CURRENT AWARENESS DOCUMENT

LINEAR AND ANGULAR FUROMARINS PSORALENS, ANGELICINS, AND RELATED COMPOUNDS

CARCINOGENICITY AND STRUCTURE ACTIVITY RELATIONSHIPS. OTHER BIOLOGICAL PROPERTIES. METABOLISM. ENVIRONMENTAL SIGNIFICANCE.

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EPA Contract No. 68-02-3948

SAIC Project No. 2-813-07-409

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June 1986

5 3 2 5 Linear and Angular Furocoumarins Psoralens, Angelicins and Related Compounds

5 3 2 5 1 Introduction

The linear and angular furocoumarins, mostly derivatives of psoralen and angelicin, constitute a special class of chemical carcinogens known as photocarcinogens Furocoumarins are naturally occurring substances found in five major plant families -- parsley, rue, legume, fig and orchid In certain plants (e g , celery, parsnips), they are produced in very high amounts, perhaps as phytoalexins, in response to fungal infection (see Section 5.3 2 5 5 2) During the last decade, furocoumarins have captured the attention of many clinicians, molecular biologists and health protection specialists because of their promise as photochemotherapeutic agents for a variety of skin diseases, as molecular probes for the study of nucleic acid structure and DNA repair mechanisms, and the use of 5-methoxypsoralen (5-MOP or bergapten) as a cosmetics ingredient

The historical development of the use of furocoumarins as photochemotherapeutic agents has been extensively described by Scott et al (1) and Fitzpatrick and Pathak (2) Furocoumarin-containing plants have been used as herbal medicine since 1200-2000 B C The ancient Indian sacred book, "Atharva Veda" and the Buddhist Bower manuscripts both mention the treatment of vitiligo (a color imperfection of the skin, also known as leukoderma or "white leprosy") with a combination of sunlight and extracts from the seeds of a plant now classified as Psoralea corylifolia L Another important plant, Ammi majus, a weed found in the Nile Valley, has similarly been used in Egypt for centuries as a cure for this socially unacceptable skin disease. It was not until the 1940's that El Mofty (3) showed that the active photochemothera-

peutic principle in seeds of Ammi majus is 8-methoxypsoralen (8-MOP, also known as Methoxsalen, Xanthotoxin) In 1974, a new milestone was set when Parrish et al (4) reported that a combination of oral administration of 8-MOP and dermal exposure to UVA* is highly effective in the treatment of a virtually incurable skin disease, psoriasis This new PUVA (acronym for psoralens plus UVA) therapy has stirred widespread interest throughout the world Since 1974, several thousand psoriasis patients have received PUVA treatment in clinical trials in Europe and the United States On May 20, 1982, the U S Food and Drug Administration approved the use of PUVA for the treatment of psoriasis The effective use of psoralens in the treatment of vitiligo has also led to the controversial use of 5-MOP as a melanogenic agent in several suntan lotions (5) Oil of bergamot, a citrus extract which contains 5-MOP, was also used in perfumery in Eau de Cologne (6, 7). Owing to its unique capacity to intercalate, photoreact and form both monoadducts and crosslinks with DNA, psoralens have also proved to be a very useful probe for the study of nucleic acid structure (8-11) and DNA repair mechanism (8, 12)

The potential phototoxicity of furocoumarins and furocoumarin-containing plant substances has been known for centuries The phototoxicity of furocoumarins was even mentioned in a 18th century German fairy tale in which the central character was described to have suffered from photodermatitis after ingesting figs and exposing himself to sunlight (see 1) Many incidents of phototoxicity in livestock and in humans exposed to furocoumarin-containing

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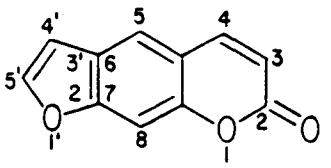
*Owing to their differential biological activities, the UV spectrum is generally divided into three regions UVA, which occupies the 320-400 nm region and is also known as long wave (wavelength) UV, near UV or black light UVB, which occupies the 280-320 nm region and is sometimes called intermediate UV or "sunburn" UV UVC, which occupies the region below 280 nm and is also called short wave UV, far UV or germicidal UV

plant products have been reported in the literature (see Section 5 3 2 5 2 1) The photocarcinogenicity of 8-MOP was discovered by O'Neal and Griffin (13) in 1957 in a study originally designed to explore the possible protective effect of the compound The interest in the study of photocarcinogenicity of furocoumarins has been rekindled recently as the concern over potential long-term health hazard of human use of furocoumarins as drugs and cosmetics ingredients grows To date, at least 11 furocoumarins have been tested for in vivo or in vitro carcinogenic activity The intense interest in these compounds have generated many recent publications as reflected by the large number of recent reviews (1, 8, 9, 12, 14, 15) on the subject Two recent symposia were devoted to review studies of the photobiologic, toxicologic and pharmacologic aspects of psoralens (16, 17)

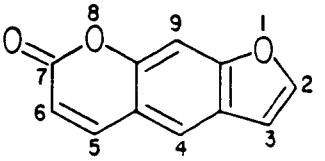
5 3 2 5 2 Physicochemical Properties and Biological Effects

5 3 2 5 2 1 PHYSICAL AND CHEMICAL PROPERTIES

The physical and chemical properties of psoralens and related furocoumarin compounds have been extensively discussed in a number of reviews (1, 8, 15, 18) Synthetically, there are 12 different ways a furan ring can be fused to a suitably substituted coumarin derivative to form different types of furocoumarins Naturally occurring furocoumarins of plant origin consist of two types, psoralens and angelicins In psoralens the furan ring is condensed linearly to the coumarin molecule (the 3,2-position of furan to the 6,7-position of coumarin), whereas in angelicins the furan ring is condensed angularly (the 3,2-position of furan to the 7,8-position of coumarin) The systematic, IUPAC name for the parent compound, psoralen, is 7H-furo[3,2-g]benzopyran-7-one Two different systems are in use for numbering the carbon and oxygen atoms in the psoralen molecule In the conventional system the original numbering sequence of both the coumarin and the furan moieties are



(Conventional)



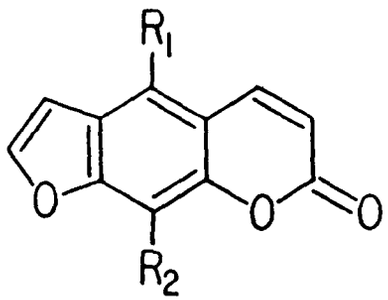
(IUPAC)

retained, but the furan positions are distinguished by the prime sign, such as 1', 2', In the IUPAC system, the atoms on the periphery of the molecule are numbered in a clockwise direction starting from the oxygen atom in the furan ring The conventional system is still widely used in the literature on psoralens and is the one adopted in this section

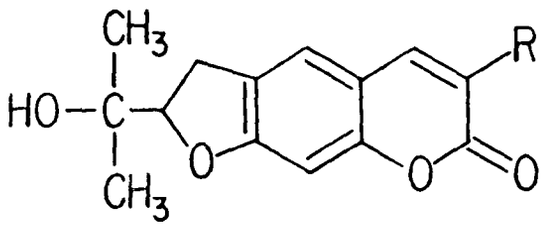
Close to 30 different derivatives of psoralen and angelicin have been isolated from natural sources (1, 9) The chemical structures of the eleven compounds which have been tested for carcinogenic activity are depicted in Table LXVIII Some physical and chemical properties of eight of these compounds are summarized in Table LXIX Most naturally occuring psoralens and angelicins are white to cream-colored, odorless, needle-like crystals at room temperature They are practically insoluble in cold water, sparingly soluble in ether or boiling water, and soluble in hot ethanol, acetone, vegetable oils, propylene glycol, benzene and chloroform The UV absorption spectra of most psoralens and angelicins show one or more absorption maxima in three regions less than 225 nm, 230-270 nm and 290-330 nm (18, 23) The lactone ring in furocoumarins is susceptible to alkaline hydrolysis. However, if the alkaline treatment is mild, acidification will reverse the reaction to reform furocoumarins. Furan-2,3-dicarboxylic acid is obtained when either psoralen or angelicin derivatives are oxidized with peroxide under alkaline conditions. Treatment of an alkoxy furocoumarin, other than a methoxy derivative, with an acetic acid-sulfuric acid mixture leads to cleavage of the ether bond (18).

Table LXVIII

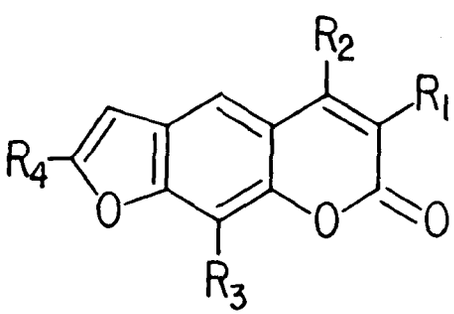
Structural Formulas of Psoralen and Related Compounds



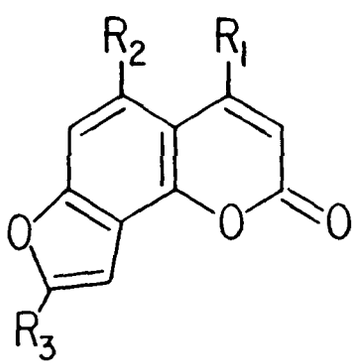
Psoralen ($R_1 = R_2 = -H$)
5-MOP or Bergapten ($R_1 = -OCH_3$, $R_2 = -H$)
8-MOP or Methoxsalen ($R_1 = -H$, $R_2 = -OCH_3$)
Imperatorin ($R_1 = -H$, $R_2 = -OCH_2CH{=}C(CH_3)_2$)



Marmesin ($R = -H$)
Chalepin ($R = -C(CH_3)_2-CH{=}CH_2$)



3-Carbethoxypsoralen* ($R_1 = -COOC_2H_5$, $R_2 = R_3 = R_4 = -H$)
4,5',8-Trimethylpsoralen or Trioxsalen ($R_1 = -H$, $R_2 = R_3 = R_4 = -CH_3$)



Angelicin ($R_1 = R_2 = R_3 = -H$)
5-Methylangelicin ($R_1 = R_3 = -H$, $R_2 = -CH_3$)
4,5'-Dimethylangelicin ($R_1 = R_3 = -CH_3$, $R_2 = -H$)

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*Synthetic compound

Table LXIX

Physicochemical and Photophysicochemical Properties of Psoralen and Related Compounds[a]

Compound (synonyms and abbreviations)	m p (C)	Solubility (μg/ml H_2O)	UV-absorption[b]			Fluorescence[c]		Quantum yield		
			λ_{max}	ϵ_{max}	$\epsilon_{365\ nm}$	λ_{excit}	λ_{emis}	Triplet state[d]	Singlet oxygen[e]	Superoxide anion[f]
Psoralen	163-164	37	295	--	--	--	--	0 45, 0 12	3 3, 7 6, +++	+++
8-Methoxypsoralen (8-MOP, methoxsalen, Xanthotoxin)	148	23	302	12,010	1,150	360	460	0 06, 0 14	1, +	+
5-Methoxypsoralen (5-MOP, Bergapten)	--	5	312	14,200	950	350	460	<0 001	0 4-0 5, +	+
4,5',8-Trimethylpsoralen (Trioxsalen)	235	1	298	7,950	1,680	360	430	--	5 3, +	++
3-Carbethoxypsoralen	153-154	13	317	10,900	6,230	365	460	0 32, 0 35	26, ++++	+
Angelicin	--	20	299	9,870	--	--	--	0 33	0, +++	++
5-Methylangelicin	189-190	--	307	10,900	--	--	--	--	++	++
4,5'-Dimethylangelicin	185	8	298	9,350	125	330	425	--	+	+

[a]Summarized from data compiled by Grossweiner (19), Rodighiero and Dall'Acqua (20), Joshi and Pathak (21), Averbeck et al (22), Guiotto et al (23), Merck Index (24)

[b]UV-Absorption in the 290-330 nm region ϵ_{max} = molar extinction coefficient at λ_{max}, $\epsilon_{365\ nm}$ = molar extinction coefficient at 365 nm

[c]The numbers shown are the optimal wavelength for excitation and emission, respectively

[d]Quantum yield of triplet state formation in water, data from Grossweiner (19)

[e]Relative quantum yield of singlet oxygen The numerical data (normalized to 8-MOP as 1 0) are from Grossweiner (1984) The semiquantative data (+, ++,) are from Joshi and Pathak (21).

[f]Relative ability to yield superoxide anion, data from Joshi and Pathak (21).

The photochemistry of psoralens has been extensively studied, the readers are referred to reviews by Song and Tapley (8), Pathak (25), Rodighiero and Dall'Acqua (20) and Grossweiner (19) for details The essential features of the photochemical changes in psoralen brought about by photoactivation are schematically summarized in Fig 16 Psoralen in ground state (P_{so}) may be photoactivated to an excited singlet state (see Section 3 2 2, Vol I) by two possible types of electronic excitation (a) excitation of the nonbonding electron in the carbonyl group to antibonding π molecular orbital (π^*) to produce the $^1n,\pi^*$ state and (b) excitation of a π-electron to the antibonding π^* state to produce the $^1\pi,\pi^*$ state The resulting excited singlet state (^1P_s) is extremely reactive, short-lived ($\sim 10^{-8}$ sec) and readily undergoes spontaneous deactivation by radiative decay resulting in fluorescence, or undergoes intersystem crossing (electronic spin flip) to the excited triplet state (^3P_s) The excited triplet state ($^3n, \pi^*$ or $^3\pi, \pi^*$), although still highly reactive and short-lived, has a considerably longer lifetime ($\sim 10^{-3}$ sec) and may allow three competing deactivating processes to take place These are (a) spontaneous radiative decay resulting in phosphorescence (see Section 3 2 2 and Suppletory Note 3, Vol I), (b) type I photochemical reactions, and (c) type II photochemical reactions The type I (anoxic) reactions involve nonradiative transfer of energy from ^3P_s to substrates resulting in direct reaction between ^3P_s and substrates In biological systems, these reactions include C_4 cycloaddition of P_s to pyrimidines, interstrand cross-linking, and conjugation to amino acid moieties of proteins These reactions are believed to play a major role in contributing to the phototoxic, photomutagenic and photocarcinogenic activities of psoralens (see Section 5 3 2 5 4) The type II (photodynamic) reactions involve indirect, sensitized transfer of energy from ^3P_s to molecular oxygen (in triplet state) to produce

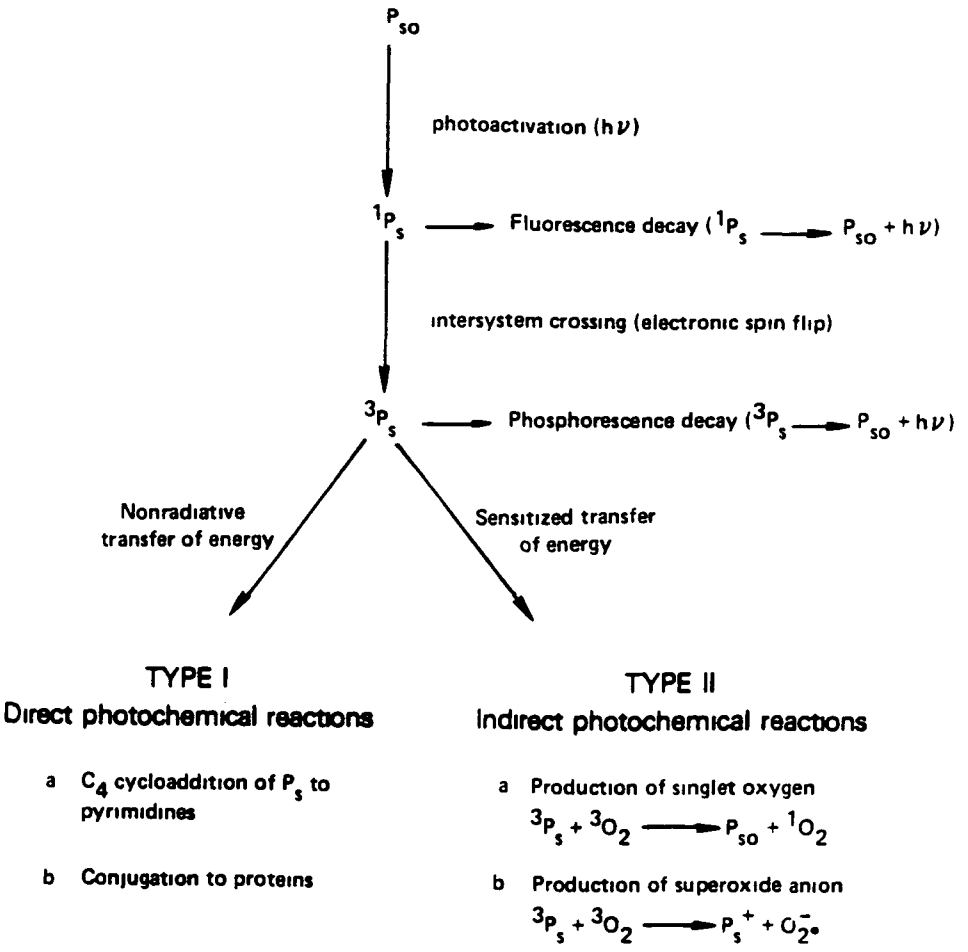

Fig. 16. Photoexcited states of psoralen and their photophysical radiative decays (fluorescence and phosphorescence) and photochemical reactions. Two types of photochemical reactions are shown: type I (direct photochemical reaction), anoxic and involving nonradiative transfer of energy, and type II (indirect photochemical reaction), oxygen-dependent and involving sensitized transfer of energy to molecular oxygen. Abbreviations: P_{so}, psoralen in ground state; ^1P_s, psoralen in singlet state; ^3P_s, psoralen in triplet state, $^3O_2$, molecular oxygen in triplet state; $^1O_2$, molecular oxygen in singlet state; $O_2^{-\bullet}$, superoxide anion. [Adapted from M.A. Pathak: NCI Monog. 66, 41 (1984).]

reactive oxygen species such as singlet oxygen ($^1O_2$), superoxide anion ($O_2^{-}\cdot$) and hydroxy radicals ($\cdot OH$) Compared to type I reactions, type II reactions are relatively unimportant in contributing to the photobiological effects of psoralens Psoralens have high tendency to intercalate into DNA, once intercalated, psoralens are less efficient in producing $^1O_2$ Furthermore, psoralens themselves can react with $^1O_2$ to form oxidation products thus effectively removing $^1O_2$ from the system (8) The relative abilities of several psoralens and angelicins to yield triplet state, singlet oxygen and superoxide anions are summarized in Table LXIX

5 3 2.5 2 2 BIOLOGICAL EFFECTS OTHER THAN CARCINOGENICITY

Toxicity and Photobiological Effects Most of the toxicity studies of furocoumarin compounds focus on their phototoxicity Relatively little data are available on their systemic toxicity. The acute LD_{50} of 8-MOP in rats by i p injection is 470 mg/kg body weight (26) Lower i p LD_{50} values of 189 and 158 mg/kg for male and female Sprague-Dawley rats, and about 250 mg/kg for CD-1 mice, were found using micronized suspension of 8-MOP (27). The corresponding oral LD_{50} values of micronized 8-MOP suspension were 791, 699 and 566 mg/kg body weight (27) In a comparative study (route not specified) by Herold et al (28), 5-MOP was substantially less toxic than 8-MOP, with reported LD_{50} values of 8,100, >30,000 and 9,000 mg/kg body weight for 5-MOP and 875, 4,400 and 505 mg/kg body weight for 8-MOP in NMRI mice, Wistar rats and Hartley guinea pigs, respectively. A 90-day subchronic toxicity study of orally administered 8-MOP in F344 rats was conducted by Dunnick et al (29), toxic effects (decreased weight gain, fatty changes in liver and adrenal cortex, testicular atrophy and death) were observed in two of the high-dose (200 and 400 mg/kg) groups with the liver, adrenal glands and testes being the most affected organs

The phototoxicity of psoralen and related compounds has been extensively studied and was the subject of several recent reviews (1, 30, 31) In mammalian skin the most striking features of acute phototoxicity due to psoralens are delayed erythema and pigmentation Under some conditions, edema and blistering is also produced The dose-response curve of the phototoxicity of psoralens is usually very steep The phototoxic effects of psoralens are manifested by either oral or parenteral administration or by topical application The action spectrum* of psoralens lies in the 340-380 nm region, the higher end of their absorption spectrum Unlike for many photosensitizers, the photosensitizing effect of psoralens is essentially independent of oxygen (1, see also Section 5 3 2 5 2 1)

Sporadic outbreaks of psoralen-induced photosensitization in livestock have been noted after grazing or feeding on plants (e g , Cymopterus watsonii, Ammi visnaga, Ammi majus) of the parsley family (32) Incidents of dermatitis in celery workers, lime pickers, and consumers using the perfume "Eau de Cologne" have also been reported In celery workers' dermatitis, it was established that the very high amount of 8-MOP and 4,5',8-trimethylpsoralen produced in celery in response to fungal disease ("pink rot") is responsible for the phototoxicity (33, 34) The presence of 5-MOP in lime oil and bergamot oil (used as an ingredient in Eau de Cologne) may account for the occasional dermatitis in the workers and consumers (1, 6)

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*The action spectrum of a photosensitizing compound is the region of wavelengths capable of eliciting photobiological response from sensitized subjects The action spectrum of a compound is generally expected to parallel its absorption spectrum However, for furocoumarin compounds, the two spectra are not in good agreement.

The structure-phototoxicity relationships of furocoumarin compounds have been extensively studied More than 150 furocoumarins and related compounds have been tested for photosensitizing activity on mammalian skin (rev in 1) Some of these results are summarized in Table LXX and compared to their ability to photoreact with DNA In general, linear furocoumarins are much more active than angular furocomarins. Saturation of the 4',5'-double bond completely abolishes activity The introduction of a bulky substituent (e g , carbethoxy) at one of the two critical double bond (3,4- and 4',5'-) of psoralen molecule yields a compound (e g , 3-carbethoxypsoralen) with monofunctional character, having little or no phototoxicity. Compounds with a large number of substitutents having ionic character tend to ionize in water and are less stable and less phototoxic Substitutents that alter the resonance of the furocoumarin molecule greatly affect its activity The introduction of electron-donating substituents such as a methyl group in the 5'- or 8-position enhances the phototoxicity of psoralen, whereas methyl substitution at 3-, 4-, and 4'-position decreases the activity Introduction of electron-withdrawing substituents (e g , -OH, -CN, $-NH_2$) at the same position exhibits an opposite effect

Comparison of the phototoxicity of furocoumarins with their photobinding activity to DNA (see Table LXX) indicates a very good correlation between phototoxicity and ability to form interstrand cross-links Apparently, the formation of monoadduct is insufficient to cause significant phototoxicity * This hypothesis is supported by the findings of Gange and Parrish (31) that blue light (>380 nm) photoactivates bifunctional 8-MOP to form monoadducts

*Some exceptions to this general rule exist Certain monofunctional psoralens such as 4,6,4'-trimethylangelicin are phototoxic (25)

Table LXX
Phototoxicity of Furocoumarin Compounds and Comparison with Their Ability to Photobind to DNA[a]

Compound	Phototoxicity in mice[b]	Ability to form monoadducts	Ability to form interstrand crosslinks
Psoralen	++++	strong	very strong
5-MOP	++	strong	strong
8-MOP	+++	strong	strong
4,5',8-Trimethylpsoralen (TMP)	++++	strong	very strong
4'-Aminomethyl-TMP	++++	strong	very strong
3,4'-Dimethyl-8-MOP	++	strong	low
5-Diethylaminobutoxypsoralen	+	strong	moderate
3-Carbethoxypsoralen	-[c]	strong	absent
Angelicin	-	weak	absent
5-Methylangelicin	-	strong	absent
4,5'-Dimethylangelicin	-	moderate	absent

[a]Adapted from P C. Joshi and M.A Pathak [Biochem. Biophys. Res Commun 112, 638 (1983)]

[b]Tested by skin photosensitization in mice in response to topical application of the compound followed by UVA exposure

[c]Data from M.P Mullen, M.A Pathak, J D. West, T.J. Harrist, and F. Dall'Acqua [NCI Monogr. 66, 205 (1984)].

with DNA, but without forming any crosslink Mice treated with 8-MOP plus blue light showed no sign of phototoxicity However, if these mice were treated with UVA 48 hours later to convert monoadducts into crosslinks, phototoxicity became evident

Mutagenicity The mutagenicity of psoralen and related compounds has been extensively studied The readers are referred to an excellent review by Scott et al (1) for studies published prior to 1976 Numerous studies have been reported since that time, mostly in vitro assays using a variety of test organisms such as prophages, bacteria, algae and yeasts The following discussion focuses mainly on studies using the Ames test and on comparative studies. It should be emphasized that the mutagenicity of furocoumarin and related compounds may be affected by a number of unusual factors as discussed below

a) Light As may be expected from the photochemical reactivity of furocoumarin compounds, the mutagenicity of these compounds may differ significantly when assayed in the dark (or under subdued light) or when assayed with exposure to light (particularly long wave UV or UVA) Their mechanism of action is believed to involve noncovalent complexing (intercalation) with DNA for "dark" mutagenicity and covalent photobinding to DNA for photomutagenicity (see Section 5 3 2 5 4)

b) Phototoxicity A number of furocoumarin compounds cause cell death after photoactivation The bifunctional furocoumarins are particularly phototoxic in this respect because of their crosslinking activity The mutagenicity testing of these compounds is often complicated by their cytotoxicity (35, 36)

c) Induction of lysogenic virus Mutagenicity assays using bacteria as the test organism may be affected by the induction (activation) of lysogenic virus by furocoumarin compounds leading to lysis of bacteria (37, 38) Interpretation of bacterial mutagenicity data should take this factor into consideration

d) DNA repair proficiency of test organisms The proficiency of DNA repair capability of test organisms plays a critical role in determining the mutagenicity of furocoumarins in *Salmonella typhimurium* (39), *Escherichia coli* (12, 40), *Aspergillus nidulans* (41) and *Saccharomyces cerevisiae* (42) It has been suggested that the repair contributes to mutation by preventing cell lethality and by introducing error during the unscheduled DNA repair synthesis

Over 20 furocoumarin compounds have been tested for mutagenic activity using the Ames test The results of these studies are summarized in Table LXXI When assayed in the absence of UV-photoactivation, most furocoumarins are either inactive or are weak mutagens toward frameshift mutants With a few exceptions, most mutagenic compounds do not require metabolic activation The weak, direct-acting frameshift mutagenic activity of these compounds is consistent with the hypothesis that, by virtue of their favorable molecular size, they produce mutations by noncovalently complexing with (intercalating into) DNA Among the compounds tested for "dark" mutagenicity, 8-MOP has been by far the most extensively studied, it was found inactive by most investigators and weakly mutagenic in two studies (44, 50) Psoralen-8-glycidol ether is an extremely potent mutagen (probably one of the most potent mutagen ever synthesized), a concentration of 0 03 μmole per plate was sufficient to produce positive results It is probably the presence of a reactive functional group (epoxide) coupled with the molecular shape and size of the

Table LXXI
"Dark" and Photoactivated Mutagenicity of Psoralen and Related Compounds in the Ames Test

Compound[a]	Without UV-photoactivation[d]		With UVA-photoactivation
	- S-9	+ S-9	
Psoralen	- (43)	- (43)	+ (39)
5-MOP (Bergapten)	- (44)		+ (39,45)
8-MOP (Methoxsalen)	- (37, 43, 46-49)	- (37, 43, 46-48)	- (37, 47-48)
	w+ (44, 50)		+ (39)
Imperatorin	+ (46)	+ (46)	
Heraclenin[b]	- (46)	- (46)	
	w+ (51)		
Oxypeucedanin[c]	- (46)	- (46)	
	w+ (51)		
Psoralen-8-glycidol ether[b]	+ (51)		
3-Carbethoxypsoralen	- (44)		
4,5',8-Trimethylpsoralen (Trioxsalen)	- (47, 48)	- (47, 48)	- (47, 48)
	w+ (44)		
Pyrido(3,4-c)furo(3,2-g)-coumarin[b]	w+ (44)		
7-Methylpyrido(3,4-c)furo-(3,2-g)coumarin[b]	w+ (44)		
Marmesin	+ (46)	+ (46)	
Chalepin	- (46)	w+ (46)	
Angelicin	- (43)	- (43)	+ (39)
5-Methylangelicin	w+ (43)	w+ (43)	
4'- or 5'-Methylangelicin	- (43)	- (43)	

Table LXXI (continued)

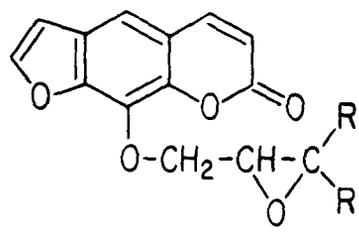
Compound[a]	Without UV-photoactivation[d] - S-9	Without UV-photoactivation[d] + S-9	With UVA-photoactivation
4,4'- or 5,5'- or 4,5'- or 4',5-Dimethylangelicin	- (43)	- (43)	
4,5'-Dimethyl-4'-hydroxymethylangelicin	- (43)	w+ (43)	+ (43)
4,5'-Dimethyl-4'-acetoxyangelicin	- (43)	w+ (43)	
4,5'-Dimethyl-4'-dimethylaminoethoxymethylangelicin	- (43)	w+ (43)	
Esculin (6,7-Dimethoxycoumarin)	- (46)	- (46)	

[a]Except where otherwise indicated, see Table LXVIII for structural formula.

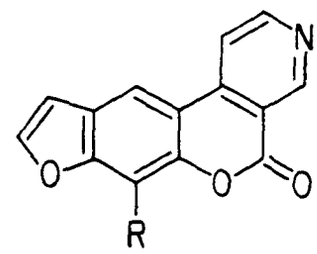
[b]See below for structural formula.

[c]Oxypeucadanin is an isomer of heraclenin with the substituent in the 5-position instead of the 8-position.

[d]Tested in the dark or under subdued light. w+ = weakly mutagenic.



Heraclenin (R=-CH_3)
Psoralen-8-glycidol ether (R=-H)



Pyrido(3,4-c) furo(3,2-g)-coumarin (R=-H)
7-Methylpyrido(3,4-c) furo-(3,2-g) coumarin (R=-CH_3)

psoralen molecule, favorable for intercalation, which makes it highly mutagenic This is shown by the fact that replacement of the psoralen moiety with a phenyl ring yields phenyl glycidol ether, which is 30 times less mutagenic Introduction of two sterically hindering methyl groups at the terminal epoxide group yields heraclenin, a naturally occurring compound, which is 1,000 times less mutagenic Interestingly, imperatorin, the unepoxized parent compound of heraclenin, is a moderately active mutagen with or without metabolic activation

The presence of both photoreactive double bonds (3,4- and 4',5'-) in psoralen molecules does not seem to be an absolute requirement for "dark" mutagenicity Blockade of the 3,4-double bond by a pyridine ring yields compounds (pyridofurocoumarins) which still retain some mutagenic activity Likewise, saturation of 4',5'-double bond does not necessarily abolish "dark" mutagenic activity In fact, marmesin is a moderately active "dark" mutagen Uwaifo (46) postulated that the introduction of a tertiary hydroxypropyl group at the 5'-position may activate the 3,4-double bond, however, the mechanism is not clear. Among the angular furocoumarins, only 5-methylangelicin is mutagenic in the dark Three derivatives of 4,5'-dimethylangelicin, bearing a hydrophilic group at 4'-position, are all mutagenic after metabolic activation (see Table LXXI)

Only six furocoumarin compounds have been tested for photomutagenic activity in the Ames test A number of studies (see Table LXXI) found both 8-MOP and 4,5',8-trimethylpsoralen nonmutagenic in the commonly used tester strains (TA98 and TA100) However using a new tester strain (TA102), which is highly sensitive to oxidative and cross-linking agents and is proficient in UV-excision repair, Levin et al (39) found several furocoumarins mutagenic after photoactivation by UVA Quantitatively, the bifunctional furocoumarins

(8-MOP, 5-MOP and psoralen) are considerably more mutagenic than the monofunctional furocoumarins (angelicins) 4,5'-Dimethyl-4'-hydroxyangelicin appears to be an unusual compound, it is mutagenic toward TA98 after metabolic activation in the dark but becomes mutagenic toward TA100 and nonmutagenic toward TA98 after UVA-photoactivation (43)

Besides the Ames test, furocoumarin compounds have been assayed in a variety of other test systems With few exceptions, remarkably similar results have been obtained from these studies When assayed in the dark, psoralen, 5-MOP, 8-MOP and angelicin are all weak, direct-acting frameshift mutagens in various strains of Escherichia coli, whereas 4,5',8-trimethylpsoralen appears to be nonmutagenic (5, 50, 52-54). There is some evidence that 8-MOP and 4,5',8-trimethylpsoralen can be metabolically activated to a base-pair substitution mutagen in the dark (47) When assayed with UV-photoactivation using UV-excision repair-proficient test organisms, bifunctional furocoumarin compounds are consistently more mutagenic than monofunctional furocoumarin compounds In E coli, the reported relative order of photomutagneic potency order is psoralen > 8-MOP > 5-MOP > angelicin in one study (54), and 8-MOP > psoralen > angelicin > 4,5'-dimethylangelicin in another (40) In the algal system Chlamydomonas reinhardii, both 5-MOP and 8-MOP are equally strong photomutagens, imperatorin is also strongly photomutagenic after prolonged UV irradiation, whereas 5'-methylangelicin is a weak photomutagen (55, 56) In the yeast (Saccharomyces cerevisiae), 4,5',8-trimethylpsoralen is highly photomutagenic, the relative photomutagenic potency is 4,5',8-trimethylpsoralen > 5-MOP > 8-MOP > 4,5'-dimethylangelicin > 3-carbethoxypsoralen in the induction of nuclear mutation, and 4,5'8-trimethylpsoralen > 5-MOP > 3-carbethoxypsoralen > 8-MOP = 4,5'-dimethylangelicin in the induction of cytoplasmic petite mutation (57)

Teratogenicity In contrast to the extensive mutagenicity data, there is little or no information available on the teratogenicity of furocoumarin compounds Herold et al (28) reported that 5-MOP exhibits no teratogenic effects in rabbits and rats, the details of the study are not available Colombo et al (58, 59) treated sea urchin sperms with psoralen and long wavelength UV, just before fertilizing untreated eggs, and observed abnormal effects (formation of giant cells, polynucleated cells, impairment of cleavage, shrinkage of cytoplasm, irregular blastomeres, and blastulas and cytolysis of embryo) in various subsequent developmental stages Neither psoralen nor long wavelength UV alone exhibited any significant effect on the development of sea urchin eggs

5 3 2 5 3 Carcinogenicity and Structure-Activity Relationships

The carcinogenicity of psoralen and related furocoumarin compounds was first studied by O'Neal and Griffin (13) in 1957 using 8-MOP The interest in furocoumarin compounds was renewed recently because of their therapeutic value in the treatment of psoriasis and other skin disorders and the commercial use of 5-MOP in suntan lotion To date, 11 furocoumarin compounds have been tested for in vivo or in vitro carcinogenic activity Early studies led to the conclusion that bifunctional furocoumarins (such as 8-MOP, 5-MOP) are more carcinogenic than monofunctional furocoumarins (such as angelicin, 3-carboethoxypsoralen). However, more recent studies using derivatives of angelicin indicate that the above conclusion is not necessarily true for all cases, some monofunctional furocoumarins can be more carcinogenic than 8-MOP Like their photomutagenicity, the carcinogenicity of furocoumarin compounds can be affected by a variety of factors as exemplified by the results of studies on 8-MOP.

5 3 2 5 3 1 CARCINOGENICITY OF 8-METHOXYPSORALEN (8-MOP)

8-Methoxypsoralen (Methoxsalen, Xanthotoxin) was first found to be photocarcinogenic in 1957 by O'Neal and Griffin (13) in mice by i p injection, in a study intended to explore the potential of the compound as a protective agent against ultraviolet light-induced skin carcinogenesis Subsequently, the compound was extensively studied in various strains of mice, particularly in the past several years, because of its use in combination with UVA in the "PUVA" treatment of psoriasis The carcinogenicity studies of 8-MOP reported prior to 1980 were reviewed by the International Agency for Research on Cancer (15) The results of these and a number of more recent studies are summarized in Table LXXII At the time of this writing, 8-MOP was also being tested under the U.S National Toxicology Program The carcinogenicity of 8-MOP is affected by a variety of factors such as (a) the route of administration, (b) the type, the intensity and the timing of irradiation used to activate the compounds, and (c) the strain of the animals used.

The route of administration has an important modulating effect on the photocarcinogenicity of 8-MOP Topical application and i p injection are substantially more effective for the induction of skin tumors than oral administration By oral administration, 8-MOP has no significant carcinogenic effect in mice of various strains as observed in several independent studies (13, 60-64). In fact, in one of these studies (13), 8-MOP appeared actually to protect against far-UV-induced skin carcinogenesis (25% incidence in 8-MOP plus far-UV-exposed mice vs. 62% in control mice exposed to far-UV alone) The doses administered in the above-cited studies ranged from 0 6 to 40 mg/kg body weight by gavage or 200 to 1,000 ppm in the diet Various forms of irradiation (far-UV, near-UV, fluorescent light) were used to photoactivate 8-MOP, usually one hour after dosing The duration of these studies ranged

Table LXXII
Photocarcinogenicity of 8-Methoxypsoralen (8-MOP) in Mice of Various Strains[a]

Strain	Route	Irradiation	Principal organs affected	References
Various strains	oral	UV (250-400 nm)	None[b]	(13, 60-64)
Swiss albino	oral	UVA	Skin[c]	(60, 65)
Swiss albino	i p.	UV (250-320 nm, 320-400 nm)	Skin[c]	(13, 60)
ICR Swiss	i p	UV (280-360 nm)	Skin[c]	(66)
Swiss	i.p.	UV (365 nm)	Skin	(67)
C3H⁻/HeN	i.p.	UVA	Skin	(68)
ICR Swiss	topical to ear	UV (280-360 nm)	Skin[c]	(66)
SKH-hairless-1	skin painting	SSR[d] (>290 nm)	Skin	(69-71)
		UVA	Skin	(72)
SKH-hairless-1 or HRS/J/Anl	skin painting	UV (various sources)	Skin	(73, 74)
Albino hairless	skin painting	SSR[d]	Skin	(75)
XVIInc/z	topical to ear	UV (365 nm)	Skin[c]	(67, 76)
Swiss albino	skin painting	UV (300-400 nm)	Skin	(77, 78)

[a]In all these studies, 8-MOP was found noncarcinogenic without photoactivation. The tumors were primarily squamous cell or basal cell carcinomas

[b]The duration of these studies ranged from 4 to 12 months.

[c]Ear and/or eye region.

[d]Simulated solar radiation, spectrum includes UVA, UVB, visible and IR light

from 4 to 12 months, although the duration was relatively short, it should be noted that in studies of similar length, but using topical or i p route, 8-MOP did prove to be carcinogenic A weak photocarcinogenic effect (skin tumor incidences of 20-35% vs 0% for control) was observed in one study (60, 65) in which Swiss albino mice were given 500 ppm 8-MOP in the diet and exposed to UVA 10 minutes to 2 hours a day, 5 days a week for 6 weeks to 3 months In contrast to its weak carcinogenicity or lack thereof by oral administration, 8-MOP was consistently found to be photocarcinogenic by i p or topical administration Daily i p injection of 0 4 mg 8-MOP to 20 Swiss albino mice 1 hour before 10-minute exposure to UVA for 6 weeks (total UVA exposure 43 joules/cm^2) induced skin tumors (mainly in ear and eye regions) in virtually all the animals, none of the irradiated control mice bore such tumors (60) Prolonged UVA exposure was less effective because of severe phototoxicity. Significant increase in the incidence of skin tumors was also observed in mice of several other strains given i p injection of 0 4 mg 8-MOP and irradiation with near or intermediate UV (see Table LXXII) Although no direct comparative studies have been conducted, topical application of 8-MOP appears to be equally effective as (if not more than) i p injection for inducing photocarcinogenesis For example, Nagayo et al (72) induced skin tumors in 20 of 20 albino SKH-hairless-1 mice after 40 weeks of daily skin painting with 40 µg 8-MOP, 30-60 minutes prior to UVA irradiation For comparison, none of the 36 control mice that received 8-MOP alone or UVA exposure alone developed tumors Dubertret et al (67) found skin tumors in 92% of 40 XVIInc/Z mice given 115 topical applications of 15 µg/cm^2 8-MOP on each ear and irradiated with UV (365 nm) About 15% of these mice also had secondary tumors Significant increase in the incidence of skin tumors (43% treated vs 15% control) was observed in Swiss albino mice skin-painted with as low as 5 µg 8-MOP 15-60 minutes before UV (300-400 nm) exposure (77, 78).

The importance of the nature, dose and timing of irradiation in the activation of 8-MOP to its photocarcinogenic form was first demonstrated by Griffin and associates. In contrast to the induction of skin tumors in nearly 100% of mice by a combination of i p injections of 8-MOP and 10-minute exposures to UVA, a combination of 8-MOP and short wavelength UV (254 nm) induced fewer tumors than exposure to UV-254 nm alone (60) Prolonging the time of UVA exposure or reducing the distance between the light source and skin decreased the tumor incidence because of excessive phototoxicity causing early death and severe erythemal responses (60) Delaying the onset of UVA exposure from 1 hour to 20-22 hours after 8-MOP injection completely abolished the photocarcinogenic effect of the treatment (13). The spectral dependence of 8-MOP photocarcinogenicity has been recently further explored Grube et al (73) topically applied 250 μg 8-MOP onto the skin of SKH-hairless-1 mice and exposed them to UV of three different wavelengths (300-400 nm, 320-400 nm, 365 nm) The respective skin tumor (squamous or basal cell carcinoma) incidences were 17/20, 15/19 and 8/19 and the latent period was considerably shorter in the first two groups indicating that UV of broader spectrum is more effective in eliciting a photocarcinogenic response Additional exposure to filtered fluorescent light (280-400 nm) either before or after combined treatment of 8-MOP and UV-365 nm increased the skin tumor incidence to 100% and shortened the latent period, suggesting a possible interaction involving the 365 nm-induced 8-MOP photoadducts and other molecular lesions induced by emissions between 280-400 nm (see Section 5 3 2 5 3 3).

In addition to the demonstration of spectral dependence of 8-MOP photocarcinogenicity, Grube, Fry and Ley (73, 74) also noted strain difference in carcinogenic response to 8-MOP In marked contrast to the high incidence of skin tumors in SHK-hairless-1 mice, 8-MOP plus UV (300-400 nm) produced no

skin carcinomas and 8-MOP plus UVA induced only a low incidence (4/19) of skin carcinomas (and after a prolonged latent period) in HRS/J/Anl hairless mice The HRS/J/Anl hairless mice were also less susceptible than SKH-hairless-1 mice to the carcinogenic effect of a combination treatment of 8-MOP, UV-365 nm and filtered fluorescent light (280-400 nm) However, no significant strain difference was observed in mice receiving 8-MOP plus UV-365 nm The strain difference in response to 8-MOP plus UVA is annulled by treating the HRS/J/Anl mice with 12-O-tetradecanoylphorbol-13-acetate (TPA), a typical tumorigenesis promotor, suggesting that the strain difference is associated with a difference in expression of dormant tumor cells rather than with tumor initiation

5 3 2 5 3 2 CARCINOGENICITY OF PSORALEN AND RELATED COMPOUNDS STRUCTURE-ACTIVITY RELATIONSHIPS

In Vivo Carcinogenicity The search for a safer drug for treatment of psoriasis, the concern over human exposure to naturally occurring furocoumarin compounds used in suntan lotion or folk herbal medicine have led to extensive studies on derivatives and structural analogs of 8-MOP At least six derivatives of psoralen and its isomer angelicin have been tested for photocarcinogenic activity in mice The results of these studies are summarized in Table LXXIII

The carcinogenicity of five furocoumarins has been studied by Zajdela, Bisagni and associates (67, 76, 80) using inbred XVIInc/Z albino mice The compounds (dissolved in acetone) were topically applied on the ears of each animal over an approximately 1 cm^2 surface at the dose of 10 or 15 $\mu g/cm^2$, and irradiated 15 minutes later with UVA or UV-365 nm at the radiation dose of 1 68 joules/cm^2 High incidence of skin tumors were observed in mice given the three bifunctional furocoumarins -- psoralen, 8-MOP and 5-MOP (76) The

Table LXXIII
Comparative Photocarcinogenicity of Psoralen and Related Compounds in Mice After Skin Painting[a]

Compound	Mouse strain	Dosing regimen	Irradiation		Skin tumor		Reference
			Type	Dose (J/cm^2)	Incidence	Latency[f]	
Psoralen[b]	XVIInc/z	115 x 10 $\mu g/cm^2$	UV-365 nm	1 68	97%	av = 40 wk	(76)
8-MOP[c]	XVIInc/z	115 x 10 $\mu g/cm^2$	UV-365 nm	1 68	92%	av = 58 wk	(76)
	Albino hairless	34 x 0 01% solution	SSR[e]	1 5	100%	T_{50} = ~ 22 wk[g]	(75)
		34 x 0 03% solution	SSR[e]	1.5	100%	T_{50} = ~ 18 wk[g]	(75)
	SKH-hairless-1	156-195 x 5 $\mu g/cm^2$	UVA	1 0	65%	T_{50} = 56 wk	(79)
		156-195 x 50 $\mu g/cm^2$	UVA	1 0	70%	T_{50} = 60 wk	(79)
		156-195 x 50 $\mu g/cm^2$	UVA	2 5	54%	T_{50} = 60 wk	(79)
		200 x 40 μg	UVA	0.72	100%	T_{50} = 24 wk	(72)
5-MOP	XVIInc/z	115 x 10 $\mu g/cm^2$	UV-365 nm	1.68	85%	av = 69 wk	(76)
	Albino hairless	34 x 0 01% solution	SSR[e]	1.5	100%	T_{50} = ~ 25 wk[g]	(75)
		34 x 0 03% solution	SSR[e]	1 5	100%	T_{50} = ~ 21 wk[g]	(75)
3-Carbethoxy-psoralen[d]	XVIInc/z	106 or 196 x 15 $\mu g/cm^2$	UVA	1 68	0%	--	(66)
	SKH-hairless-1	156-195 x 50 $\mu g/cm^2$	UVA	1.0	0%	--	(79)

Table LXXIII (continued)

Compound	Mouse strain	Dosing regimen	Irradiation		Skin tumor		Reference
			Type	Dose (J/cm^2)	Incidence	Latency[f]	
Angelicin	XVIInc/z	106 or 196 x 15 $\mu g/cm^2$	UVA	1 68	"moderately carcinogenic"		(cited in 80)
	SKH-hairless-1	156-195 x 50 $\mu g/cm^2$	UVA	2 5-5 0	20%	T_{50} = >60 wk	(79)
5-Methyl-angelicin	SKH-hairless-1	156-195 x 5 $\mu g/cm^2$	UVA	1 0	78%	T_{50} = 53 wk	(79)
		156-195 x 50 $\mu g/cm^2$	UVA	1 0	97%	T_{50} = 33 wk	(79)
4,5'-Dimethyl-angelicin	SKH-hairless-1	156-195 x 50 $\mu g/cm^2$	UVA	2 5	97%	T_{50} = 40 wk	(79)
		156-195 x 50 $\mu g/cm^2$	UVA	7 5	100%	T_{50} = 42 wk	(79)
Aflatoxin B_1	SKH-hairless-1	200 x 40 μg	UVA	0 72	0%	--	(72)

[a]All compounds listed in this table are noncarcinogenic without photoactivation. At the radiation doses applied (expressed in joules/cm^2), irradiation alone is also without carcinogenic effects

[b]Psoralen is not photocarcinogenic after oral administration (61)

[c]See Table LXXII for other carcinogenicity studies of 8-MOP.

[d]3-Carbethoxypsoralen is also not photocarcinogenic after i p. injection (67)

[e]SSR simulated solar radiation Wavelength spectrum includes UVA, UVB, visible and IR

[f]Latent period for tumor induction T_{50} = time for appearance of tumor in 50% of the surviving mice

[g]Estimated from graph published in the reference

two monofunctional furocoumarins -- angelicin and 3-carbethoxypsoralen (3-CP) -- were found to be "moderately carcinogenic" (cited in 80) and inactive (67), respectively Based on comparison of tumor incidence and latent period, the relative photocarcinogenic potency of the five compounds follows the order psoralen > 8-MOP > 5-MOP > angelicin > 3-carbethoxypsoralen (inactive) This relative order is generally in good agreement with the relative photomutagenic potency of these compounds and led to the suggestion that monofunctional furocoumarins are less carcinogenic than bifunctional furocoumarins, and may therefore be potentially safer therapeutic agents for treatment of psoriasis The finding of photocarcinogenicity of 5-MOP is also of great importance because the compound was, at one time, used an active ingredient, to promote pigmentation, in many widely used suntan preparations at concentrations of 12 to 50 μg/ml (5) Based on photomutagenicity data, 5-MOP was earlier predicted to be photocarcinogenic (5) Like 8-MOP, the photocarcinogenicity of 5-MOP can be promoted by the classical tumorigenesis promotor TPA (see Section 5 3 2 5 3 3) suggesting that photosensitized 5-MOP can initiate a population of "dormant" tumor cells, which are normally not expressed but can persist over a long period of time and can progress towards macroscopic tumors if and when promoted

The photocarcinogenicity of 5-MOP has been confirmed by Young et al (75) using albino hairless mice The compound was skin-painted on the flanks of the mice (applied as a 0 01 or 0 03% solution in a mixture of arachis oil and isopropyl myristate, the volume applied was not specified), and photoactivated by exposure to simulated solar radiation Compared to 8-MOP, 5-MOP induced skin tumors with a slightly longer latent period, but the difference was not considered significant and the two compounds were considered to be equally potent photocarcinogens

The conclusion that bifunctional furocoumarins are more carcinogenic than their monofunctional counterparts has recently been challenged by Mullen et al (79) using SKH-hairless-1 mice Compounds were topically applied (0 05 ml) on the backs of the mice in 0 01 or 0 1% ethanol solutions and photoactivated 45 minutes later with UVA These investigators did confirm the non-carcinogenicity of 3-carbethoxypsoralen and the weak carcinogenicity of angelicin However, two methyl derivatives of angelicin have been found to be even more carcinogenic than 8-MOP, inducing more skin tumors with a much shorter latent period under similar conditions (see Table LXXIII) The relative photocarcinogenic potency of the five furocoumarins tested by Mullen et al (79) follows the order 5-methylangelicin $\geq$ 4,5'-dimethylangelicin > 8-MOP > angelicin > 3-carbethoxypsoralen (inactive) The photocarcinogenicity of 5-methylangelicin appears to be directly dependent on the dose of the compound applied, whereas that of 8-MOP is not Increasing the irradiation dose has no effect on the photocarcinogenicity of 4,5'-dimethylangelicin but decreases the 8-MOP-induced tumor incidence due to higher phototoxicity Mice receiving 0 01 and 0 1% solutions of 8-MOP exhibit severe skin irritation, scabbing, and epidermal necrosis No sign of phototoxicity was observed in mice receiving either of the other four monofunctional furocoumarins These findings have led the investigators to hypothesize that the DNA photoadducts formed with both the monofunctional furocoumarins (monoadducts) and the bifunctional furocoumarins (crosslinks as well as monoadducts), undergo an error-prone excision-repair process which is potentially carcinogenic The increased lethality associated with the formation of cross-links by bifunctional furocoumarins may actually reduce the carcinogenic potential of the affected cells by killing the cells before somatic mutation can take place The lack of carcinogenicity of 3-carbethoxypsoralen was attributed to its

photolability According to some unpublished observations (P C Joshi and M A Pathak, cited in 79), 3-carbethoxypsoralen undergoes rapid transformation to a less reactive molecule upon irradiation It should, therefore, not be considered as a typical monofunctional furocoumarin compound

In addition to the compounds listed in Table LXXIII, 4,5',8-trimethylpsoralen (trioxsalen) was reported to be carcinogenic in albino mice (M A Pathak, D M Kramer and T B Fitzpatrick, cited in 79), the details of the study were not available at the time of this writing Aflatoxin B_1, a difurocoumarocyclopentenone compound (see Section 5 3 1 1) structurally related to furocoumarins, is not carcinogenic after topical application to SKH-hairless-1 mice followed by UVA irradiation (72) Actually, aflatoxin B_1 can be photoactivated to bind to DNA However, the nature of the photobinding of AFB_1 to DNA differs from that of metabolically activated DNA binding (see Section 5 3 1.1 4) and is apparently unrelated to the steps leading to carcinogenesis

In Vitro Carcinogenicity Four furocoumarin compounds have been tested for in vitro carcinogenic activity by the cell transformation assay Evans and Morrow (81) showed that a 8-MOP plus UV treatment of primary mouse, rat and hamster embryo cultures and several established cell lines (BHK_{21}/Cl_{13}, NIH/3T3 and Balb/c 3T3) caused a high percentage of cell transformation as judged by altered cell morphology and growth characteristics Subcutaneous inoculation of 8-MOP-plus-UV-transformed hamster embryo fibroblasts into 2-day-old newborn Syrian hamsters produced palpable tumors after 3 days The same transformed cells grew into spindle cell sarcomas 10-14 days after inoculation into the cheek pouch of adult hamsters The capability of 8-MOP plus UV to induce in vitro cell transformation has been confirmed by Uwaifo et al (82) using mouse fibroblast $C3H/10T_{1/2}$ cells With UVA activation, 8-MOP induced "type II foci" at concentrations of 1 0, 1 5 and 2 5 μg/ml No such

transformation by 8-MOP was observed without UVA exposure Among the other three naturally occurring furocoumarins investigated in the same study, imperatorin (8-isoamylenoxypsoralen) induced "type III foci" at a concentration of 10 μg/ml but was inactive at lower concentrations Marmesin (which is highly cytotoxic) and chalepin did not induce any foci when tested at concentrations of up to 1 0 μg/ml and 5 0 μg/ml, respectively 8-Methoxypsoralen, imperatorin, marmesin and chalepin have all been isolated from two Nigerian medicinal plants and are suspected to contribute to the high incidence of skin cancer in Nigeria and other tropical countries (82)

5 3 2 5 3 3 MODIFICATION OF CARCINOGENESIS BY FUROCOUMARIN COMPOUNDS

There is a scarcity of information on the modification of carcinogenesis by furocoumarins and the interaction of furocoumarins with other carcinogens in animals Fry et al (74) reported that SKH-hairless-1 mice are considerably more susceptible than HRS/J/Anl hairless mice to skin carcinogenesis induced by 8-MOP plus UVA However, treatment of HRS/J/Anl mice with 12-O-tetradecanoylphorbol-13-acetate (TPA), a typical tumorigenesis promotor, eliminates this strain difference rendering the two strains equally susceptible The promoting effect of TPA on the photocarcinogenicity of 5-MOP was also observed by Zajdela and Bisagni (76) using XVIInc/Z mice Treatment with TPA 8 months after the termination of 5-MOP plus UVA treatment raised the tumor incidence from 85% to 100% and increased the percentage of animals with multiple tumors from 25% to 61% An analysis of the distribution of latent periods indicated that the additional tumors observed in the promotion experiment arose from accelerating the emergence of tumors which would have had very long latent periods without promotion. These results indicate that both 8-MOP and 5-MOP can, after photoactivation, initiate "dormant" tumor cells which can persist over a long period of time and evolve towards macroscopic tumors if and when promoted

As mentioned in Section 5 3 2 5 3 1, Grube et al (73) demonstrated that additional exposure to simulated solar radiation either before or after 8-MOP plus UV-365 nm (PUVA) treatment substantially potentiated the photocarcinogenic effects Mice treated with the simulated solar radiation plus UV-365 nm alone did not develop any tumors The simulated solar radiation (280-400 nm) contains the UVB (280-320 nm) portion which is known to be photocarcinogenic (see 83) The results suggest a possible interaction (e g , synergism or promotion) between PUVA and UVB in photocarcinogenesis In this respect, it is interesting to note that Roberts et al (84) found that mice treated with subcarcinogenic doses of PUVA were tumor-susceptible when challenged with UVB-induced "regressor" tumors cells * The PUVA treatment appeared to generate a suppressor cell response in mice and apparently allowed the growth of "regressor" tumor cells through immunosuppression Besides animal data, there are some epidemiologic evidence and a variety of case reports to indicate that PUVA therapy may potentiate or promote tumor induction in patients who had a history of prior exposure to substantial amounts of known carcinogens (see Section 5 3 2 5 5 1). Apparent suppression of cell-mediated immune response in human skin by PUVA treatment also has been observed (85)

5 3 2 5 4 Metabolism and Mechanism of Action

5 3.2.5 4 1 METABOLISM

The pharmacokinetic properties of psoralens have been exensively discussed in a recent symposium (17) with emphasis on development of dosing

*Tumor cells induced by UVB in mice are of two immunologic types -- the "progressor" cells which grow rapidly when transplanted into normal syngenic hosts, and the "regressor" cells which are highly immunogenic and are usually rejected unless transplanted into immunosuppressed syngenic hosts

regimens to achieve optimal therapeutic efficacy The metabolism of the two most widely used psoralen drugs, 8-MOP (methoxsalen) and 4,5',8-trimethylpsoralen (trioxsalen), has been studied by various investigators (25, 86-90) Seven metabolites have been isolatd from the urine of SKH-hairless-1 mice or of human volunteers given an oral dose of 8-MOP (25, 87) Three major metabolites have been tentatively identified as (a) 8-hydroxypsoralen (only in human urine), (b) methoxyfurocoumaric acid, the hydrolyzed open-chain form of 8-MOP, which can be cyclized to the lactone form of 8-MOP, and (c) a glucuronide which, upon hydrolysis, regenerates 8-MOP. Both 8-hydroxypsoralen and the methoxyfurocourmaric acid are not phototoxic. A fourth major metabolite may be either (a) the 4',5'-epoxide or 4',5'-dihydrodiol derivative of 8-MOP, or (b) the 3,4-epoxide or 3,4-dihydrodiol derivative of 8-MOP, or (c) 6-carboxymethyl-7-hydroxy-8-methoxycoumarin The identity of the other three minor metabolites remains to be elucidated The metabolites identified in the urine of dogs given an i v dose of 8-MOP include the methoxyfurocoumaric acid, and 6-carboxymethyl-7-hydroxy-8-methoxycoumarin and its conjugate (88) *In vivo* studies showed little or no metabolism of 8-MOP by mouse liver homogenates or by microsomes (25) However, rat liver preparations effectively metabolize 8-MOP *in vitro*, and the metabolic rate is enhanced by pretreatment of the animals with the typical enzyme inducers, phenobarbital or β-naphthoflavone (90) The metabolism of 4,5',8-trimethylpsoralen in human volunteers and mice mainly involves oxidation of the 5'-methyl group (86, 89). The major urinary metabolite is 4,8-dimethyl-5'-carboxypsoralen which is not phototoxic Using *in vitro* systems, it has been established that the 5'-methyl group is first hydroxylated and then further oxidized to carboxy group. Both the 5'-hydroxy and 5'-carboxy derivatives of 4,5',8-trimethylpsoralen may be further hydroxylated With the possible exception of epoxida-

tion, all the above metabolic pathways appear to represent detoxification For both 8-MOP and 4,5',8-trimethylpsoralen, there is no evidence that metabolism would enhance the mutagenicity of the compound in the Ames test (see Table LXXI) Photoactivation appears to be the major, if not the sole, pathway in the activation of most psoralens to toxic, mutagenic or carcinogenic intermediates

5 3 2 5 4 2 MECHANISM OF ACTION

Despite extensive studies (rev in 8, 12, 14, 19, 20, 25), the mechanism of carcinogenesis and mutagenesis by psoralens is not well understood It appears that, for most psoralens, at least three types of mechanisms (a) intercalation complexing with DNA, (b) photoreaction with DNA, and (c) epigenetic mechanisms, either alone or in combination, may contribute to their carcinogenic or mutagenic action

It is now generally believed that intercalation complexing with DNA is probably responsible for the "dark" mutagenicity of most psoralens, and represents an important initial step in the photobinding of these compounds to DNA (see discussion below) The tricyclic structure of psoralens, and to a lesser extent of angelicins, provides a favorable molecular size and shape for intercalation complexing with DNA Polynuclear hydrocarbons, as well as polynuclear heteroaromatics structurally related to furocoumarins, are known to intercalate into DNA, resulting in increased solubility, DNA viscosity, and melting temperature (T_m) and spectral shifts (see Section 5 1 1 6 2 3 in Vol IIA and Sections 5 1 5 1 and 5 1.5 2 in Vol IIB).

The evidence supporting the existence of furocoumarin-DNA intercalation complexes has been reviewed (1, 20, 91) The usually low solubilities of furocoumarins in water (see Table LXIX) is significantly enhanced by the addi-

tion of DNA Likewise, the solubility of DNA in aqueous solution can be increased by inclusion of furocoumarin compounds such as 5-MOP Paralleling this increased solubility, both the viscosity and melting temperature (T_m) of DNA solution are elevated A spectral shift in the absorption maxima and a decrease in extinction coefficient are observed after furocoumarin-DNA complexing (1) There is also evidence that complexing causes slight unwinding of the DNA helix (91) The furocoumarin-DNA noncovalent binding constants of several furocoumarins have been determined (20, 91) There appears to be an inverse relationship between the binding (association) constants of furocoumarins and their water solubilities, suggesting that mainly hydrophobic forces are involved in the complexes (20) just as with the polynuclear hydrocarbons The stability of the furocoumarin-DNA intercalation complexes is low and this is consistent with the observation that most furocoumarins are either inactive or weak, direct-acting, frameshift mutagens when assayed in the dark (see Section 5 3 2 5 2 2) Despite the weak intercalation complexing of the psoralen molecule with DNA, such intercalation plays an important role if the molecule is coupled with a reactive functional group For example, psoralen-8-glycidol ether is one of the most potent synthetic mutagens known, substitution of the psoralen nucleus with a phenyl moiety reduces the activity by more than 30 times (see Section 5 3.2 5 2 2) Furocoumarins show a much lower capacity to form noncovalent complexes with RNA, and photobind to RNA less effectively than to DNA Moreover, there is some evidence that furocoumarins do not intercalate randomly but do so preferentially in regions having a high density of repetitive alternations of purines and pyrimidines (20) A sequence-specific noncovalent complexing has also been shown to occur before the covalent binding of the potent carcinogen, aflatoxin B_1, to DNA (see Section 5 3 1.1 4 2)

The mechanism of photobinding of psoralens and angelicins to DNA has been extensively investigated (rev in 1, 8, 9, 19, 20, 25) Figure 17 summarizes the proposed mechanism for bifunctional linear furocoumarins Once the furocoumarin-DNA complex is established, covalent bonds between the furocoumarin molecule and DNA are formed upon irradiation Firstly, a C_4-cycloaddition reaction involving the 5,6-double bond of pyrimidines and the exicted (triplet state) 3,4- or 4',5'-double bond of the psoralen molecule occurs, forming 3,4- or 4',5'-monoadducts Then, the 3,4- or 4',5'-monoadducts may be further photoactivated to react with a second pyrimidine base to give a diadduct When a diadduct is formed with the two strands of DNA, a cross-link is formed Photoreaction of angelicins with DNA yields mainly 4',5'-monoadduct (20, 92) because the angular structure of angelicins forecloses the possibility of photoreaction of the 3,4-double bond with a second pyrimidine base in the sister DNA strand The photoreaction of 3-carbethoxypsoralen is also expected to yield 4',5'-monoadduct because of the presence of bulky substituent in the 3-position, however, the identity of this photoadduct has not been clearly defined (20) The relative ability of psoralens and angelicins to photoreact with DNA, in terms of total photobinding (mostly monoadducts plus some crosslinking), ranks as follows 4,5',8-trimethylpsoralen >> 4,5'-dimethylangelicin > psoralen > 8-MOP > 5-MOP > angelicin > 3-carbethoxypsoralen (19, 20). For crosslinking, the ranking is 4,5'8-trimethylpsoralen >> psoralen > 8-MOP > 5-MOP, whereas 3-carbethoxypsoralen and angelicin show little or no crosslinking activity (19) For 4,5',8-trimethylpsoralen, 5-MOP and 8-MOP, the ratio of monoadduct to crosslink formation is about 9 1 (20)

The role of monoadduct formation and crosslinking in photocarcinogenesis and photomutagenesis by furocoumarin compounds is not clearly understood Crosslinks are expected to be more effective than monoadducts for inducing

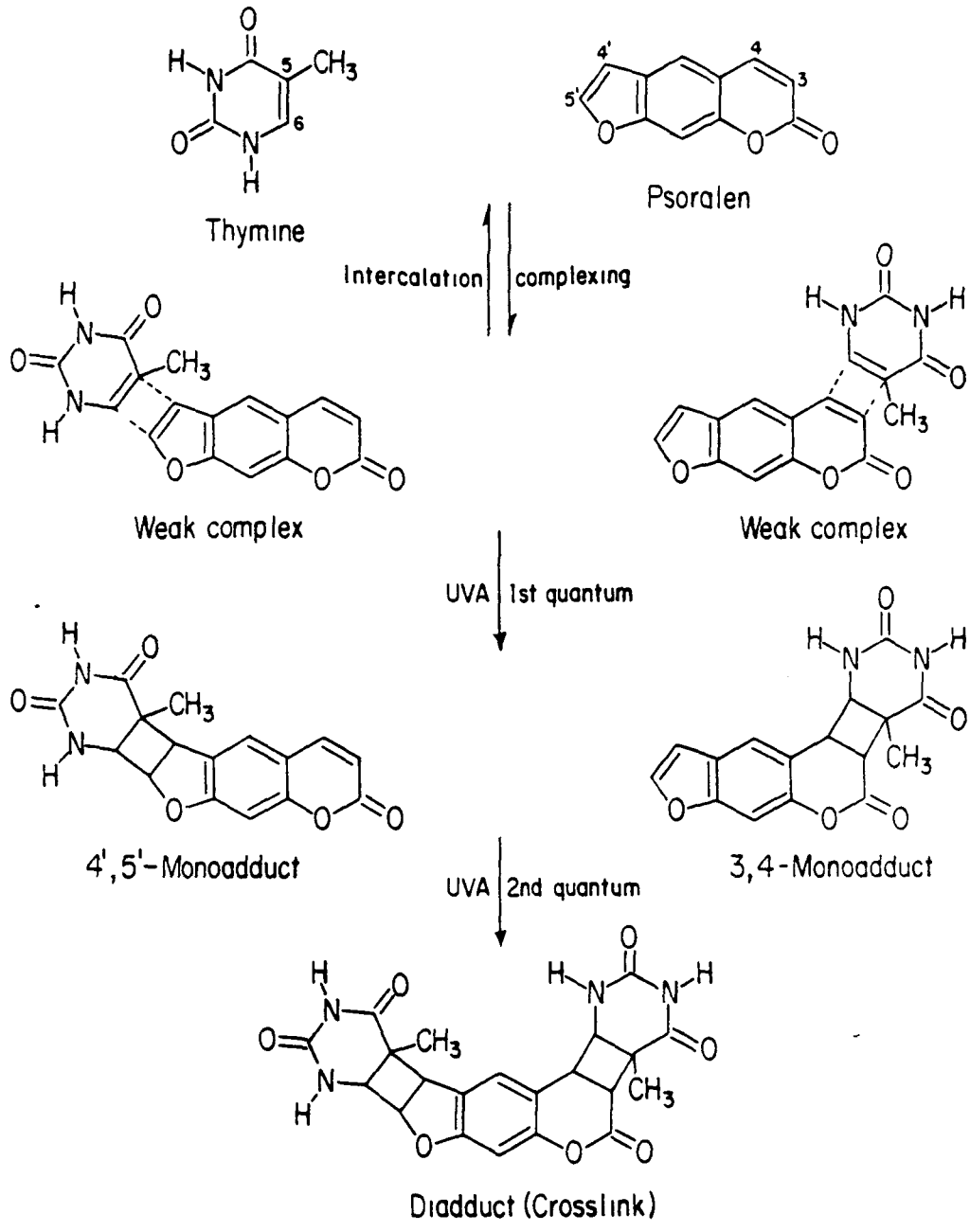


Fig. 17. Proposed mechanism for photobinding of psoralen to DNA. The first step is believed to involve weak, intercalation complexing between psoralen and pyrimidine (e.g., thymine) residues in DNA. Upon UVA-irradiation, a C_4-cycloaddition reaction takes place, forming 3,4- or 4',5'-monoadduct. Further addition of a second molecule of pyrmidine to either monoadduct gives rise to diadduct (crosslink). [Modified from B R Scott, M.A. Pathak, and G.R. Mohn Mutat. Res. 39, 29 (1976).]

potentially more genotoxic large-scale changes in the genome (93) However, crosslinks are also more difficult to repair (94) and, consequently, are more likely to lead to cell lethality than monoadducts Photomutagenicity studies using various microbial test organisms have consistently shown that bifunctional furocoumarins are more mutagenic than monofunctional furocoumarins. Furthermore, the presence of one or more proficient DNA repair pathways appears to be a requirement for the expression of photomutagenicity of some furocoumarins in various microbial test organisms (see Section 5 3 2 5 2 2) These results suggest that at least one probable mechanism of photomutagenesis is through "fixation" of the furocoumarin-induced DNA lesions by DNA repair mechanisms The usually error-prone SOS repair mechanisms, triggered by furocoumarin-DNA photoadducts (both crosslinks and monoadducts), contribute to photomutagenesis by promoting cell lethality and by introducing errors during the repair synthesis Photocarcinogenicity studies of 8-MOP in mice indicate that DNA crosslinking does not play a role in carcinogenesis (73) and actually appears to reduce the carcinogenic potential of 8-MOP by causing cell death (see Section 5 3 2 5 3 1) Certain monofunctional furocoumarins (e g , 5-methylangelicin, 4,5'-dimethylangelicin) are in fact more photocarcinogenic than bifunctional furocoumarins (see Section 5 3 2 5 3 2) The ranking of photocarcinogenic potency follows the order 4,5'-dimethylangelicin > psoralen > 8-MOP $\geq$ 5-MOP > angelicin > 3-carbethoxypsoralen, which is poorly related to the relative crosslinking activities of the compounds, but correlates well with their relative ability to form monoadducts The lack of correlation between mammalian photocarcinogenicity and microbial photomutagenicity is intriguing, the possibility that mammalian and microbial cells may differ in their ability to repair furocoumarin-DNA photoadducts should be investigated

The mechanism(s) by which photoadducts of furocoumarins initiate the process of carcinogenesis is not known It has been shown that both the structure and the template functions of DNA are altered by photoreaction with furocoumarins (rev in 1, 9, 10), and such changes are expected to be potentially genotoxic The contributory role of DNA repair pathways to photocarcinogenesis is not clear The repair of photoadducts is error-prone and it is possible that it would introduce errors during repair synthesis and lead to somatic cell mutation However, there is evidence that the excision repair of both monoadducts (94, 95) and especially of crosslinks (94) of furocoumarins is relatively inefficient in mammalian cells Certain portions of the mammalian genome (e g., a specific region of DNA in monkey cells called α-DNA) are particularly poorly repaired after photoreaction with furocoumarins (95) Cultured cells from xeroderma pigmentosum patients lack the capacity to repair photoadducts of furocoumarins in DNA (94-96). Yet, xeroderma pigmentosum patients appear to be particularly susceptible to the photocarcinogenic effect of PUVA treatment (97, 98) suggesting that the lack of DNA repair may be more important for photocarcinogenesis by furocoumarins

Besides direct interaction with DNA, photoactivated psoralens may also exert carcinogenic or tumorigenesis-promoting action by a variety of indirect mechanisms often loosely categorized as epigenetic mechanisms Animal data (Section 5 3.2 5 3 3) and epidemiologic evidence (Section 5.3.2 5 5 1) both indicate that psoralens plus UVA treatment significantly enhances the tumor incidences in animals or humans previously exposed to other known carcinogens, suggesting synergism or promotion of expression of preexisting dormant tumor cells. Strauss et al (85) and Kripke (99) have shown that PUVA treatment causes immunosuppression in humans and in mice In mice the PUVA treatment appears to suppress the specific immune response to contact-sensitizing anti-

gens, by inducing antigen-specific T-suppressor cells Roberts et al (84) have shown that transplanted tumor cells, which normally do not grow in immune-proficient mice, grow rapidly in PUVA-treated mice Thus, it appears that the immunosuppression brought about by PUVA treatment promotes dormant tumor cells or allows their growth and expression Consistent with the promotion of carcinogenesis by PUVA treatment, Gange (100) showed that PUVA treatment of hairless albino mice induces epidermal ornithine decarboxylase, the enzyme which is believed to play a role in tumor promotion by the typical promotor, 12-O-tetradecanoylphorbol-13-acetate (101). Another possible indirect mechanism through which PUVA treatment may induce carcinogenesis is the induction (activation) of latent tumor virus(es) Coohill and Moore (102) reported that treatment of Simian virus 40-transformed hamster kidney cells with PUVA leads to the induction of the SV40 virus, an oncogenic DNA tumor virus (103) In addition to the specific indirect mechanisms exemplified above, psoralens can photoconjugate with proteins leading to the inactivation of cellular enzymes (19) It is possible that random inhibition of some key enzymes can lead to disturbance of gene expression and loss of control of nuclear function. Granger et al (104, 105) have shown recently that PUVA treatment inactivates two of the three enzymic functions of DNA polymerase of Escherichia coli, whether this finding has any relevance to mammalian carcinogenesis remains to be studied The reactive oxygen species ($^1O_2$, $O_2^{-\cdot}$, $\cdot OH$, etc) produced by the interaction of triplet state psoralens with molecular oxygen (see Section 5 3 2 5 2 1) are also potentially carcinogenic They can cause direct DNA damage or can initiate membrane lipid peroxidation (19) leading to the generation of other potentially carcinogenic hydroperoxides and unsaturated aldehydes (see Vol. IIIA, Section 5.2 1.1 4 1.1) There is also some evidence that the singlet oxygen may be responsible for at least a part of the photomutagenic effect of 8-MOP (21, 106).

5 3 2 5 5 Environmental Significance

5 3 2 5.5.1 EPIDEMIOLOGIC EVIDENCE

Psoralen-containing herbal medicine, in combination with sunlight, have been used in the treatment of vitiligo in Egypt and in India since 1200-2000 B C (2) This form of photochemotherapy has gained widespread interest in modern medicine in the last decade, as oral psoralens plus UVA (PUVA) therapy has been found to be highly effective in the treatment of psoriasis, and mycosis fungoides as well as vitiligo Since 1974 several thousand patients have received PUVA treatment in clinical trials in Europe and the United States The safety of such therapy has been a subject of great concern as animal carcinogenicity data indicate increased incidence of skin cancer in mice given 8-MOP (topically or intraperitoneally) plus UVA Several prospective epidemiologic studies are currently being undertaken to investigate the potential excessive risk of skin cancer in patients undergoing PUVA therapy Considering the long latent period required for the induction of most human cancer, all of these studies can only be considered to be at the beginning stage at the time of this writing. The interim results of these studies are summarized in Table LXXIV It should be noted that some of the patients selected for PUVA therapy had prior exposure to other treatments (e g , therapeutic doses of X-ray, UVB, arsenical compounds, nitrogen mustard) that are themselves potentially carcinogenic These patients should be considered as a separate subset in the assessment of carcinogenic risk of PUVA therapy Moreover, the susceptibility of individual patients to the potential cancer risk of PUVA therapy is expected to be affected by a variety of host factors, such as degree of pigmentation, skin type and DNA-repair efficiency

Table LXXIV
Summary of Epidemiologic Studies on Induction of Skin Cancer in Patients Receiving PUVA Therapy[a]

Authors	Study group	Cumulative dose[b]	Follow-up	Results
Honigsmann et al. (107)	318 psoriasis patients in Innsbruck, Austria	660 J/cm^2	27 months	None of 246 patients with no prior exposure to other carcinogens developed cancer (cancer incidence of 0 08 expected) Four of 72 patients with prior exposure to arsenic developed skin cancer (an incidence higher than that of the general population).
Wilson (108)	300 psoriasis patients in Utah, USA	Not specified	4 years	Two patients had skin cancer, one of the patients had a previous history of skin cancer
Lassus et al. (109)	568 psoriasis patients in Helsinki, Finland	<500 J/cm^2	2 1 years (range, 1-3.6 years)	No significant increase in cancer risk in patients with or without prior exposure to other carcinogens
Roenigh and Caro (110)	631 psoriasis patients in various treatment centers in the U S A	1,000 J/cm^2	4 years	Ten patients (3 with prior exposure to ionizing radiation) developed skin tumors The incidence of skin cancer in patients with no prior carcinogen exposure was not higher than that of the general population

Table LXXIV (continued)

Authors	Study group	Cumulative dose[b]	Follow-up	Results
K. Wolff, unpublished results (cited in ref 111)	4,600 psoriasis patients in Europe	Not specified	6 years	Skin tumor incidence in 3,450 patients with no prior exposure to other carcinogens apparently lower than expected (0 03 vs 0 08) Incidence in 1,150 patients with prior exposure to carcinogen 5 times higher than the general population
Stern et al. (112), Stern (113)	1,380 psoriasis patients in 16 treatment centers in the U S A	1,600 J/cm^2	5 years	No significant increase in cancer risk in patients who had no prior carcinogen exposure and who received fewer than 80 PUVA treatments Significant increase (9 times higher) in risk of developing squamous cell carcinomas in patients with prior carcinogen exposure
Harrist et al (114)	230 vitiligo patients in India	Not specified	55 months	No evidence of increase in skin cancer risk
Henseler and Christopher (115)	381 psoriasis patients in the Federal Republic of Germany	Not specified	Since 1976	No evidence of increase in skin cancer risk

[a]With one exception, all the studies involved the use of 8-MOP plus UVA In the study of Harrist et al (115), 8-MOP, psoralen, and 4,5',8-trimethylpsoralen were used as the photosensitizer, and the patients were exposed to natural sunlight

[b]Cumulative PUVA expressed in terms of Joules/cm^2 The data shown were those calculated by Stern (113)

As the results summarized in Table LXXIV indicate, there appears to be no evidence, thus far, that PUVA therapy leads to increased incidence of skin cancers in patients who had no history of prior exposure to other carcinogenic treatment In fact, in some of these studies, the incidence of skin cancer was apparently slightly lower than those of the control or general population However, considering the short follow-up time in all these studies and the low cumulative dose in some of these studies, it would be premature to draw any conclusion at this point Even if PUVA therapy is carcinogenic, a rise in the skin cancer rates in these patients would not be expected until the 1990s

In contrast to patients with no prior carcinogen exposure, patients who had received treatments with X-ray, UVB or arsenicals for their skin disease, before being subjected to PUVA treatment, developed significantly higher incidences of skin cancer than the control or general population It is not clear to what extent the increase may be attributed to PUVA therapy. Reviewing the data from five different studies, Halprin et al (111) calculated that the maximum incidence of skin cancer in these patients was 2% per year This rate was not considered to be any higher than that expected from these patients without PUVA therapy (116) Stern (113), however, is of the opinion that prolonged exposure to high doses of PUVA can lead to substantial increase in the risk of developing skin cancer, particularly in patients previously treated with ionizing radiation In some patients the tumors appeared within 2 years after the start of PUVA therapy (112) After 4 years of follow-up, the patients were nine times more likely to develop squamous cell carcinomas than the control group ($p < 0.001$) and two times more likely to develop basal cell carcinomas He cautioned that the negative results seen in other studies could be due to shorter follow-up time and lower cumulative dose.

In addition to the above studies, there are a number of individual case reports of rapid induction of skin cancer in patients after receiving PUVA therapy In all these cases, the patients either had previous carcinogen exposure or were believed to be "intrinsically susceptible " Moller and Howitz (117) reported that a 43-year-old vitiligo patient, who had been previously treated with arsenic trioxide 8 years before, developed multiple basal cell carcinomas 2 months after receiving 8-MOP plus sunlight treatment for 91 days Verdich (118) detected multiple squamous cell carcinomas in mycosis fungoides patients who received PUVA therapy after exposure to electron beam or nitrogen mustard Stern et al (119) observed an increase in skin cancer rate in patients, with previous histories of very high skin exposure to tar and far-UV, within 2-7 years after the start of PUVA therapy. Reed and associates (97, 98) found that xeroderma pigmentosum patients (deficient in DNA-repair enzymes) could develop skin tumors within 1 month after starting PUVA therapy The extremely short induction time in these cases, together with animal data, led Bridges and Strauss (120) to hypothesize that PUVA may act as a "pseudopromotor," allowing the expression of preexistent neoplastic foci that would have otherwise remained dormant This hypothesis was supported by the observation by Strauss et al. (85) that PUVA therapy can reduce or abolish cell-mediated immunity in human skin The totality of the above epidemiologic evidence and case reports clearly indicate that, at least in certain types of patients, PUVA therapy can present a substantial increase in cancer risk Thus, it is imperative to conduct a risk-benefit analysis for each individual patient selected for PUVA therapy

5 3 2.5 5 2 ENVIRONMENTAL SOURCES, OCCURRENCE AND EXPOSURE

Furocoumarin compounds have a wide distribution in the plant kingdom (rev. in 1, 9, 32, 121, 122) Over twenty-eight naturally occurring deriva-

tives of psoralen or angelicin have been isolated from five major botanical families (a) Umbeliferae (parsley family) which includes parsley, parsnip, celery, Ammi majus (bishop's weed), Angelica archangelica, (b) Rutaceae (rue family) which includes bergamot fruits, lime, gas plants, cloves, common rue, (c) Leguminosae (legume family) which includes Psoralea corylifolia, Xanthoxylum flavum, (d) Moraceae (fig family) which includes Ficus caria (fig), and (e) Orchidacea (orchid family) They are present in the fruits, seeds, leaves or roots of the plants The most frequently found furocoumarins include 8-MOP (xanthotoxin, methoxsalen), 5-MOP (bergapten), isopimpinellin (5,8-dimethoxypsoralen), 4,5',8-trimethylpsoralen (trioxsalen), psoralen, imperatorin (8-isoamylenoxypsoralen) and angelicin (1, 121). The quantitative data on concentration are relatively scant Ivie et al (123) reported that parsnip (Pastinaca sativa) root contains an average of 26 ppm 8-MOP, 10 5 ppm psoralen and 3 2 ppm 5-MOP These compounds are not destroyed by usual cooking procedures (boiling or microwave) Carrot (Daucus carota) root contains negligible amounts (<0 3 ppm) of the above three compounds Trace amounts of psoralen, 5-MOP, 8-MOP and isopimpinellin were detected in several varieties of healthy celery (Apium graveolens), their total concentration did not exceed 1 3 ppm (124) In contrast to healthy celery, celery infected with fungus (e g , Sclerotinia sclerotiorum) produces high concentrations of psoralens, perhaps as phytoalexins, in response to disease infection Levels of psoralens (8-MOP, 4,5',8-trimethylpsoralen) in such cases can reach hundreds or perhaps thousands of parts per million (9, 33, 125)

Human exposure to furocoumarin compounds may originate from a variety of sources which include (a) photochemotherapeutic or medicinal uses, (b) application of cosmetics, (c) occupational exposure and (d) ingestion of psoralen-containing foodstuffs The use of psoralen-containing plant extracts in

photochemotherapy dates back to 1200-2000 B C when boiled extracts of the fruit of Ammi majus and Psoralea corylifolia was used in Egypt and India, respectively, along with sunlight, to treat vitiligo (2) The interest in this photochemotherapeutic method has surged since 1974 when Parrish et al (4) discovered that oral 8-MOP plus UVA (PUVA) therapy is highly effective in the treatment of psoriasis Therapy with PUVA has subsequently been found effective in the treatment of a number of other skin diseases which include vitiligo, mycosis fungoides (a form of malignant lymphoma which arises in the skin), polymorphous light eruption, palmar/plantar vesicular dermatitis and eczema (2) In some cases, PUVA therapy is the only effective treatment available Currently, over 7,500 patients in the United States and Europe are receiving PUVA therapy in clinical trials In addition to photochemotherapy, psoralen-containing plants are also used as folk medicine in some tropical countries Concoctions of roots of Clausena anista (wild Rutaceae), Ruta chalepensis, and Afraegle paniculata (Rutaceae) are used in the treatment of various diseases ranging from hemorrhoids to measles, scarlet fever, headaches and heart ailment in Guatemala, Nigeria and other tropical countries (126). These preparations contain 8-MOP, imperatorin, chalepin and marmesin (see 82)

Human exposure to furocoumarin compounds may occur through the use of some cosmetics products which contain oil of bergamot, a citrus extract with 5-MOP or bergapten content (6, 7). Oil of bergamot was and may still be used in perfumery in Eau de Cologne which is responsible for the so-called "Eau de Cologne dermatitis" (erythema and blistering of the skin) in some consumers (6) 5-Methoxypsoralen was or is perhaps still used as an active ingredient in many widely used suntan lotions, at concentrations of 12 to 50 μg/ml, to promote skin pigmentation (5) With the demonstration of photocarcinogenicity of 5-MOP in animals (see Section 5 3 2 5 3 2), it is obvious that such unnecessary exposure should be avoided

Besides cosmetics, dermal exposure to psoralens may also occur to professional fruit or vegetable pickers Celery workers in Massachusetts (USA) were reported to develop severe dermatitis after handling rotten celery It was subsequently established that extracts of celery rot ("pink rot") contained high amounts of 8-MOP and 4,5',8-trimethylpsoralen (33) which, in combination with sunlight, produced the characteristic phototoxic effects (see 34) *Occasional dermatitis has also been reported in lime pickers and in workers in* contact with fig leaves, both of which contain psoralens (1, 6)

The extent of human exposure to psoralens from food sources is not known and is dependent on the individual's diet Human foodstuffs known to contain psoralens include figs, parsley, parsnip roots and celery (1, 123, 124) Ivie et al (123) estimated that consumption of 0 1 kg of either raw or cooked parsnip root, which contains about 40 ppm psoralens, could expose an individual to 4 to 5 mg of psoralens Such exposure might be expected to cause pharmacological effects in some individuals (the prescribed dosage of 4,5',8-trimethylpsoralen in PUVA therapy for vitiligo is 5 mg) It is important to note that individuals consuming food which contain rotten celery or parsnip could have substantially higher exposure, because the concentration of psoralens in such products can reach hundreds and perhaps thousands of parts per million Persons in the high risk group (e g , xeroderma pigmentosum patients, persons with history of prior exposure to carcinogen) should minimize or avoid the consumption of such food products, particularly if they have a high exposure to sunlight.

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