Photoreactivity (3655 Å) of various skin-photosensitizing furocoumarins with nucleic acids

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The photoreaction at 3655 Å between skin-photosensitizing furocoumarins and DNA leads to a photo-C₄-cyclo-addition reaction in which the 5—6 double bond of the pyrimidine bases and the 3—4 or the 4’—5’ double bond of the furocoumarins are involved. In continuing the research in this field, the authors have investigated the photoreactions (3655 Å) of psoralen, xanthotoxin (8-methoxy-psoralen), bergapten (5-methoxy-psoralen) and 8-methyl-psoralen with native calf thymus DNA, with heat-denatured DNA and with ribosomal yeast RNA, using labelled furocoumarins and enzyme labels. After exposure of the irradiated DNA to the enzymes the influence of the temperature at which the irradiation was made. At room temperature (22°—30 °C) psoralen photoreacts in almost equally extent with each of the three nucleic acid samples, while xanthotoxin, bergapten and 8-methyl-psoralen photoreact to a greater extent with native DNA than with denatured DNA and RNA.

The temperature during the irradiation (in the range examined: 2°—30 °C) does not affect the rate of the photoreactions between the four furocoumarins and native DNA, whereas it has an evident influence on the photoreactions with denatured DNA and with RNA. In the photoreaction of psoralen this influence concerns the double bond (3—4 or 4’—5’) of the furocoumarin which is engaged in the C₄-cycloaddition to pyrimidine bases. In the case of the other furocoumarins, the rate of the photoreactions is affected: at 2°—8 °C it is greater than at 30 °C. The preliminary formation (in the dark) of a complex between the furocoumarins and the macromolecules is involved in this temperature influence.

It is known ¹–⁵ that furocoumarins photoreact with DNA by irradiation at 3655 Å and that these photoreactions appear to be able to explain the photosensitizing properties that the same furocoumarins exert on various biological substrates ⁶–¹².

When added to an aqueous solution of DNA, furocoumarins, out of irradiation, form complexes in which they are weakly linked to the macromolecule ¹³–¹⁵, ¹⁹. By subsequent irradiation of the solutions, furocoumarins form covalent bonds with DNA. They give a C₄-cyclo-addition reaction to the 5—6 double bond of the pyrimidine bases and may react either a) with their 4’—5’-double bond or b) with their 3—4 double bond, giving therefore two types of photoadducts.

As an example of the structures of these photoadducts, we may consider those obtained from psoralen (1) and thymine*. They are indicated by the formulas 5 and 6 for the type a), 7 and 8 for the type b). We point out that the photoadducts of


* These photoadducts were obtained by irradiating at 3655 Å psoralen and the simple components of DNA, i. e. pyrimidine bases, nucleosides and nucleotides. However we have found them also among the products of hydrolysis of DNA irradiated in the presence of psoralen ¹⁵.
type a) have a brilliant violet fluorescence when observed in long wavelength ultraviolet light, while those of type b) are not fluorescent.

Our preceding experiments \(^3,19\) performed using \(^{14}\)C labelled bergapten (5-methoxy-psoralen) (3) and irradiating it in the presence of native DNA, denatured DNA, ribosomal RNA and s-RNA at room temperature, have shown that this furocoumarin has a photoreactivity with native DNA much higher than with the other nucleic acids.

On the contrary however Krauch, Krämer and Wacker \(^20\), using \(^{14}\)C labelled psoralen (1) and irradiating it in the presence of native DNA, and RNA at room temperature, or in the presence of denatured DNA at 0—5 °C found an almost equal photoreactivity with each of the three nucleic acids samples.

As these results are apparently in contrast to those obtained by us, they can only be explained by assuming different behaviour by the two furocoumarins (psoralen and bergapten) or the influence of the temperature at which the irradiation is made. For this reason we have made a screening in this field, with the aim of clarifying if furocoumarins may fotoreact effectively, not only with native DNA, but also with denatured DNA and RNA.

We have examined 4 furocoumarins: psoralen (1), xanthotoxin (2), bergapten (3) and 8-methyl-psoralen (4), which were irradiated at 3655 Å in the presence of native DNA, denatured DNA and RNA.

After the irradiation we have determined both the amount of furocoumarin linked to the nucleic acids, and the fluorescence acquired by the macro-molecule as a consequence of this addition.

For testing the influence of the temperature at which the irradiation was made, the solutions during the irradiation were kept at a thermostatically controlled temperature in the range between 2 and 30 °C.

Materials and Methods

Native DNA: from calf thymus, highly polymerized (Mann Research Laboratories, New York). Hypochromicity was higher than 37%, \(T_m^*\) 87°.

Denatured DNA: obtained by heating for 15' in a water boiling bath a 0,1% aqueous solution of above mentioned native DNA and then quenching it in ice.

RNA: from yeast, highly polymerized (Calbiochem, Los Angeles, California).

Bergapten: 5-methoxy-psoralen (3): obtained by methylation of 5-hydroxy-psoralen (bergapten) with \(^{14}\)CH\(_3\); specific activity 8,5 \(\times\) 10\(^8\) dpm/mM \(^3\).

Psoralen, xanthotoxin and 8-methyl-psoralen: Psoralen was extracted from leaves of Ficus carica \(^26\); xanthotoxin was purchased from Chinoin S.p.A. Milano, purified by sublimation and by crystallization from ethyl alcohol, m. p. 148°; 8-methyl-psoralen was prepared by synthesis \(^25\). They were titrated by the Wilzbach method \(^22\). After a long contact (2 months) with tritium in a sealed glass tube, each furocoumarin was purified through the following successive steps: dissolution in aqueous 10% NaOH and precipitation with 10% HCl; crystallization from aqueous ethyl alcohol (40%); sublimation in high vacuum and crystallization from benzene + petrol ether; separation by preparative thin layer chromatography on silica gel (F254 Merck; solvent: ethyl acetate 34%, cyclohexane 66%); elution with absolute ethyl alcohol and filtration trough a Millipore Mitef 5 µ filter. After this step, the u.v. absorption spectra of the substances were identical to those of the pure compounds. The radiochemical purity of the substances was tested by means of thin layer chromatography both on silica gel (see above) and on cellulose powder (water 95%, dioxane 5%).

Specific activities: psoralen 4,5 \(\times\) 10\(^8\) dpm/mM; xanthotoxin 1,36 \(\times\) 10\(^8\) dpm/mM; 8-methyl-psoralen 2,8 \(\times\) 10\(^8\) dpm/mM.

Preparation of the solutions: Aqueous solutions of nucleic acids (0.1%), containing NaCl 2 mM, were used. Furocoumarins were added in ethanolic solutions; the final concentration of psoralen, xanthotoxin and 8-methyl-psoralen were 20 µg/ml, that of bergapten was 5 µg/ml (bergapten is less soluble than the other furocoumarins). The final concentration of ethyl alcohol was always lower than 1 percent. (Solutions A.)

The solutions of native DNA and of RNA, after the addition of furocoumarins, were slowly shaken for 30 minutes at room temperature and then filtered through a 5 µ Millipore filter.

- Determined as Marmur and Doty \(^21\).
To the denatured DNA solution, the ethanolic solutions of furocoumarins were added just after the heat denaturation. The warm solutions were shortly shaken and then rapidly quenched in ice, left in the cold for 30 minutes and then filtered through a 5 μ Millipore filter.

Irradiation of the solutions: The solutions A prepared as above described (2 ml), were placed into glass calibrated tubes, 1.2 cm in diameter, immersed into a cell (cm 7 x 4 x 6) with glass walls, in which thermostatically controlled water circulated.

The irradiation was made with two HPW 125 Philips lamps, which emit almost exclusively at 3655 Å placed on both sides of the cell, at a distance of 3.5 cm.

Using a chemical actinometer 24, with a ferrioxalate 0.15 M solution, it was determined that the incident irradiation on the 2 ml of used solutions was equivalent to 2.9 \times 10^{18} \text{ quanta/sec}.

Irradiations were made at the following temperatures: 2°, 8°, 15°, 22°, 30 °C.

To the irradiated solutions solid NaCl was added to a 1 M final concentration, and after NaCl was dissolved, two volumes of ethyl alcohol were also added. Precipitated nucleic acid was separated by centrifugation, washed with 80% ethyl alcohol and dissolved in 2 ml of distilled water. (Solutions B.)

Determination of the radioactivity: Small portions of the solutions B (0.2 ml) were added with 1 ml of distilled water and 10 ml of dioxane-base scintillator *, and finally counted with a Beckman CPM 100 liquid scintillation spectrometer.

For check and reference purposes also the solutions of nucleic acids precipitated from the unradiated samples and the solutions A, kept in the dark, were counted under the same conditions.

Determination of the fluorescence: Other portions of the solutions B (0.5 ml) were added with 2 ml of 0.1 M phosphate buffer pH 7 and then used for determining the activating and the fluorescence spectra and the fluorescence intensities with an Aminco Bowman spectrophotofluorimeter.

The maximum activating wavelength for all the samples examined was 330 m/z. The maximum fluorescent wavelengths, which were used in determining the fluorescence intensities, are reported in Table I.

All the fluorescence intensity values reported in this paper were obtained using a same reference standard and therefore they are comparable.

Spectrophotometric determinations: The spectrophotometric determinations were made, using a recording Beckman DB spectrophotometer, on aqueous solutions of the furocoumarins alone (concentration 4 μg/ml for bergapten, 6 μg/ml for the other furocoumarins), using water as blank, and on the same solutions of furocoumarins added with the various nucleic acids (concentration 0.1%), using in this case as blank an aqueous solution of the same nucleic acid at the same concentration.

The solution were thermostatically controlled at the temperatures of 2°, 15° and 30 °C.

Results and Discussion

Photoreaction between native DNA and psoralen, xanthotoxin, bergapten, 8-methyl-psoralen

In the photoreactions which native DNA all furocoumarins showed an analogous behaviour and the results now obtained are in a satisfactory agreement with those previously found with bergapten 3. Among the four furocoumarins tested, 8-methyl-psoralen showed the highest photoreactivity 23.

The temperature of irradiation exerts rather no influence both on the rates of the photoreactions and on the fluorescence acquired by DNA. Only in some case they appear to be a little higher when the irradiation is made at lower temperature (see Table I and figs. 1 and 2).

<table>
<thead>
<tr>
<th>Furocoumarin</th>
<th>Nucleic acid</th>
<th>Maximum fluorescent wavelength [μm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psoralen</td>
<td>native DNA</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>denatured DNA</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>RNA</td>
<td>400</td>
</tr>
<tr>
<td>Bergapten</td>
<td>native DNA</td>
<td>420</td>
</tr>
<tr>
<td></td>
<td>denatured DNA</td>
<td>420</td>
</tr>
<tr>
<td></td>
<td>RNA</td>
<td>450</td>
</tr>
<tr>
<td>Xanthotoxin</td>
<td>native DNA</td>
<td>420</td>
</tr>
<tr>
<td></td>
<td>denatured DNA</td>
<td>450</td>
</tr>
<tr>
<td></td>
<td>RNA</td>
<td>450</td>
</tr>
<tr>
<td>8-methyl-psoralen</td>
<td>native DNA</td>
<td>395</td>
</tr>
<tr>
<td></td>
<td>denatured DNA</td>
<td>395</td>
</tr>
<tr>
<td></td>
<td>RNA</td>
<td>395</td>
</tr>
</tbody>
</table>

Table I. Maximum fluorescent wavelength shown by nucleic acid solutions (B) after irradiation in the presence of various furocoumarins.

Photoreactions of psoralen with RNA and denatured DNA

In the photoreactions of psoralen with RNA and denatured DNA it appears immediately that in these

22 K. E. Wiltzchak, J. Amer. chem. Soc. 79, 1013 [1957].
23 G. Caporale and A. M. Bareggi, Gazz. chim. ital. 98, 444 [1968].
* PPO g 4, POPOP g 0.075, naphthalene g 120, dioxane up to ml 1000.--.
cases the photoreactivity is very similar to that with native DNA. The results are therefore in agreement with those obtained by Krauch, Krämer and Wacker\textsuperscript{20}.

Another fact is also evident, that is an influence of the temperature at which the irradiation was made on the fluorescence intensities acquired by nucleic acids after the irradiation. (The rates of the photoreactions on the contrary are only a little affected.)

This influence appears clearly from Tables III and IV and from fig. 1. While the percentage of

<table>
<thead>
<tr>
<th>Temperature of irradiation [$^\circ$C]</th>
<th>Psoralen irradiated for minutes</th>
<th>Xanthotoxin irradiated for minutes</th>
<th>Bergapten irradiated for minutes</th>
<th>8-methyl-psoralen irradiated for minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>14.84 22.45 31.41 40.07</td>
<td>11.04 19.16 27.71 43.12</td>
<td>6.10 11.27 22.73 40.11</td>
<td>34.23 52.90 67.71 83.30</td>
</tr>
<tr>
<td>8</td>
<td>13.73 22.98 30.03 41.14</td>
<td>8.24 16.98 27.64 40.32</td>
<td>5.84 11.16 19.51 36.48</td>
<td>38.12 53.91 69.50 83.58</td>
</tr>
<tr>
<td>15</td>
<td>12.31 22.62 31.46 41.15</td>
<td>7.75 15.24 26.10 40.00</td>
<td>5.60 11.89 21.86 36.48</td>
<td>38.34 53.51 68.47 83.33</td>
</tr>
<tr>
<td>22</td>
<td>11.98 20.39 31.47 40.10</td>
<td>7.32 13.50 25.14 39.54</td>
<td>5.16 11.73 21.05 37.40</td>
<td>36.79 51.92 68.42 81.17</td>
</tr>
<tr>
<td>30</td>
<td>11.99 20.96 31.10 41.20</td>
<td>6.81 12.80 23.50 37.00</td>
<td>4.90 9.92 17.50 33.04</td>
<td>31.50 47.70 65.37 75.00</td>
</tr>
</tbody>
</table>

a) Percentages of the furocoumarins, initially present in the solutions, which were found linked to native DNA after irradiation.

<table>
<thead>
<tr>
<th>Temperature of irradiation [$^\circ$C]</th>
<th>Psoralen irradiated for minutes</th>
<th>Xanthotoxin irradiated for minutes</th>
<th>Bergapten irradiated for minutes</th>
<th>8-methyl-psoralen irradiated for minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2.30 2.90 3.30 3.00</td>
<td>0.20 0.24 0.28 0.35</td>
<td>0.33 0.45 0.60 0.85</td>
<td>4.00 4.50 4.95 4.50</td>
</tr>
<tr>
<td>8</td>
<td>2.40 2.60 3.20 3.00</td>
<td>0.19 0.22 0.27 0.34</td>
<td>0.33 0.48 0.68 0.84</td>
<td>4.00 4.70 5.00 4.50</td>
</tr>
<tr>
<td>15</td>
<td>2.10 2.70 3.10 3.00</td>
<td>0.21 0.25 0.31 0.36</td>
<td>0.35 0.53 0.70 0.91</td>
<td>4.24 4.80 5.30 4.70</td>
</tr>
<tr>
<td>22</td>
<td>2.10 2.80 3.30 3.20</td>
<td>0.20 0.25 0.30 0.35</td>
<td>0.39 0.58 0.67 0.90</td>
<td>4.24 4.99 5.30 4.40</td>
</tr>
<tr>
<td>30</td>
<td>2.20 2.90 3.35 3.20</td>
<td>0.15 0.20 0.25 0.30</td>
<td>0.40 0.53 0.68 0.90</td>
<td>4.11 4.86 5.21 4.58</td>
</tr>
</tbody>
</table>

b) Intensity of fluorescence (arbitrary units) acquired by native DNA after irradiation in the presence of furocoumarins.

Table II. Photoreactions with native DNA.

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Fig. 1. Photoreactions between psoralen and nucleic acids: 20 minutes of irradiation (3655 Å) at various temperatures (2—30°). Fluorescence intensities (arbitrary units) acquired by nucleic acids as a consequence of the irradiation.

Fig. 2. Photoreactions of xanthotoxin and 8-methyl-psoralen with nucleic acids; 20 minutes of irradiation (3655 Å) at various temperatures (2—30°). The amounts of furocoumarins linked to the nucleic acids are expressed as percentages of the amounts initially present in the solutions.
Table III. Photoreactions with denatured DNA.

<table>
<thead>
<tr>
<th>Temperature of irradiation [°C]</th>
<th>Psoralen irradiated for minutes</th>
<th>Xanthotoxin irradiated for minutes</th>
<th>Bergapten irradiated for minutes</th>
<th>8-methyl-psoralen irradiated for minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2.90 6.00 8.30 6.50</td>
<td>0.40 0.55 0.65 0.75</td>
<td>0.60 1.05 1.65 2.00</td>
<td>4.07 6.00 5.76 4.95</td>
</tr>
<tr>
<td>8</td>
<td>1.60 2.90 3.90 2.80</td>
<td>0.12 0.20 0.22 0.27</td>
<td>0.30 0.48 0.63 0.74</td>
<td>4.51 5.62 5.55 4.50</td>
</tr>
<tr>
<td>15</td>
<td>1.00 1.60 1.90 1.60</td>
<td>0.04 0.09</td>
<td>0.18 0.25 0.33 0.35</td>
<td>2.83 3.33 3.49 2.73</td>
</tr>
<tr>
<td>22</td>
<td>0.80 1.20 1.40 1.30</td>
<td>—</td>
<td>0.08 0.13 0.18 0.18</td>
<td>1.33 1.79 1.92 1.60</td>
</tr>
<tr>
<td>30</td>
<td>0.75 1.10 1.20 1.20</td>
<td>—</td>
<td>—</td>
<td>0.93 1.17 1.26 0.99</td>
</tr>
</tbody>
</table>

a) Percentages of the furocoumarins, initially present in the solutions, which were found linked to RNA after irradiation.

Table IV. Photoreactions with RNA.

<table>
<thead>
<tr>
<th>Temperature of irradiation [°C]</th>
<th>Psoralen irradiated for minutes</th>
<th>Xanthotoxin irradiated for minutes</th>
<th>Bergapten irradiated for minutes</th>
<th>8-methyl-psoralen irradiated for minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>8.17 15.23 24.41 35.23</td>
<td>2.78 4.75 8.53 12.92</td>
<td>2.50 5.04 10.16 18.61</td>
<td>25.47 41.58 57.97 72.04</td>
</tr>
<tr>
<td>8</td>
<td>8.01 15.56 24.98 33.61</td>
<td>2.55 4.53 7.74 12.10</td>
<td>2.30 5.31 9.47 17.46</td>
<td>23.14 40.47 53.20 70.27</td>
</tr>
<tr>
<td>15</td>
<td>7.60 14.17 22.80 32.47</td>
<td>1.43 2.93 5.41 8.48</td>
<td>1.37 2.71 5.18 9.88</td>
<td>18.63 32.69 43.34 59.24</td>
</tr>
<tr>
<td>22</td>
<td>7.33 12.29 20.90 30.45</td>
<td>0.78 1.72 2.66 3.77</td>
<td>0.94 1.88 3.32 6.14</td>
<td>17.70 25.54 36.84 43.59</td>
</tr>
<tr>
<td>30</td>
<td>6.85 11.00 19.90 25.52</td>
<td>0.73 1.53 2.65 3.69</td>
<td>0.49 0.91 1.53 2.92</td>
<td>13.02 20.00 33.53 47.72</td>
</tr>
</tbody>
</table>

b) Intensity of fluorescence (arbitrary units) acquired by RNA after irradiation in the presence of furocoumarins. — Signifies that the fluorescence intensity was too low for a correct determination.

Table III. Photoreactions with denatured DNA.
psoralen linked to the nucleic acids is almost the same at the various temperatures, fluorescence intensities on the contrary show strong differences; after 20 minutes of irradiation at $2^\circ$, denatured DNA and RNA have fluorescence intensities respectively 8 times and 4 times higher than those after irradiation at $22 - 30^\circ$.

In order to give an explanation of this fact, we must recall what we have already said in the introduction, that is psoralen may photoreact with the pyrimidine bases of nucleic acids forming either fluorescent photoadducts (when its $4' - 5'$ double bond is involved) or not fluorescent ones (when the $3 - 4$ double bond is involved). In preceding research we have found that in native DNA both these types of photoadducts are formed; recent experiments showed that after irradiation of native DNA at room temperature in the presence of psoralen, the fluorescent photoadducts can be obtained after acid hydrolysis of the same DNA in an amount 3 times greater than that of the not fluorescent ones.

After the present results we must conclude that in the photoreactions between psoralen and RNA or denatured DNA the temperature of irradiation has influence on the type of photoaddition which occurs. It is evident that at $2^\circ$ the ratio fluorescent photoadducts/not fluorescent photoadducts must be higher than that at $22^\circ - 30^\circ$.

**Photoreactions of xanthotoxin and bergapten with RNA and denatured DNA**

Xanthotoxin and bergapten showed a very similar behaviour.

We observed in these cases an influence of the temperature at which the irradiation was made on the rates of the photoreactions. By contrast with psoralen, the fluorescence of the nucleic acids after the irradiation is not affected: its intensity is always rather proportional to the amount of furocoumarin which was linked, if we consider a given period of irradiation.

As we can see from Tables III and IV and from fig. 2, the photoreactivity of xanthotoxin and bergapten at $22^\circ - 30^\circ$ is very low, about 10 times lower than that with native DNA. This confirms what we had previously found about the photoreactivity of bergapten with various nucleic acids at room temperature and extends the conclusion to xanthotoxin.

However as the temperature of irradiation decreases, the photoreactivity of xanthotoxin and bergapten progressively increases: with denatured DNA at $2^\circ$ it is rather the same than that with native DNA, while with RNA it remains always at a lower level.

**Photoreactions of 8-methyl-psoralen with RNA and denatured DNA**

The results of the photoreactions of 8-methyl-psoralen with RNA and denatured DNA are analogous to those obtained with xanthotoxin and bergapten; also in this case we observed an evident influence of the temperature of irradiation on the rates of the photoreactions (see Tables III and IV and fig. 2) while the influence on the fluorescence is lacking.

However we point out that the high photoreactivity of 8-methyl-psoralen observed with native DNA, appears evident also in the photoreactions with RNA and denatured DNA. Therefore the photoreactivity with these nucleic acids at room temperature ($22^\circ - 30^\circ$), even if much lower than that with native DNA, still has a high level.

**Influence of the temperature on the formation of complexes between furocoumarins and nucleic acids**

In order to explaining how the temperature of irradiation can affect the rate of the photoreactions of xanthotoxin, bergapten and 8-methyl-psoralen with RNA and with denatured DNA, we have suggested that it exerts its influence on the preliminary formation of complexes between furocoumarins and nucleic acids.

In a previous research we had studied the dark-interaction between furocoumarins and nucleic acids. By operating at $20^\circ$ we had found that complexes are well formed by bergapten with native DNA, but with denatured DNA, ribosomal

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27 F. Bordin, L. Musajo, and R. Bevilacqua, This Journal, in press.
RNA and s-RNA they take place in a very reduced extent.

Even if the formation of these complexes occurs out of any irradiation, from much evidence we have reached the conclusion that they are in very useful condition for the subsequent photoreaction, which, of course, takes place when the solution is irradiated.

As the formation of a complex between a small molecule and a macromolecule is generally accompanied by a modification of the optical properties of the simple compound (a decrease of the optical density and sometimes a bathochromic shift of the \( \lambda_{\text{max}} \) is observed), we have determined the u.v. spectra (in a range of 300 – 390 nm) of aqueous solutions of our furocoumarins in the presence of nucleic acids at various temperatures, that is at 30\(^\circ\), 15\(^\circ\) and 2\(^\circ\)C.

The results obtained with bergapten in the presence of native DNA, denatured DNA and RNA are reported in Table V. With xanthotoxin and 8-methyl-psoralen the results were analogous.

They show that in the presence of native DNA the complex is well formed even at 30\(^\circ\) and a decrease of temperature has rather no influence.

Different is the case in the presence of denatured DNA and also, even if less evident, in the presence of RNA: at 30\(^\circ\) there is only a little decrease of the optical density of bergapten, but the decrease becomes more and more evident by decreasing the temperature. A bathochromic shift may also be observed.

We have also ascertained that these modifications of the u.v. spectra of furocoumarins are completely reversed if we, after the successive determinations at 30\(^\circ\), 15\(^\circ\) and 2\(^\circ\), reverse the process repeating the determinations at 15\(^\circ\) and at 30\(^\circ\).

These facts indicate that the ability of forming complexes with furocoumarins in RNA and denatured DNA is dependent on the temperature, and it is much higher at 2\(^\circ\) than at 30\(^\circ\). By contrast in native DNA it does not depend on the temperature.

We think that these findings may explain why the photoreactions between xanthotoxin, bergapten and 8-methyl-psoralen with RNA and denatured DNA are affected by the temperature of irradiation, while those with native DNA are independent from it.

Less well explainable from this point of view is the behaviour of psoralen. The spectrophotometric determinations performed on the solutions of psoralen in the presence of native and denatured DNA and of RNA in no case showed evident modifications at the various temperatures. This is in agreement with the fact that the photoreactions of psoralen with denatured DNA and RNA, as well as with native DNA, are almost independent from the temperature. However until now we have found no evidence to explain the variation on the type of photoaddition to RNA and to denatured DNA that psoralen gives at various temperatures.

**Conclusion**

From the data reported in this paper it is evident that the tested furocoumarins, i.e. psoralen, xanthotoxin, bergapten and 8-methyl-psoralen, by irradiation at 3655 Å may photoreact, not only with native DNA, but also with denatured DNA and with RNA. However in these cases the photoreactions are influenced by the temperature at which the irradiation is performed. This influence concerns the type of photoaddition that the furocoumarin gives with nucleic acids in the case of psoralen, and the rate of the photoreactions in the case of the other three furocoumarins. In particular it appears that xanthotoxin and bergapten photoreact with RNA and denatured DNA in a very reduced extent at 30\(^\circ\), but at a much higher rate at low temperatures (2\(^\circ\) – 8\(^\circ\)). On the contrary in the photoreactions with native DNA, no influence of the irradiation temperature was observed.

<table>
<thead>
<tr>
<th>Temperature ( [^\circ \text{C}] )</th>
<th>Bergapten 4 ( \mu \text{g/ml} ) in water</th>
<th>Aqueous solutions of bergapten 4 ( \mu \text{g/ml} ) containing 0.1% native DNA</th>
<th>Aqueous solutions of bergapten 4 ( \mu \text{g/ml} ) containing 0.1% denatured DNA</th>
<th>Aqueous solutions of bergapten 4 ( \mu \text{g/ml} ) containing 0.1% RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( E ) ( \lambda_{\text{max}} ) m( \mu )</td>
<td>( E ) ( \lambda_{\text{max}} ) m( \mu )</td>
<td>( E ) ( \lambda_{\text{max}} ) m( \mu )</td>
<td>( E ) ( \lambda_{\text{max}} ) m( \mu )</td>
</tr>
<tr>
<td>30</td>
<td>0.284 313</td>
<td>0.190 323.5</td>
<td>0.262 313</td>
<td>0.236 313</td>
</tr>
<tr>
<td>15</td>
<td>0.283 313</td>
<td>0.186 323.5</td>
<td>0.218 316.5</td>
<td>0.214 314.5</td>
</tr>
<tr>
<td>2</td>
<td>0.282 313</td>
<td>0.182 323.5</td>
<td>0.180 320.5</td>
<td>0.196 315</td>
</tr>
</tbody>
</table>

Table V. Influence of the temperature on the spectrophotometric properties of bergapten (aqueous solution) in the presence of native and denatured DNA and of RNA.
Among the four furocoumarins, 8-methyl-psoralen has shown the highest photoreactivity towards all the three nucleic acid samples. This fact is not surprising if one assumes that a correlation exists between the \textit{in vitro} photoreactions and the \textit{in vivo} photosensitizing properties of furocoumarins. In fact we recall that 8-methyl-psoralen was found to be the more active furocoumarin in the guinea-pig skin photosensitization\textsuperscript{25}.

Moreover from all the data it appears evident that the behaviour of a single furocoumarin in the photoreactions with the various nucleic acids may show remarkable differences from those of the other components of this group.

Therefore general conclusions concerning the whole group of furocoumarins cannot be drawn from results obtained in experiments performed with only one of these compounds.

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**Peptidsynthesen mit symm. Anhydiden, II\textsuperscript{1}**

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(Z. Naturforschg. 21 b, 314—317 [1969]; eingegangen am 23. Oktober 1968)

Das Hexapeptid Ser-Ser-Thr-Ser-Ala-Ala wurde über die symm. Anhydride von \(N\)-Boc-O-(Z-TF)-Ser und \(N\)-Boc-O-(Z-TF)-Thr stufenweise aufgebaut. Die Abspaltung der Z-TF-Reste erfolgte durch katalytische Hydrierung.


Auch für die nachfolgenden Kupplungsreaktionen wurden aus \(N\)-Boc-O-(Z-TF)-Ser-OH bzw. aus \(N\)-Boc-O-(Z-TF)-Thr-OH mit Methyläthinyldiäthyamin die symm. Anhydride hergestellt, die ohne Isolierung eingesetzt wurden. Die Ninhydrinreaktion wurde stets fast negativ. Daß die Ausbeuten manchmal wesentlich geringer als 100% sind, dürfte damit zusammenhängen, daß infolge der Z-TF-Reste, die ein asymm. C-Atom besitzen, ein Gemisch von Diastereoisomeren vorliegt, von denen zwecks Charakterisierung der Verbindungen z. T. kristalline, auf jeden Fall aber feste Anteile isoliert wurden.

Die Schlußabspaltung der Z-TF-Gruppen sowie der Carboxylsäurgruppe (BzI) erfolgte durch katalytische Hydrierung in Eisessig. Beim Eindampfen der Lösung bei höherer Temperatur wurde auch der N-Boc-Rest entfernt. Das Endprodukt Ser-Ser-Thr-Ser-Ala-Ala lieferte eine korrekte Aminosäureanalyse.

Wie in dem Schema angegeben ist, wurden bei den ersten drei Kupplungsreaktionen neben der Methode der symm. Anhydride auch andere eingesetzt, die aber z. T. wesentlich geringere Ausbeuten lieferten.

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\textsuperscript{1} F. WEYGAND, P. HUBER u. K. WEISS, Z. Naturforschg. 22 b, 1034 [1967].


\textsuperscript{3} K. KAPPFELER u. R. SCHWYZER, Helv. chim. Acta 44, 1136 [1961].