Mutation Research, 79 (1980) 73—77

Short Communication

MUTAGENICITY OF PSORALEN EPOXIDES

G. WAYNE IVIE [a], JAMES T. MACGREGOR [b] and BRUCE D. HAMMOCK [c]

[a] *Veterinary Toxicology and Entomology Research Laboratory, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, College Station, TX 77840,* [b] *Western Regional Research Laboratory, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Berkeley, CA 94710, and* [c] *Department of Entomology, University of California, Riverside, CA 92502 (U.S.A.)*

(Received 14 January 1980)
(Revision received 31 March 1980)
(Accepted 2 May 1980)

Linear furocoumarins (psoralens) occur widely in nature as constituents of hundreds of plant species from several families (Ivie, 1978b; Pathak et al., 1962). Many psoralens are potent photosensitizers, and some are used medicinally as light-activated drugs for the treatment of certain skin disorders, including skin depigmentation (leukoderma) and psoriasis (Pathak et al., 1962; Scott et al., 1976). Humans are also exposed to psoralens by the consumption of certain foods, and dermally through perfumes and fragrances, skin-tanning preparations, and the exposure of field workers and food processors (Pathak et al., 1962; Scott et al., 1976). Many cases of psoralen phototoxicity in humans have been documented as a result of these interactions (Pathak et al., 1962), and livestock and poultry are sometimes likewise affected (Ivie, 1978b).

Psoralens crosslink with DNA in the presence of activating ultraviolet light and are mutagenic, but have little (Bridges and Mottershead, 1977) or no (Scott et al., 1976) mutagenicity in the absence of light. For this reason, psoralens are not generally considered to pose a significant genetic, mutagenic, or carcinogenic hazard to man (Scott et al., 1976). However, some plant psoralens have α-epoxy ether substituents that could dramatically affect their light-independent mutagenic potential. Many epoxides are mutagenic (Wade et al., 1978, 1979), and because psoralens readily intercalate between pyrimidine bases of DNA strands (Scott et al., 1976), the potential for subsequent light-independent alkylation reactions by the epoxide moiety of psoralen epoxides may be greatly increased. We report here that 2 naturally occurring psoralen epoxides are only weakly mutagenic in the dark, but that a closely related synthetic psoralen epoxide is a potent mutagen, and in fact may be the most mutagenic alkyl epoxide yet studied.

Mutagenicity studies were conducted with histidine-dependent strains (TA100, TA1535, TA98 and TA1537) of the bacterium *Salmonella typhimu-*

rium, using the plate-incorporation assay developed by Ames et al. (1975). Mutagenicity of the test compounds was evaluated both with and without the addition of rat liver enzyme (S-9) preparations (Ames et al., 1975) and in some cases with added mouse-liver epoxide hydrase enzymes (Hammock et al., 1979).

The natural psoralen epoxides studied were heraclenin and oxypeucedanin (Fig. 1), which are the major psoralen epoxides occuring in most plants. Oxypeucedanin was isolated from the ripened seed of *Ammi majus* (Ivie, 1978a), and herclenin was synthesized by reported procedures (Ivie, 1978a). Psoralen-8-glycidyl ether (PSGE, Fig. 1) was prepared by the reaction of epibromohydrin with xanthotoxol (8-hydroxypsoralen) (Ivie, 1978a). Glycidol and phenyl glycidyl ether (PGE) were obtained commercially, and the dimethyl analog of glycidol (glycidol-DiMe, Fig. 1) was prepared by epoxidation of commercially obtained 3-methyl-2-buten-1-ol. Structures of all compounds were confirmed by nuclear magnetic resonance (NMR) and mass spectral analysis, and each appeared to be pure on the basis of NMR and/or thin-layer chromatography. Appropriate levels of these compounds were added to the test plates via 100 μl of DMSO (Ames et al., 1975). Control plates received DMSO only, and aflatoxin B_1, MNNG, or 9-aminoacridine were used as positive controls to confirm proper functioning of the assay system (Table 1). To minimize the possibility of light-induced mutations by the test compounds, all phases of the assays were conducted either in the dark or under low intensity artificial lighting that emitted essentially no long- or short-wave ultraviolet radiation.

Heraclenin is a weak mutagen in *S. typhimurium* strains TA100, TA98 and

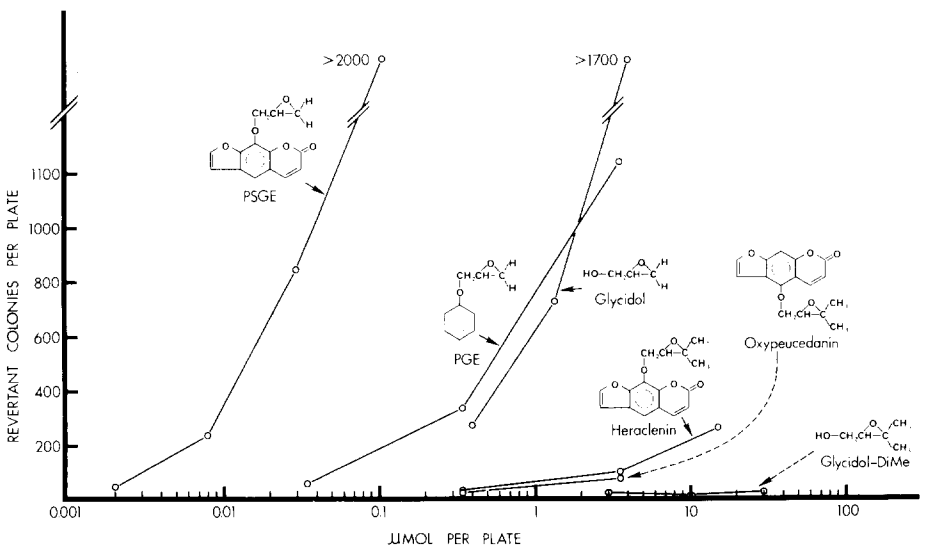

Fig. 1. Mutagenicity of psoralen epoxides and related compounds in a histidine-dependent strain of *Salmonella typhimurium* (TA100). The numbers of revertant colonies occurring in untreated (control) plates ($\bar{X}$ ± S.D. = 119 ± 13) have been subtracted. Aflatoxin B_1 was used as a positive control (see Table 1, footnote b). Data obtained on assays performed with added rat-liver enzymes are not included in the figure. Addition of these enzymes had no effect on the mutagenicity of any of the compounds studied, with the exception of PSGE and PGE where mutagenic activity was decreased by as much as 1 order of magnitude.

TABLE 1

MUTAGENICITY OF PSORALEN EPOXIDES AND RELATED COMPOUNDS IN 3 HISTIDINE-DEPENDENT STRAINS OF *Salmonella typhimurium*

Compound [a]	Revertant colonies per plate [b,c]			
	μmol per plate	TA1535	TA98	TA1537
Heraclenin	0.35	0	10	1
	1.05	0	23	13
	3.50	0	63	51
	10.50	TOX [d]	211	TOX [d]
Oxypeucedanin	0.35	ND [e]	10	7
	3.50	ND [e]	14	9
PSGE	0.002	0	8	0
	0.008	29	32	4
	0.031	206	64	34
	0.105	660	192	187
PGE	0.033	32	8	1
	0.33	215	0	2
	1.00	588	5	5
	3.33	1216	13	5
Glycidol	0.40	261	12	0
	1.35	721	0	0
	4.05	>1500	0	0
	13.51	>2600	13	4
Glycidol-DiMe	0.98	0	0	0
	2.94	0	0	3
	9.80	0	0	1
	29.40	0	0	1

[a] Structures of the compounds are shown in Fig. 1.

[b] Average of 2—3 determinations. The numbers of revertant colonies occurring in untreated (control) plates have been subtracted. These were ($\bar{X}$ ± S.D.) 16 ± 3 (TA1535), 25 ± 7 (TA98), and 8 ± 2 (TA1537). Data obtained from assays performed with added rat-liver enzymes are not included in the table, but with every compound and bacterial strain tested, mutagenic activity was either not affected or was significantly decreased.

[c] The numbers of revertant colonies occurring in positive control plates were as follows: TA100 and TA98 (aflatoxin B_1, 0.5 μg/plate, >1000); TA1535 (MNNG, 5.0 μg/plate, >2500); TA1537 (9-amino-acridine, 100 μg/plate, >2000).

[d] Growth inhibition of the background lawn.

[e] Not determined.

TA1537, and is not mutagenic in strain TA1535 (Fig. 1, Table 1). Its 5-substituted isomer, oxypeucedanin, was available in only very small quantities, but the limited studies conducted with oxypeucedanin suggested that it is no more active, and possibly even less so, than heraclenin. On the other hand, the synthetic psoralen epoxide PSGE, which differs from the natural psoralens only in that it lacks dimethyl substitution at the epoxide moiety, is a potent mutagen in strains TA100 and TA1535 (Fig. 1, Table 1) and, considering the low levels of spontaneous reversions in strains TA98 and TA1537 (Table 1), is quite mutagenic in these strains as well. These data suggest that psoralen epoxides, in the dark, act both as base-pair substitution and frameshift mutagens, whereas the absence of appreciable activity of PGE and glycidol in strains TA98 and

TA1537 suggests that these epoxides have base-pair mutagen specificity (Ames et al., 1975). Our data on PGE and glycidol agree with those previously reported by others (Greene et al., 1979; Wade et al., 1979).

Studies in which the epoxide moiety of PSGE was converted to the diol synthetically or by the incorporation of mouse-liver epoxide hydrase (Hammock et al., 1979) revealed that the diol has greatly reduced mutagenicity and thus confirms that the epoxide is largely if not totally responsible for the mutagenicity observed in these tests. Dimethyl substitution at the epoxide moiety dramatically diminishes mutagenicity, as evidenced by comparisons of the data obtained with PSGE and its naturally occurring dimethyl analogs and of glycidol with its dimethyl analog (Fig. 1). These dimethyl-substituted epoxides are as much as 3 orders of magnitude less mutagenic than their unsubstituted analogs. Electron-donating effects of the methyl substituents, leading to lower reactivity of the epoxide (Wade et al., 1978), and perhaps steric effects probably account for the reduced activity observed.

While these studies indicate that naturally occurring psoralen epoxides have low mutagenic potential and are thus unlikely to be of significant toxicological significance in man, they further show that a synthetic psoralen epoxide, PSGE, is highly mutagenic in certain strains of *S. typhimurium* (Fig. 1, Table 1). It appears, in fact, that PSGE may be the most mutagenic alkyl epoxide yet studied, and its mutagenicity is clearly enhanced by the presence of the psoralen nucleus. The related epoxide PGE differs from PSGE only in the nature of the aromatic substituent (phenyl versus psoralen), yet PSGE is as much as 30-fold more mutagenic than PGE (Fig. 1). The most likely explanation for the enhanced activity of PSGE is that the psoralen moiety, which readily forms molecular complexes with DNA (Scott et al., 1976), greatly facilitates the intercalation of PSGE into DNA strands, where the epoxide reacts at some as yet undetermined site. Because the psoralen nucleus complexes with and, in the presence of light, forms mono- or di-adducts with pyrimidine bases (Scott et al., 1976), PSGE and its analogs represent unique trivalent alkylating agents that should be very useful models for mechanistic studies of both light and dark reactions of psoralens and epoxides with DNA.

Acknowledgment

We thank Robert E. Wilson for technical assistance in performing the mutagenicity assays.

References

Ames, B.N., J. McCann and E. Yamasaki (1975) Method for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test, Mutation Res., 31, 347—364.

Bridges, B.A., and R.P. Mottershead (1977) Frameshift mutagenesis in bacteria by 8-methoxypsoralen (methoxalen) in the dark, Mutation Res., 44, 305—312.

Greene, E.J., M.A. Friedman, J.A. Sherrod and A.J. Salerno (1979) In vitro mutagenicity and cell transformation screening of phenylglycidyl ether, Mutation Res., 67, 9—19.

Hammock, B.D., S.S. Gill, S.M. Mumby and K. Ota (1979) Comparison of epoxide hydrases in the soluble and microsomal fractions of mammalian liver, in: R.S. Bhatnagar (Ed.), Molecular Basis of Environmental Toxicity, Ann Arbor Science Pub., Ann Arbor, pp. 229—272.

Ivie, G.W. (1978a) Linear furocoumarins (psoralens) from the seed of Texas *Ammi majus* L. (bishop's weed), J. Agric. Food. Chem., 26, 1394—1403.

Ivie, G.W. (1978b) Toxicological significance of plant furocoumarins, in: R.F. Keeler, K.R. van Kampen and L.R. James (Eds.), Effects of Poisonous Plants on Livestock, Academic Press, New York, pp. 475—485.

Pathak, M.A., F. Daniels and T.B. Fitzpatrick (1962) The presently known distribution of furocoumarins (psoralens) in plants. J. Invest. Dermatol., 39, 225—239.

Scott, B.R., M.A. Pathak and G.R. Mohn (1976) Molecular and genetic basis of furocoumarin reactions, Mutation Res., 39, 29—74.

Wade, D.R., S.C. Airy and J.E. Sinsheimer (1978) Mutagenicity of aliphatic epoxides, Mutation Res., 58, 217—223.

Wade, M.J., J.W. Moyer and C.H. Hine (1979) Mutagenic action of a series of epoxides, Mutation Res., 66, 367—371.