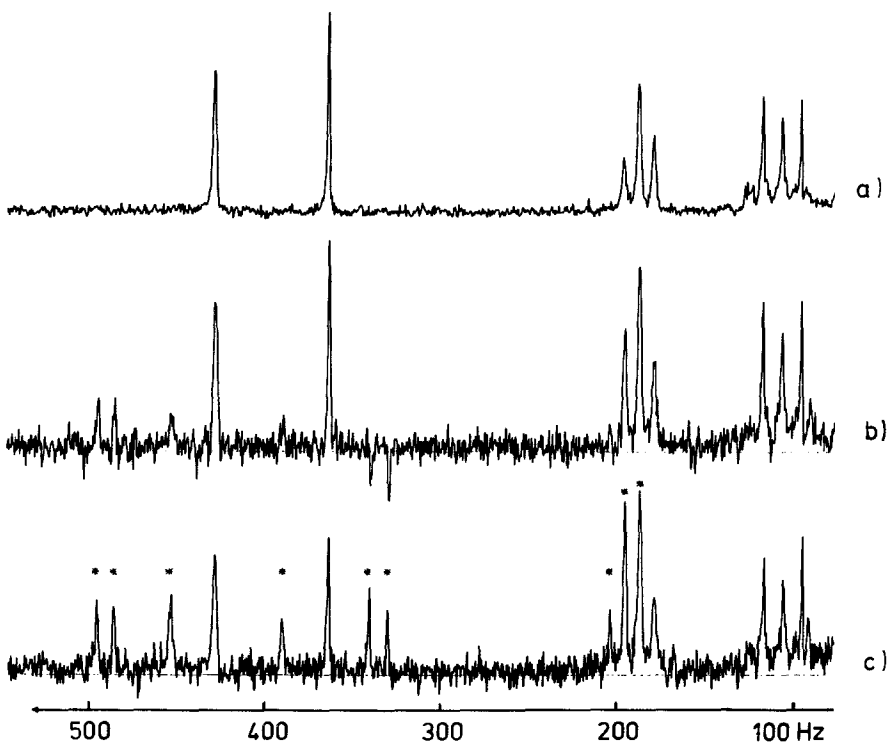

Fig. 6. **a** spectrum of 4′,5′-dihydropsoralen dimer obtained at 90 MHz, **b** spectrum obtained upon irradiation (20 scans). The high field emission is assigned to the proton at C_3 and the low field enhanced absorption to the proton at C_4 of the corresponding monomer, **c** dark spectrum of dimer irradiated for 15 min in the spectrometer. The asterisk denotes the signal of the monomer

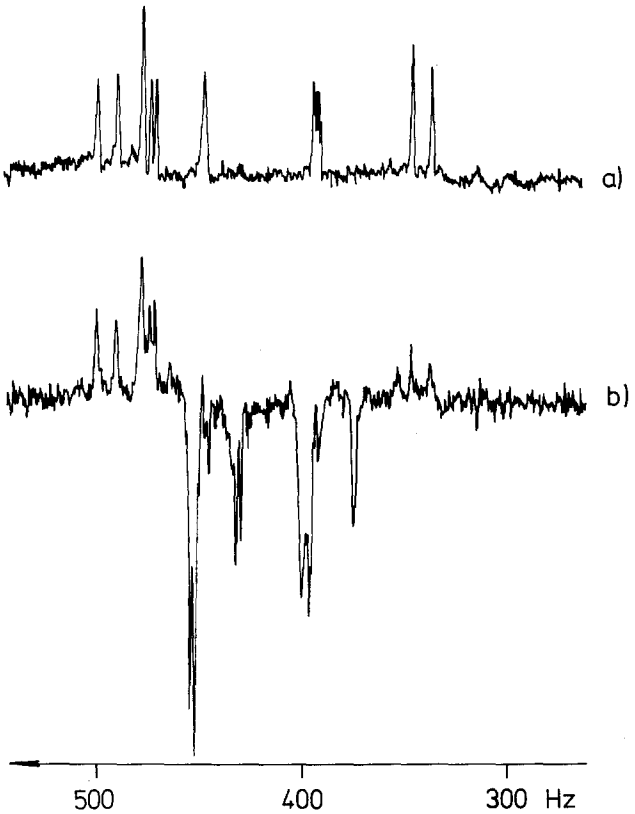

Fig. 7a and b. Spectrum of 3 mM psoralen in 80% D_2O and 20% DMSO at 343 K; **a** dark spectrum, 200 scans, **b** 200 scans + $h\nu$

H_4 of the monomer. Again H_3 appears in emission and H_4 in enhanced absorption.

After irradiation of 4′,5′-dihydropsoralen or psoralen, new weak lines are observed in the NMR spectra which are due to photolysis products. They do not show nuclear spin polarization. Irradiation of the photodimer for the same length of time causes new resonance lines which are nearly of the same intensity as the signals of the remaining dimer. The chemical shifts and splittings are identical with those of the monomer of 4′,5′-dihydropsoralen.

Remarkable solvent effects on the intensity of the polarized signals were observed. The intensity decreases in the sequence DMSO; dimethylformamide, acetonitrile and methanol. In dioxane and chloroform, no nuclear polarization could be detected. A comparable solvent effect on the yield of dimerization of coumarins has been reported where the rate of dimerization is fast in DMSO and dimetyhlformamide, but no dimerization occurs in dioxane (Hammond et al. 1964).

Because of the biological relevance of the detected intermediated products, psoralens inD_2O-solutions (with 20% DMSO) were also used. In addition to the nuclear spin polarization of the protons of the 3,4-double bond, emission signals

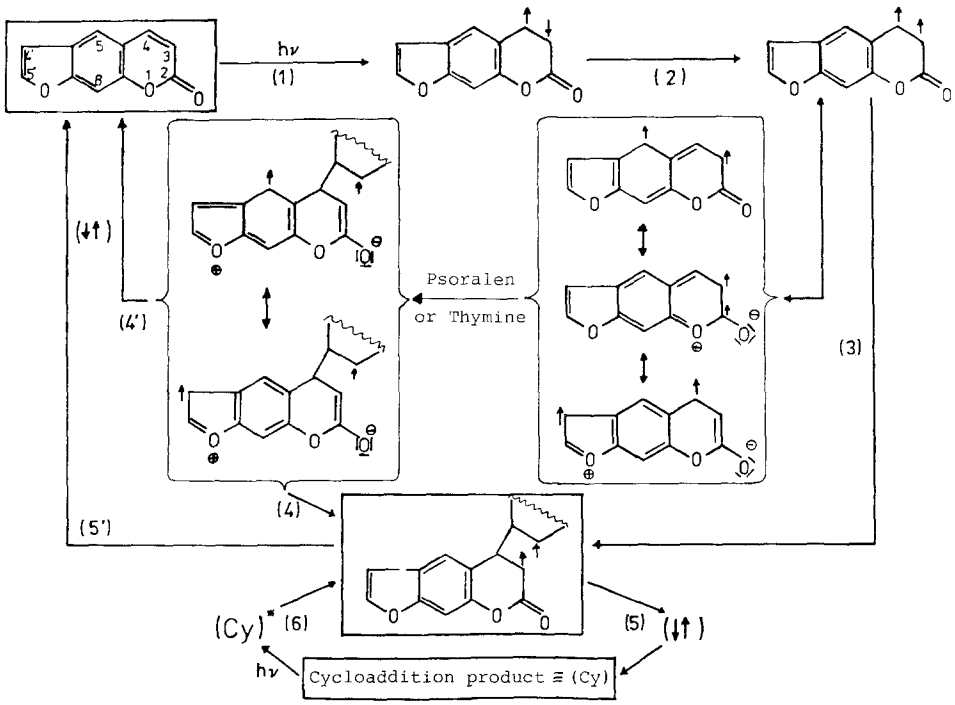

Fig. 8. Reaction scheme for the observed CIDNP-effects (see text)

of protons at C_5, C_8, and C_4' and of the corresponding ones of the dimer were measured for psoralen, 4',5'-dihydropsoralen and 8-MOP (Fig. 7). 8-hydroxypsoralen gave no CIDNP effect in D_2O. This is remarkable because this dye is normally biologically inactive (Song et al. 1975). Preliminary experiments (in DMSO-D_2O mixture) in the presence of thymine led to the same polarized signals as in the case of the dimerization of psoralens.

As an explanation of the experimental results, we propose a reaction scheme shown in Fig. 8. Irradiation of the monomer psoralen leads to an excited triplet state with spin densities localized on mainly C_3 and C_4, as calculations have shown. This molecule can react with an unexcited psoralen molecule (or thymine) to a biradical dimer. This transient product can either revert to the monomer or it can form a second σ-bond giving the cyclobutane type dimer. Only those molecules in which electron spin conversion of the intermediate state takes place are able to form the dimer. Molecules of the transient product in which no spin conversion takes place, react under cleavage of the newly formed σ-bond to two monomer molecules. Simultaneously, a singlet-triplet-mixing of the remaining free electrons occurs and causes the nuclear spin polarization. Irradiation of the dimer results in a cleavage of one σ-bond of the cyclobutane ring, thus forming the same intermediate, and therefore identical CIDNP-effects for the photo-dimerization and the photochemical cleavage of the dimer are observed.

Table 4. Biological and photodynamic activities of some psoralens, given in an absolute and relative measure. The data of DNA binding and the sensibilization of skin are taken from Musajo and Rodighiero 1972 and 1974. Our data were obtained in D_2O-solutions (except of trimethylpsoralen where 5% methanol was added). $\lambda > 315$ nm; concentrations: I^- : 0,15 M; lysozyme: 25 μM; trypsine: 10 μM

Furocoumarin (C = 20 μM)	Quantum efficiency of I_3^- formation		Inactivation quantum efficiency of Lysozyme		Inactivation quantum efficiency of Trypsin		Quantum efficiency of DNA binding		Relative skin sensitization
	($\times 10^{-3}$)	Relativ	($\times 10^{-3}$)	Relativ	($\times 10^{-3}$)	Relativ	($\times 10^{-3}$)	Relativ	
Psoralen	1.88	100	2.9	100	0.33	100	5.5	100	100
4,5',8-Trimethyl Psoralen	2.1	111	1.3	45	0.13	39	–	–	270
8-MOP	3.2	170	1.66	58	0.19	57	4.6	83	71
Bergapten	0.95	51	0.44	15	0.042	13	2.9	52	61
Xanthotoxol	0.1	5	0.17	6	0.025	8	0	0	0
Angelicin	1.2	63	2	68	0.22	67	2	36	12

Table 5. Quantum efficiencies of triplet-(ϕ_0^T) and singlet oxygen-(ϕ') formation

Furocoumarin	ϕ'	ϕ_0^T
Psoralen	0.048	(0.18–0.08)
Trimethylpsoralen	0.054	(0.21–0.09)
8-Methoxypsoralen	0.083	(0.2)
Bergapten	0.025	(0.1–0.04)
Xanthotoxol	0.003	(0.01–0.005)
Bergaptol	0.003	(0.01–0.005)
Angelicin	0.031	(0.12–0.05)

At this stage of experiments, the analysis of the signs of the polarizations must remain open. Therefore, reactions from an excited singlet biradical state cannot be completely excluded. Radical intermediate products of psoralens which were measured in this work with the CIDNP method were proposed earlier by Löber and Kittler (1977).

4. Inactivation of Enzymes and Oxidation of I^-

8-methoxypsoralen inactivates enzymes via a singlet-oxygen-mechanism (type II-mechanism) as measured by Poppe (1977). In Table 4 the results of enzyme inactivation processes of further psoralens are shown and compared with oxidation of Iodide ions via a singlet-oxygen-mechanism under irradiation (Grossweiner 1975). Furthermore, the data are compared with type I reactions (binding of psoralens to DNA and photosensitization of skin) (Musajo and Rodighiero 1974, 1972).

Taking the quantum efficiency for I_3^- formation via singlet oxygen as a measure of the formation of psoralen molecules in the triplet state, a competition between skin sensitization and enzyme inactivation can be observed. Apparently, the triplet formation probability of OH substituted psoralens (e.g. Xanthotoxol) is very low, possibly because of a ketone-enol tautomerization.

Table 5 gives the quantum efficiencies ϕ' of singlet-oxygen-formation calculated with eosine as a reference; (Kepka and Grossweiner 1973) in comparison to the quantum efficiencies for triplet formation. The triplet states were identified by flash photolysis in aqueous solution described by Poppe and Grossweiner (1975) and Poppe (1977). For 8-MOP, a value of $\phi_0^T = 0.2$ was measured in water. Sa E Melo et al. (1979) determined a value of $\phi_0^T = 0.06$ for 8-MOP in ethanol.

Conclusion

The molecular orbital calculations, in agreement with experimental results, indicate that the differences in biological effectivity of different psoralens are based on variations in triplet formation probability and various reactivities of the

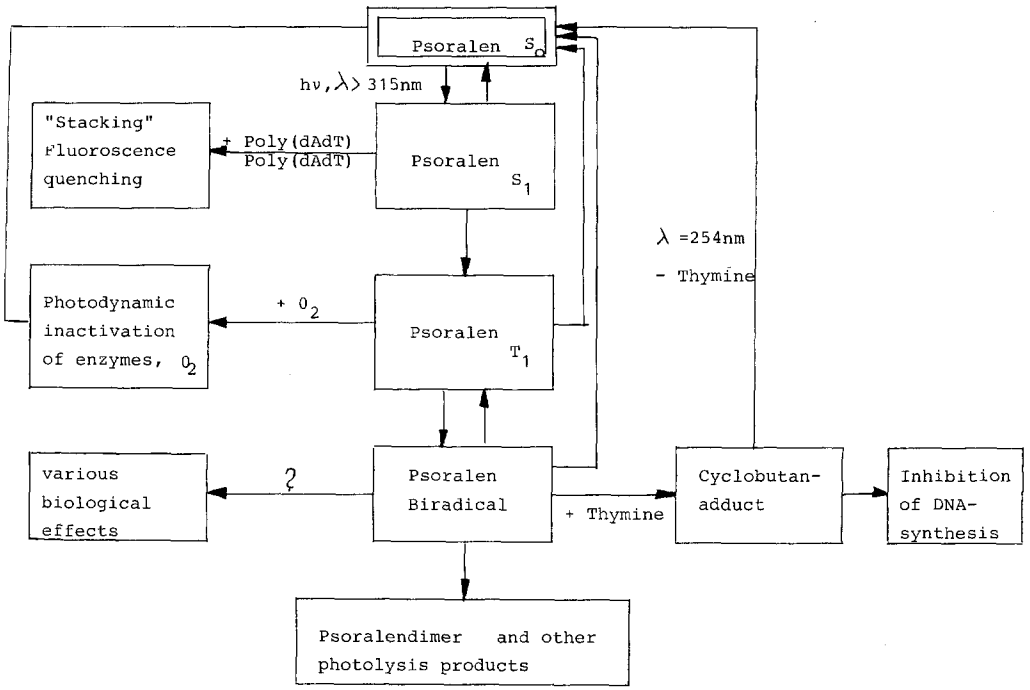

Fig. 9. Overall reaction scheme of psoralens (see text)

3,4-double bond. It has been shown that the formation of a biradical through the triplet state is the decisive step for psoralen dimer formation, as well as for cyclobutane addition with thymine, while singlet oxygen production is responsible for enzyme inactivation (e.g., lysozyme and trypsin).

Figure 9 represents an overall reaction scheme of psoralens, including enzyme inactivation by singlet oxygen, biradical formation and the production of adducts. From the first excited singlet state, intersystem crossing to the triplet state takes place. From the excited triplet state, the psoralen molecules undergo their photodynamic action. The differences in biological activity of psoralen derivatives are caused by various triplet quantum yields and various reactivities of the 3,4-double bond. The 3,4-double bond is the reactive site of the biologically active dyes as demonstrated by theoretical calculations and CIDNP-measurements. The triplet state may be deactivated in the following ways:

Triplet energy transfer from the excited psoralen molecules to molecular oxygen produces singlet oxygen and the psoralen relaxes to the ground state. Singlet oxygen causes various photodynamic effects like the above mentioned enzyme inactivation or oxidation. A relaxation from the triplet to the ground state can occur after collision with a molecule other than oxygen. Polarization of the electron spins lead to the formation of a reactive biradical. This is the decisive step for psoralen dimer formation, as well as for cyclobutan addition

with thymine. On the other hand, the biradical can react with other biomolecules or relax after spin conversion to the ground state.

In DNA-dye complexes base sequence dependent effects were observed, i.e. different fluorescence lifetimes or energy transfer processes appear when the dyes are intercalated between different kinds of base pairs (Andreoni et al. 1980; Anders 1979). The investigation of psoralen-DNA complexes under this aspect could be of interest, too.

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