Handbook of

PLANT *and* FUNGAL TOXICANTS

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J.P. Felix D'Mello

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12 Linear Furanocoumarins

M. M. Diawara and J. T. Trumble

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12.1 INTRODUCTION

The linear furanocoumarins are plant metabolites that have been used since ancient times to treat skin disorders such as psoriasis, conditions of skin depigmentation (such as leprosy, vitiligio, and leukoderma), mycosis fungoides, polymorphous dermatitis, and eczema.[1,2,3,4,5,6] Use of furanocoumarin-containing plants for medicinal purposes dates as far back as 2000 B.C.[4] The legume *Psorelea coryfolia* and the umbelliferous plant *Ammi majus*, for example, have been used since ancient times in North African civilizations, in the Hindu culture, and by the Chinese.[1,4,7] The increased use of the linear furanocoumarins in medicine has occasionally been linked, however, to higher incidence of skin cancer.[1,2,3,8] and other disorders such as sister chromatid exchanges, gene mutation, and chromosomal aberrations in humans.[9,10] Because these biosynthetic compounds are active against herbivores (including humans) and distributed among both wild and domesticated plant species,[8,11] they have garnered substantial scientific attention and thus have been the subject of a great deal of research in the last several decades. To date, the linear furanocoumarins have been characterized and identified in at least 15 plant families: Amaranthaceae, Compositae, Cyperaceae, Dipsacaceae, Fabaceae, Goodeniaceae, Guttiferae, Leguminosae, Moraceae, Pittosporaceae, Rosaceae, Rutaceae, Samydaceae, Solanaceae, and Umbelliferae (Apiaceae).[8,11,12]

From an evolutionary standpoint, the driving force(s) leading to the production of furanocoumarins has generated considerable speculation. In Apiaceae, the presence of furanocoumarins is suspected to have evolved in response to several physical and biological stress factors. Beier and Oertli,[13] Zangerl and Berenbaum,[14] and Zobel and Brown,[15] found that furanocoumarins were induced by UV light, suggesting that these chemicals may provide protection against mutagenic UV radiation. Zobel and associates[16] recently reported that a significant portion of these compounds can be exuded on the plant surface, where they may act as "sunscreens" (UV blockers).

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The furanocoumarins have been reported to be active against a wide variety of organisms. Inhibition of bacterial, fungal, as well as viral infections have been associated with increased concentrations of linear furanocoumarins in plants.[17,18,19,20,21,22,23] Recent studies also suggest the potential allelopathic role of furanocoumarins against nearby plants through retardation of germination and growth.[16,24] The furanocoumarins have also been shown to provide the chemical basis for feeding deterrency in *Apium graveolens* (celery) cultivars selected for, or found to have, resistance to insects[13,25,26,27] or, in other cases, they prevented feeding adaptation by specialist herbivores.[28] In addition, furanocoumarins have proven to be toxic to a broad spectrum of insects, suggesting that they may have evolved, at least in parts, in response to herbivory.[8,29,30] The allocation of a high proportion (>60%),[31] of the available complement of linear furanocoumarins to the outer leaves of celery (as opposed to the interior leaves, petioles, or roots), appears to support all of the previously mentioned rationales for the development and/or maintenance of these compounds (e.g., UV radiation, fungi, bacteria, herbivory), at least in celery.

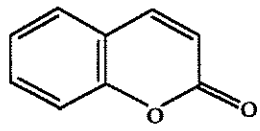
Due to the diverse array of documented biological activities (from medicinal to agricultural to ecological) of the linear furanocoumarins, reporting in detail on all of the available literature is not feasible. Therefore, this chapter has been organized into a selection of what we believe are key topic areas including the biosynthesis of linear furanocoumarins, their toxicity to a wide range of organisms, the factors affecting production and toxicity, metabolic detoxification, and the outlook for research on linear furanocoumarins.

12.2 BIOSYNTHESIS OF LINEAR FURANOCOUMARINS

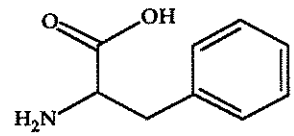
Like many of the more than 800 coumarin (**1**) derivatives that have been identified and characterized primarily from green plants,[8] the linear furanocoumarins are structurally derived from shikimic and chorismic acids via phenylalanine (**2**).[8,11] Their biosynthesis "begins with the transformation, catalyzed by phenylalanine ammonia lyase, of phenylalanine to *trans*-cinnamic acid (**3**)."[8] *Trans*-cinnamic acid is first ortho-hydroxylated to 2-hydroxycinnamic acid. Umbelliferone (or 7-hydroxycoumarin) (**4**), which is considered to be the mother compound of all linear furanocoumarins,[8,32] is derived when cinnamic acid is hydroxylated at the 4′ position to p-coumaric acid (**5**) and then at the 2′ position.

Several workers have reviewed the biosynthesis of coumarins.[8,11,32,33,34] The specific biosynthetic pathway varies among plant taxa.[11,32,33,34,35,36,37,38,39] In Rutaceae and Apiaceae, demethylsuberosin (**6**) is the coumarin widely reported to be the intermediate in the conversion of umbelliferone to marmesin (**7**) via prenylation.[8,32,39,40,41] This reaction is reportedly catalyzed by an enzyme referred to as dimethylallylpyrophosphate: umbelliferone dimethylallyl-transferase.[41] Ebel[42] recently proposed that marmesin loses its hydroxypropyl group via oxidation to yield psoralen (**8**); it is believed that this conversion is catalyzed by a P450 monooxygenase. Psoralen is hydroxymethylated to 5-methoxypsoralen (bergapten) (**9**), 8-methoxypsoralen (xanthotoxin) (**10**), or 5,8-methoxypsoralen (isopimpinellin) (**11**) in the presence of site-specific methylases.[8,32,37,38,40] Psoralen, bergapten, xanthotoxin, and isopimpinellin are the four linear furanocoumarins that have been widely characterized and identified in green plants. Except for some reported fungal toxicity,[23,43] isopimpinellin has generally not proved to have the photosensitizing properties of the other three linear furanocoumarins;[44] consequently, this compound has received less attention. In addition to the linear furanocoumarins, some plant species also have angular furanocoumarins such as angelicin (**12**), but the linear furanocoumarins usually constitute a higher proportion of their total furanocoumarins.[8]

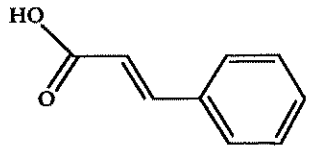
Murray and colleagues[11] reported that coumarin synthesis occurred primarily in younger leaves of legumes. In contrast, the buds and seeds of *Pastinaca sativa* (Apiaceae) had the highest concentrations of these compounds.[8] Leaves of celery have been shown to have much higher concentrations of furanocoumarins than petioles.[25,26,27,31,45] Similar results were found



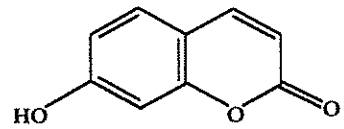
1. Coumarin



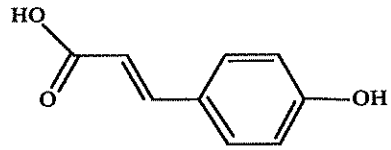
2. Phenyalanine



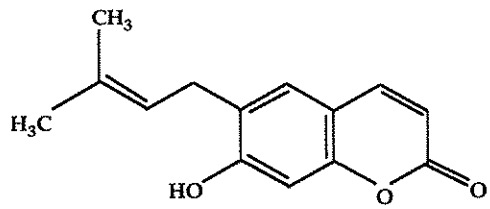
3. *trans*-Cinnamic acid



4. Umbelliferone



5. p-Coumaric acid



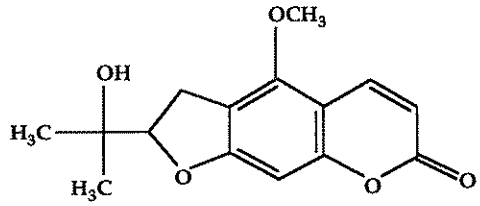
6. Demethylsuberosin

for other species of Apiacae and among Rutaceae; upper green leaves of *Ruta graveolens* contained more furanocoumarins than lower green leaves and green leaves of the plant had more furanocoumarins than yellow leaves, which also contained more than dry leaves.[46] Lime pulp was shown to have much less furanocoumarins than the peel.[47] Coumarins are, however, generally found in all plants parts.[8,31] As for their biosynthesis, the composition[21,25,31,45,46] as well as the localization[8,31,48] of linear furanocoumarins within specific plant structures vary among and within plant taxa. To date, it remains unclear whether the furanocoumarins are translocated between different parts of the plant either during or after synthesis; this has been previously discussed (see Diawara et al.[31]).

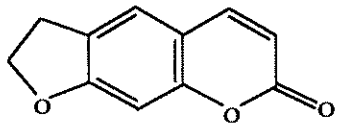
12.3 TOXICITY OF LINEAR FURANOCOUMARINS

12.3.1 Mechanism of Toxicity

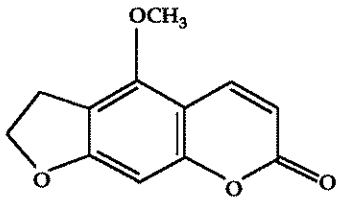
Like most other coumarins, the linear furanocoumarins are photoactivated plant biosynthetic compounds.[8,11,14,29,46,49,50,51,52] The effective ultraviolet A (UVA) wavelength range for this photoreactivity is between 320 and 400 nm;[11,52,53,54,55,56] the addition of UVB radiation does



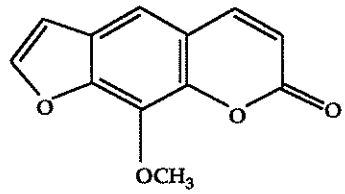
7. (+)-Marmesin



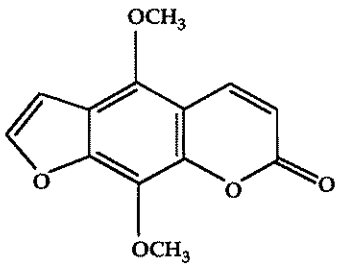
8. Psoralen



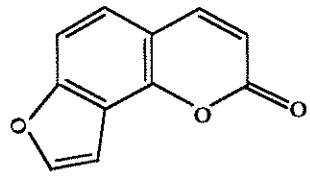
9. Bergapten



10. Xanthotoxin



11. Isopimpinellin



12. Angelicin

not seem to significantly affect activity.[57] Following absorption of a photon, the furanocoumarins form an excited triplet state which can react with molecules such as pyrimidine bases or with ground state oxygen, resulting in the formation of singlet oxygen or toxic oxyradicals such as superoxide anion radicals or hydroxyradicals.[8] All of these molecules can react with DNA, RNA, proteins, and lipids. The furanocoumarins have been shown to bind to the pyrimidine base of DNA.[53,58] This binding can result in formation of monoadducts, where furanocoumarins bind to a single pyrimidine base, and thus cause cytoplasmic mutations.[59] Recent studies by Laquerbe and colleagues[60] comparing data obtained from normal human lymphoblasts, rodents, and yeast cells suggest that the mutagenic potential of the monoadducts vs. diadducts (also called cross-links) may be species-specific. Diadducts, which cross-link complementary strands of DNA and prevent their separation,[52,61] are formed when UV-activated monoadducts react with additional pyrimidine bases in opposing strands of DNA.[50,58] The furanocoumarins have also been shown to inhibit enzymes by degrading protein constituents due to production of singlet oxygens and photobinding.[62,63] They can be photoactive with proteins and lipids in both oxygen-dependent and oxygen-independent reactions.[8] The DNA

binding ability of furanocoumarins and their reactivity with and ability to damage lipids, proteins, RNA, as well as DNA, constitute the basis for their toxicity to a wide range of organisms including mammals, insects and other arthropods, nematodes, viruses, and bacteria, and even plants and fungi.

Despite the vast volume of literature on toxicity of furanocoumarins, the actual mechanism(s) of their action at the molecular level is not well understood; for instance, it is unclear how pigment deposition in photodermatitis (see toxicity to mammals) is affected by cross-linking of DNA. However, it is known that activity of tyrosinase in pigment cells is stimulated by the compound trimethylpsoralen.[8] Xanthotoxin and other furanocoumarins inhibit both mammalian[64,65,66,67] and insect cytochrome P450s,[68,69] which are among the most important insect enzymes involved in metabolism of allelochemicals.[70] During a recent study designed to test coumarin, benzofuran, and 16 furanocoumarins for inhibitory effects on the insect *Manduca sexta* midgut cytochrome P450-catalyzed *O*-demethylation of *p*-nitroanisole, Neal and Wu[69] found that "all of the inhibitory furanocoumarins tested were mechanism-based irreversible inhibitors" and proposed that "the furanocoumarin is oxidized by cytochrome P450 at the double bond of the furan ring forming an unstable epoxide that can react with cytochrome P450."

12.3.2 Toxicity to Mammals

The most commonly reported manifestation of linear furanocoumarin toxicity to higher animals is phytophotodermatitis, an epidermal reaction symptomized by bullous eruptions, pigmentation, erythema, and potential vesicle formation.[8,32,51,71,72] These manifestations may be seen simply at the point of contact with high-furanocoumarin content material or over the entire body of an individual, depending on whether there was dermal contact only or an oral ingestion, respectively. Thus, furanocoumarins can reach the skin by direct contact or by blood-borne transfer to the skin following ingestion. Most humans show little reaction, or at least symptoms are not visible in the absence of UVA light exposure; thus the terms "photosensitization" and "photoactivation" are sometimes used to describe the reaction in the medical literature.

Scientific interest in phytophotodermatitis started in Europe in the 17th century. According to Brown,[32] a major step toward understanding the role of furanocoumarins in the causation of dermatitis in humans was a study reported in 1938 which demonstrated that synthetic pure bergapten and xanthotoxin induced the effects of furanocoumarin-containing plants when directly applied to the skin; this was later confirmed by several workers in Italy and the U.S.

Crop plants that have been reportedly associated with human health hazards as a result of high contents of linear furanocoumarins belong to four plant families: Apiaceae (anise, caraway, carrot, celery, chervil, dill, fennel, lovage, parsley, and parsnip), Moraceae (figs), Rutaceae (grapefruit, lemon, lime, and orange), and Solanaceae (potato).[11,12,8,45,47,73,74,75,76] Of these, celery has been among the most extensively studied because of the occasionally high concentrations of linear furanocoumarins in the plant and risks of phytophotodermatitis associated with harvesting, handling, or ingestion.[45,75,76,77,78,79] These hazards apparently are even more serious when plants are infected with disease-causing pathogens.[17,20]

The chemicals responsible for crop plant-induced phytophotodermatitis have been known since the mid-1970s to be the linear furanocoumarins psoralen, 5-methoxypsoralen (bergapten), and 8-methoxypsoralen (xanthotoxin).[13,18,77] The threshold level for toxicity to humans was determined to be 18 μg g^{-1} fresh weight for development of acute dermatitis,[77] and 7–9 μg g^{-1} for repeated or chronic exposures.[78] This may vary for different body regions.[80]

The furanocoumarins have been shown to be both mutagenic and carcinogenic.[45,55,60,81,82,83,84,85,86,87,88] *In vitro* bioassays with bacterial and mammalian cells demonstrated that these chemicals are lethal and carcinogenic.[81] Beier[47] recently reported the death of a 45-year-old woman due to complications from severe burns that the patient received in a tanning salon while under psoralen medication. The World Health Organization recognizes these

psoralens as causal agents of skin cancer in humans.[89] Further, these compounds can interact with other medications: furanocoumarins have been demonstrated to induce hypothermic activity and anticonvulsive activity in combination with several drugs when injected into rats.[90] These compounds have also been shown to deter feeding by grazing animals.[91]

Like other DNA-damaging agents, the furanocoumarins hypersensitize several rare hereditary and tumor-prone disorders in humans, including Fanconi anemia (see Bredberg et al.[92] and references therein). It is also well established that they can cause conjunctival hyperemia and decreased lacrimation[10] and increase plasma melatonin levels by inhibiting metabolism of this compound (see Garde et al.[93] and Rosselli et al.[94] and references therein). Thus, potential effects of these plant compounds on mammals are not only diverse, but occasionally debilitating or even lethal.

12.3.3 Toxicity to Insects, Plants, Fungi, Bacteria, and Viruses

Berenbaum[8] recently reviewed the toxicity of the furanocoumarins to insects, plants, fungi, bacteria, and viruses; the reader is referred to her article for details on earlier studies. In reference to furanocoumarin toxicity to plants, Berenbaum did suggest the involvement of furanocoumarins in regulation of seed germination because of their localization in seed coats in many species, and their absence from the endosperm. More recent reports have confirmed that the furanocoumarins can exert allelopathic effects against nearby plants through retardation of germination and growth.[16,95] Kupidlowska and co-workers[24] suggested that this allelopathy may be due to the furanocoumarins' ability to retard mitosis, to decrease oxygen uptake by meristematic cells, and to cause structural and physiological alterations in the mitochondrial matrix, as observed in *Allium cepa*. New advances in activity of furanocoumarins against fungi, bacteria, and viruses that attack plants include reports on their toxicity to several species of fungus,[20,21,22,23] to the yeast *Saccharomyces cerevisiae*,[96,97] and the green alga *Chlamydomonas reinhardtii*.[98]

Studies on furanocoumarin-containing plants and their relationships to herbivores have provided excellent model systems of plant-insect interactions. A prospective case of coevolution based on these interactions has been described.[8] Since Berenbaum's[8] report on toxicity of furanocoumarins to arthopods, other studies have confirmed that these chemicals cause loss of fitness through delayed developmental times or growth reductions,[30,99,100] and in some cases have demonstrated activity as feeding deterrents.[30]

Some recent studies suggest that some chemical precursors of linear furanocoumarins may be more toxic to fungi than the linear furanocoumarins. Afek et al.[23] observed that marmesin, an immediate precursor to the linear furanocoumarins, played a more important role than psoralen, bergapten, xanthotoxin, and isopimpinellin in celery resistance to pathogenic agents during storage. If this pattern is repeated for other plant pathogens, it would provide substantial evidence regarding the driving forces responsible for the evolution of the linear furanocoumarins. Also, such a pattern would suggest that the linear furanocoumarins provide additional advantages that outweigh those of the precursors such as stability, storability, lack of autotoxicity, etc.

One such possible advantage of linear furanocoumarins over the precursors is an increased activity against herbivores. In tests with the coumarin derivatives ostruthin and osthol, no effects on insect survival or growth were found.[99] However, there is ample evidence that the linear furanocoumarins can effectively limit insect herbivory. Although psoralen has been reported to be the most photodynamically active compound among furanocoumarins according to earlier studies,[1,54,102,103] a growing number of recent studies[30,66,67,69] suggest that xanthotoxin is more toxic.

12.4 FACTORS AFFECTING LINEAR FURANOCOUMARIN PRODUCTION AND TOXICITY

Ultraviolet A radiation is certainly a major factor determining the toxicity of linear furanocoumarins to most organisms.[8,11,14,29,49,50] In addition to UV light, a number of other factors can increase the toxic effects of furanocoumarins. Exposure to fungal, bacterial, and viral agents has been associated with increased plant furanocoumarin-content, and consequently increased human and animal health hazards. Infection of celery with the disease-causing pathogens such as *Sclerotinia sclerotiorum* or *Fusarium oxysporum* resulted in induction of a new furanocoumarin and/or an increased production of existing ones compared with healthy plants.[13,17,20,22] This initially led scientists to suggest that only diseased celery contained linear furanocoumarins (perhaps produced by the pathogen, rather than the plant), but it has now been established that healthy celery contains furanocoumarins and can cause photodermatitis.[45,75,76,78] Higher production of linear furanocoumarins in plants infected with pathogenic agents compared with healthy ones has also been documented in parsnip,[19] parsley,[104] citrus, and fig leaves.[74] Because of the increased concentrations of linear furanocoumarins in plants following exposure to pathogens, these compounds have also been referred to as phytoalexins.[105] Increased production of furanocoumarins in plants as a response to environmental stress has been confirmed at the molecular level (see Berenbaum[8] and references therein).

A variety of anthropogenic stresses also have the potential to induce furanocoumarin production in plants. Experiments by Dercks and colleagues,[106] showed significant increases (more than 500%) in linear furanocoumarin production in celery as a result of a 4 h exposure to acidic fogs. Beier and Oertli[13] and Beier and Nigg[12] demonstrated that application of copper sulfate served as a general elicitor in celery. Mechanical damage occurring during harvesting and storage has also been shown to increase concentrations from about 2 $\mu g\ g^{-1}$ to 95 $\mu g\ g^{-1}$.[56] Other factors such as temperature,[13,23] cold storage practices,[56] and growing conditions such as light and nutrient regime,[8,14] have all been implicated in increased furanocoumarin production in plants. Purohit and colleagues[107] recently demonstrated that tissue culturing induced higher production of xanthotoxin in *Ammi majus* than any other technique previously described. Thus, human activities related to production, storage, and pollutant generation can substantially increase the hazards associated with furanocoumarins.

In oral medicinal use, the potential carcinogenicity and toxicity of furanocoumarins to humans can be influenced by the environmental factors related to the ingestion of the compounds. When used in skin phototherapy, the eating habits of patients under psoralen treatment have been shown to impact treatment efficacy. During a study designed to determine the impact of food consumption vs. fasting conditions on the pharmacokinetics of bergapten, Ehrsson et al.[6] observed that administration of bergapten tablets with food greatly increased the bioavailability of the medication. Dietary omega-3 and omega-6 fatty acid sources decreased inflammatory responses and allowed relatively rapid repair of psoralen-induced cutaneous toxicity, but these lipids did not affect psoralen-induced tumorigenesis.[108]

12.5 METABOLIC DETOXIFICATION OF LINEAR FURANOCOUMARINS

The ability of certain insect species to specialize on furanocoumarin-containing plants[8] sparked a series of studies designed to document the metabolic detoxification mechanisms of these compounds by arthropods. The chief metabolic detoxification pathway appears to be through mixed function oxidases (MFOs), a series of enzymatic reactions which allow organisms to break down complex molecules into smaller ones that are more easily degraded or excreted.[109]

In one early study, Brattsten et al.[110] reported that plant secondary compounds increased MFOs in larvae of *Spodoptera eridania*. Both the xanthotoxin-tolerant *Papilio polyxenes* and the xanthotoxin-susceptible *Spodoptera frugiperda* were found to metabolize this chemical by oxidative cleavage of the furan ring, but the rate of the metabolism was much higher in *P. polyxenes*.[111] Ingestion of xanthotoxin has also been shown to increase enzymatic activity in larvae of *Trichoplusia ni*[112] and *Depressaria pastinacella*.[113] Several studies[113,114,115,116] indicate involvement of cytochrome P450 in excretion and metabolism of furanocoumarins. The observation that the furanocoumarins deter feeding by herbivores might be linked to the fact that a slow feeding rate would allow herbivores to better metabolize toxic chemicals by either detoxifying them in the midgut prior to absorption[109,111,115,117] or by efficiently excreting them.[117]

However, such metabolic detoxification of plant defensive compounds by herbivores reportedly is not without costs. For example, nicotine can be detoxified by P450s in *Spodoptera eridania* larvae, but concentrations of 0.05% dietary nicotine have proven to significantly reduce relative growth rates and the efficiency of food conversion.[118] In a series of experiments conducted with increasing concentrations of proteins, Berenbaum and Zangerl[115] recently evaluated the cost of cytochrome P450-mediated detoxification of xanthotoxin by *Depressaria pastinacella*, an insect restricted to feeding on plants in two genera of Apiaceae. They noted a progressive decline in growth rates with decreasing protein levels, but silk spinning and detoxification rates were only affected with 0% protein in the artificial diet. Much higher (almost threefold) metabolism of xanthotoxin was also induced when there was no protein in the diet; this, however, resulted in nearly 80% reduction in growth rates. As reported by the authors, these results suggest that "xanthotoxin detoxification capacity is maintained at the expense of growth."

Using natural concentrations co-occurring in fruits of *Pastinaca sativa*, Berenbaum and co-workers[119] observed a synergism between six different furanocoumarins in their toxicity to the insect *Helicoverpa zea*. This synergistic interaction was confirmed by subsequent studies designed to test co-occurring natural concentrations of *Pastinaca sativa* furanocoumarins for toxicity against the insect *Papilio polyxenes*. Rates of cytochrome P450-mediated metabolism were significantly reduced when equimolar concentrations of the linear furanocoumarins xanthotoxin and bergapten and the angular furanocoumarin angelicin were combined.[120] Conversely, using much higher artificial dietary concentrations of these chemicals based rather on LC_{50} values, Diawara and colleagues[30] found that the combination of bergapten and xanthotoxin produced an additive effect on *Spodoptera exigua* mortality, but combining psoralen with either bergapten, xanthotoxin, or both resulted in significant antagonistic effects.

In mammals, the toxicity of furanocoumarins is primarily reduced through quick excretion after ingestion[3] and metabolic breakdown into nonphototoxic compounds.[121] For instance, the phototoxic 4,8-dimethylpsoralen is degraded into the nonphototoxic 4,8-dimethyl-5′ carboxypsoralen in both humans and mice.[121] The skin photosensitizing property of furanocoumarins is believed to be due to psoralen ring system;[54] therefore, the furanocoumarins lacking methyl substituents also lack potency to photosensitize.[8] Ma and colleagues,[116] recently observed that the nature of substituents on the benzene ring determines the efficiency of metabolism of furanocoumarins by larvae of *Papilio polyxenes*. Presence of nonmetabolizable angular furanocoumarins also proved to inhibit metabolism of linear furanocoumarins,[116] and this may increase the latter's toxicity.

Plant extracts have also been shown to detoxify linear furanocoumarins. Rizzi and colleagues[122] reported *in vitro* antimutagenetic effect against xanthotoxin-induced photomutagenesis to *Salmonella typhimurium* from unspecified compounds in extracts and chromatographic fractions of the bark of the plant *Uncaria tomentosa*.

12.6 OUTLOOK FOR LINEAR FURANOCOUMARIN RESEARCH

The furanocoumarins will certainly continue to receive attention in skin therapy due to their proven medicinal value. In addition to the use of furanocoumarins in the treatment of psoriasis, xanthotoxin likely will continue to be a successful skin photochemotherapy agent for use against several other skin disorders including T-cell lymphoma. Because psoralen derivatives are used in 1) nucleic acid research, 2) in human immunodeficiency syndrome (AIDS) research as possible treatments of this condition or its related complications (see Danheiser and Trova[123] and references therein) and 3) in cancer research, more studies can be expected in the near future. In addition, new psoralen analogs[124,125] and derivatives[87,126,127] are being synthesized and tested for potential use in skin phototherapy in an attempt to reduce the phototoxic side effects associated with the use of psoralens. An increasing number of studies are reporting on the repair of psoralen plus UV-induced DNA damage.[97,128,129]

The concerns of potential human and animal health hazards associated with furanocoumarins can be significantly reduced by continued research efforts to better understand the mechanisms of their toxicity and localization of these compounds in specific plant parts and structures. For instance, it has recently been observed that over 60% of the linear furanocoumarins in celery occurs in leaves on outer plant petioles.[31] These highly localized concentrations of furanocoumarins are of considerable importance given a trend in marketing intact organically-grown celery (leaves not trimmed) rather than the more common "topped" celery (outer petioles and most leaves removed) marketed by most commercial producers.[106] Consequently, potential hazards to consumers could be greatly minimized by avoiding contact with these plant parts. Finally, we expect more studies on photochemotherapy techniques, such as the newly described method where xanthotoxin is administered in a "relaxing" bath, which may be more effective and have fewer side effects than standard ingestion therapy.[130]

Currently, little is known about the biological activity of the immediate chemical precursors of the furanocoumarins. Elucidation of the biological effects of the precursors seems likely to provide insight into the evolution of these compounds. To date, much of the research on toxicity of furanocoumarins has focused on testing of single compounds, particularly 8-methoxypsoralen (xanthotoxin). Continued efforts to study chemical combinations that occur naturally in plants under natural conditions would also further our understanding of their activity. Some of these studies will require investigation at the molecular level.

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REFERENCES

1. Musajo, L. and Rodighiero G., The skin-photosensitizing furocoumarins, *Experientia*, 18, 153, 1962.
2. Van Scott, E. J., Therapy of psoriasis. *J. Am. Med. Assoc.*, 235, 197, 1976.
3. Scott, B. R., Pathak, M. A., and Mohn, G. R., Molecular and genetic basis of furanocoumarin reactions, *Mutat. Res.*, 39, 29, 1976.
4. Pathak, M. A. and Fitzpatrick, T. B., The evolution of photochemotherapy with psoralens and UVA (PUVA): 2000 BC to 1992 AD, *Photochem. Photobiol. B*, 14, 3, 1992.

5. Chadwick, C. A., Potten, C. S., Cohen, A. J., and Young, A. R., The time of onset and duration of 5-methoxypsoralen photochomoprotection from UVR-induced DNA damage in human skin, *Br. J. Dermatol.*, 131, 483, 1994.
6. Ehrsson, H., Wallin, I., Ros, AM., Eksborg, S., and Berg, M., Food-induced increase in bioavailability of 5-methoxypsoralen, *Eur. J. Clin. Pharrnacol.*, 46, 375, 1994.
7. Pathak, M. A. and Fitzpatrick, T. B., Relationship of molecular configuration to the activity of furocoumarins which increase the cutaneous responses following long wave ultraviolet radiation, *J. Invest. Dermatol.*, 32, 255, 1959a.
8. Berenbaum, M. R., Coumarins, in *Herbivores Their Interactions with Secondary Plant Metabolites, The Chemical Participants, Vol. I,* Rosenthal, G. A. and Berenbaum, M. R., Eds., Academic Press, New York, 1991, chap. 6.
9. Bredberg, A. and Lambert, B., Induction of SCE by DNA cross-links in human fibroblasts exposed to 8-MOP and UVA irradiation, *Mutat. Res.*, 118, 191, 1983.
10. Calzavara-Pinton, P., Carlino, A., Manfedi, E., Semeraro, F., Zane, C., and Panfilis, G., Ocular side effects of PLTVA-treated patients refusing eye sun protection, *Acta. Derm. Venereol. Suppl. Stockh.*, 186, 164, 1994.
11. Murray, R. D. H., Mendez, J., and Brown, S. A., *The Natural Coumarins: Occurrence, Chemistry and Biochemistry.* J. Wiley and Sons, Chichester, UK., 1982.
12. Beier, R. C. and Nigg, H. N., Toxicity of Naturally occurring chemicals in food, in *Foodborne Disease Handbook*, 3, Hui, Y. H., Richard Gorham, J., Murrell, K. D., and Cliver, D. O., Eds., Marcel Decker, New York, 1994.
13. Beier, R. C. and Oertli, E. H., Psoralen and other linear furanocoumarins as phytoalexins in celery, *Phytochemistry*, 22, 2595, 1983.
14. Zangerl, A. R. and Berenbaum, M. R., Furanocoumarins in wild parsnip: effects of photosynthetically active radiation, ultraviolet light, and nutrients, *Ecology*, 68, 516, 1987.
15. Zobel, A. and Brown, S., Influence of low-intensity ultraviolet radiation on extrusion of furanocoumarins to the leaf surface, *J. Chem. Ecol.*, 19, 939, 1993.
16. Zobel, A. M., Crellin, J., Brown, S. A., and Glowniak, K., Concentration of furanocoumarins under stress conditions and their histological localization, in *International Symposium on Natural Phenols in Plants, Vol. II Acta Hort.* 381, Giebel, M., Treutter, D., and Feucht, W., Eds., Weihenstephan, 1994, pages 510-516.
17. Scheel, L. D., Perone, V. B., Larkin, R. L., and Kupel, R. E., The isolation and characterization of two phototoxic furanocoumarins (psoralens) from diseased celery, *Biochemistry*, 2, 1127, 1963.
18. Ashwood-Smith, M. J., Ceska, O., and Chaudhary, S. K., Mechanism of photosensitivity reactions to diseased celery, *Br. Med. J.*, 290, 1249, 1985.
19. Desjardins, A. E., Spencer, G. F., Plattner, R. D., and Beremand, M. N., Furanocoumarin phytoalexins, trichothecene toxins, and infection of *Pastinaca sativa* by *Fusarium sporotrichioides*, *Phytopathol.*, 79, 170, 1989.
20. Heath-Pagliuso, S., Matlin, S. A., Fang, N., Thompson, R. H., and Rappaport, L., Stimulation of furanocoumarin accumulation in celery and celeriac tissues by *Fusarium oxysporum* F sp. *apii.*, *Phytochemistry*, 31, 2683, 1992.
21. McCloud, E., Berenbaum, M., and Tuveson, R. W., Furanocoumarin content and phototoxicity of rough lemon (*Citrus jambhiri*) foliage exposed to enhanced UVB irradiation, *J. Chem. Ecol.*, 18, 1125, 1992.
22. Afek, U., Aharoni, N., Carmeli, S., and Roiser, L., A suggestion for new mechanism of celery resistance to pathogens, *Acta Hort.*, 342, 357, 1993.
23. Afek, U., Aharoni, N., and Carmeli, S., The involvement of marmesin in celery resistance to pathogens during storage and the effect of temperature on its concentration, *Phytopathology*, 85, 711, 1995.
24. Kupidlowska, E., Kowalec, M., Sulkowski, G., and Zobel, A., The effect of coumarins on root elongation and ultrastructure of meristematic cell protoplast, *Annals Botany*, 73, 525, 1994a.
25. Trumble, J. T., Dercks, W., Quiros, C. F., and Beier, R. C., Host plant resistance and linear furanocoumarin contents of *Apium* accessions, *J. Econ. Entomol.*, 83, 519, 1990.
26. Diawara, M. M., Trumble, J. T., Quiros, C. F., and Millar, J. G., Resistance to *Spodoptera exigua* in *Apium prostratum*, *Entomol. Exp. Appl.*, 64, 125, 1992.

27. Diawara, M. M., Trumble, J. T., and Quiros, C. F., Linear furanocoumarins of three celery breeding lines: implications for integrated pest management, *J. Agric. Food Chem.*, 41, 819, 1993.
28. Berenbaum, M. R., Zangerl, A. R., and Lee, K., Chemical barriers to adaptation by a specialist herbivore, *Oecologia*, 80, 501, 1989.
29. Trumble, J. T., Moar, W. T. Brewer, M. J., and Carson, W. G., Impact of UV radiation on activity of linear furanocoumarins and *Bacillus thuringiensis* var *kurstaki* against *Spodoptera exigua*: implications for tritrophic interactions, *J. Chem. Ecol.*, 17, 973, 1991.
30. Diawara, M., Trumble, J., White, K., Carson, W., and Martinez, L., Toxicity of linear furanocoumarins to *Spodoptera exigua*: evidence for antagonistic interactions, *J. Chem. Ecol.* 19, 2473, 1993.
31. Diawara, M. M., Trumble, J. T., Quiros, C. F., and Hansen, R., Implications of distribution of linear furanocoumarins within celery, *J. Agric. Food Chem.*, 43, 723, 1995.
32. Brown, S, A., Biochemistry of the coumarins, in *Recent Advances in Phytochemistry Vol. 12*, Swain, T., Harborne, J. B., and Sumere, C. H., Eds., Plenum Press, New York, 1978. pages 249–286.
33. Edwards, K. G. and Stoker, J. R., Biosynthesis of coumarin: the isomerization stage, *Phytochemistry*, 6, 655, 1967.
34. Floss, H. G., Biosynthesis of furanocoumarins, in *Recent Advances in Phytochemistry Vol. 4*, Runeckles, V. C. and Watkin, J. E., Eds., Appleton-Century-Crofts, New York, 1972, pages 143–164.
35. Brown, S. A., Towers, G. H. N., and Wright, D., Biosynthesis of the coumarins. Tracer studies on coumarin formation in *Hierochloe odorata and Melilotus officinalis, Can. J. Biochem. Physiol.*, 38, 143, 1960.
36. Floss, H. G. and Mothes, U., Zur Biosynthese von Furanocumarinen in *Pimpinella magna*, *Z. Naturforsch.* 19b: 770, 1964.
37. Caporale, G., Dall'Acqua, F., Capozzi, A., Marciani, S., and Crocco, R., Studies on the biosynthesis of some furocoumarins present in *Ruta graveolens, Z. Naturforsch.*, 26b, 1256, 1971.
38. Caporale, G., Dall'Acqua, F., Capozzi, A. and Marciani, S., Studies on the biosynthesis of furocoumarins in the leaves of *Ficus carica* L., *Ann. Chim. (Rome)*, 62, 536, 1972.
39. Games, D. E. and James, D. H., The biosynthesis of the coumarins of *Angelica archangelica, Phytochemistry*, 11, 868, 1972.
40. Brown, S. A. and Steck, W., 7-Demethylsuberosin and osthenol as intermediates in furanocoumarin biosynthe*sis, Phytochemistry, 12*, 1315, 1973.
41. Ellis, B. E. and Brown, S. A., Isolation of dimethylallylpyrophosphate: umbelliferone dimethylallyltransferase from *Ruta graveolens, Can. J. Biochem.*, 52, 734, 1974.
42. Ebel, J., Phytoalexin synthesis: the biochemical analysis of the induction process, *Ann. Rev. Phytopathol.*, 24, 235, 1986.
43. Martin, J. T., Baker, E. A., and Byrde, R. J. W., The fungitoxicities of cuticular and cellular components of citrus lime leaves, *Ann. Appl. Biol.*, 57, 491, 1966.
44. Ashwood-Smith, M., Poulton, G. A., and Liu, M., Photobiological activity of 5,7-dimethoxycoumarin, *Experientia*, 39, 262, 1983.
45. Berkley, S. F., Hightower, A. W., Beier, R. C., Fleming, D. W., Brokopp, C. D., Ivie, G. W., and Broome, C. V., Dermatitis in grocery workers associated with high natural concentrations of furanocoumarins in celery, *Ann. Intern. Med.*, 105, 351, 1986.
46. Zobel, A. M. and Brown, S. A., Psoralens in senescing leaves of *Ruta graveolens*, *J. Chem Ecol.*, 17, 1801, 1991.
47. Beier, R. C., Natural pesticides and bioactive components in foods, *Rev. Environ. Contam. Toxicol.*, 113, 47, 1990.
48. Zobel, A. M. and March R., Autofluorescence reveals differential histological localizations of furanocoumarins in fruits of some Umbelliferae and Leguminosae, *Annalas Botany*, 71, 251, 1993.
49. Dall'Acqua, F., Marciani, S., and Rodighiero, G., The action spectrum of xanthotoxin and bergapten for the photoreaction with native DNA, *Z. Naturforsch.*, 24b, 667, 1969.

50. Cole, R. S., Light-induced cross-linking of DNA in the presence of a furocoumarin (psoralen). Studies with phase l, *Escherichia coli* and mouse leukemia cells, *Biochim. Biophys. Acta*, 217, 30, 1970.
51. Parrish, J. A., Fitzpatrick, T. B., Tanenbaum, L., and Pathak, M. A., Photochemotherapy of psoriasis with oral methoxsalen and longwave ultraviolet light, *New Engl. J. Med.*, 291, 1207, 1974.
52. Ashwood-Smith, M. J. and Grant, E., Conversion of psoralen DNA monoadducts in *E. coli* to interstrand DNA cross links by near UV light (320-360 nm): Inability of angelicin to form cross links, in vivo, *Experientia*, 33, 384, 1977.
53. Musajo, L., Rodighiero, G., and Dall'Acqua, F., Evidence of a photoreaction of the photosensitizing furocoumarins with DNA and with pyrimidine nucleosides and nucleotides, *Experientia*, 21, 24, 1965.
54. Pathak, M. A., Worden, L. R., and Kaufman, K. D., Effect of structural alterations on the photosensitizing potency of furocoumarins (psoralens) and related compounds, *J. Invest. Dermatol.*, 48, 103, 1967.
55. Igali, S., Bridges, B. A., Ashwood-Smith, M. J., and Scott, B. R., Mutagenesis in *Escherichia coli* IV. Photosensitization to near ultraviolet light by 8-methoxypsoralen, *Mutat. Res.*, 9, 21, 1970.
56. Chaudhary, S. K., Ceska, O., Warrington, P. J., and Ashwood-Smith, M. J., Increased furanocoumarin content of celery during storage, *J. Agric. Food Chem.*, 33, 1153, 1985.
57. Granstein, R., Morison, W., and Kripke, L., Carcinogenicity of combined ultraviolet B radiation and psoralen plus ultraviolet A irradiation treatment of mice, *Photoderm. Photoimm. Photomed.*, 9, 198, 1993.
58. Dall'Acqua, F., Marciani, S., Ciavatta, L., and Rodighiero, G., Formation of interstrand crosslinkings in the photoreactions between furocoumarins and DNA, *Z. Naturforsch.*, 26b, 561, 1971.
59. Rodighiero, G. and Dall' Acqua, F., Biochemical and medical aspects of psoralens, *Photochem. Photobiol.*, 24, 647, 1976.
60. Laquerbe, A., Moustacchi, E., and Papadopoulo, D., Genotoxic potential of psoralen cross-links versus monoadducts in normal human lymphoblasts, *Mutat. Res.*, 346, 173, 1994.
61. Cole, R. S. and Zusman, D., Sedimentation properties of phage DNA molecules containing light-induced psoralen cross-links, *Biochim. Biophys. Acta*, 224, 660, 1970.
62. Veronese, F. M., Schiavon, O., Bevilacqua, R., Bordin, F., and Rodighiero, G., Photoinactivation of enzymes by linear and angular furocoumarins, *Photochem. Photobiol.*, 36, 25, 1982.
63. Wagner, S., White, R., Wolf, L., Capman, J., Robinette, D., Lawlor, T., and Dodd, R., Determination of residual 4'-aminomethyl-4,5',8-trimethylpsoralen and mutagenicity testing following psoralen plus UVA treatment of platelet suspensions, *Photochem. Photobiol.*, 57, 819, 1993.
64. Letteron, P., Descator, V., Larrey, D., Tinel, M., Geneve, J., and Pessayre, D., Inactivation and induction of cytochrome P450 by various psoralen derivatives in rats, *J. Pharmacol. Exp. Ther.*, 238, 685, 1986.
65. Mays, D. C., Hilliard, J. B., Wong, D. D., and Gerber, D. E., Activation of 8-methoxypsoralen by cytochrome P450, *Biochem. Pharmacol.*, 38, 1647, 1989.
66. Maenpaa, J., Sigusch, H., Raunio, H., Syngelma, T., Vuorela, P., Vuorela, H., and Pelkonen, O., Differential inhibition of coumarin 7-hydroxylase activity in mouse and human liver microsomes, *Biochem. Pharmacol.*, 45, 1035, 1993.
67. Maenpaa, J., Juvonen, R., Raunio, H., Rautio, A., and Pelkonen, O., Metabolic interactions of methoxsalen and coumarin in humans and mice, *Biochem. Pharma.*, 48, 1363, 1994.
68. Zumwalt, J. G. and Neal, J. J., Cytochrome P450 from Papilio polyxenes: adaptations to host plant allelochemicals, *Comp. Biochem. Physiol.*, 106C, 111, 1993.
69. Neal, J. and Wu, D., Inhibition of insect cytochromes P450 by furanocoumarins, *Pest. Biochem. Phys.*, 50, 43, 1994.
70. Brattsten, L. B., Metabolic defenses against plant allelochemicals, in *Herbivores: Their Interactions with Secondary Plant Metabolites*, 1, Rosenthal, G. A. and Berenbaum, M. R., Eds., Academic Press, New York, 1991, 175.
71. Giese, A. C., Photosensitization by natural pigments, *Photophysiology*, 6, 77, 1971.

72. Musajo, L. and Rodighiero, G., Mode of photosensitizing action of furocoumarins, *Photophysiology*, 7, 115, 1972.
73. Legrain, P. M. and Barthe, R., Dermite des mains et des avant-bras chez un ramasseur de celeris, *Bull. Fr. Dermatol. Syphiligr.*, 33, 662, 1926.
74. Zaynoun, S. T., Alfimos, B., G., Ali, L. A., Tenekjian, K. K., Khalidi, U., and Kurban, A. K., *Ficus carica*: isolation and quantification of the photoactive components, *Contact Dermatitis*, 11, 21, 1984.
75. Ljunggren, B., Severe phototoxic burn following celery ingestion, *Arch. Dermat.*, 126, 1334, 1990.
76. Finkelstein, E., Afek, U., Gross, E., Aharoni, N., Rosenberg, L., and Havely, S., An outbreak of phytophotodermatitis due to celery, *Internat. J. Dermat.*, 33, 116, 1994.
77. Austad, J. and Kavli, G., Phototoxic dermatitis caused by celery infected by *Sclerotinia sclerotiorum, Contact Dermatitis*, 9, 448, 1983.
78. Seligman, P. J., Mathias, C. G., O'Malley, M. A., Beier, R. C., Fehrs, L. J., Serril, W. S., and Halperin, W. E., Photodermatitis from celery among grocery store workers, *Arch Dermatol.*, 123, 1478, 1987.
79. Fleming, D., Dermatitis among grocery workers associated with high natural concentrations of furanocoumarins in celery, *Allergy Proc.*, 11, 125, 1990.
80. Kuusilehto, A., Lehtinen, R., and Jansen, C., Comparison of the minimal phototoxic dose in topical 4,5′ 8-trimethylpsoralen PUVA treatment of Caucasian skin and of oral mucous membrane, *Acta. Derm. Venereol.*, 70, 508, 1990.
81. Ashwood-Smith, M. J., Poulton, G. A., Barker, M., and Mildenberger, M., 5-methoxypsoralen, an ingredient in several suntan preparations, has lethal, mutagenic and cleistogenic properties, *Nature*, 285, 407, 1980.
82. Bauluz, C., Paramio, J. M., and de-Vidania, R., Further studies on the lethal and mutagenic effects of 8-methoxypsoralen-induced lesions on plasmid DNA, *Cell. Mol. Biol.*, 37, 481, 1991
83. Boesen, J. J., Stuivenberg, S., Thyssens, C. H., Panneman, H., Darroudi, F., Lohman, P. H., and Simons, J. W., Stress response induced by DNA damage leads to specific, delayed and untargeted mutations, *Mol. Gen. Genet.*, 234, 217, 1992.
84. Mathews, M. M., Comparative studies of lethal photosensitization of *Sarcina lutea* by 8-methoxypsoralen and by toluidine blue, *J. Bacteriol.*, 85, 322, 1963.
85. Roelandts, R., Mutagenicity and carcinogenicity of methoxypsoralen plus UV-A, *Arch. Dermatol.*, 120, 662, 1984.
86. Papadopoulo, D., and Moustacchi, E., Mutagenic effects photoinduced in normal human lymphoblasts by a monofunctional pyridopsoralen in comparison to 8-methoxypsoralen, *Mutat. Res.*, 245, 259, 1990.
87. Yang, S. C., Lin, J. G., Chion, C. C., Chen, L. Y., and Yang, J. L., Mutation specificity of 8-methoxypsoralen plus two doses of UVA radiation in the hprt gene in diploid human fibroblasts, *Carcinogenesis*, 15, 201, 1994.
88. Young, A. R., Photocarcinogenicity of psoralens used in PUVA treatment-present status in mouse and man, *J. Photochem. Photobiol. B-Biol.*, 6, 237, 1990.
89. International Agency for Research on Skin Cancer, *Evaluation of the Carcinogenic Risk of Chemicals to Humans, Supplement 4: Chemicals, Industrial Process and Industries Associated with Cancer in Humans*, International Agency for Research on Cancer, Lyon, 1983.
90. Chandhoke, N. and Ghatak, G. J., Pharmacological investigations of angelicin: a tranquillosedative and anticonvulsant agent, *Indian J. Med. Res.*, 63, 833, 1975.
91. Ashkenazy, D., Kashman, Y., Nyksa, A. and Friedman, J., Furanocoumarins in shoots of *Pituranthos triadiatus* (Umbelliferae) as protectants against grazing by hyrax (Procaviidae: *Procavia capensis syriaca*), *J. Chem. Ecol.*, 11, 231, 1985.
92. Bredberg, A., Sandor, Z., and Brant, M., Mutational response of Fanconi anemia cells to shuttle vector site-specific psoralen cross-links, *Carcinogenesis*, 16, 555, 1995.
93. Garde, E., Micic, S., Knudsen, K., Angelo, H., and Wulf, H., 8-Methoxypsoralen increases daytime plasma melatonin levels in humans through inhibition of metabolism, *Photochem. Photobiol.*, 60, 475, 1994.

94. Rosselli, F., Duchaud, E., Averbeck, D., and Moustacchi, E., Comparison of the effects of DNA topoisomerase inhibitors on lymphoblasts from normal and Fanconi anemia donors, *Mutat. Res.*, 325, 137, 1994.
95. Kupidlowska, E., Dobrzynska, K., Parys, E., and Zobel, A., Effect of coumarin and xanthotoxin on mitochondrial structure, oxygen uptake, an succinate dehydrogenase activity in onion root cells, *J. Chem. Ecol.*, 20, 2471, 1994b.
96. Saffran, W., Greenberg, R., Thaler-Scheer, M., and Jones, M., Single strand and double strand DNA damage-induced reciprocal recombination in yeast. Dependence on nucleotide excision repair and RADI recombination, *Nuc. Acids Res.*, 22, 2823, 1994.
97. Dardalhon, M. and Averbeck, D., Pulsed-field gel electrophoresis analysis of the repair of psoralen plus LJVA induced DNA photoadducts in *Saccharomyces cerevisiae, Mutat. Res.*, 336, 49, 1995.
98. Schimmer, O. and Kuhne, I., Mutagenic compounds in an extract from *Rutae Herba* (*Ruta graveolens* L.), *Mutat. Res.* 243, 57, 1990.
99. Hadacek, F., Muller, C., Wemer, A., Greger, H., and Proksch, P., Analysis, isolation and insecticidal activity of linear furanocoumarins and other coumarin derivatives from *Peucedanum* (Apiaceae: Apioideae), *J. Chem. Ecol.*, 20, 2035, 1994.
100. Brewer, M., Meade, T., and Trumble, J., Development of insecticide-resistant nd susceptible *Spodoptera exigua* (Lepidoptera: Noctuidae) exposed to furanocoumarins found in celery, *Environ. Entomol.*, 24, 392, 1995.
101. Berenbaum, M., Toxicity of a furanocoumarin to armyworms: a case of biosynthetic escape from insect herbivores, *Science*, 201, 532, 1978.
102. Musajo, L., Rodighiero, G., and Caporale, G., L'activité photodynamique des coumarines naturelles, *Bull. Soc. Chim. Biol.*, 36, 1213, 1954.
103. Pathak, M. A. and Fitzpatrick, T. B., Bioassay of natural and synthetic furocoumarins (psoralens), *J. Invest. Dermatol.*, 32: 509, 1959b.
104. Knogge, W., Kombrink, E., Schmelzer, E., Hahlbrock, K., Occurrence of phytoalexins and other putative defense-related substances in uninfected parsley plants, *Planta*, 171, 279, 1987.
105. Paxton, J., A new working definition of the term "phytoalexin," *Plant Dis.*, 64, 734, 1980.
106. Dercks, W., Trumble, J. T., and Winter, C., Impact of atmospheric pollution on linear furanocoumarin content in celery, *J. Chem. Ecol.*, 16, 443, 1990.
107. Purohit, M., Pande, D., Datta, A., and Srivastava, P., Enhanced xanthotoxin content in regenerating cultures of *Ammi majus* and micropropagation, *Planta. Med.*, 61, 481, 1995.
108. Yen, A., Black, H., and Tschen, J., Effect of dietary omega-3 and omega-6 fatty acid sources on PUVA-induced cutaneous toxicity and tumorigenesis in the hairless mouse, *Arch. Dermatol. Res.*, 286, 331, 1994.
109. Ivie, G. W., Bull, D. L., Beier, R. C., Pryor, N. W., and Oertli, E. H., Metabolic detoxification: mechanism of insect resistance to plant psoralens, *Science*, 221, 374, 1983.
110. Brattsten, L. B., Wilkinson, C. F., and Eisner, T., Herbivore-plant interactions: mixed function oxidases and secondary plant substances, *Science*, 196, 1349, 1977.
111. Bull, D. L., Ivie, G. W., Beier, R. C., Pryor, N. W., and Oertli, E. H., Fate of photosensitizing furanocoumarins in tolerant and sensitive insects, *J. Chem. Ecol.*, 10, 893, 1984.
112. Lee, K. and Berenbaum, M. R., Action of antioxidant enzymes and cytochrome P450 monooxygenases in the cabbage looper in response to plant phototoxins, *Arch. Insect Biochem. Physiol.*, 10, 151, 1989.
113. Nitao, J. K., Enzymatic adaptation in a specialist herbivore for feeding on furanocoumarin-containing plants, *Ecology*, 70, 629, 1989.
114. Cohen, M. B., Berenbaum, M. R., and Schuler, M. A., Induction of Cytochrome P450-mediated detoxification of xanthotoxin in the black swallowtail, *J. Chem. Ecol.*, 15, 404, 1989.
115. Berenbaum, M. and Zangerl, A., Costs of inducible defense: protein limitation, growth, and detoxification in parsnip webworm, *Ecology*, 75, 2311, 1994.
116. Ma, R., Cohen, M., Berenbaum, M., and Schuler, M., Black swallowtail (Papilio polyxenes) alleles encode cytochrome P450s that selectively metabolize linear furanocoumarins, *Arch. Biochem. Biophys.*, 310, 332, 1994.
117. Nitao, J. K., Metabolism and excretion of the furanocoumarin xanthotoxin by parsnip webworm, *Depressaria pastinacella*, *J. Chem. Ecol.*, 16, 417, 1990.

118. Cresswell, J. E., Merritt, S. Z., and Martin, M. M., The effect of dietary nicotine on the allocation of assimilated food to energy metabolism and growth of fourth instar larvae of the southern armyworm, *Spodoptera eridania* (Lepidoptera: Noctuidae), *Oecologia (Berlin)*, 89, 449, 1992.
119. Berenbaum, M. R., Nitao, J. K., and Zangerl, A. R., Adaptive significance of furanocoumarin diversity in *Pastinaca sativa* (Apiaceae), *J. Chem. Ecol.*, 17, 207, 1991.
120. Berenbaum, M. and Zangerl, A., Furanocoumarin metabolism in *Papilio polyxenes*: biochemistry, genetic variability, and ecological significance, *Oecologia*, 95, 370, 1993.
121. Mandula, B. B., Pathak, M. A., and Dudek, G., Photochemotherapy: identification of a metabolite of 4,5',8-trimethylpsoralen, *Science*, 193, 1131, 1976.
122. Rizzi, R., Re, F., Bianchi, A., De-Feo, V., de-Simone, F., Bianchi, L., and Stivala, L. A., Mutagenic and antimutagenic activities of *Uncaria tomentosa* and its extracts, *J. Ethnopharmacol.*, 38, 63, 1993.
123. Danheiser, R. and Trova, M., Synthesis of linear furocoumarins via photochemical aromatic annulation strategy. An efficient total synthesis of bergapten, *Synlett.*, 573, 1995.
124. Vedaldi, D., Caffieri, S., Miolo, G., Dall'Acqua, F., Baccichetti, F., Guiotto, A., Benetollo, F., Bombieri, G., Recchia, G., and Cristofolini, M., Azapsoralens: new potential photochemotherapeutic agents for psoriasis, *Farrnaco.*, 46, 1407, 1991.
125. Caffieri, S., Moor, A., Beijersbergen van Henegouwen, M., Acgua, F., Guiotto, A., Chilin, A., and Rodighlero, P., Difurocoumarins, psoralen analogs: synthesis and DNA photobinding, *Chem. Sci.*, 50, 1257, 1995.
126. Baccichetti, F., Bordin, F., Simonato, M., Toniolo, L., Marzano, C., Rodighiero, P., Chillin, A., and Carlassare, F., Photobiological activity of certain new methylazapsoralens, *Farrnaco.*, 47, 1529, 1992.
127. Zagotto, G., Gia, O., Baccicbetti, F., Uriarte, E., and Palumbo, M., Synthesis and photobiological properties of 4-hydroxymethyl-4'-methylpsoralen derivatives, *Photochem. Photobiol.*, 58, 485, 1993.
128. Spielmann, H., Dwyer, T., Sastry, S., Hearst, J., and Wemmer, D., DNA Structural reorganization upon conversion of a psoralen furan-side monoadduct to an interstrand cross-link: Implications for DNA repair, *Proc. Natl. Acad. Sci. U.S.A.*, *92*, 2345, 1995.
129. Wang, G. and Galzer, P., Altered repair of targeted psoralen photoadducts in the context of an oligonucleopeptide-mediated triple helix, *J. Biol. Chem.*, 270, 22595, 1995.
130. Kerscher, M., Lehmann, P., and Plewig, G., PUVA bath therapy. Indications and practical implementation, *Hautarzt*, 45, 526, 1994.