additional ligand. Surprisingly, a Fe(III) complex is not observed under our conditions, but cannot be excluded in solutions of other composition[9-11], e.g. the Cotton effect described previously[9] was measured in a solution of **1** and $FeCl_3$ in water (concentration of both $3 \cdot 10^{-3}$ M). The chelating properties of the 2 related amino acids nicotianamine (**1**) and mugineic acid (**2**) show remarkable differences. Apart from the comparable stability of their Cu(II) complexes, the stability constants for the Fe(II)- and Zn(II)-complexes of **2** are much smaller than those for the complexes of **1**. The stability constant for the Fe(III) complex of **2** is relatively high (log K = 18.1)[11]. The different complexing behavior of the amino acids may be due to the presence of a terminal hydroxy group in **2** instead of a primary amino group in **1**.

Because of the different coordination behavior of nicotianamine (**1**) towards Fe(II) and Fe(III) it seems reasonable to assume that the cellular iron transport is mediated in the ferrous and not in the ferric form. On the other hand it could be supposed that **1** is biochemically converted into a compound of the mugineic acid type, which forms a stable complex with Fe(III), but the normalizing effect of the antipode of **1**, (+)-nicotianamine[4], might be an argument against this assumption. Finally in further physiological investigations the idea should not be neglected that perhaps **1** has only an indirect influence on iron transport.

1 Part 13 in the series 'On the 'normalizing factor' for the tomato mutant *chloronerva*', for part 12 see Ripperger et al.[4].
2 Visiting scientist from the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 16610 Prague 6 (Czechoslovakia).
3 Address for reprint requests to K.S., Institute of Plant Biochemistry, Academy of Sciences of the GDR, Weinberg 3, DDR-4020 Halle/S.
4 Ripperger, H., Faust, J., and Scholz, G., Phytochemistry *21* (1982) 1785.
5 Böhme, H., and Scholz, G., Kulturpflanze *8* (1960) 93.
6 Scholz, G., Kulturpflanze *13* (1965) 239.
7 Scholz, G., Kulturpflanze *15* (1967) 255.
8 Buděšínský, M., Budzikiewicz, H., Procházka, Ž., Ripperger, H., Römer, A., Scholz, G., and Schreiber, K., Phytochemistry *19* (1980) 2295 and references therein.
9 Ripperger, H., and Schreiber, K., Heterocycles *17* (1982) 447.
10 Scholz, G., Biochem. Physiol. Pfl. *161* (1970) 358.
11 Sugiura, Y., Tanaka, H., Mino, Y., Ishida, T., Ota, N., Inoue, M., Nomoto, K., Yoshioka, H., and Takemoto, T., J. Am. chem. Soc. *103* (1981) 6979.
12 Fushiya, S., Sato, Y., Nakatsuyama, S., and Nozoe, S., 23rd Symp. Chem. nat. Products, p. 173. Nagoya 1980.
13 We thank Dr R. Krauspe, Halle, for the glass electrode.
14 Gmelins Handbuch der anorganischen Chemie, Syst. No. 59, Eisen, part B, p. 321.
15 Schwarzenbach, G., Willi, A., and Bach, R.O., Helv. chim. Acta *30* (1947) 1303.
16 Ackermann, H., and Schwarzenbach, G., Helv. chim. Acta *32* (1949) 1543.

0014-4754/83/030261-02$1.50 + 0.20/0

Photobiological activity of 5,7-dimethoxycoumarin

M. J. Ashwood-Smith, G. A. Poulton and M. Liu

Department of Biology and Department of Chemistry, University of Victoria, Victoria (British Columbia, Canada V8W 2Y2), August 28, 1981

Summary. Some biological properties of 5,7-dimethoxycoumarin (DMC) include dark induced frameshift mutagenesis in bacteria, lethal photosensitization and the formation of sister chromatid exchanges in Chinese hamster cells. The number of sister chromatid exchanges per unit of cell lethality produced by DMC is almost the same as observed with 5-methoxypsoralen.

The photobiology of furocoumarins and their biochemical reactions with nucleic acids have been extensively studied in recent years[1-3]. Furocoumarins are known to intercalate within the DNA strands in a dark reaction[4,5], and UV-radiation of this complexed material can lead to monoadduct formation (photocyclization at the 3,4- or 4′,5′-bonds) and to di-adduct formation (interstrand cross-links)[6-8]. The mutagenic and carcinogenic effects of furocoumarins are attributed mainly to interstrand cross-link formation, monoadducts being assumed to be much less active[9,10].

Psoralen use in conjunction with UV-A radiation (PUVA therapy) for treatment of psoriasis and mycosis fungoides has been remarkably successful[11-14], although concern was raised regarding the possibility of skin cancer associated with such treatment[15,16]. Recent results[17,18] indicate that this concern was justified, there being an increased risk of squamous cell carcinoma (2.7-times higher than expected) in patients treated with PUVA who had been previously exposed to other potential carcinogens, with the relative risk increasing with the number of PUVA treatments. In order to reduce the possibility of such side effects, non-cross-linking compounds such as 3-carbethoxypsoralen (3-CPs) and 5,7-dimethoxycoumarin (DMC) have been investigated as potential substitutes in PUVA therapy[9,19-21]; the results of clinical trials with monofunctional reagents are still incomplete. Since no evidence was available for the photobiological effects of DMC, such a study was undertaken.

DMC is found naturally in several citrus oils, including oils of bergamot, lime and lemon[22-24], in concentrations varying from 0.46% (lime) to 0.053% (lemon)[22]. To what extent DMC contributes to the well-known photosensitizing effects of lime oil is not known as this oil contains a substantial number of other coumarins and furocoumarins[23].

This communication reports that DMC is a frameshift mutagen in the dark, that it lethally photosensitizes mammalian cells and induces in them sister chromatid exchanges (SCEs).

Materials and methods. Chemicals. The furocoumarins used in this study were characterized and their purity established as previously described[25]. DMC (Aldrich Chemical Co., USA) was twice recrystallized (85% ethanol) prior to use. 3-CPs was a gift from Dr E. Moustacchi, Paris, France.

Bacterial mutation studies. Frameshift mutagenesis studies in the dark were conducted on *E. coli lac-, z, thiamine* as

described by Bridges and Mottershead[26] and by Ashwood-Smith[27]. Cells were grown for 12 h at 37 °C with aeration in brain-heart infusion supplemented with thiamine. They were harvested by centrifugation and washed twice with phosphate buffer (0.07 M, pH 7.0) before being plated at a density of 2×10^7 cells per plate; furocoumarins and DMC were present in the medium for the complete incubation period as this is necessary for the detection of frame shift mutagens[26]. There is no evidence of interaction between the media and test chemicals. After 3 days at 37 °C *lac*+ mutants were counted.

Table 1. Frameshift mutagenesis in *E. coli*, by DMC, 5-MOP, 8-MOP and combinations in the dark

Treatment Chemicals at 40 µg/ml	Induced LAC+ mutants per 1×10^8 cells (± SE)
1 Control (ethanol only)	15.2 (3.3)
2 DMC	88.0 (12.5)
3 5-MOP	70.2 (13.9)
4 8-MOP	220.4 (10.6)
5 5-MOP + DMC	210.2 (4.5)
6 5-MOP + 8-MOP	288.2 (29.4)
7 DMC + 8-MOP	263.0 (31.3)
p-Values by t-tests; between 1 and 2	0.0002
between 1 and 3	0.001
between 1 and 4	> 0.0001
between 2 and 5	0.004
between 3 and 5	0.006
between 3 and 6	> 0.0001
between 4 and 6	0.005
between 2 and 7	0.0001
between 4 and 7	0.0432

Table 2. Photosensitized induction in CHO cells of sister chromatid exchanges by DMC and 3-CPs

	Dose of UVA/B (J/m²)	SCEs per cell ± SE
DMC (40 µg/ml)	0	4.92 ± 0.35
	1000	13.52 ± 0.36
	1608	15.72 ± 0.74
	2412	19.32 ± 0.59
	3216	44.84 ± 1.88
	4020	53.76 ± 1.09
	4500	79.52 ± 0.71
	4824	88.60 ± 2.39
3-CPs (40 µg/ml)	0	3.85 ± 0.31
	402	6.20 ± 0.35
	1206	7.38 ± 0.35
	2010	16.11 ± 0.59
	2412	17.47 ± 0.72
	3216	24.67 ± 0.67

SCEs were measured in CHO cells as described by Ashwood-Smith et al.[25]. Cells were grown, after irradiation, for 30 h in the presence of bromodeoxyuridine according to the method of Perry and Wolff[29].

Table 3. Comparative induction of sister chromatid exchanges at equivalent lethality of DMC and several other furocoumarins

Chemical (40 µg/ml)	LD_{90} in J/m² (UVA/B)	SCEs per cell at LD_{90}
3-CPs*	2090	13.5
Angelicin*	965	38.1
DMC*	5628	76.0
5-MOP	730	60.6
8-MOP	500	66.0

*Monoadduct formation only. Data for angelicin, 5-MOP and 8-MOP from Ashwood-Smith[30].

Photosensitization. The light source has been described previously[28], and consisted of 2 parallel black light bulbs (GEC F20T12 · BLB) emitting 13.4 J/m², measured by chemical actinometry (ferrous oxalate). More than 95% of the light was emitted between 320 and 380 nm and showed a Gaussian distribution with a peak at 350–355 nm.

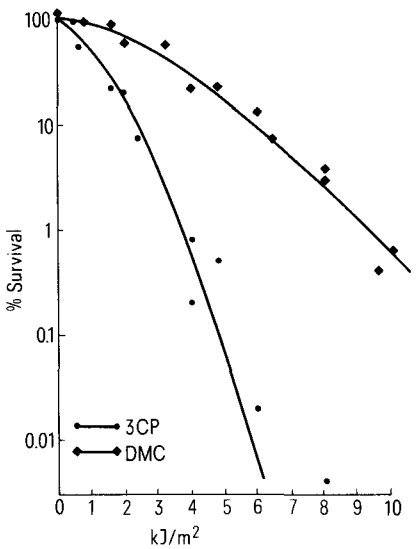
Tissue culture. Tissue culture studies (CHO studies) were carried out as described previously[25]. Irradiation alone, or in the presence of 2% ethanol had no effect on cell survival. None of the chemicals tested in this study affected survival at the concentration used within the time period of the experiment.

Sister chromatid exchanges (SCEs) were measured as originally described by Perry and Wolfe[29] and modified as outlined by Ashwood-Smith et al.[25].

Results and discussion. Three criteria were used to evaluate the biological action of DMC in comparison with several furocoumarins. The 1st, dark-induced frame shift mutagenesis, is a function of DNA intercalation which is normally regarded as a necessary prerequisite to photobinding. The 2nd criterion involved measuring the relative lethal photosensitization of mammalian cells, in vitro. The 3rd criterion was an assessment of the photoinduced damage to the chromosomes of mammalian cells, in vitro, and this was done by measuring sister chromatid exchanges. These methods of comparative assessment are well established procedures[16,25–30].

Ou et al.[31] have demonstrated that DMC intercalates with, photobinds to, and affects template activity of calf thymus DNA. DMC was the only coumarin tested to show significant complex formation. It would be expected to be a frameshift mutagen and this was, in fact, the case (table 1). Intercalation by 1 coumarin or furocoumarin might be thought to interfere with the action of another, more active one which co-occurs with it (e.g. in citrus oils[22–24]). At the dose level used here there is no evidence of interference; on the contrary, the evidence is of additivity (table 1).

The relative inactivity of 3-CPs and DMC in yeast cells, both in terms of lethality and mutation induction has been described[9,19]. In CHO cells DMC is less active than 3-CPs as a lethal photosensitizer by a factor of two (fig.) with an LD_{99} of approximately 9500 J/m² compared to 3-CPs at 4000 J/m², angelicin at 1600 J/m² and psoralen at 326 J/m²



Survival of CHO cells following photosensitization with 3-CP and DMC. Both compounds tested at 40/µg/ml.

and is clearly then one of the least active sensitizers in terms of radiation dose. Survival curves for CHO cells with a number of furocoumarins have been published previously[25,30].

A marked difference exists between the formation of SCEs with 3-CPs and DMC. With DMC a typical dose response similar to that seen with furocoumarins[30] was observed (table 2); 3-CPs had considerably less effect and this may be related to its low mutagenic activity and lack of carcinogenicity[9,19].

The parallel behavior of DMC, which cannot form cross-links, is very interesting. DMC causes nearly the same number of SCEs per cell at the same level of cell survival as 5-MOP although considerably more energy was required to produce the same kill (about 8 times as much at LD_{90}, table 3).

DMC is, therefore, a very potent producer of SCEs in contrast to the non-carcinogenic 3-CPs. Angelicin which is only weakly active as a skin photocarcinogen (F. Zajdela; personal communication) produces fewer SCEs per unit lethal dose than the DNA cross-linking linear furocoumarins. Neither compound alone without light, nor light itself, produces cell death.

If DMC should turn out to be a photocarcinogen, and there is no information on this point, then its wide distribution in a number of plants and essential oils would not be without interest.

1 Song, P.-S., and Tapley, Jr, K.J., Photochem. Photobiol. *29* (1979) 1177.
2 Parsons, B.J., Photochem. Photobiol. *32* (1980) 813.
3 Cahn, J., Forlot, P., Grupper, C., Meybeck, A., and Urbach, F., eds. Psoralens in Cosmetics and Dermatology. Pergamon Press, Paris 1981.
4 Beaumont, P.C., Land, E.J., Navaratnam, S., Parsons, B.J., and Phillips, G.O., Biochim. biophys. Acta *608* (1980) 182.
5 Sherman, W.V., and Grossweiner, L.I., Photochem. Photobiol. *34* (1981) 579.
6 Dall'acqua, F., Marciani, S., and Rodighiero, G., FEBS Lett. *9* (1970) 121.
7 Dall'acqua, F., Marciani, S., Ciavatta, L., and Rodighiero, G., Z. Naturforsch. *B26* (1971) 561.
8 Cole, R.S., Biochim. biophys. Acta *217* (1970) 30.
9 Averbeck, D., and Moustacchi, E., Photochem. Photobiol. *31* (1980) 475.
10 Belogurov, A.A., and Zavilgelsky, G.B., Mutation Res. *84* (1981) 11.
11 Parrish, J.A., Fitzpatrick, T.B., Tanenbaum, L., and Pathak, M.A., N. Engl. J. Med. *291* (1974) 1207.
12 Melski, J.W., Tanenbaum, L., Parrish, J.A., Fitzpatrick, T.B., and Bleich, H.L., J. invest. Derm. *68* (1977) 328.
13 Tronnier, H., and Lohning, R., Castellania *2* (1974) 267; in: Photochemotherapy, Basic Technique and Side Effects, p.71. Ed. E.G. Jung. Schattauer Verlag, Stuttgart 1976.
14 Roenigk, Jr, H.H., in: Psoralens in Cosmetics and Dermatology, p.211. Eds J. Cahn et al. Pergamon Press, Paris 1981.
15 Ashwood-Smith, M.J., and Grant, E.L., B. med. J. *1* (1979) 342.
16 Ashwood-Smith, M.J., Grant, E.L., Heddle, J.A., and Friedman, G.B., Mutation Res. *43* (1977) 377.
17 Stern, R.S., Thibodeau, L.A., Kleinerman, R.A., Parrish, J.A., and Fitzpatrick, T.B., N. Engl. J. Med. *300* (1979) 809.
18 Stern, R.S., in: Psoralens in Cosmetics and Dermatology, p.203. Eds J. Cahn et al. Pergamon Press, Paris 1981.
19 Averbeck, D., Moustacchi, E., and Bisagni, E., Biochim. biophys. Acta *518* (1978) 464.
20 Ou, C.-N., Song, P.-S., Harter, M.L., and Felkner, I.C., Photochem. Photobiol. *24* (1976) 487.
21 Harter, M.L., Felkner, I.C., and Song, P.-S., Photochem. Photobiol. *24* (1976) 491.
22 Stanley, W.L., and Vannier, S.H., J. Am. chem. Soc. *79* (1957) 3488.
23 Stanley, W.L., and Vannier, S.H., Phytochemistry *6* (1967) 585.
24 Latz, H.W., and Madsen, B.C., Analyt. Chem. *41* (1969) 1180.
25 Ashwood-Smith, M.J., Poulton, G.A., Barker, M., and Mildenberger, M., Nature, Lond. *285* (1980) 407.
26 Bridges, B.A., and Mottershead, R.P., Mutation Res. *44* (1977) 305.
27 Ashwood-Smith, M.J., Mutation Res. *58* (1978) 23.
28 Ashwood-Smith, M.J., and Grant, E.L., Experientia *33* (1977) 384.
29 Perry, P., and Wolff, S., Nature, Lond. *251* (1974) 156.
30 Ashwood-Smith, M.J., Natarajan, A.T., and Poulton, G.A., in: Psoralens in Cosmetics and Dermatology, p.117. Eds J. Cahn et al. Pergamon Press, Paris 1981.
31 Ou, C.-N., Tsai, C.-H., Tapley, Jr, K.J., and Song, P.-S., Biochemistry *17* (1978) 1047.

0014-4754/83/030262-03$1.50 + 0.20/0

Evidence to support the hypothesis that ATP is a co-transmitter in rat vas deferens

A.M. French and N.C. Scott

Pharmacology Section, Department of Pharmacy, Heriot-Watt University, Edinburgh EH1 2HJ (Scotland), July 13, 1982

Summary. Application of exogenous ATP or of noradrenaline (NA) produced responses in bisected rat vas deferens which mimicked the biphasic responses to nerve stimulation, and these actions were modified by nifedipine and verapamil in a manner similar to the modification of the 2 phases of the responses of the vas to nerve stimulation. It is proposed that sufficient evidence now exists to support the hypothesis that in this tissue, ATP is released along with NA from the motor nerves and that ATP may indeed be a co-transmitter.

Considerable controversy has surrounded the nature of neurotransmission in the vas deferens of several animal species. There is ample evidence that noradrenaline (NA) is involved[1-3]: however the existence of a 2nd nonadrenergic-noncholinergic (NANC) transmitter is suggested since there is a component of the contractile response to nerve stimulation which is resistant to adrenoceptor blockade or to depletion of NA from the motor nerve terminals. It has been suggested that adenosine triphosphate (ATP), which is present in the storage vesicles of adrenergic nerves may be involved[4,5]. Single pulse electrical field stimulation of the rat vas deferens results in a contraction of the tissue which shows 2 distinct phases, or peaks. The early peak, which occurs 250–280 msec after stimulation, is virtually unaffected by the presence of α-adrenergic blocking agents such as prazosin, or by depletion of neuronal stores of NA, due to prior treatment of the rats with reserpine: the later peak, occurring at 650–700 msec, is reduced or abolished by both