zygotes might show a diverse susceptibility towards growth retardation. For PGM_1 and ACP_1, in fact, the excess of PGM_1-2 and B in LFD (compared to adults) appears much stronger than that of other homozygous phenotypes.

Some 'normal' polymorphisms may also influence the duration of gestation. The distorsions of ACP_1 and ADA distributions, observed in PT infants, may be expression of these interactions.

It is well known that survival rate is strongly related to birth weight. Assuming that the proportion of LFD is equal to 0.05 and that their probability of survival as compared to other babies is equal to 0.7[7], we have calculated in the sample from Rome an approximate mean relative fitness of 0.993 for homozygotes. However, small differences of fitness among homozygotes would influence the polymorphic frequencies considerably. The study of a consecutive series, including 'normal' and 'heavy weight' infants, could allow a more precise evaluation of selection intensity.

A correlation between growth retardation and homozygosity for allelic types of enzymes and antigens may have a major role in the maintenance of 'normal' polymorphisms.

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5-Methylangelicin: a new highly photosensitizing angular furocoumarin

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Summary. 5-Methylangelicin, a new highly photosensitizing angular furocoumarin, was studied in 2 different biological systems, the T_2 phage and Ehrlich ascites tumor cells; in comparison with angelicin, the parent compound, it was several times more active.

Furocoumarins are well-known photosensitizing drugs which on irradiation with long wave UV-light react with the pyrimidine bases of DNA[2]. 2 different classes of furocoumarins have been described; psoralen derivatives, which have a linear molecular structure, and are able to form both monofunctional adducts and inter-strand cross-links with DNA, and angelicin derivatives, which have an angular structure and can form only monoadducts. The biological consequences of these types of damage have been widely studied[3]; in particular, cross-links produce heavy genetic damage with a high killing effect. Angelicin, the parent angular compound, is not very useful for biochemical and medical applications, even though it is an interesting monofunctional reactive, because of its poor photobinding ability towards DNA and therefore its low sensitizing activity.

Studying several angelicin derivatives we have already described the interesting properties of 4,5′-dimethylangelicin[4]; in this paper we deal with another new angular furocoumarin showing high photosensitizing activity, i.e. 5-methylangelicin.

Materials and methods. 5-Methylangelicin was prepared by chemical synthesis, which will be described elsewhere; angelicin was a gift of the Franco-Indian Pharmaceutical Co. (Bombay). ^{3}H-thymidine (21 Ci/mM) and ^{3}H-uridine (25 Ci/mM) were purchased from the Radiochemical Centre (Amersham, England). All irradiations were performed, as already described[4], in Hanks' solution containing the furocoumarin, using Petri dishes (5 cm in diameter; 5 ml aliquots) and a Philips HPW 125 lamp (365 nm; irradiation intensity 2×10^6 quanta/sec). T_2 bacteriophage was grown in brain-heart infusion (Difco Laboratories) using *E. coli* B_{48} as host bacteria; irradiations were performed at concentrations of 10^9 phages and 4 µg of drug per ml. Virus titers were determined according to Adams[5]. Ehrlich ascites tumor was routinely transferred by i.p. injection of 2×10^6 cells into Swiss mice; the 50% lethal dose corresponded to the injection of 5×10^2 cells/mouse. After irradiation (2×10^7 cells/ml), DNA and RNA syntheses were studied as previously described[4], by incubating cells (samples of 2×10^6) at 37 °C for 15 min in Hanks' solution (0.5 ml) in the presence of labelled nucleoside (1 µCi). The trichloroacetic-precipitable radioactivity was then determined using a Beckman LS 150 liquid scintillation spectrometer. The DNA and RNA contents were determined by the diphenylamine[6] and orcinol[7] reactions respectively. The results, calculated as a percentage of the radioactivity

Effect of irradiation (365 nm) in the presence of 5-methylangelicin or of angelicin on the tumor transmitting capacity of Ehrlich ascites cells

Furocoumarins	µg/ml	Irradiation time (min) 8	16	32
5-Methylangelicin	4	100 (9.2±0.13)	40 (9.4±0.26)	0 (60)
Angelicin	4	100 (9.2±1.3)	100 (9.4±0.26)	100 (11.2±0.32)
	20	100 (11.2±0.7)	100 (14±1.7)	30 (29.6±3.5)

After irradiation the tumor cells were injected i.p. into Swiss mice (5×10^6/animal) which were then observed for 60 days. The mortality percentage (due to the tumor growth) and, in brackets, the mean survival time (days), calculated excluding the survivors, and the S.E., are reported. Ehrlich cells incubated in the dark in the presence of the drugs, or irradiated in their absence, when injected in the same amount into healthy mice produced a 100% mortality and a mean survival time equal to that of the untreated cells (about 7±0.5 days).

incorporated into nucleic acid of control cells (about 2000 dpm/μg for DNA and 2400 dpm/μg for RNA), were submitted to the probit analysis and expressed as the ID_{50}, i.e. the UV-radiation dose which in the presence of the drug produces a 50% inhibition. To study the effect on tumor growth, the irradiated cells were injected i.p. into Swiss mice (5×10^6/mouse; groups of 10 animals). The animals were observed for 60 days, and the mortality due to tumor growth was scored.

Results a) Inactivation of T_2 bacteriophages: Figure 1 shows the survival-curves obtained after irradiating T_2 phages in the presence (4 μg/ml) of 5-methylangelicin and of angelicin, used as a reference compound. 5-Methylangelicin showed a high killing activity; in fact, after about 10 min of irradiation the T_2 phage was practically inactivated. On the other hand, angelicin proved to be less effective: after 10 min of irradiation in the presence of this drug, about a half of the virus particles retained their infectivity.

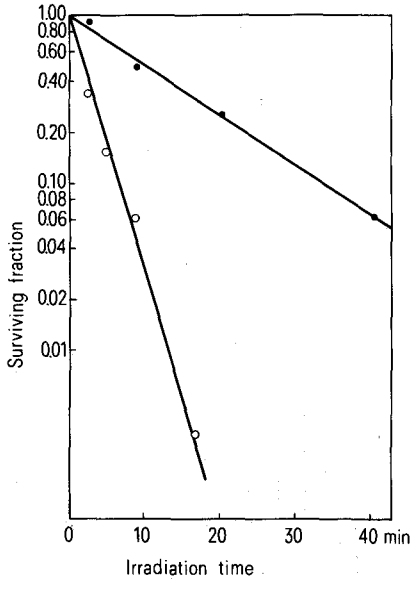

Fig. 1. Inactivation of T_2 phage by irradiation (365 nm) in the presence (4 μg/ml) of 5-methylangelicin (–○——○–) or of angelicin (–●——●–). After irradiation the plaque-forming units per ml were scored using *E. coli* B_{48} as indicator bacteria. Incubation in the dark in the presence of the drugs or 40 min irradiation in their absence, were both unable to reduce the surviving fraction significantly.

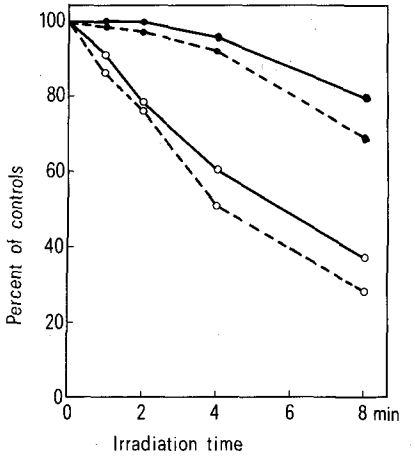

Fig. 2. Inhibition of the synthesis of DNA (solid lines) and of RNA (dotted lines) in Ehrlich ascites tumor cells by irradiation (365 nm) in the presence of 4 μg/ml of 5-methylangelicin (○) or of angelicin (●). The irradiated cells were incubated in the presence of ^{3}H-thymidine or of ^{3}H-uridine; the radioactivity incorporated into the acid-insoluble fraction was then determined. The results are expressed as a percentage of the incorporation of untreated cells. Incubation in the dark in the presence of the drugs or irradiation in their absence were both ineffective.

b) Experiments with Ehrlich ascites tumor cells: DNA and RNA syntheses were assayed in Ehrlich ascites cells after irradiation in the presence (4 μg/ml) of 5-methylangelicin and of angelicin, for comparison; figure 2 shows the results of one of these experiments. 5-Methylangelicin photosensitization produces a strong inhibition of both DNA and RNA syntheses, while angelicin proved noticeably less effective. Using the data from several experiments and the probit analysis, we calculated the ID_{50}, i.e. the UV-radiation dose that in the presence of the drug yields a 50% inhibition, which gave the following results (quanta $\times 10^{-18}$ ± confidence limits for $P = 0.05$):

	DNA	RNA
5-methylangelicin	9.07 ± 1.2	8.3 ± 1.4
angelicin	25.00 ± 1.1	23.3 ± 1.6

From these data, it appears that using angelicin as photosensitizer, to obtain a 50% inhibition, radiation doses almost 3 times higher than that with 5-methylangelicin are required.

To test the activity on cell replication, samples of Ehrlich cells irradiated in the presence of 5-methylangelicin (4 μg/ml) were injected i.p. into healthy mice, and the mortality due to tumor development was scored. In this test, a killing effect on the treated cells will result in a lowering of the tumor growth and therefore of the mortality of the injected mice. The results of these experiments together with the data obtained with angelicin are reported in the table. 5-Methylangelicin appeared very effective both in reducing the mortality and in increasing the mean survival time of the injected mice, while angelicin showed a very poor activity, except when it was employed at a concentration 5 times higher (20 μg/ml).

Remarks in conclusion. On the basis of these results, obtained in experiments on 2 different biological systems - a virus, the T_2 phage, and eukaryotic cells, the Ehrlich tumors - 5-methylangelicin appears as a new angular furocoumarin showing high photosensitizing activity. In comparison with angelicin, the parent compound, it seems to be several times more active. Angular furocoumarins are well-known monofunctional reagents for double-stranded DNA, forming only monoadducts with pyrimidine bases; actually, in preliminary experiments (data not shown here), 5-methylangelicin, although photobinding to DNA, proved to be incapable of inducing cross-links in DNA. Therefore, 5-methylangelicin appears to be a very interesting compound, being a monofunctional reagent for DNA able to produce strong photoinduced biological effects.

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