4,5′-Dimethylangelicin, a New Very Active Monofunctional Furocoumarin

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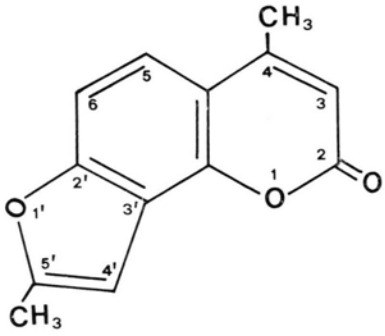
Photochemotherapy, Angular Furocoumarins, Monoadducts, DNA Synthesis

4,5′-dimethylangelicin is a new angular furocoumarin that by irradiation at 365 nm shows a very high photoreactivity towards DNA *in vivo*, without forming any cross-linkage; by photosensitization of Ehrlich ascites tumor cells a strong inhibition of DNA synthesis was observed.

Furocoumarins are drugs used in the photochemotherapy of vitiligo [1] and recently of psoriasis [2] and some other skin-diseases. Their biological activity is due to the covalent linkages they form with the pyrimidine bases of DNA by irradiation with longwavelength UV light [3]; in a living cell, the main result is the inhibition of nucleic acid synthesis [4] and consequently of cell replication [5]. The furocoumarins generally used in photochemotherapy (8-methoxypsoralen and 4,5′,8-trimethylpsoralen) have a molecular structure resulting from a linear condensation of their three rings; they form with DNA both monofunctional adducts and inter-strand cross-linkages [3]. Both these damages can be repaired by the cellular enzymes [6, 7], but in restoring the original properties of DNA the repair of monofunctional adducts appears to be more efficient than the cross-linkage repair, which seems on the other hand to be error-prone [8]. Therefore, a drug forming only monoadducts would be safer for therapeutic use.

At present, we know some furocoumarin derivatives having an angular structure which, for geometrical reasons, are able to form only monofunctional adducts, angelicin [9] for instance. However, this drug reacts poorly wih DNA and therefore its biological activity is low [10].

With the aim of enhancing the photosensitizing properties of angular furocoumarins, we have begun to study some new methylderivatives of angelicin; in this paper we report on the first results obtained with one of them, *i.e.* 4,5′-dimethylangelicin, using the Ehrlich ascites tumor cells for the bioassay.



4,5′-dimethylangelicin

Materials and Methods

4,5′-Dimethylangelicin was prepared by chemical synthesis which will be described elsewhere; angelicin was extracted from the roots of *Angelica archangelica* [11]. Both were labeled by the Radiochemical Centre, Amersham, England (specific activity: 4,5′-dimethylangelicin 6 mCi/mM; angelicin 5 mCi/mM). From the same Radiochemical Centre [^{3}H]thymidine (21 Ci/mM) and [^{14}C]thymidine (62 mCi/mM) were purchased. Psoralen was obtained by chemical synthesis [12].

Ehrlich ascites tumor was routinely transferred in our laboratory by i.p. injection of 2×10^6 cells into NCl mice.

For the irradiation, the tumor cells, diluted up to 2×10^7 cells/ml with Hank's solution containing the furocoumarin, were kept in the dark for 15 min and then 5 ml aliquots were irradiated, following a procedure already described [4] using a Philips HPW 125 lamp (365 nm; irradiation intensity 2×10^{16} quanta/sec).

For the sedimentation studies, the cells were labeled by incubating for an hour at 37 °C in Hank's medium in the presence of 10 μCi/ml of [^{3}H]thymidine (specific activity was about 3500 dpm/μg). Sedimentation experiments were carried out as already described [8], using alkaline 5–20% sucrose gradients and a Spinco Model L centrifuge. As an internal marker, [^{14}C]DNA from untreated cells incubated in the presence of 0.5 μCi/ml of [^{14}C]thymidine, was used (specific activity about 500 dpm/μg).

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DNA synthesis was studied by incubating the irradiated cells at 37 °C for 15 min in Hank's solution in the presence of 3 μCi/ml of [^{3}H]thymidine and DNA was extracted and its specific activity determined [4] (with untreated cells it was about 2000 dpm/μg). DNA extraction was performed by the Szybalska and Szybalski method [13] and its quantitative determination according to Burton [14].

The radioactivity was determined by diluting samples up to 1 ml with distilled water, mixing with 10 ml of Instagel (from Packard Instruments Company, Inc.) and counting by means of a Beckman liquid scintillation spectrometer Model LS 150. In the double labeling experiments, narrow windows were used with a low ^{14}C-spillover (less than 10%) and care was taken so that ^{3}H-counts were about ten times higher than ^{14}C-counts; in such a manner, less than 1% of counts monitored by the ^{3}H-counting was due to the ^{14}C-radioactivity.

Results and Discussion

Photobinding capacity to DNA of Ehrlich ascites cells

Ehrlich ascites cells were irradiated for increasing times in the presence of 4 μg/ml of [^{3}H]4,5′-dimethylangelicin; after this, DNA was extracted and counted. Similar experiments were carried out with [^{3}H]angelicin. The results (Fig. 1) clearly show that 4,5′-dimethylangelicin photoreacts with the cellular DNA more efficiently than angelicin; the amounts of 4,5′-dimethylangelicin covalently linked to DNA were about six times greater than those of angelicin.

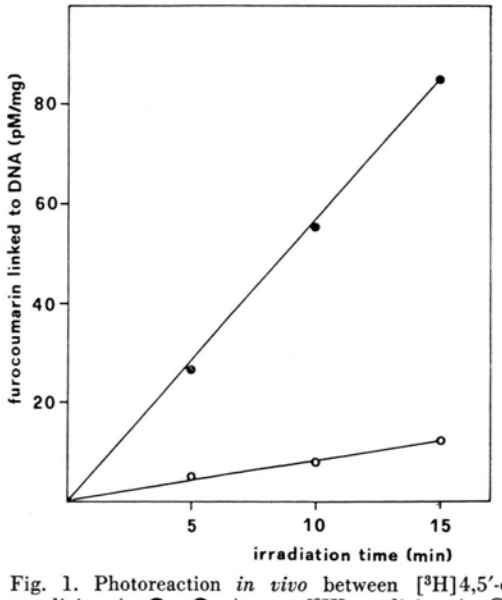

Fig. 1. Photoreaction *in vivo* between [^{3}H]4,5′-dimethylangelicin (—●—●—) or [^{3}H]angelicin (—○—○—) and DNA of Ehrlich ascites tumor cells; cells were irradiated for increasing times in the presence of 4 μg/ml of the tested drug, their DNA was extracted and its radioactivity determined.

Assay for cross-linkage formation

As 4,5′-dimethylangelicin has an angular structure, one could reasonably assume that, like angelicin, it was unable to induce cross-linkages in DNA. To verify this assumption, sedimentation studies were performed. Ehrlich ascites cells were labeled by growing in the presence of [^{3}H]thymidine and then irradiated for increasing times in the presence of 10 μg/ml of 4,5′-dimethylangelicin. DNA was assayed by velocity sedimentation in alkaline 5–20% sucrose gradients. Fig. 2 shows the data related to one of these experiments, from which it appears that no cross-linkages were induced in DNA by

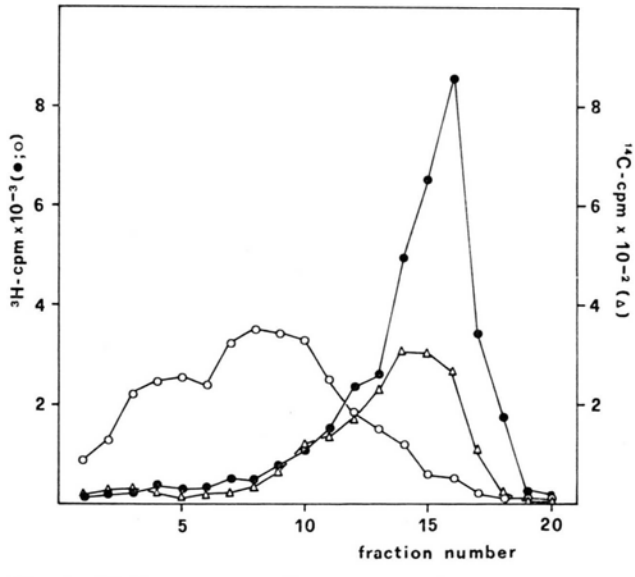

Fig. 2. Alkaline sucrose sedimentation profiles of Ehrlich ascites cell DNA.
—●—●—●—: ^{3}H-labeled DNA obtained from cells irradiated for 10 min in the presence of 10 μg/ml of 4,5′-dimethylangelicin;
—○—○—○—: ^{3}H-labeled DNA from cells irradiated as above but in the presence of 10 μg/ml of psoralen;
—△—△—△: ^{14}C-labeled DNA from untreated cells, used as an internal marker.
Gradients were spun for 105 min at 38,000 rpm, 4 °C in a SW-39 rotor; then they were fractionated and the ^{14}C- and ^{3}H-radioactivity determined.

4,5′-dimethylangelicin; in fact the sedimentation profile is practically the same as the control [^{14}C]-DNA from untreated cells used as an internal marker. No cross-linkages were detected even after an irradiation time of 30 min. For a comparison, in Fig. 2 is also reported the sedimentation profile of a sample of DNA from Ehrlich ascites cells cross-linked by irradiating cells in the presence of psoralen.

Effect of DNA synthesis

To study the biological effects of the photosensitization by 4,5′-dimethylangelicin, the DNA synthesis was determined in Ehrlich ascites cells after irradiation in the presence of 4 μg/ml of the drug. In these experiments, in addition to untreated cells, the controls were samples kept in the dark in the presence of the drug and samples irradiated in its absence; as already observed all these treatments failed to affect DNA synthesis. Fig. 3 shows the results of one of these experiments together with the data obtained with angelicin, as a comparison. 4,5′-dimethylangelicin resulted more effectively in inhibiting DNA synthesis than angelicin; in fact, the D_{50}, *i.e.* the UV radiation dose that in the presence of 4 μg/ml of the drug under examination yielded 50% of inhibition of DNA synthesis, was 10.8×10^{18} quanta for 4,5′-dimethylangelicin and 25×10^{18} quanta for angelicin. These values were calculated by probit analysis using the data of various experiments.

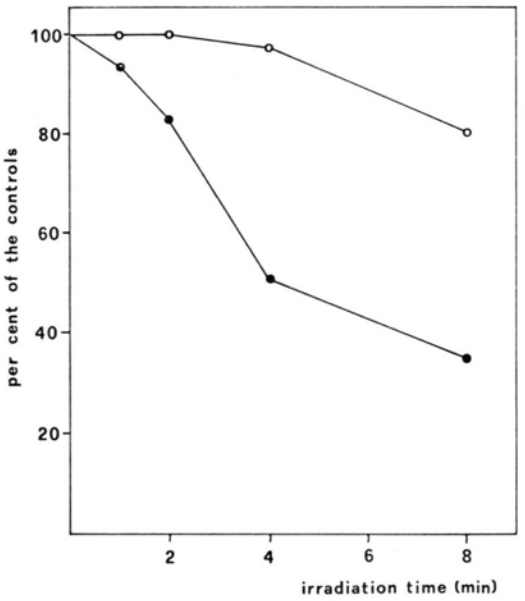

Fig. 3. DNA synthesis in Ehrlich ascites tumor cells after irradiation in the presence of 4 μg/ml of 4,5′-dimethylangelicin (—●—●—) or of angelicin (—○—○—); cells were then incubated at 37 °C in the presence of [^{3}H]thymidine, DNA was extracted and its radioactivity determined.

On the basis of these preliminary results, 4,5′-dimethylangelicin appears to be a pure monofunctional reagent, *i.e.* unable to induce cross-linkages in DNA and shows a much higher photoreactivity with DNA *in vivo* than angelicin. The ability to inhibit DNA synthesis resulted 2.5 times higher than that of angelicin and this fact is clearly related to the enhanced photoreactivity of 4,5′-dimethylangelicin.

This biological effect was tested immediately after irradiation, before starting the DNA repair; studies on late effects in which DNA repair can occur are now in progress.

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