Synthesis, structure-activity relationships and photochemical studies of novel coumarins, dihydropsoralens and dihydroangelicins as photo-activated agonists of the psoralen receptor.

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Synthesis, Structure-Activity Relationships And Photochemical Studies of Novel Coumarins, Dihydropsoralens and Dihydroangelicins as Photo-Activated Agonists of the Psoralen Receptor

By

Marilyn Schulte Whittemore

A Dissertation

Presented to the Graduate Committee

of Lehigh University

In Candidacy for the Degree of

Doctor of Philosophy

In Pharmaceutical Chemistry

Lehigh University

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CERTIFICATE OF PRESENTATION

This dissertation is respectfully submitted to the Graduate Faculty of Lehigh University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Marilyn Schulte Whittemore

CERTIFICATE OF APPROVAL

Approved and recommended for acceptance as a dissertation in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 7, 1998

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ABSTRACT

Psoralens are tricyclic photochemically active compounds used in the treatment of psoriasis, vitiligo and eczema. Psoralens can cross the cell membrane, intercalate and photo-link nuclear DNA by forming mono and diadducts. Carcinoma induction has been implicated as a delayed side effect of Psoralen Ultra Violet A (PUVA) therapy in recent clinical studies, with psoralen-DNA cross-links as the molecular promoter. An alternative mechanism of action not involving DNA is also responsible for the biological activity. Laskin identified specific high affinity receptors for psoralens on the cell membrane of epidermal cells.

Development of psoralens that are incapable of cross-link formation or have reduced ability to cross the cell membrane may increase cell surface effects and possibly reduce the mutagenic and carcinogenic effects of PUVA therapy. Novel synthetic routes were developed to the 5'-halomethyl-4,8-dimethyl-4',5'-dihydropsoralens, the 4'-halomethyl-4,8-dimethyl-4',5'-dihydropsoralens, and the 5'-iodomethyl-4-methyl-4',5'-dihydroangelicins, as precursors to amine substitution. The quaternary pyridinium derivatives and the hydrophilic amino derivatives were designed to minimize membrane penetration by increasing water solubility. These compounds are incapable of forming DNA crosslinks because they lack a reactive double bond in the furan ring. The halogenated psoralens showed activity with IC_{50}'s in the 0.5 - 10 uM range in the PAM 212 keratinocyte cell line bioassay. The high biological activity of the quaternary compounds, with IC_{50}'s from 1-300 uM, suggests that a biological event occurs at the cell

surface. It is not yet known if the amino derivatives, with IC_{50}'s from $3.5 \cdot 10^{-3}$ - 2 IC_{50}'s *u*M, act at the cell surface or through DNA intercalation. The angelicins and their derivatives showed a greater reduction in biological activity than either of the psoralens. The sensitivity of the receptor to small changes in the psoralen structure allowed structure-activity relationships to be determined. Bulky substituents on the 3-position reduced biological activity.

The 5'-X-mercurimethyl psoralens showed impressive dark activity, with IC_{50}'s in the 4-30 *u*M range, which were increased two fold by light activation. Their toxicity did not depend on receptor binding. The antifungal, antibacterial and antituberculosis properties of these mercurated compounds were established, with the 2-iodomercurimethyl dihydrobenzofuran showing 99% inhibition of growth against *Mycobacterium tuberculosis*.

GENERAL INTRODUCTION

Both naturally occurring and synthetic psoralens in combination with UVA light are useful in the treatment of psoriasis, eczema and vitiligo. Recent years have seen their application to cancer (T cell lymphoma), autoimmune diseases and viral inhibition. Clinicians use such terms as PUVA (psoralen ultraviolet A radiation), photopheresis, photochemotherapy, and photosensitization to designate these more specialized therapeutic applications of psoralens. Though considered the most effective treatment for some diseases, clinicians worry that psoralens cause genetic mutations induced by DNA damage.

Several limitations exist in PUVA therapy. One of the major concerns about PUVA therapy is possible DNA damage from monoadduct or diadduct formation with DNA. Genetic mutations can result because cellular level repair processes for bifunctional DNA cross-links are highly error prone and mutagenesis or carcinogenic events may result (Smith, 1988). This represents a significant post-treatment risk of cancer induction. A recent report in The New England Journal of Medicine notes that, although PUVA therapy is currently the most effective treatment for many skin diseases, increased risk of subsequent squamous cell skin cancer, up to a five fold increase risk for melanomas and for irregular pigmented skin lesions (Stern, 1997) all arise. The possibility of melanoma formation increases with the numbers of treatments, the length of treatments and the length of time post treatment, with a delay of up to 15 years before incidence of cancer. If left untreated, melanoma can be fatal, as it was for two of the nine patients in a recent study (Stern, 1997). Responding to fears of patients and doctors, the

National Psoriasis Foundation evaluated the risks of long term effects of PUVA therapy and guidelines were offered for further evaluation of long term effects of PUVA therapy (Morison, 1998). Dihydropsoralens and angelicins, capable of only monoadduct formation, potentially have reduced mutagenic properties and may decrease the long-term negative effects of PUVA therapy.

Concern for reducing the toxic side effects of PUVA therapy has presented an interesting challenge to the synthetic chemist to maintain the effectiveness of the psoralen light activation while minimizing the potentially mutagenic/carcinogenic properties. Previous studies from our laboratories have shown that psoralens can retain phototherapeutic activity and can anchor to a target membrane receptor even after major structural alteration to the furan ring (Heindel, 1991). Work done by Van Dongen and Heindel showed that linear psoralens, which can only form monoadducts, could kill malignant cells when activated by UV light (Heindel, 1991). In their series of compounds, dihydrofurocoumarins were synthesized and were shown to be effective. Further work showed that charged molecules, such as quaternized psoralens have photoactivated therapeutic properties. It was shown that neither cross-linking minimized by the removal of the second double bond on the furan ring portion of the psoralen moiety nor membrane penetration prevented by the presence of a charge on the molecule is required for biological activity. Increasing the water solubility of the molecule by forming quaternary compounds or substituting an amine functionality limits passage through the cell membrane, resulting in increased cell surface effects, thereby selectively targeting the psoralen receptor. Compounds synthesized were evaluated by Laskin at the University of Medicine and Dentistry of New Jersey Robert Wood Johnson Medical

School using an assay for photoactivated inhibition of cell growth in PAM 212 keratinocytes, a model cell line employed as a test system to indicate the concentration to effect an epidermal cytotoxicity of 50% (the IC_{50}). Samples generated were submitted for biological evaluation against algae and fungi at Buckman Laboratories. Furthermore, the unique mercurated psoralens prepared herein showed effectiveness against *A. niger*, a fungi, and *Chlorella vulgaris*, an algae, without light activation. Evaluation by the National Institute of Allergy and Infectious Diseases showed impressive activity against *Mycobacterium tuberculosis* by a psoralen mimic, the 2-iodomethyl-2,3-dihydrobenzofuran. This compound has been selected for further screening. Additionally, several of the 5'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralens and other iodinated compounds evidenced modest activity.

The ultimate goal of this work was to develop novel synthetic pathways to unique psoralens whose structural targets are selected by consideration of earlier structure-activity studies and the desire to probe and expand new structure activity principles in the therapeutic photobiology of psoralens. An improved synthesis for the 5'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralens using different electrophiles to promote ring closure and design of a synthesis for the novel 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen were the primary synthetic goals. The search for more active analogs which would selectively target the specific psoralen receptor in the PAM keratinocyte cell line focused on the amine substitution of the halogenated precursors. Compounds, some with 3-substitution, were generated to analyze trends in substitution to allow structure-activity relationships to be deduced. Guidance in the appropriate biological studies to be performed was

provided by our pharmacological collaborator whose own mechanistic investigations have been essential to the molecular targets chosen to date.

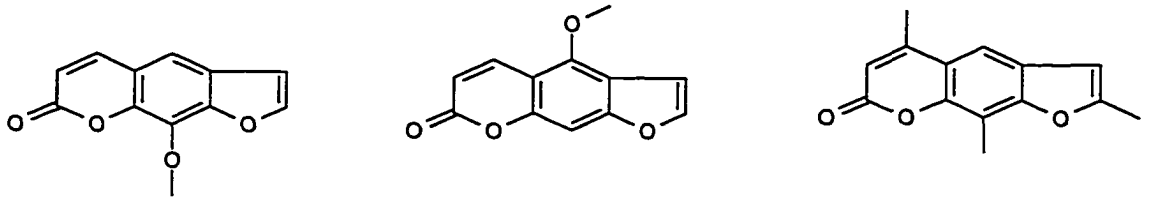
BACKGROUND

HISTORICAL

Psoralens and angelicins (both naturally occurring and synthetic) are tricyclic heterocycles, useful in combination with UVA light, in the treatment of psoriasis, vitiligo, eczema and mycosis fungoides. Psoralens, linear furocoumarins from plant extracts of *Umbelliferae, Rutaceae, Leguminosae* and *Mimosae*, have been used since ancient times both topically and internally for treatment of skin conditions (Pathak, 1967). Angelicins, angular furocoumarins, are obtained from roots of the family *Umbelliferae* and *Pimpinella* (Bordin, 1991). In the 1940's Fahmy and Abu-Shady began to study these crude botanical extracts. Subsequently there were publications on the isolation of the compounds (Fahmy, 1947), clinical studies (El-Mofty, 1950) and toxicity studies (Elvi, 1950). For fifty years, research has been ongoing in photochemistry and photobiology of psoralens, including seminal work done by American scientists Fitzpatrick, Kaufman and Gasparro and also by European scientists such as Rodighiero and Dall'Acqua. Kaufman synthesized 8-methoxypsoralen and 4,5',8-trimethylpsoralen, with the latter proving to be more active than the naturally occurring product in producing hyperpigmentation without skin inflammation. Initial UVA irradiations were at 360 nm but later work showed the best therapeutic dose to be 340 nm (Cripps, 1982) and the 334-346 nm range proved to be optimal for 8-methoxypsoralen-inhibition of DNA synthesis in human lymphocytes (Gasparro, 1984).

CURRENT CLINICALS AND NEW DEVELOPMENTS

Pharmaceutically utilized psoralens include (Figure 1):



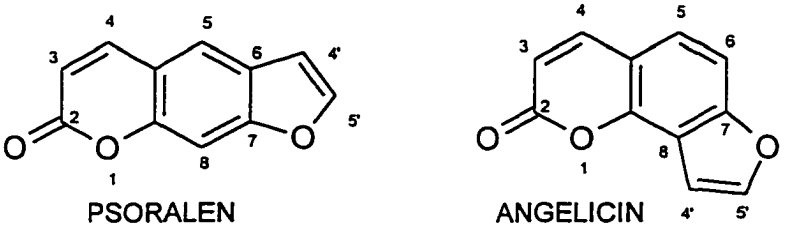
8-METHOXYPSORALEN
8-MOP

5-METHOXYPSORALEN
5-MOP

4,5',8-TRIMETHYLPSORALEN
TMP

Figure 1 Commercial psoralens

TMP and 8-MOP are available in the United States from ICN Pharmaceuticals only by prescription. These clinically used psoralens are lipophilic, non-nitrogenous, small uncharged molecules with limited water solubility. All contain two photoactivatable functionalities, the pyrone double bond and the furan double bond, which absorb in the UV range. The numbering system for psoralens and angelicins is presented below (Figure 2).



PSORALEN

ANGELICIN

Figure 2 Psoralen and angelicin structures

Many psoralen analogs have been synthesized for evaluation of antitumor, antipsoriatic, anticancer and antiviral properties. Psoralens have proven to be effective research tools for the elucidation of nucleic acid structure and function and considerable research has been done on psoralen-nucleic acid photochemistry and photobiology

(Cimino, 1985). The antiviral properties of psoralens have been utilized in the irradiation of blood products in the presence of amino-halogenated psoralens (Goodrich, 1996). The varying properties of psoralens acting on different cell types have been exploited in the treatment of many diseases. In the same tissue type, in treatment of psoriasis and vitiligo, rapid keratinocyte growth is suppressed by PUVA while proliferation in melanocytes is stimulated (Parrish, 1974 and Nordlund, 1982). The tanning effects of PUVA therapy are the result of stimulating melanogenesis and pigment transfer to keratinocytes (Pathak, 1962 and Rosdahl, 1980). In the treatment of T-cell lymphoma, feedback mechanisms regulating tumor growth are affected when the patient's psoralen containing blood was irradiated extracorporally and then returned to the patient generating a high immune response (Edelson, 1983 and 1987). Psoralens have broad pharmacology.

MODES OF ACTION

DNA INTERCALATION

Several modes of action for psoralens have been investigated. Initially believed to be the sole mode of action, intercalation of DNA by the planar molecules, followed by UVA light activation results in the formation of mono and diadducts. It has been shown that psoralens, as highly lipophilic agents, penetrate the cell's membrane and intercalate into the DNA. Psoralens then photo cross link the double helix though bis-cyclobutane dimers at the 3,4-double bond of the pyrone ring and the 4',5'-double bond of the furan ring in the psoralen molecule to the double bonds of the pyrimidine bases in the DNA (Figure 3 and Figure 4) (Hearst, 1989). Furan-side mono adducts form in the greatest

yield, with a second UVA photon causing formation of a cycloaddition product at the pyrone portion of the psoralen (Kanne, 1982). When the DNA is unable to uncoil and serve as a template for gene expression, the target cell cannot reproduce. It was initially believed that cells were incapable of repairing DNA crosslinks, now believed to be repairable, but evidence shows monoadducts can induce extensive cell damage (Smith, 1988). Angelicins and dihydropsoralens bind after intercalation and photoactivation to form only monoadducts (Figure 3).

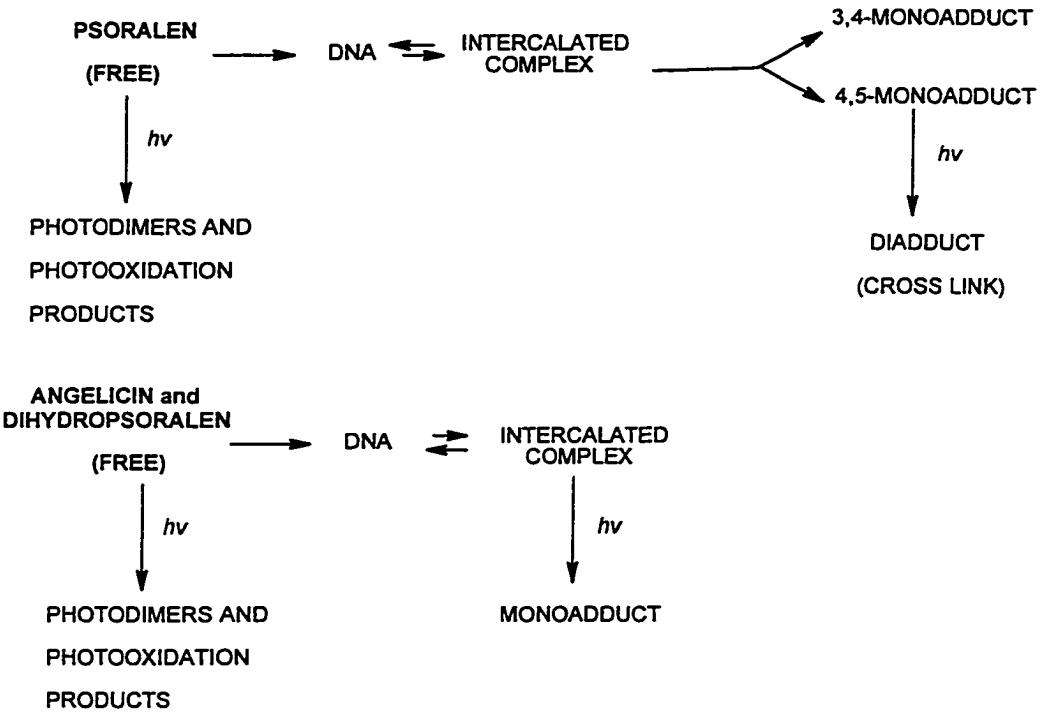


Figure 3 Ability to form di and monoadducts

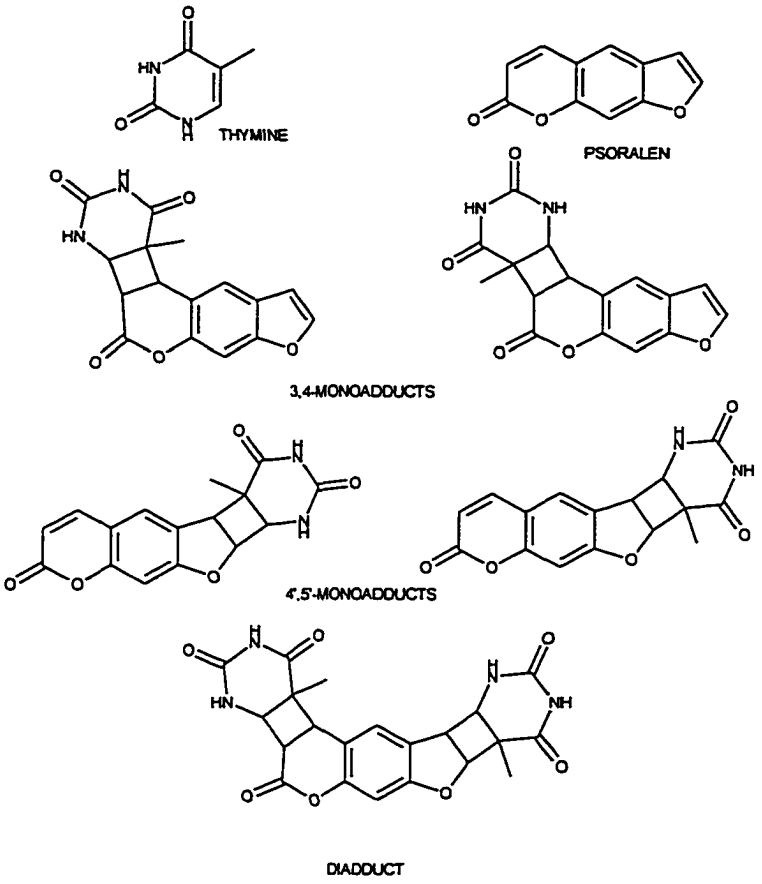


Figure 4 Structures of psoralen, thymine, monoadducts and diadduct

DNA binding between base pairs has been verified by: isolation of photoadducts formed after intercalation, fluorescence anisotropy and nuclear magnetic resonance (NMR) studies (Straub, 1981, Dall'Acqua, 1978 and Romer, 1985). DNA-psoralen binding constants may be measured by partitioning a psoralen between chambers separated by a semi-permeable membrane, where one chamber contains a buffered DNA solution without psoralen and the other chamber contains psoralen in a buffered solution with no DNA. After the system has equilibrated, the concentration of the psoralen in the DNA chamber indicates the affinity of the psoralen molecule for DNA (Isaacs, 1977). The extent of intercalation between DNA and psoralen can be measured

spectrophotometrically by detecting changes in absorbance or fluorescence spectra of the psoralen after DNA intercalation (Dall'Acqua, 1981). Data may be presented in Scatchard plots of Fraction Bound/Nucleotide plotted against Fraction Bound (Scatchard, 1949). Often such plots show two linear regions, possibly an indication of two binding sites, a strong and a weak, with each having a K (Computational Binding Constant). Another possible interpretation is that once a single psoralen molecule binds, the binding of additional psoralens may be affected (Gasparro, 1988).

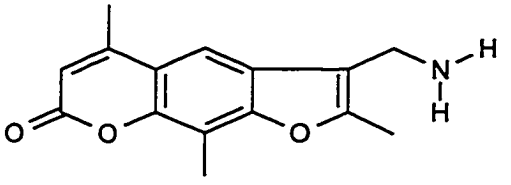
Initial testing showed an inability of angelicins to photosensitize skin, and the compounds were assumed to be inactive or poorly active (Musajo, 1962). It was later found that the angelicins showed DNA photobinding though to a lesser extent than psoralens (Rodighiero, 1969). Angelicins were shown to inhibit DNA and RNA synthesis for Erlich ascites tumor cells, also to a lesser extent than psoralens. However, it is now known that even monofunctional DNA adducts can result in cell lethality (Bordin, 1975). Angelicins, initially assumed to be less active because they can only form such monoadducts with DNA and generate no crosslinks, have actually shown strong antiproliferative effects with reduced erythema (Bordin, 1991). Development of angelicins has been pursued, in the hope of reducing PUVA side effects such as mutagenicity and the risk of skin cancer (Cole, 1973). The addition of methyl functionalities to the angelicin ring system – such as the 4,6,4'- trimethylangelicin has increased DNA photobinding accompanied by greater antiproliferative activity (Rodighiero, 1969 and 1970).

Structure-activity studies have shown that increasing lipophilicity (such as by the addition of methyl groups to psoralens and angelicins) increases DNA binding.

Increasing water solubility by addition of charged moieties or polar groups greatly affects DNA binding (Guiotto, 1984). The negative electric field around the DNA, from the anionic phosphate groups, helps prevent binding of electron-rich species (Matthew, 1984). Of the currently used clinical psoralens, 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP) and 4,5',8-trimethylpsoralen (TMP) all have similar water solubilities (38, 5, and 0.6 *u*g/ml) but yet have different DNA binding constants (770, 2800 and 7700 M^{-1}) (Cimino, 1985 and Kanne, 1982). Even the adduct forming ability of these compounds differs: 8-MOP having 75% adducts occuring at the 4',5'- double bond (26% cross links and 46% furan adducts) with only 19% adducts at the 3,4 - double bond; TMP having 39% cross links, 55% furan adducts and 2.5% adducts at the 3,4 - double bond (Cimino, 1985). Adding a methyl group to the 4-position in the pyrone ring of TMP reduces the tendency to form pyrone-side adducts.

A cationic analog, 4'-aminomethyl-4,5',8-trimethylpsoralen (AMT), is the most water soluble of the compounds studied, is protinated at physiological pH, and has a high DNA binding constant (Figure 5). Nevertheless, it forms the fewest cross-links, giving strong evidence that not all intercalations are at crosslinking sites (Wu, 1984). Alas, there is no obvious rational for correlating the variety of physical constants and biological (or clinical) behavior. The types and numbers of photoadducts formed may result from conformation restrictions such as DNA winding and packing around chromosomal proteins. The presence of a bulky substituent may inhibit the steric orientation necessary for crosslink formation or membrane penetration. Even in such a simple functional platform as the psoralen nucleus, modest changes in what is attached around the

periphery are well known to profoundly alter the biological activity. Psoralen structure-activity relationships are still in the empirical, data-gathering stage.



4'-AMINOMETHYL-4,5',8-TRIMETHYLPSORALEN
AMT

Figure 5 AMT structure

A SPECIFIC PSORALEN RECEPTOR

In an alternative mechanism of action to that which presumes a DNA target, a 22,000 Da protein present in psoralen-sensitive cells has been found to be a specific binding site for psoralens and to affect cell growth regulation (Laskin, 1985; and Yurkow and Laskin, 1987). On responsive cells, biological responses are triggered when psoralens bind to specific receptors, are activated by UV light, and then intracellular signals are promoted. The signals interact with growth factor receptors such as epidermal growth factor (EGF) receptor, a 170,000 Da transmembrane glycoprotein that possesses tyrosine-specific protein kinase activity when activated by EGF, a low molecular weight polypeptide. After a psoralen binds to its receptor, EGF binding is prevented. Activation of the EGF receptors and the accompanying tyrosine kinase activity is modulated by EGF and by its generation of regulatory signals (Mermelstein, 1989). The EGF controls many cellular activities, including nutrient transport, phospholipid turnover, protein phosphorylation, glycolysis, ornithine decarboxylase activation, endocytosis and

synthesis of DNA, RNA and proteins (Carpenter 1976 and 1979, and Das, 1982). The origin of the cell determines what will be the biological response to psoralen or EGF binding. Different cell types respond differently: EGF inhibits growth of A431 epidermoid cells but stimulates growth of human fibroblasts (Barnes, 1982 and Milstone, 1984). Thus, psoralen binding can inhibit or promote growth of different cell types after photoactivation (Laskin, 1988). The psoralen binding occuring at other sites than the psoralen receptor may also promote different biological effects of PUVA. This is a case of multiple targets and multiple effects for the same class of pharmaceuticals.

Specific, high affinity, saturable binding sites for the psoralens have been identified in culture for different cell types: HeLa cells, an epithelial cell line derived from human cervical carcinoma, and PAM 212 keratinocytes, the KB oral carcinoma and the B16 and G-361 melanomas (Laskin, 1988). Characterization of the nonnuclear psoralen binding sites used high specific activity radiolabeled [^{3}H]-8-MOP. In saturation studies, two independent psoralen binding sites with different affinities for 8-MOP were found: a higher-affinity site with a Kd of 19 nM and 1.8×10^5 receptor sites per cell and a lower-affinity site with a Kd of 4 μ M and 7.1×10^6 receptor sites per cell (Laskin, 1988). The presence of psoralen receptors in melanocyte and keratinocyte-derived cells is consistent with biological activity of the psoralens in the skin. The number of psoralen receptors varies with cell type, with fibroblast cells having five to ten times more psoralen receptors than epithelial cells of the skin (Laskin, 1985). With the radiolabel studies, Laskin determined that psoralen uptake into the cell was rapid, reaching equilibrium within thirty minutes. Using unlabeled psoralen prior to the addition of the radiolabeled psoralen

was seen (Laskin, 1985). Further work by Laskin proved that non-specific binding of psoralens to the cells was not saturable, but receptor sites were saturable reversibly. The binding of analogs to psoralen receptors had strong structure activity correlations, with TMP being the most effective inhibitor of radiolabeled 8-MOP binding to HeLa cells. Psoralen and 8-MOP were also effective, though angelicin was not (Laskin, 1988). Thus, it is clear that the psoralen receptor is highly specific and that modifications in psoralen structure greatly affect binding and ultimate clinical response.

Laskin has presented evidence that the psoralen receptor is not associated with the cell' s DNA: psoralens are present in the cytoplasm and cell surface membranes of treated cells, as detected by their fluorescence properties. When fractionating HeLa cells, psoralen binding occurred in the membrane fractions, therefore, not all psoralen molecules interact with DNA. After equilibration, and UVA treatment, 8-MOP was found covalently bound in the nuclear material and the cytoplasm (Yurkow, 1987). In competitive binding studies, psoralen binding was found to be saturable in the cytoplasmic and membrane portions of the cell, but saturation of covalent binding of psoralen to DNA has not been proven (Laskin, 1985). Direct interaction with DNA by psoralens is not involved in the receptor-mediated biological activity, for the receptor was found to be solely in the cytoplasm and the cell membrane of HeLa cells in gel electrophoresis and fluorography studies (Kitten, 1983). The psoralen-receptor complex was resistant to DNAase and RNAase treatment, but showed sensitivity to proteases (Laskin, 1988). This means that candidate drugs whose design minimizes uptake in nuclear DNA may target the non-DNA receptor and therefore have a unique photochemistry.

After binding of the EGF to the receptor, the EGF-receptor complex is internalized. Studies of binding with radiolabeled EGF to the receptor showed decreased binding in HeLa cells in the presence of 8-MOP and UV light (Laskin, 1986). This phenomenon was comparable with the action of other inhibitors of EGF binding, such as the phorbol ester, 12-O-tetradecanoyl phorbol-13-acetate, (TPA) (Lee, 1978). Unlike the TPA inhibition of EGF binding, the PUVA inhibition of EGF binding to HeLa cells required light. Psoralen binding occurred rapidly, and was evidenced by several active psoralen analogs. Mouse and human skin cell types, known to have psoralen receptors, showed similar inhibition following PUVA treatment. Later work proved the psoralens are not competitive inhibitors for the EGF (Laskin, 1986). PUVA inhibition of binding was also shown to be temperature dependent. Psoralens did not inhibit EGF binding in broken cell fractions, suggesting that cellular metabolic activity is necessary for the biological activity of PUVA and inhibition of EGF binding is probably an indirect mechanism, similar to the action of the tumor promoter TPA on EGF binding inhibition (Laskin, 1988). Laskin developed a model for the mechanism of action for psoralens binding to a specific receptor localized at the cytoplasm or other cell components such as DNA (Figure 6). Biological responses are initiated by psoralens activated by UVA light, unlike TPA binding to a specific receptor in the cytoplasm which initiates a response without light activation being required. Both psoralen and TPA receptors interact with the EGF receptors to control EGF binding and the resultant protein kinase activity, altering the cell's response to a growth stimulus (Laskin, 1988).

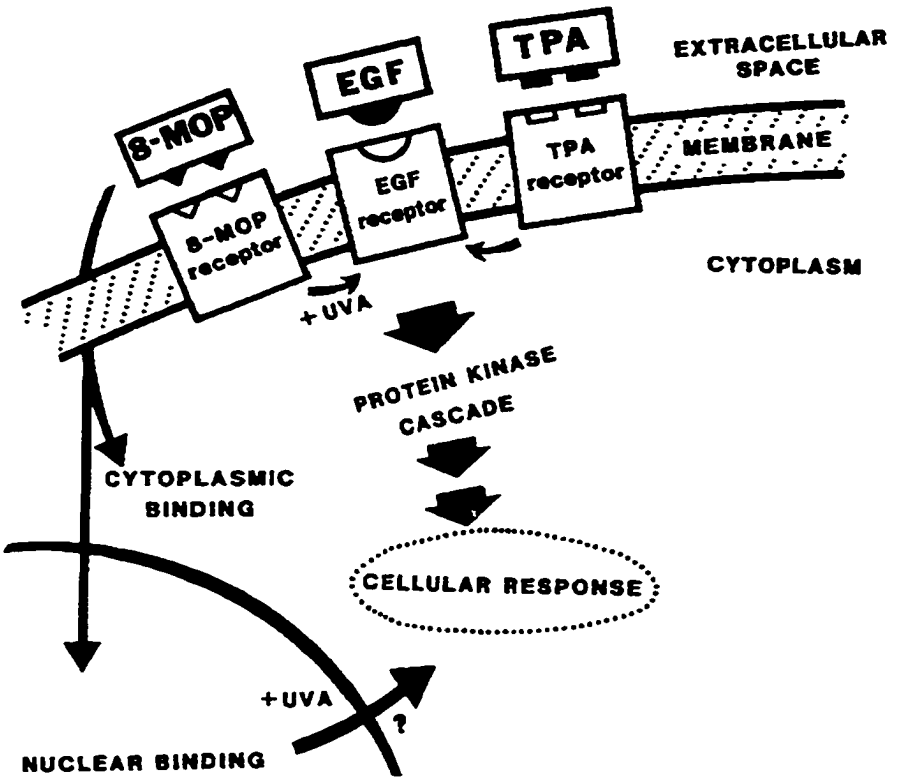


Figure 6 Model of psoralen receptor

A PROTEIN INTERACTION SITE

It is clear that there are several *in vivo* targets for psoralens. Other research groups concluded that proteins, keratin, collagen and lipoproteins could be targets for psoralen, along with DNA, after finding ^{3}H labeled 8-MOP in the cytoplasm and the nucleus of HeLa cells by autoradiography (Bredberg, 1977 and Bertaux, 1981). Other targets than DNA binding are indicated by electron microscopy of guinea pig skin (Cech, 1979).

Protein damage may be responsible for the formation of cataracts, a side effect in PUVA therapy, because the turnover rate for proteins in the eye is slower than for other tissues (Lerman, 1982 and Grossweiner, 1984).

A PHOTOCHEMICAL INTERACTION (RADICALS AND SINGLET OXYGEN)

In addition to the multiple biological targets for psoralens, there are also multiple molecular chemical mechanisms. When psoralens interact with proteins, two chemical processes may occur: formation of singlet oxygen and formation of radicals. Singlet oxygen formation, produced by psoralens has been shown, but the effects on living organisms are uncertain (Poppe, 1975). Psoralen excited states may be quenched by amino acids and other cellular components, in addition to quenching by oxygen. Molecules in the singlet excited state move into a triplet state that can itself generate free radicals and cause biological photosensitization. At least one researcher believes that the radical formation may promote some of the beneficial effects of PUVA, such as pigment formation (Craw, 1984).

FATTY ACID AND LIPID INTERACTION

Covalent adducts between unsaturated fatty acids and psoralens have been observed upon activation by UV light (Kittler, 1988). Using a competitive kinetics technique monitoring formation of psoralen-fatty acid adducts and disappearance of fatty acid, singlet oxygen is not evidenced as a reaction intermediate, though some incidental singlet oxygen forms (Wang, 1983). Cycloaddition of unsaturated fatty acids with psoralens occurs much more rapidly than the reaction of unsaturated fatty acids with

oxygen. Thus, product formation: adduct or oxidized, depends on competing rates of reactions under the conditions studied.

In addition to covalent binding to unsaturated fatty acids, oxidation of fatty acids may also occur. Cellular membrane degradation and cell death can occur following lipid peroxidation, which has been linked to cancer, inflammation and arthritis (Fantone, 1985). The PUVA treatments side effects, such as erythema, inflammation and vesiculation of the skin may be caused by lipid oxidation (Pathak, 1983).

AMINO ACID INTERACTION

From a chemistry viewpoint, the unanswered question is "what biomolecule or molecules form the covalent bond to the psoralen when a protein receptor is the target?" CIDNP, chemically induced dynamic polarization, techniques were used to study the protein-like associations with psoralens. The nuclear spin polarizations of triplet excited states of psoralens were investigated with and without aromatic amino acids. This method has demonstrated that psoralens probably react with amino acids by free radical reactions. Work has shown that triplet excited states of some psoralens have been quenched at receptor sites (Bensasson, 1987). Mono and difunctional psoralens were evaluated for their photoactivation as a predictor for photosensitizing drug properties (Marko, 1988). The polarizations on N-acetyltyrosine in acetonitrile when irradiated with a psoralen were dependent on the psoralen structure. When irradiating 5-MOP, a bifunctional psoralen, the strongest effects were seen at the 3,4-pyrone double bond. Marko suggested that the increased emission and absorption signals resulted from formation of a radical ion pair. Formation of both semi-oxidized and semi-reduced psoralens resulted:

$$5\text{-MOP}^* + 5\text{-MOP} \rightarrow \overline{5\text{-MOP}^{+\cdot}\ 5\text{-MOP}^{-\cdot}}$$

When a psoralen (Ps) was irradiated with an amino acid (AA) this lead to a psoralen triplet reduction due to a reversible electron transfer.

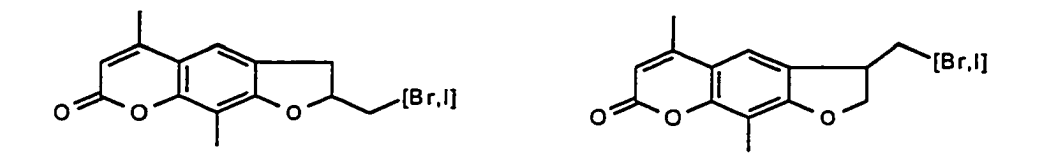
$$^3Ps + AA \rightarrow Ps^{-\cdot} + AA^{+\cdot}$$

This was similar to the pattern seen for amino acids with dyes capable of attaining a triplet state. Marko presented his results as confirmation that psoralens and amino acids react through a radical pathway.

As new understanding of the psoralen mode of action emerges, synthetic analogs may be designed with the goal of enhanced activity, greater receptor interaction, and reduced carcinogenicity/mutagenicity. Interest in the mechanism of action of the psoralens continues, because of the combination of beneficial and harmful effects of PUVA, as does hope for drug development without accompanying mutagenicity and severe side effects.

RESULTS AND DISCUSSION

The design and synthesis of displaceable functionalities on the furan ring was a prime goal of this study. Such displaceable functionalities provide critical reactive psoralen electrophiles from which sets of candidate drugs can be readily generated and studied. Furthermore, to minimize unwanted DNA cross-linking, it is preferable that the furan ring be saturated. The 4,8-dimethyl-4',5'-dihydropsoralen precursors with possible substitution at the 3-position by hydrogen, bromine or iodine chosen as pharmaceutical building-blocks include (Figure 7):



4,8-DIMETHYL-5'-HALOMETHYL-4',5'-DIHYDROPSORALEN 4,8-DIMETHYL-4'-HALOMETHYL-4',5'-DIHYDROPSORALEN

Figure 7 Target psoralens for synthesis

The quaternary compounds of dihydropsoralen have proven difficult to synthesize. The known method of synthesis did not allow formation of 5'-(pyridiniummethyl)-4',5'-dihydropsoralens, compounds which in fully aromatic forms had tested as most active in the HeLa cell screen. (Heindel, 1995 and Heindel, 1993). A facile synthesis of 5'-bromomethyl and 5'-quaternary ammonium psoralens was discovered but hydrogenolysis of the pendant leaving-group-to-carbon bond, not hydrogenation of the 4',5'-double bond, is the dominant outcome when catalytic hydrogenation or exchange hydrogenation are attempted (Heindel, 1995). This

unfortunate consequence leads to the recovery of the parent psoralen, not to the 4',5'-dihydropsoralen analogues (Figure 8).

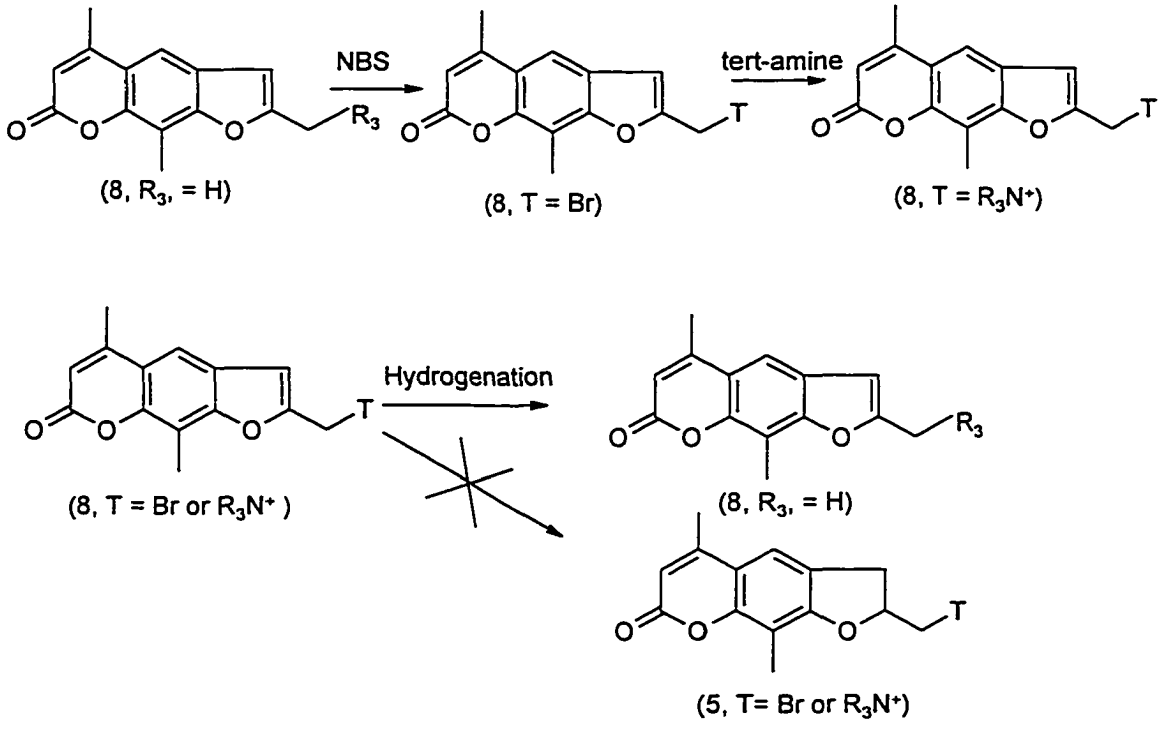


Figure 8 Hydrogenation fails to yield desired product

Preparation of a tertiary aminomethylpsoralen less labile to hydrogenolysis, reduction and subsequent methylation can produce methylated quaternary compounds in low overall yields (Heindel, 1994). Obviously, however, neither 5'-N-pyridiniummethyl dihydropsoralens -- nor any quaternary psoralens derived from aromatic heterocyclic amines – can be made available by this route (Figure 9).

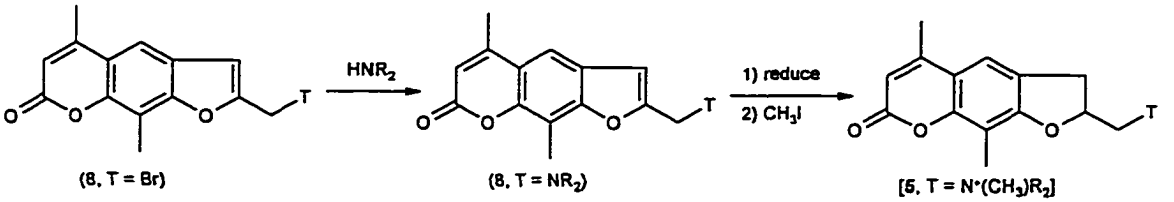


Figure 9 Tertiary aminomethylpsoralen

Previous research in the Lehigh University laboratories created 4',5'-dihydro-5'-bromomethyl moieties by this route (Figure 10). Three major difficulties limit the utility of this route: (1) sometimes mixtures containing the bromopyran (Compound B) result from the ring closure step, (2) nucleophilic displacement upon a bromomethyl moiety was frequently unsuccessful, and (3) some synthetic manipulations of the dihydrobromomethyl (Compound A) resulted in dehydrohalogenation.

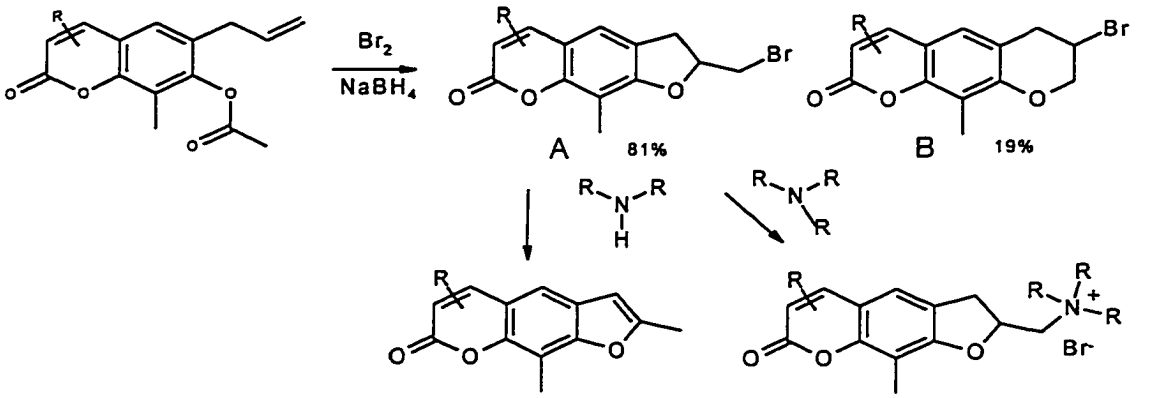


Figure 10 Mixed furan and pyran formation with some amine substitution

5'-HALOMETHYL-4,8-DIMETHYL-4',5'-DIHYDROPSORALENS

In general, cyclization reactions may occur through two transition states, the *exo* or *endo* cyclization mode. This effect has been widely studied (Figure 11). Use of

halogenation and mercuration/hydration with intramolecular nucleophiles as a route to cyclic compounds is reported in many reviews (Frederickson, 1997; Bartlett, 1984 and Cardillo, 1990). Natural products containing tetrahydrofuran and tetrahydropyran ring systems have been synthesized by halonium ion or mercuronium ion mediated cyclization of precursor alkenols. NBS has been employed in the cyclization reaction of a hydroxyalkene to generate a precursor in the synthesis of picrotoxinin (Corey, 1979). Iodine in $NaHCO_3$ was used to generate analogues of muscarine from fluorinated allyl alcohols (Bravo, 1990) as was mercury trifluoroacetate in tetrahydrofuran (Bravo, 1989). Researchers at Merck have used mercury acetate to induce cyclization of a 2-allyl phenol substituent to generate a 2-chloromercurimethyldihydrofuran in good yield in a series of novel 5-lipooxygenase inhibitors (Hutchinson, 1992).

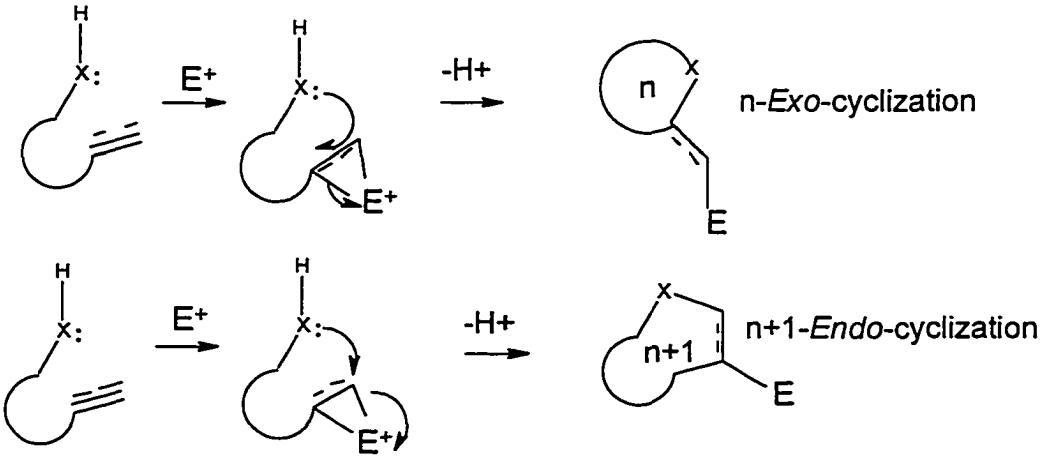


Figure 11 *Exo* and *Endo* cyclization

This work presents our alternative cyclization routes to new members of the essential 4,8-dimethyl-4',5'-dihydro-5'-halomethylfurocoumarin ring system (some

carrying substituents in the 3-position) motivated largely by the known limited alkylation reactivity of the existing bromomethyl compounds. The formation of the 5'-iodomethyl derivative facilitated the formation of the amine and quaternary derivatives by displacement, for the iodide is a more reactive leaving group. Dehydrohalogenation was nevertheless evidenced with some amines.

A precursor to various synthetic routes was 4,8-dimethyl-7-hydroxycoumarin (Figure 12) (Panetta, 1982). The 7-allyloxy-4,8-dimethylcoumarin was converted to the Claisen product, 6-allyl-7-hydroxy-4,8-dimethylcoumarin (Kaufman, 1980). The ring closures to the novel 4,8-dimethyl-4',5'-dihydro-5'-halomethylfurocoumarins were accomplished by pathways noted in Table 1 (Figure 12). Not one of these closures has been reported as a route to psoralens but some precedent exists for examples in other heterocyclic systems. The references to model compounds are cited in the table.

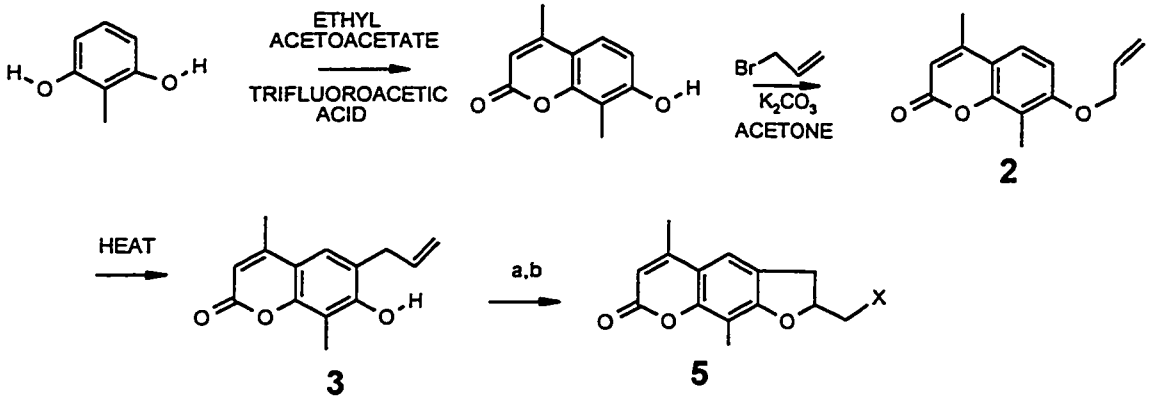
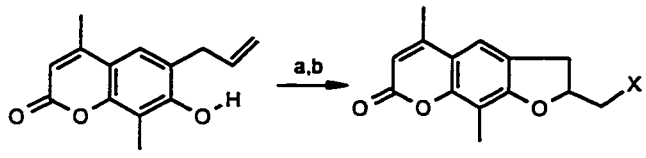


Figure 12 New synthetic routes to 4,8-dimethyl-5'-X-methyl-4',5'-dihydropsoralen



a) REACTANT		b) FURTHER TREATMENT	FINAL PRODUCT	REFERENCE
$HgCl_2$	HgCl	I_2 / KI /ETHANOL	I	Nesmejanow, (1935)
$Hg(OCOCH_3)_2$	$Hg(OCOCH_3)$	I_2 / KI	I	Mills, (1923)
$Hg(OCOCF_3)_2$	$Hg(OCOCF_3)$	I_2 / KI	I	Reitz, (1987)
3 moles N-Bromosuccinimide	Br with 3-Br			Corey (1979), Tanaka,
1.5 moles N-Iodosuccinimide	I			Reitz, (1987)
I_2 / $SnCl_4$	I			Orito, (1997)
I_2 / $NaHCO_3$ / CH_3CN	I			

Table 1 Ring closures to 4,8-dimethyl-5'-X-methyl-4',5'-dihydropsoralens

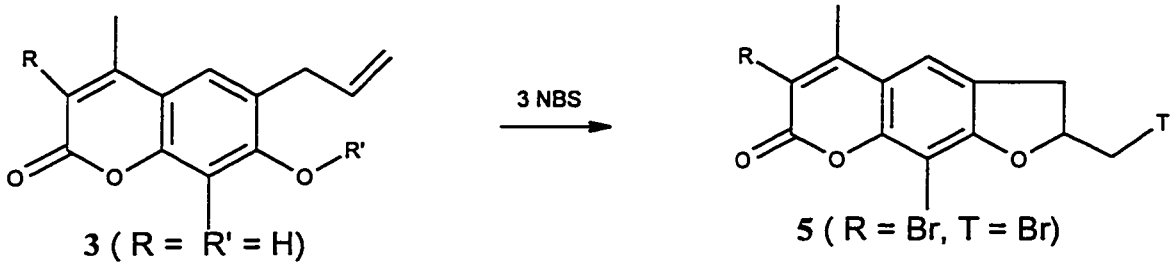


Figure 13 3 NBS ring closure of 4,8-dimethyl-6-allyl-7-hydroxycoumarin

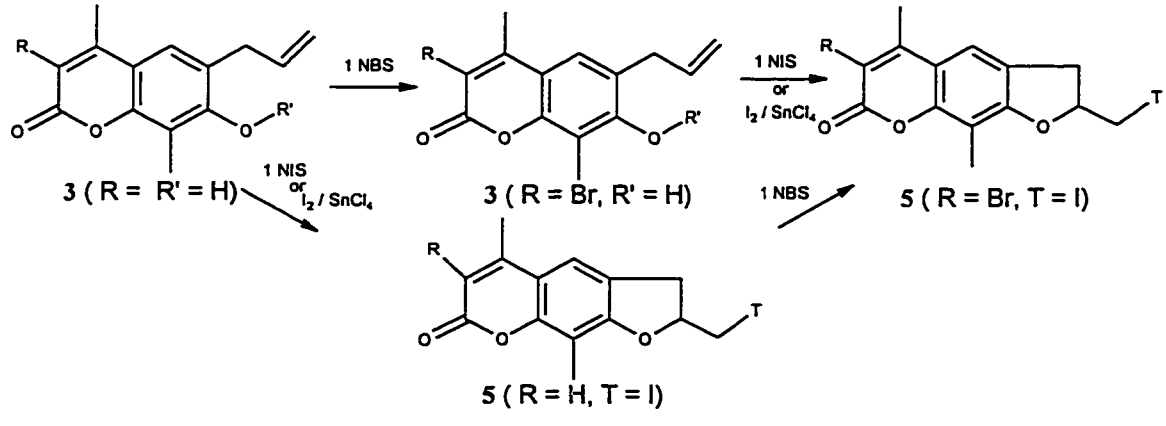


Figure 14 Variations in synthesis of 4,8-dimethyl-5'-halomethyl-4',5'-dihydropsoralen

Because the bromine facilitated cyclization on the 4,8-dimethyl-6-allyl-7-hydroxycoumarin led to benzopyran formation in addition to the desired dihydrofuran formation (Figure 10), alternative electrophiles were explored in the halocyclization reaction to form the dihydrofuran portion of the psoralen ring with higher regioselectivity. Literature references offer many possibilities for electrophiles which may be useful in catalyzing the closure of 7-hydroxy-6-allylcoumarin to psoralens (Corey, 1979; Tanaka, 1983; Reitz, 1987; Orito, 1997 and Bravo, 1990). One reasonable principle might be that the use of more sterically-bulky electrophiles might force more discrimination in closure to form five membered over six membered rings.

Ring closure did occur with the selected electrophiles with the absence of benzopyran (six membered ring) formation. One regiospecific bromination-cyclization used three molar equivalents of NBS, thereby generating 3-bromo-4,8-dimethyl-5'-bromomethyl-4',5'-dihydropsoralen, selectively brominating the 3-position and ring closing to the dihydrofurocoumarin in high yields. The absence of the benzopyran formation is a significant advantage (Figure 13). Variations in temperature from -80°C to 25°C and the presence or absence of light did not appear to affect this reaction.

Use of one mole of NBS in chloroform or one mole of pyridinium hydrobromide perbromide in acetic acid selectively gave 3-bromo-4,8-dimethyl-6-allyl-7-hydroxycoumarin, previously generated by the addition of bromine from one mole of Br_2. No ring closures occurred under these conditions when electrophile concentration was only equimolar.

Development of alternative routes to the 4,8-dimethyl-4',5'-dihydro-5'-halomethylfurocoumarin ring system (some with 3-position substitutions) were

successful. The iodomethylfurocoumarins proved very useful, for the iodomethyls were considerably more reactive to alkylation than had been the bromomethyls. The formation of the 5'-iodomethyl derivative facilitated the formation of the tertiary amine and quaternary derivatives. One route employed N-iodosuccinimide (NIS) to selectively ring close 4,8-dimethyl-6-allyl-7-hydroxycoumarin in a single step to the 4,8-dimethyl-5'-iodomethyl-4',5'-dihydropsoralen with the absence of benzopyran formation and absence of halogenation at the 3-position (Figure 14). Initial reactions showed trace iodination at the 3-position but further attempts to isolate the 3-iodo-4,8-dimethyl-5'-iodomethyl-4',5'-dihydropsoralen by this route were not successful. Use of a two-fold excess of NIS yielded only 4,8-dimethyl-5'-iodomethyl-4',5'-dihydropsoralen. The 3-iodo-4,8-dimethyl-5'-iodomethyl-4',5'-dihydropsoralen was generated by reacting 4,8-dimethyl-5'-iodomethyl-4',5'-dihydropsoralen with ICl in acetic acid at 50°C overnight.

A second route to the 4,8-dimethyl-5'-iodomethyl-4',5'-dihydropsoralen by tin (IV) chloride assisted iodocyclization had modest literature precedent in the synthesis of the 2-iodomethyl-2,3-dihydrobenzofurans from 2-allylphenol. That reaction occurred regioselectively in a 5-*exo*-Trig type cyclization (Orito, 1997). Applied to psoralen synthesis, this route gave a similar advantage to the NBS and NIS cyclizations in that reactions proceed in high yield with no competing benzopyran formation (Figure 14). Additional iodinating reagents used to successfully ring-close include iodine / $NaHCO_3$ / CH_3CN, ICl / CH_2Cl_2 and IBr / CH_2Cl_2, with the latter two prone to multiple products. Attempts to ring close using N-chlorosuccinimide failed. Bromination at the 3-position may be done before or after ring closure of the 6-allyl-7-hydroxy coumarin. The bromine and iodine based cyclization reactions offer new methods for the synthesis of readily

substituted dihydropsoralens to form tertiary amines or quaternary compounds, with the iodide-based cyclizations being preferred for their increased reactivity over the bromides.

Some limitations are seen in that the quaternary amine from triethylamine could not be prepared, and attempts to react 5'-iodomethyl-4,8-dimethyl-4',5'-dihydrofurocoumarin with dodecyl amine also did not prove successful. The elimination product 4,8,5'-trimethylpsoralen was recovered when similar displacements were attempted with 4,8-dimethyl-5 '-iodomethyl-4',5'-dihydrofurocoumarin and imidazole, diisopropanolamine, piperidine, dodecyl morpholine or an amine in the presence of a strong base in an attempt to force the alkylation of that amine. Thus, elimination dominated over substitution when more basic nucleophiles were employed.

All of the above discussed pathways were applied to the synthesis of various 3-substituted dihydropsoralens via the highly reactive 3-R-5'-bromomethyl-4',5'-dihydropsoralens or via the 3-R-5'-iodomethyl-4',5'-dihydropsoralens. A very useful expansion of the halogen-promoted cyclization which generated these halomethyl compounds to other affinity-labeled psoralens has been discovered. The use of mercury (II) salts to ring-close 6-allyl-7-hydroxycoumarins has generated a therapeutically promising family of 5'-halomercurimethyl psoralens.

A halomercurimethyl electrophile on a psoralen might provide an active site directed affinity label to anchor that psoralen to its native target. Laskin has shown the existence of a psoralen receptor on epidermal cells, and this can be exploited in diagnostic and therapeutic pharmacology by placing a "masked" reactive electrophile on a receptor ligand (Katznellenbogen, 1974; Rando, 1974 and Taylor, 1993). Pharmaceutical examples include compounds developed for site binding to components

in oncologic pathways such as receptor targeted steroidal-based alkylating agents (Katznellenbogen, 1974), haloacyl nucleosides (Montgomery, 1983) and amino acid mustards (Sneader, 1985). Histological tissue markers and imaging agents are possibilities for site-directed alkylating agents that are too toxic for systemic use. Employing electrophilic ring closure to generate 4',5'-dihydropsoralens directly from the Claisen precursor is a one step method to form these novel-site alkylating moieties. Many times an already formed core molecule will have an electrophile attached to it in a subsequent reaction; thus a direct synthesis which produces both in one step represents an improvement.

Other electrophilic promoters of cyclization, which act by coordinating with the pi-bond in the allyl side chain, may promote direct closure to the 5'-X-methyl-4',5'-dihydropsoralen. Previously mercury-based cyclizations had been developed which used mercury salts as electrophilic agents, and these share similarities with the iodocyclizations in that they proceeded rapidly without benzopyran formation (Nesmejanov, 1935; Mills, 1923; Reitz, 1987 and Adams, 1923). Prior studies have demonstrated the formation of 1-halomercurimethyl-1,2-dihydrobenzofurans from allylphenol reacted with mercuric acetate or mercuric chloride (Figure 15)(Adams, 1923 and Nesmejanow, 1935). Conversion to a 1-iodomercury-1,2-dihydrofuran was achieved by treatment with one equivalent of potassium iodide in ethanol or water. Formation of the 1-iodomethyl-1,2-dihydrobenzofuran, a model compound for this work, was accomplished by heating the iodomercury compound in a solution of iodine and potassium iodide (Figure 15) (Adams, 1923). Formation of oxygen heterocycles with mercury-based cyclization is the intramolecular equivalent of solvomercuration, similar

to the iodocyclizations discussed in this dissertation (Bartlett, 1984). Normally the reaction is a synchronous process where the electron-donating group reacts with the weakly polarized complex formed with mercury salt and the allyl double bond. Ring closure is usually directed by electronic factors, though the electrophile and solvent can affect product outcome (Cardillo, 1990). Analogues of muscarine (a tetrahydrofuran) with high stereoselectivity, are formed by cyclization of fluorinated alcohols using Hg(II) trifluoroacetate in THF (Bravo, 1989).

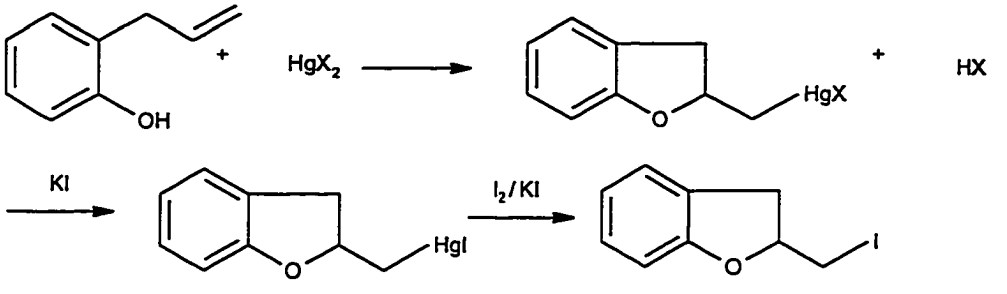


Figure 15 Mercury salt ring closure of 2-allylphenol with conversion to 1-iodomethyl-1,2-dihydrobenzofuran

Sastri documented formation of a mercury chloride addition product of 6-allyl-4,8-dimethyl-7-hydroxy coumarin with mercury (II) chloride at room temperature based on chloride analysis of the product (Figure 16) (Sastri, 1962). We have shown that a direct cyclization leading to the hitherto unknown 4,8-dimethyl-5'-chloromercurimethyl-4',5'-dihydropsoralen occurs in refluxing alcohol in the presence of mercury salts. NMR and elemental analysis show ring closure with no formation of an addition product (Figure 17). Significant cytotoxicity was found in members of this family, in which the chloro moiety is substituted by other anion functionalities. In addition to being

therapeutically useful, these mercurimethyl compounds are synthetically useful in that they may be converted to the same 5'-iodomethyl compounds described earlier herein. Thus 4,8-dimethyl-5'-iodomethyl-4',5'-dihydropsoralen may also be generated through mercury intermediates albeit with lower yields and more reaction steps than the iodocyclizations. Initial formation of 5'-substituted mercurimethyl psoralens was accomplished using $Hg(OAc)_2$, $HgCl_2$, and $Hg(OCOCF_3)_2$ as electrophiles. Subsequent conversion of these to the iodomercurimethyl compounds gave the 4,8-dimethyl-5'-iodomethyl-4',5'-dihydropsoralen. These mercurated psoralen compounds showed strong activity, both light activated and in the dark. The improved activity in the presence of light activation implies but does not prove that the psoralen functionality might be the molecular cause of this activity. The potential for directing an alkylating agent by attaching it to a molecule with a strong affinity for receptor sites on rapidly growing cells, such as tumor cells, has great therapeutic possibilities as imaging agents.

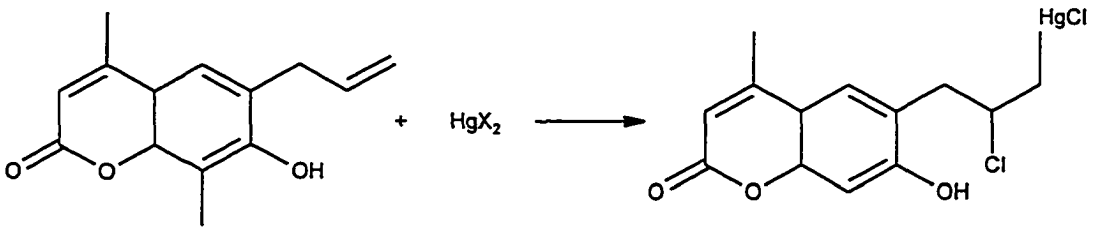


Figure 16 Mercury chloride addition product (Sastri)

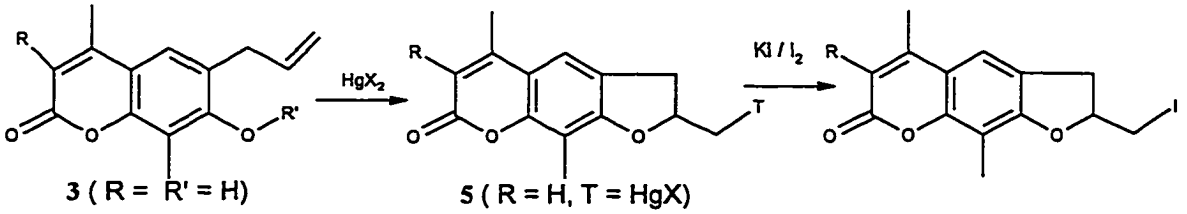


Figure 17 Mercury salt ring closure of 4,8-dimethyl-6-allyl-7-hydroxycoumarin

I proposed that the X-mercurimethyl psoralens have an affinity to the psoralen receptor, and that once they are aligned with the receptor, permanent binding by the mercury functionality to the thiols in the membrane proteins could occur. This is a logical explanation for the dark activity of the mercurated psoralens. In our hands, a mercurated psoralen linked to cysteine and to glutathione in a dark reaction. Research by Laskin has shown that glutathione may be competing for the mercurated psoralens with R-SH sites on proteins in cell membranes or in the test medium. When Laskin employed in the dark reaction bioassay an excess of D,L-buthionine-[S,R]-sulfoximine (BSI), a known inhibitor of glutathione biosynthesis and a suppressant of glutathione activity, the IC_{50}'s decreased to one-fourth or less of earlier values. Adding N-acetyl-1-cysteine, a promoter of glutathione synthesis because it is a precursor to glutathione, to confirm the mechanism, test data showed that PAM 212 keratinocytes were protected from the lethal effects of the mercurated psoralens. The effect of light activation of the organomercurials was not evaluated for light can have lethal effects on cells with reduced glutathione levels. Transient free radicals induced by BSI have increased toxicity in the presence of light.

To assess whether receptor-binding by the psoralen moiety is essential to the action of the mercurated dihydropsoralens, successively smaller fragments of the psoralen ring system with X-mercurimethyl functionalities were synthesized (Figure 15). X-mercurimethyl benzofurans were synthesized and evaluated by the psoralen pharmacological screen. Evidence of the same dark activity was present, with enhanced activity when activated by light. Ring-closure of 4-pentenol by mercury salts yielded 2-X-mercurimethyltetrahydrofurans, used to determine the effects of the X-mercurimethyl

functionality in the absence of an aromatic ring system (Figure 18). Thus it is clear that neither the light nor the dark reaction toxicity of these psoralens, or their mimics, depends on receptor binding.

Figure 18 Mercury model from 4-pentenol

Results of the studies of mercurated dihydropsoralens prompted an interest in mercurated angelicins as a way to understand the mode of action of these mercury based compounds. Angelicins have a reduced affinity for the psoralen receptor, and they provided another interesting comparison for the effect of modifying the aromatic portion of the mercurated psoralen. In fact, all the methods used in previous ring closures of the 6-allyl-7-hydroxycoumarin to generate the 5'-X-methyl-4,8-dimethyl-4',5'-dihydropsoralen were successfully applied to 8-allyl-4,8-dimethyl-7-hydroxycoumarin. Methods included ring closure by mercury salts and by $SnCl_4/I_2$ to produce 5'-X-methyl-4,8-dimethyl-4',5'-dihydroangelicins (X = $HgOCOCH_3$ and HgCl) (Figure 19) and (X = I) (Figure 20). Amino-substituted angelicins were synthesized for comparison of their activity to that of the amino substituted psoralens. Thus, psoralens capable of forming only monoadducts with increased cell surface effects while maintaining clinical effectiveness ought to have therapeutic potential with diminished side effects. When angelicins are used, they are limited in their receptor interaction by the geometry of their structures. The angelicins share a molecular level pharmacology with the 4',5'-dihydropsoralens in their inability to form cross-links.

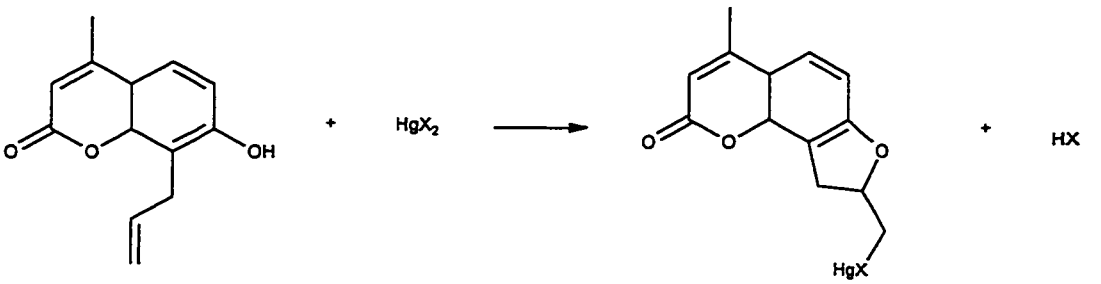


Figure 19 Synthesis of 4-methyl-5'-X-mercurimethyl-4',5'-dihydroangelicin

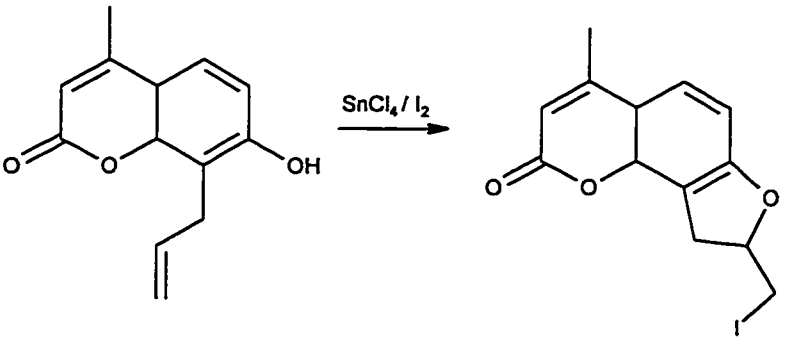


Figure 20 Synthesis of 4-methyl-5'-iodomethyl-4',5'-dihydroangelicin

4'-IODOMETHYL-4,8-DIMETHYL-4',5'-DIHYDROPSORALENS

DIAZONIUM TETRAFLUOROBORATE ROUTE

The research published by Beckwith on o-(allyloxy)benzene diazonium tetrafluoroborates as intermediates in formation of dihydrobenzofurans was the precedent for the synthesis of novel 4'-halomethyl-4',5'-dihydropsoralens using the allyloxycoumarin diazonium tetrafluoroborate salts (Figure 21). The unanswered question in our proposed synthetic approach was "will a transient diazonium ion couple onto loci in the pyrone ring or will it cyclize smoothly to a furan?" The closure of the furan ring of the psoralen was successful. In the cyclization of aryldiazonium salts, the

nucleophile transferred is derived from an added reagent such as NaI, CuCl, CuBr, CuCN or NaSPh, so that from a single diazo precursor, different products may be generated. Yields depend on the efficiency of the radical generation and cyclization. The mechanism proposed by Beckwith is shown in Scheme I where ArN_2^+ represents diazonium ions and R· represents the cyclized radical. The reduction of the diazonium ion is the initiation step, and this relies on the ability of the iodide ion to act as a one electron reductant. Step 5 involves an electron transfer and step 4 is a chain propagation step involving iodine atom transfer from I_3^- formed from iodine (Beckwith, 1987).

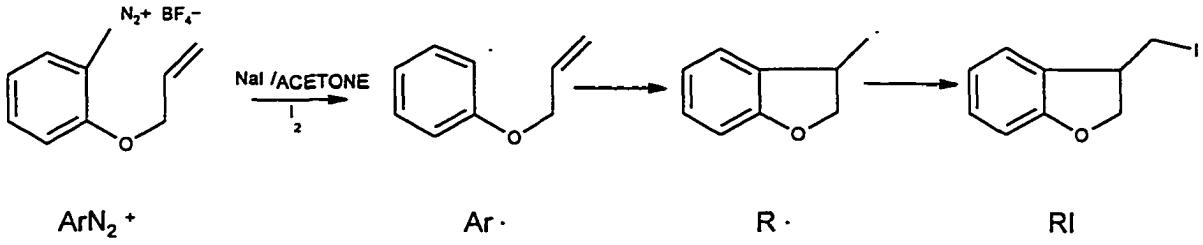


Figure 21 Diazotetrafluoroborate cyclization to 3'-iodomethyl-2',3'-dihydrobenzofuran

1	$ArN_2^+ + I^- \rightarrow ArN_2^{\cdot} + I^{\cdot}$
2	$ArN_2^{\cdot} \rightarrow Ar^{\cdot} + N_2$
3	$Ar^{\cdot} \rightarrow R^{\cdot}$
4	$R^{\cdot} + I_3^- \rightarrow RI + I_2^{\cdot -}$
5	$ArN_2^+ + I_2^{\cdot -} \rightarrow ArN_2^{\cdot} + I_2$
6	$I^{\cdot} + I^{\cdot} \rightarrow I_2$
7	$I^- + I_2 \rightarrow I_3^-$

Figure 22 Free radical reaction pathway

Reaction products recovered indicate that the rate of cyclization of the radical was faster than the iodination of the uncyclized aryl radicals. The generation of five membered rings by the *exo* trig mode occurred exclusively, in the absence of formation of six membered rings by the *endo* mode. If these parameters exist in cyclization-synthesis of psoralens, no contaminating pyranocoumarins (six-membered products) should be formed.

Applying the Beckwith scheme for the generation of dihydrobenzofurans to 6-nitro-7-hydroxycoumarin for the generation of 4'-halomethyl dihydropsoralens has proven successful (Figure 23). The reduction of the nitro compound was accomplished with tin(II) chloride, tin, and concentrated HCl in ethanol (Adams, 1944). Alternative unsuccessful reductants were Pd/C in cyclohexene and NH_4OH / $NaHSO_3$ in isopropanol. The diazotization of the amine occurred rapidly and in high yields with generation of a stable diazonium tetrafluoroborate salt (Roe, 1949). The product remained undecomposed after four weeks. The diazonium tetrafluoroborate salt cyclized readily to yield 4,8-dimethyl-4'-iodomethyl-4',5'-dihydropsoralen. Initial reactions of 4,8-dimethyl-4'-iodomethyl-4',5'-dihydropsoralen with morpholine and pyridine to generate amino derivatives indicated dehydrohalogenation to the 4,8,4'-trimethylpsoralen to be the major product (Figure 24). After reaction with pyridine, approximately 10-30% of the desired product was recovered.

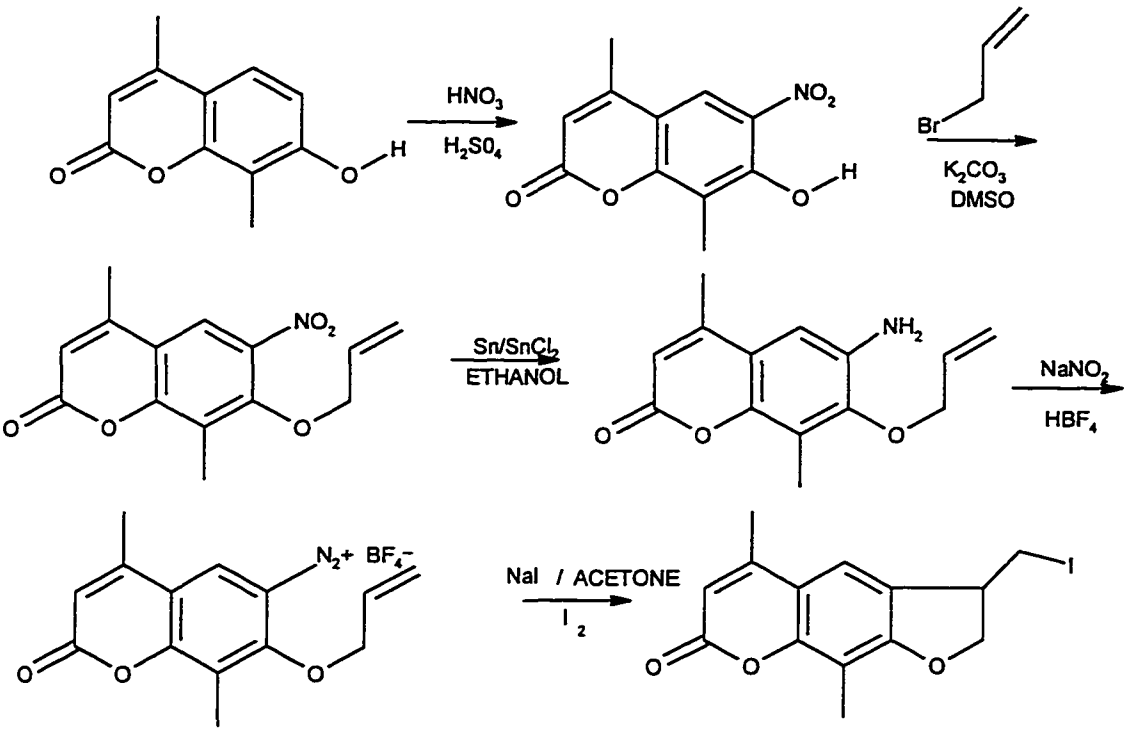


Figure 23 Diazotetrafluoroborate route to 4,8-dimethyl-4'-iodomethyl-4',5'-dihydropsoralen

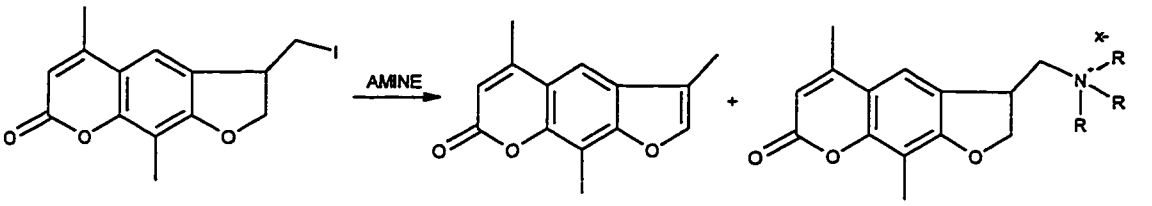


Figure 24 Amino substitution of 4,8-dimethyl-4'-iodomethyl-4',5'-dihydropsoralen

In an attempt to increase water solubility, amino derivatives of current clinical psoralens have been made. AMT, 4'-aminomethyl-4,8,5'-trimethylpsoralen hydrochloride and the 4'-pyridiniummethyl-4,8,5'-trimethylpsoralen chloride salt have been studied extensively (Isaacs, 1982). These agents have the ability to form noncovalent adducts with double stranded DNA in the absence of UVA and the ability to form covalent

adducts to DNA when activated by UVA. To maintain the desirable high water solubility combined with the inability to form covalent diadducts, the 4'-halomethyl-4',5'-dihydropsoralen served as a promising synthetic target on the pathway to 4'-aminomethyldihydropsoralens. Besides the diazotization route, other catalyzed cyclizations appear possible.

SAMARIUM IODIDE RING CLOSURE

For a synthetic alternative to the ring closure of the diazonium tetrafluoroborate as a possible precursor of the requisite 4'-halomethyls, precedent is found in the work by Curran on the samarium Grignard reaction on 2-iodo-7-allyloxyphenols for the formation of 3-iodomethyl-2,3-dihydrobenzofuran. This chemistry applied to our project employs 6-iodo-4,8-dimethyl-7-allyloxycoumarin for the formation of 4,8-dimethyl-4'-iodomethyl-4',5'-dihydropsoralen (Figure 25) (Curran, 1992).

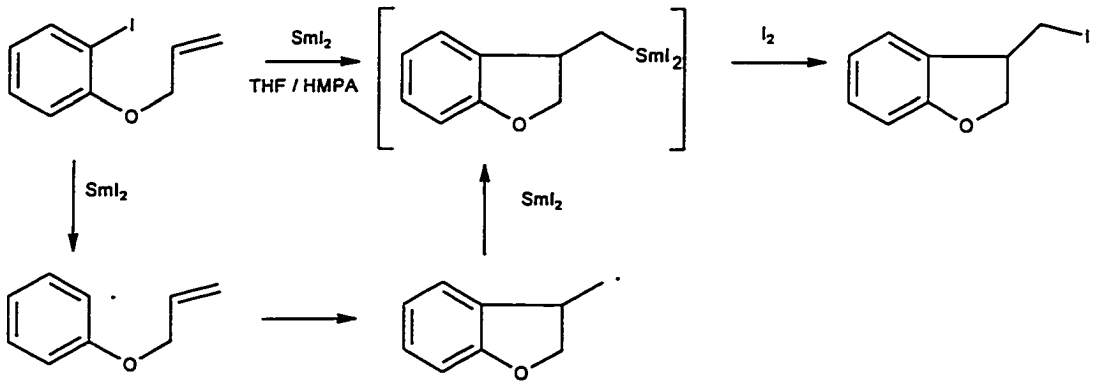


Figure 25 Samarium iodide ring closure model

The anticipated trapping of molecular iodine by the samarium iodide reagent generated from 4,8-dimethyl-6-iodo-7-allyloxycoumarin did not generate the 4,8-

dimethyl-4'-iodomethyl-4',5'-dihydropsoralen, the critical intermediate needed for subsequent conversion to amine-containing products (Figure 26). Preparation of the requisite pre-samarium starting material, 6-iodo-4,8-dimethyl-7-hydroxycoumarin, was accomplished by the addition of KI/I_2 in NH_4OH and dioxane to the non-iodocoumarin. Reacting 6-iodo-4,8-dimethyl-7-hydroxycoumarin with allyl bromide gave the 6-iodo-4,8-dimethyl-7-allyloxycoumarin. Unfortunately, attempts to generate the alkyl samarium species by the samarium Grignard reaction in THF/HMPA, as outlined in Curran's work, resulted in the recovery of the dehalogenated allyloxycoumarin, unreacted starting material, and other unidentified products. Dehalogenation may have resulted from the proton transfer from THF occurring more rapidly than radical cyclization. Work was discontinued on this route to focus on the generation of 4'-aminomethyl and quaternary dihydropsoralens when the desired 4,8-dimethyl-4'-iodomethyl-4',5'-dihydropsoralen precursor was generated through the diazonium tetrafluoroborate route. Various attempts to prepare the 6-iodo-4,8-dimethyl-7-hydroxycoumarin in high yield lead to iodinated products that were submitted for bioassay. The addition of 4,8-dimethyl-7–hydroxycoumarin to ICl in acetic acid selectively generated the unwanted 3-iodo-4,8-dimethyl-7-hydroxycoumarin. Excess ICl gave 3,6-diiodo-4,8-dimethyl-7-hydroxycoumarin from the 4,8-dimethyl-7–hydroxycoumarin, and this was converted to the 3,6-diiodo-4,8-dimethyl-7-allyloxycoumarin.

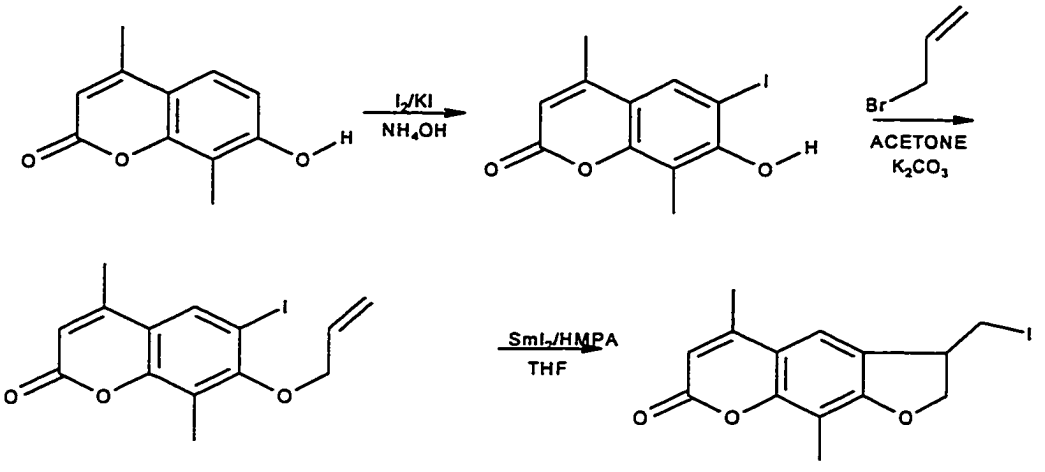


Figure 26 Samarium iodide reaction scheme designed for synthesis of 4,8-dimethyl-4'-iodomethyl-4',5'-dihydropsoralen

As an alternative samarium-mediated route, if the coumarin ring had deactivated the SmI_2 radical formation, the use of 2',4'-dihydroxy-3'-methylacetophenone as a precursor was considered (Figure 27). In our hands iodination by ICl in methylene chloride generated 2',4'-dihydroxy-3-methyl-5-iodoacetophenone. This aryl iodo ketone converted to the mono-allyl at the non-hydrogen bonded phenol. The samarium iodide reaction with and without ketal protection of the ketone functionality subsequently to generate the 3-iodomethyl-2,3-dihydrobenzofuran followed by a Wittig reaction to close the psoralen ring system failed. Work on this route was discontinued when the desired 4'-iodomethyl psoralen was obtained by the tetrafluoroborate.

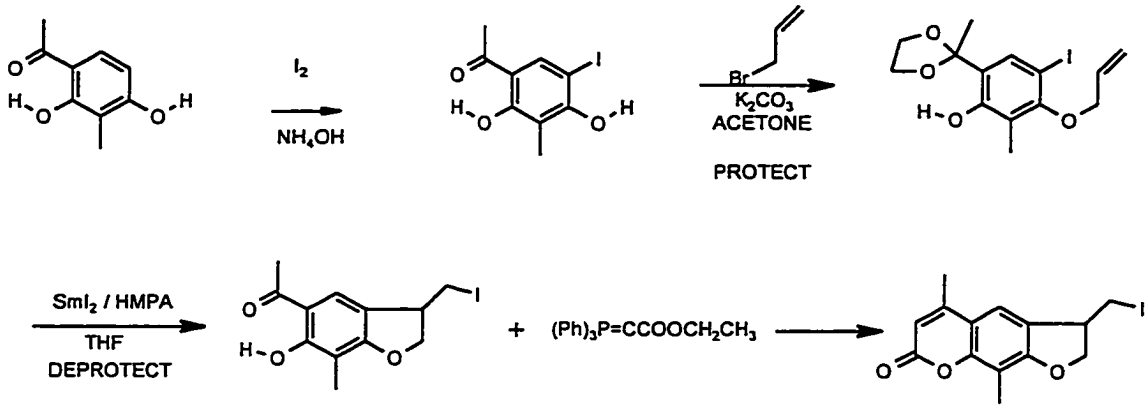


Figure 27 Alternate samarium iodide reaction scheme designed for synthesis of 4,8-dimethyl-4'-iodomethyl-4',5'-dihydropsoralen

ANTIFUNGAL ACTIVITY

The mercurated psoralens and psoralen models showed modest activity in the Buckman routine screen for antifungal activity. Though concern for the use of alkylmercury compounds is an issue, topical application, where delivery is localized to the site of infection, could be an interesting area to pursue. With enhanced activity after light activation, which is unique to this series of compounds, the ability to control resistant fungal infections may warrant further study. The development of antifungal drugs has lagged behind the development of antibacterial pharmaceuticals.

ANTITUBERCULAR ACTIVITY

A major health threat in developing countries and also in the United States in HIV infected patients is infection by drug resistant strains of *Mycobacterium tuberculosis* (Heifets, 1991). Combination therapy, including isoniazid, rifampin and pyrazinamide,

has failed to eradicate tuberculosis (TB) in the United States. TB is one of the most lethal infectious bacterial infections in the world (Gentry, 1998). The U.S. Department of Health and Human Services developed a plan to eliminate TB in the US by 2010, including pretreatment drug susceptibility testing of *Mycobacterium tuberculosis* isolates and rapid screening of new antibacterial agents.

Compounds from this work were screened in a primary *in vitro* assay by the National Institute of Allergy and Infectious Diseases against of *Mycobacterium tuberculosis* H37Rv. The 2-iodomercurimethyl-2,3-dihydrobenzofuran showed the most impressive activity with 99% inhibition of bacterial growth and has been selected as a candidate for further study. Many of the 5'-iodomethyl-4',5'-dihydropsoralen analogs with halogens at the 3-position displayed moderate activity in this screen, as did the amines such as the 5'-[(N,N-dimethylamino)methyl]-4,8-dimethyl-4',5'-dihydropsoralen hydro iodide salt and the 5'-pyridiniummethyl-4,8-dimethyl-4',5'-dihydropsoralen iodide salt .

MERCURY COMPOUNDS – POSSIBLE MODES OF ACTION

The 5'-X-mercurimethyl-4',5'-dihydropsoralens, the 2-X-mercurimethyl-2,3-dihydrobenzofurans and the 2-X-mercurimethyltetrahydrofurans all demonstrated considerable toxicity in the PAM cell assay. Not only were all of the IC_{50}'s of comparable order of magnitude -- which belies any psoralen-specific pharmacology -- but a two-level biological response was observed. Each compound displayed a dark-test IC_{50} and a considerably lower light-test IC_{50}, a fact made all the more surprising by the observation that no specific UV chromophore at the irradiation wavelength of 320-360 nm is present in the latter two classes. There is, in fact, the tailing

trail of end-absorption of both the mercurated dihydrobenzofurans and the mercurated tetrahydrofurans above 300 nm, but the observed extinction coefficients in this region are less than 15.

The photochemical behavior of alkyl mercury compounds has not been well studied but some interesting observations have been made. Mercurio iodo organometallics (i.e., R-Hg-I) have been reported to extrude elemental mercury upon irradiation above 300 nm (Abel, 1995). Others have noted that divalent mercury compounds under light-catalysis oxidize transient alkyl radicals to highly reactive carbonium ions capable of alkylating nucleophilic loci (Horspool, 1994). The most significant findings, however, are those of Russel who reported that simple ethyl, propyl, and butyl mercurio halides (i.e., R-Hg-X) generate, upon photolysis above 300 nm where they lack a specific chromophore, alkyl radicals which can substitute upon pyridine rings (Russel, 1985). Moreover, irradiation of R-Hg-Cl (R = t-butyl or neopentyl) at 350 nm produced transient alkyl radicals which could alkylate anions, extrude mercury, and self-couple (Russel, 1982). The authors of the latter paper make the interesting conclusion that, "The nature of this photoinitiation is not certain but appears not to involve a simple homoloysis of the organomercurial, since simple alkyl mercury halides do not absorb light above about 310 nm."

As we have shown herein, our psoralen-mimic organomercury compounds are capable of a dark-reaction alkylation to thiol-containing proteins, and this event is a likely molecular cause of the toxicity in the non-irradiated cell assay. While a variety of explanations of the light-augmented toxicity are possible, two seem most likely. First, as noted in Russel's work, the alkyl-carbon-to-mercury bond is cleaved to radicals by > 300

nm light even though no specific UV band exists in that region. After a first alkylation in a dark-reaction at critical cellular thiols leading to R-Hg-S-protein takes place, a second light-catalyzed cleavage at the R-Hg bond may occur producing radicals capable of a second level of cellular attack. Alternatively, after anchoring of the organo mercury in a dark reaction, light activation may induce cellular oxy-radicals (peroxide, superoxide), and these may undergo some hitherto unidentified chemistry with the mercury species to generate a photo-toxic event. There is literature on light-catalyzed effects on cellular oxygen radical species (Cantoni, 1984). In our hands the attempt to show peroxy reactivity with our alkyl mercury compounds was successful in the presence of the 2-acetomercurimethyltetrahydrofuran with hydrogen peroxide in deuterium oxide, where irradiation produced extrusion of mercury. This leads to the conclusion that a cellular parallel to the unusual photochemical behavior of alkyl mercury compounds described by Russel (photo-produced alkyl radicals attacking critical cellular components) must be taking place (Russel, 1982).

To understand the molecular mechanism of our mercury substituted compounds, the interaction with thiols was investigated. Literature methods to quantitate sulfhydryl groups in proteins include titration by organic mercury compounds such as p-chloromercuribenzoate. A slight shift in absorbance in the 230-235 nm range was seen with a two fold increase in absorbance at 254 nm as mercaptide formation was complete (Boyer, 1954). The possibility of alkylation of cellular thiols by our X-mercurimethylpsoralens and X-mercurimethyltetrahydrofurans was modeled in a UV study in which p-methoxybenzene thiol served as a cysteine substitute with a strong absorbance band in the mid-UV range (238 nm). The slight changes in absorbance

maxima and absorbance of p-methoxybenzene thiol were measured as 2-acetomercurimethyltetrahydrofuran was added. No further change was evidenced as excess 2-acetomercurimethyltetrahydrofuran was added. The bonding appears to be immediate, for delayed scanning after addition of mercury containing compounds showed no change in absorbance from the initial spectrum for that sample. Using standard solutions to plot absorbance versus concentration at various wavelengths, it is possible to mathematically separate UV spectra of overlapping absorption components when no region exists where each components predominates (Harris, 1999).

A study of cobalt mediated radical reactions, based on vitamin B_{12} which has cobalt as a core transition metal, has interesting parallels to our study. Findings indicate that cobalt forms weak covalent bonds to carbon (20-30 kcal / mol) which leads to relatively stable organocobalt compounds and homolysis by heat or light provides a source of carbon radicals (Bhandal, 1990). This has strong parallels to the psoralen synthetic routes using diazonium tetrafluoroborate and to the samarium iodide synthetic routes proposed in this work. Bhandal studied the cobalt mediated radical initiated cyclization trapping sequence which led to reduced heterocycles for synthetic purposes but also considered the interaction between the organocobalt complex with molecular oxygen and light. The cobalt peroxy complex was isolated by chromatography but was unstable. Heating caused a breakdown to a carbon radical. The 2,3-dihydro-3-methylene benzofuran generated by treatment of o-iodoallylphenol with the cobalt salen complex in Bhandal's work was isolated, and the NMR correlates with the furan portion of the exocyclic methylenedihydropsoralen intermediate formed when the 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen is treated with base in my work.

MECHANISM STUDY

As indicated in the data (Figure 29), interesting competitive elimination/substitution pathways have been observed in the reaction of pyridine with the 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen, the 5'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen and 5'-iodomethyl-4-methyl-4',5'-dihydroangelicin by following the reaction progress by proton NMR. These findings could easily be the focus of mechanistic investigation, but the primary purpose of this project was the development of novel therapeutics. Consideration of mechanistic probes permits some tentative conclusions about the findings. Many texts show pathway partitioning between E_2 (or E_{1CB}) and S_N2, a classic topic in mechanism discussions (March, 1992; Hine, 1962, Lowry, 1976). Many parallels exist with our study.

The 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen in strong base showed no deuterium incorporation in either product or starting material, rapidly converting to an elimination product containing an exocyclic methylene, as evidenced by NMR (Figure 30). The same compound with pyridine, a weaker base, gave 31% substitution and 62% elimination (Figure 29). The lack of deuterium incorporation and the presence of a methylene intermediate discount the E_{1CB} mechanism. Basic conditions favor the E_2 over E_1 option. That stronger bases give elimination exclusively with no substitution is well-established in other competitve elimination/substitution cases (March, 1992). Bhandal noted an exocyclic intermediate in the o-iodo allylphenol ring closure catalyzed by cobalt

species derived from cobalt(III) salen, where it rearranged in trifluoroacetic acid to form 3-methyl-2,3-benzofuran (Bhandal, 1990). The differences in reactivity of the 4,8-dimethyl-4'-iodomethyl-4',5'-dihydropsoralen and the 4,8-dimethyl-5'-iodomethyl-4',5'-dihydropsoralen, the 4-methyl-5'-iodomethyl-4',5'-dihydroangelicin were significant. No generation of the exocyclic methylene intermediate was detected by NMR in the 5'-substituted psoralen reactions.

A transient exocyclic methylene can be detected in the 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen route but the identity of its exact precursor is uncertain. The elimination product ultimately observed from the reaction, the 4,8,4'-trimethylpsoralen, could arise directly by HI loss from the starting material or by a pyridine elimination (presumably E_2) from the pyridinuim salt. Elimination of onium salts in basic medium is well known and in the absence of rate data we are unable to establish whether the pyridinium displacement product is a precursor of the aromatized psoralen or an incidental co-product to it.

In any case, however, a benzylic C-H at the 4'-position is being extracted to give the trimethylpsoralen, and C-H moieties adjacent to an aromatic ring are well established as prime loci for proton loss in beta-eliminations (Lowry, 1976). Hine has noted that charge dispersal in the E_2 transition state has the effect of building up electron density on the beta-carbon and generating an alkene-like transition barrier, two effects which surely benefit from proximity to and overlap with an aromatic ring (Hine, 1962). This effect is absent in the 5'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen and the 5'-iodomethyl-4-methyl-4',5'-dihydroangelicin, both of which give exclusively substitution, though at a slower rate, when reacting with pyridine. In comparison, no starting material remains in

the 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen with pyridine after four hours, but 20-30% remains with the the 5'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen and the 5'-iodomethyl-4-methyl-4',5'-dihydroangelicin after eight hours. Yields or conversion percentages per time interval are not rate constants, but nevertheless a diminished reactivity can be documented for the 5'-substituted compounds. The NMR spectra run during this study can be found in the experimental section (Figure 35, Figure 37, and Figure 38).

These 5'-iodomethyls may be modeled as beta-aryloxyethyl halides (i.e., Ar-O-CH-CH_2-I) and some interesting precedent exists. In S_N2-like displacements on alpha-aryloxymethyl halides (Ar-O-CH_2-X) versus beta-aryloxyethyl halides (Ar-O-CH_2-CH_2-X), the relative KI/acetone rates are 920:0.3, wherein the standard rate set equal to one is n-butylchloride (Conant, 1924). An interpretation is that a proximal aryloxy stabilizes charge build-up in the transition state of the displacement. However, one insulated by a single additional methylene is ineffective and even somewhat rate-retarding compared to the unhindered and unactivated primary halide standard.

While such arguments might offer explanation for the diminished reactivity toward displacement of the 5'-iodomethyls, some elimination is seen when a base such as morpholine, imidazole or dodecylmorpholine is used. March has noted that beta-haloethers seldom undergo typical dehydrohalogenation, but assisted by reactive metals such as zinc, magnesium and sodium they can eliminate both the ether and the halide units (March, 1992). The reaction is not a true E_2 but a reduction called the Boord synthesis: Cl- CH_2-CH_2-O-R + metal = C=C + metal oxide + halide.

McElvain made one of the most pertinent observations about the lack of reactivity toward elimination by beta-alkoxyethyl halides when he reported that they eliminated distal to the ether C-H (McElvain, 1942). In other words, a 2-bromobutylacetal eliminates to give a 2-butene acetal; CH_3-C(R)H-CH(Br)-CH$(OEt)_2$ gives CH_3-C(R) =CH-CH$(OEt)_2$. Hine has commented that the reason for this behavior is not known (Hine, 1962). One speculation is that the transition state for E_2 elimination requires the destabilizing build-up of electron density next to an electron-rich ether oxygen.

As has been noted, pyridine-induced functionalization of the 4'- and 5'-iodomethyldihydropsoralens in this project behaves as predicted theoretically from the behavior of similar systems. One would expect both slower rates and suppressed elimination in the 5'-iodomethyls and similarly, one would expect enhanced and competing elimination versus substitution in the 4'-iodomethyls.

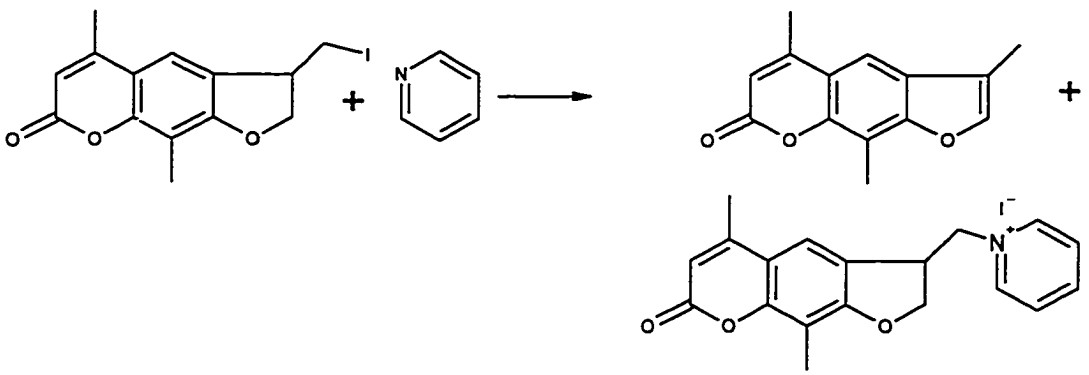


Figure 28 Amine substitution of 4,8-dimethyl-4'-iodomethyl-4',5'-dihydropsoralen

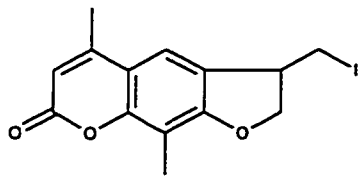
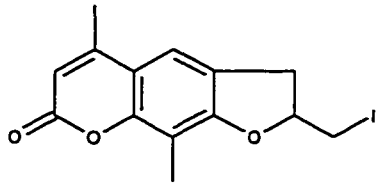
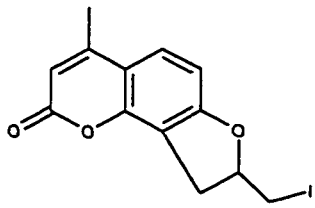
	Time	Pyridinium iodide salt	Aromatized product	Starting material
	4 hours	31%	62%	
	8 hours	78%		21%
	8 hours	62%		33%

Figure 29 Study of the mechanisms for pyridine substitution/elimination

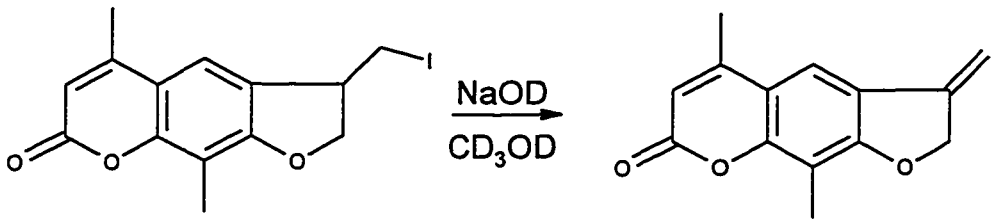


Figure 30 Base dehydrohalogenation of 4,8-dimethyl-4'-iodomethyl-4',5'-dihydropsoralen

CONCLUSIONS

GOALS ACCOMPLISHED

- Improved synthetic methods for the 5'-halomethyl-4,8-dimethyl-4',5'-dihydropsoralens
- Novel synthetic routes for the 4'-halomethyl-4,8-dimethyl-4',5'-dihydropsoralen
- Novel synthetic routes for the 5'-halomethyl-4-methyl-4',5'-dihydroangelicins
- Generation of amino and quaternary derivatives
- Better understanding of structure-activity relationships
- Photochemical studies of the mercurated compounds synthesized

SYNTHESIS OF NOVEL DIHYDROPSORALENS AND DIHYROANGELICINS

The alternative electrophiles employed to promote ring closure of the 4,8-dimethyl-6-allyl-7-hydroxycoumarin to the 5'-halomethyl-4,8-dimethyl-4',5'-dihydropsoralen represented an improvement over the previous molecular bromine catalyzed ring closure. Although when using 1 mole N-bromosuccinimide substitution occurred at the 3-position, using 3 moles caused substitution at the 3-position and closure of the ring to the dihydrofuran with no bromopyran, an undesired product in the previous route. The hydroxy functionality promoted substitution at the 3-position. When iodine was used to promote ring closure, either by N-iodosuccinimide or I_2 / $SnCl_4$, the more bulky electrophile promoted ring closure to the 5'-halomethyl-4,8-dimethyl-4',5'-

dihydropsoralen by an *exo*-trig mechanism without substitution at the 3-position. The mercury (II) salt-based ring closures also promoted cyclization before electrophilic substitution. The NBS, iodine and mercury-based ring closures may be applied to coumarins substituted at the 3-position, such as the 3-fluoro-7-hydroxycoumarin and 3-cyano-7-hydroxycoumarin. The 3-substituted compounds cyclize with equal propensity to their unsubstituted counterparts. To generate the 3-iodo-5'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen harsher conditions such as heating with ICl with acetic acid are required.

While yields and workability are comparable in the NIS and I_2 / $SnCl_4$ ring closures, cost favors the I_2 / $SnCl_4$ route. For the mercury (II) salt ring closures the disposal of mercury waste is an economic consideration. These compounds were synthesized as synthetic intermediates to the 5'-iodomethyldihydropsoralens, but lower yields and the multi-step process preclude this route. The mercury (II) salt cyclizations occurred in good yields and directly closed to the dihydrofuran system with no mercury addition product.

The ring closures applied to the 5'-iodomethydihydropsoralens are also effective in generating the 5'-iodomethyl-4-methyl-4',5'-dihydroangelicin from 4-methyl-8-allyl-7-hydroxycoumarin. As expected from Laskin's inhibition of radiolabeled 8-MOP binding to the psoralen receptor, where angelicins showed reduced affinity to the receptor, angelicins showed reduced biological activity in our bioassay.

Of the two routes outlined to ring close a 6-substituted-7-allyloxycoumarin to the 4'-halomethyl-4,8-dimethyl-4',5'-dihydropsoralen, the diazonium tetrafluoroborate route was successful. NaI was used to terminate the radical cyclicization. Use of CuBr or CuCl

instead of NaI would allow formation of different products such as the 4'-bromomethyl-4,8-dimethyl-4',5'-dihydropsoralen or the 4'-chloromethyl-4,8-dimethyl-4',5'-dihydropsoralen. The presence of a less labile leaving group on the 4'-halomethyl-4',5'-dihydropsoralen might favor the substitution versus elimination which was problematic in the the 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen where elimination was favored over substitution.

The iodine/samarium iodide/HMPA ring closure of 6-iodo-7-allyloxy-4,8-dimethylcoumarin was not successful but warrants further study. Varying reaction conditions such as the use of excess SmI_2 or HMPA might favor the ring closure before extraction of a hydrogen from the solvent that results in dehalogenated starting material as the primary reaction product. The presence of the pyrone ring of the coumarin modifies the reactivity from the iodoallyloxybenzene in Curran's work (Curran, 1992).

The study of the mechanism for pyridine substitution of the 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen and the the 5'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen and the 5'-iodomethyl-4-methyl-4',5'-dihydroangelicin showed competing E_2 and S_N2 pathways. For the the 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen the elimination to the fully aromatized 4,8,4'-trimethyl psoralen is the major product. Stronger bases and more hindered amines have a greater tendency to dehydrohalogenate through the exocyclic methylene psoralen as a transient intermediate. The amine substitutions of the the 5'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralens and the 5'-iodomethyl-4-methyl-4',5'-dihydroangelicins show slower rates but no such exocyclic methylene intermediate and the pyridinium iodide salts of these compounds readily form with no dehydrohalogenation. With strong bases or hindered amines, 5'-

iodomethyl-4,8-dimethyl-4',5'-dihydropsoralens and the 5'-iodomethyl-4-methyl-4',5'-dihydroangelicins can dehydrohalogenate to the fully aromatized system.

Although not considered to be a feasible large scale synthetic route to psoralen synthesis, the mercury ring closed products and the psoralen mimics had surprising dark activity that was enhanced by light activation, an unusual phenomenon in psoralen chemistry. With the 2-mercurimethyltetrahydrofurans having similar activity to the mercurated psoralens in the PAM 212 bioassay, receptor binding was not a factor in the toxicity of these molecules. Laskin found that addition of glutathione to the bioassay increased the IC_{50} of the mercurated psoralens, leading to the conclusion that glutathione competes with protein thiols of the mercury compounds. Addition of excess D,L-buthione-[S,R]-sulfoximine (BSI), a glutathione inhibitor, decreased the IC_{50} to one-fourth or less of earlier values. When Laskin added n-acetyl-l-cystine, a glutathione precursor, PAM keratinocytes were protected from the lethal effects of the mercury psoralens and mercury mimics.

MODE OF ACTION AND PHOTOCHEMICAL STUDY

To prove the interaction of the mercury psoralens and mercury mimics discusses herein with protein thiols several techniques were employed. Although electrospray mass spectrometry was inconclusive, a UV study confirmed mercury-sulfur bond formation. The unusual photochemical behavior of mercurated psoralens and mercury mimics leads to the conclusion that the dark activity of the mercury compounds arises from binding to thiols of proteins and once activated by light generation of free radicals which attack cell components enhances the activity. The acetomercurimethyltetrahydrofuran with

peroxide, which was irradiated, extruded elemental mercury, a phenomena documented in Russel's work (Russel, 1982 and 1985).

STRUCTURE ACTIVITY RELATIONSHIPS

The PAM keratinocyte cell line was developed for a sensitive assay of epidermal toxicity. The halogenated precursors showed impressive activity, with IC_{50}'s from 0.5 - 6 *u*M for the 5'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralens and for 0.1 to 0.5 *u*M for the 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralens. The reduced biological activity that resulted with substitution of the 3-position could be the result of steric hindrance at the receptor or changes in the lipophilicity, modifying the membrane penetration. The 5'-iodomethyl-4-methyl-4',5'-dihydroangelicin showed less biological activity than the halogenated psoralens, but it was not determined if this was from reduced receptor affinity or lack of DNA crosslink formation. The most active compounds generated were the amino derivatives of the 5'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralens , with IC_{50}'s as low as $3.5 \cdot 10^{-3}$ to 2 *u*M. Dehydrohalogenation in the 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralens prevented synthesis of that series for comparison.

The mercurated psoralens, angelicins and models have potential as topical antifungals and as antituberculosis drugs. Although concern exists for the use of mercury based drugs, the ability to control multidrug resistant tuberculosis strains, as with the 99% inhibition of growth by the 2-iodomercurimethyltetrahydrofuran could justify their use. The compound progressed to the second tier screening in a primary *in vitro* assay by The National Institute of Allergy and Infectious diseases against *Mycobacterium*

tuberculosis H37Rv. All compounds showing antituberculosis activity had a labile iodine.

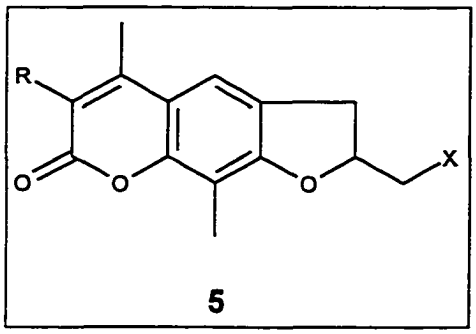
PHARMACOLOGICAL, FUNGICIDAL AND ALGICIDAL ASSAY TECHNIQUES

KERATINOCYTE ASSAY

An assay for the photobiological activity of psoralens and psoralen-like therapeutic candidates was developed and demonstrated to show positive responses to the known clinically approved psoralens by Professor Jeffrey Laskin. Mouse PAM cells were seeded in 6-well tissue culture plates at 5,000 cells/well (2 mL) in complete DMEM medium (Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin G, 10,000 *u*g/mL streptomycin sulfate, and 50 *u*g/mL gentamycin sulfate). The next day the medium was removed and replaced with 1.5 mL complete medium containing the indicated concentrations of compound. After 30 min at 37°C, the lids were removed from the plates which were then placed under a set of 4 UVA light bulbs for 10 min (0.69 J/cm^2). The medium was then removed and replaced with 2 mL fresh complete DMEM. After 4 days at 37°C, the cells were released with 0.5 mL trypsin, diluted into 9.5 mL Isotone II (Coulter Corporation), and 0.5 mL was counted with a Model ZBI Coulter Counter. The biological activity of a compound was assessed by plotting the number of cells counted in a well treated with the compound as percent of the number of cells in an untreated well versus the concentration of the compound. A range of concentrations was tested with the UVA light treatment and the highest 4 concentrations were also tested in the absence of UVA light treatment to

determine the specificity of the UVA activation of the compound. The concentration of compound required for inhibition of 50% of cell growth (IC_{50}) was then determined.

Table 2 (IC_{50} values in uM)



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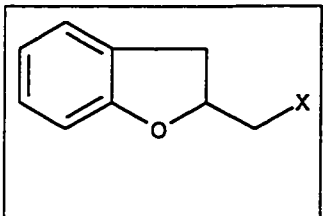
Compound	Photo-toxic activity	
R = H, X = HgI	1.3	(4.2)**
R = H, X = Hg-O-$COCF_3$	4.2	(7.0)**
R = H, X = $HgOCOCH_3$	5.0	(6.0)**
R = H, X = HgCl	6.1	(15)**
R = F, X = Hg-O-$COCH_3$	13	(14)**
R = CN, X = Hg-O-$COCH_3$	20	(30)**
R = Br, X = I	0.48	
R = H, X = I	1.0	
R = CN, X = I	4.0	
R = F, X = Br	4.0	

R = I, X = I	4.2
R = F, X = I	6.2
R = CN, X = Br	6.4

R = H, X = 2,6-dimethylmorpholino	0.01
R = H, X = N,N-dimethylamino hydroiodide salt	0.10
R = H, X = $N(CH_2CH_2OH)_2$	0.30, 1.4
R = H, X = morpholino	3.2
R = H, X = pyridinium iodide salt	0.37
R = Br, X = pyridinium iodide salt	1.6
R = CN, X = pyridinium iodide salt	>100
R = H, X = H	0.27
R = Br, X = phthalimido	3.9



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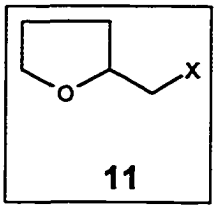
Compound		
X = Hg-O-$COCH_3$	2.7	(7)**
X = HgI	3.9	(9.5)**
X = HgCl	6	(11)**

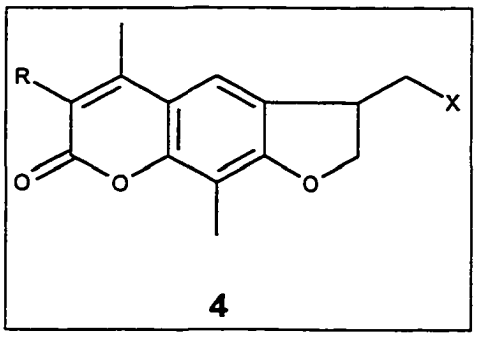


11

Compound

X = Hg-O-$COCH_3$	0.20	(4)**
X = Hg-O-$COCF_3$	0.21	(2)**

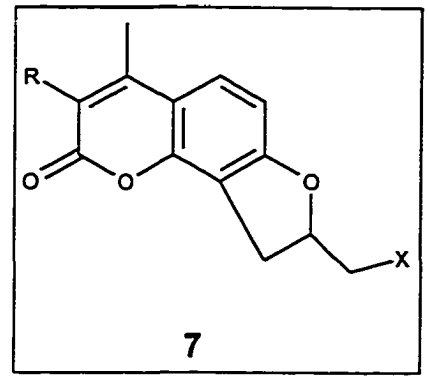
** Mercury compounds demonstrated toxicity without light but no other compounds showed this dark toxicity effect. With light all mercury compounds demonstrated an enhanced toxicity. The values shown above as ( )** are the dark-reaction toxicities in uM.



4

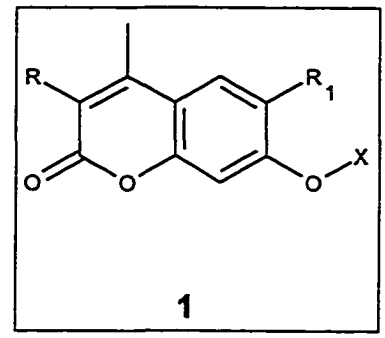
Compound

R = H, X = I	0.1	>(100)**
R = H, X = Pyridinium iodide	0.5	
R = Br, X = I	0.2, 0.5	
R = Br, X = Pyridinium iodide	>30	



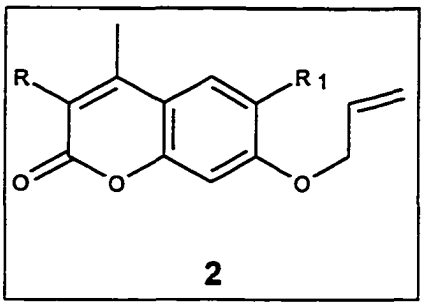
7

Compound		
$R = R_1 = H, X = I$	21	>(100)**
$R = R_1 = H, X = HgOCOCH_3$	23	(32)**
$R = R_1 = H, X = HgI$	23	(32)**
$R = R_1 = H, X =$ Pyridinium iodide salt	300	



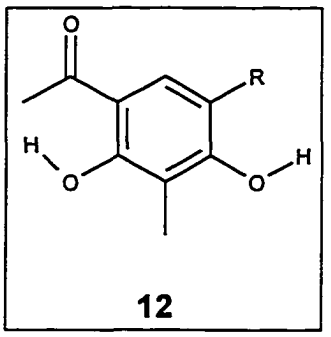
1

Compound	
$R = H, R_1 = I, X = H$	18
$R = I, R_1 = I, X = H$	>300
$R = I, R_1 = H, X = H$	61
$R = H, R_1 = H, X = CH_3$	40
$R = H, R_1 = NO_2, X = H$	>300



2

Compound		
R = H, R_1 = I	1.1	
R = I, R_1 = I	1.9	
R = H, R_1 = NO_2	5	>(100)**
R = H, R_1 = NH_2	240	>(100)**



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Compound	
R = I, X = H	>300
R = I, X = $CH_2CH{=}CH_2$	90

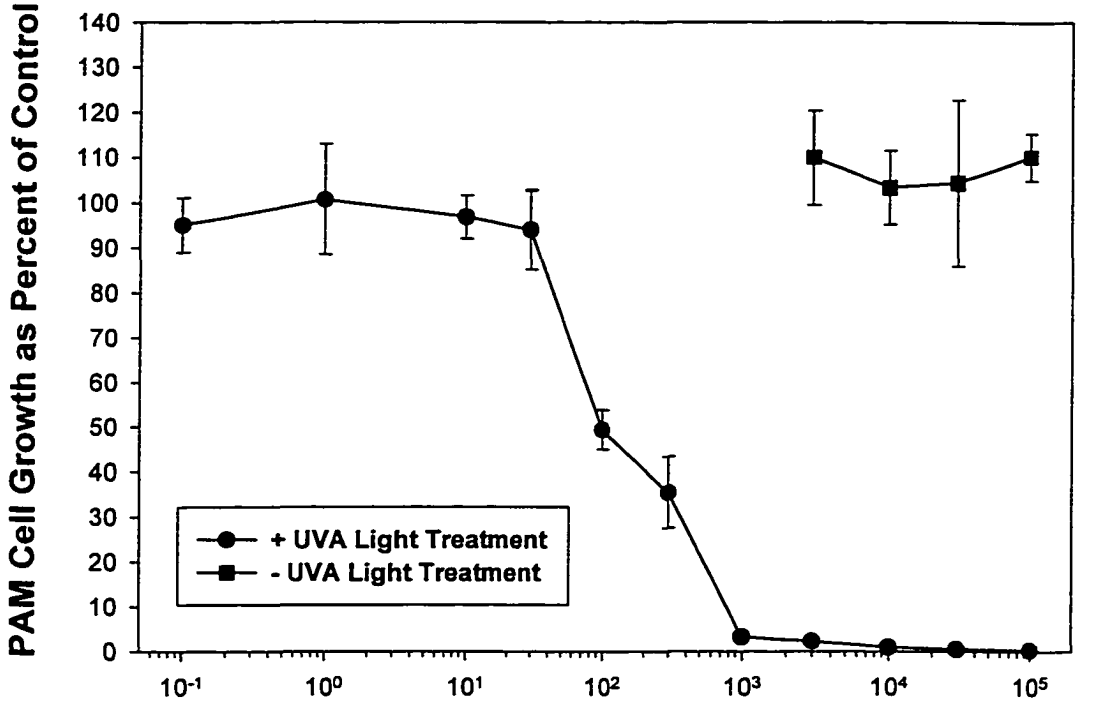


Figure 31 PAM cell assay of Biological activity

Biological Activity of 5'-[N,N-dimethylamino]methyl-4,8-dimethyl-4',5'-dihydropsoralen HI salt. The number of cells counted in a well treated with the compound as percent of the number of cells in an untreated well is plotted versus the compound concentration. Circles represent samples treated with the compound and then UVA light, and squares represent samples treated with the compound but no UVA light. This compound has an IC_{50} of about 100 nM in the presence of UVA light, but no IC_{50} could be determined in the absence of UVA light even when the cells were treated with up to 100,000 nM 5'-[N,N-dimethylamino]methyl-4,8-dimethyl-4',5'-dihydropsoralen HI salt.

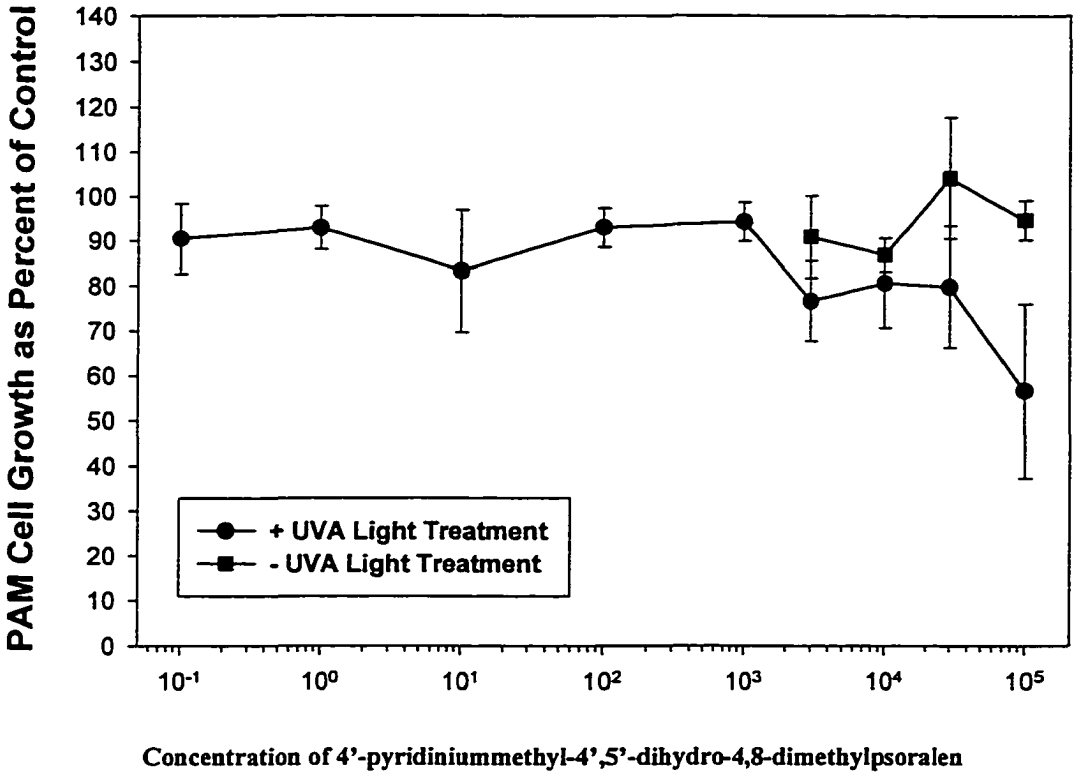


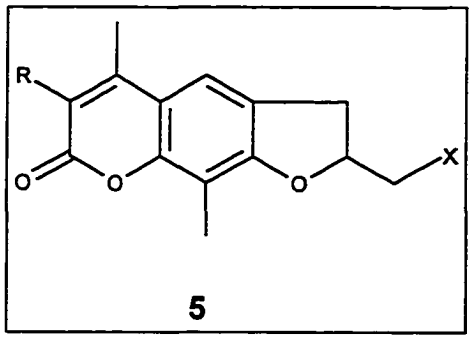
Figure 32 PAM cell assay of biological activity

Biological Activity of 4'-pyridiniummethyl-4',5'-dihydro-4,8-dimethylpsoralen. The number of cells counted in a well treated with the compound as percent of the number of cells in an untreated well is plotted versus the compound concentration. Circles represent samples treated with the compound and then UVA light and squares represent samples treated with the compound but no UVA light. This compound has no IC_{50} that could be determined either in the presence or absence of UVA light even when the cells were treated with up to 100,000 nM 4'-pyridiniummethyl-4',5'-dihydro-4,8-dimethylpsoralen.

FUNGICIDE SCREEN

The antifungal activities of compounds synthesized were measured by determining the inhibition of growth of *Aspergillus niger* in a standard liquid medium. The liquid growth medium is described as a solution of mineral salts + yeast extract (MSY). The MSY broth was prepared as described in ASTM G 21-70 (American Standard Testing Methods), and amended with glucose (10 g liter^{-1}) and yeast extract (1 g liter^{-1}). An aliquot (250 *u*L) of sterile medium was dispensed into each test well of a standard 96-well microplate (Corning No. 430247). Stock solutions of test compounds were prepared by dissolving the materials in 9:1 (v/v) solution of acetone:methanol. Appropriate volumes of stock solutions were added to the test wells to achieve the desired ppm levels. Each test well (plus controls) was then inoculated with 5 *u*L of a standardized suspension of *Aspergillus niger*. The cell suspension was prepared by suspending viable cells from a slant of potato dextrose agar into sterile, deionized water. The suspension was then adjusted to provide $OD_{686} = 0.28$. This density contains approximately 2.5×10^7 CFU (colony forming units) mL^{-1}. The microplates were incubated in the dark for four days at 28° C. The optical density for each well at 686 nm was then recorded automatically using a SpectraMax 340 microplate reader (Molecular Devices Corp., Sunnyvale, CA). All wells were visually inspected to corroborate data from the instrument. Test wells with an OD ≤ 0.05 were judged to exhibit complete inhibition of cellular growth.

Table 3 MIC (parts per million) against *Aspergillus niger*



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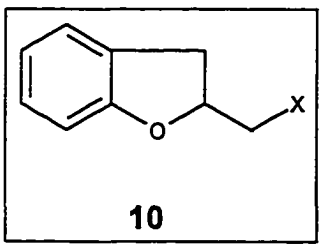
Compound	*Aspergillus niger* MIC (parts per million)
R = H, X = HgCl	<8
R = H, X = HgI	<8
R = H, X = HgOAc	<8
R = H, X = Hg-O-$COCF_3$	6



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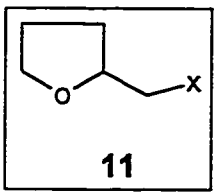
Compound	
X = HgCl	24
X = HgI	<8
X = HgOAc	16



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Compound	
X = HgOAc	<8
X = Hg-O-$COCF_3$	<8

ALGICIDE SCREEN

Test tubes containing modified Allen's medium are amended with increasing dosages of a compound to be tested and inoculated with a suspension of algal cells. After fourteen days of incubation, algal growth is rated as positive or negative. The minimum inhibitory concentration (MIC) of the compound is the smallest dosage showing negative algal growth.This test method, developed by Dr. Graciela H. Vunk at Buckman Laboratories, provides a technique for screening newly synthesized compounds for their effectiveness to inhibit algal growth. A correlation between test findings and in-field results is presumed but the precise degree of correlation between this test and field tests has not yet been determined.

The apparatus required consisted of sterilized 18-150 mm test tubes and an incubator capable of a constant (± 2° C) temperature within 26 to 28° C.

Reagents And Materials

Stock solutions (g/200 g deionized water each) were generated with: K_2HPO_4, 1.50 g; $MgSO_4 \cdot 7H_2O$, 1.50 g; Na_2CO_3, 0.80 g; $CaCl_2 \cdot 2H_2O$, 0.50 g; $Na_2SiO_3 \cdot 9H_2O$, 1.16 g and Citric acid, 1.20 g.

PIV metal stock solution (g/1000 g deionized water) was generated by the addition of: Na_2EDTA, 0.750 g; $FeCl_3 \cdot 6H_2O$, 0.097 g; $MnCl_2 \cdot 4H_2O$, 0.041 g; $ZnCl_2$, 0.005 g; $CoCl_2 \cdot 6H_2O$, 0.002 g and $Na_2MoO_4 \cdot 2H_2O$, 0.004 g.

The modified Allen's medium is prepared in a volumetric flask by the addition of 1.5 g $NaNO_3$, 5.0 mL stock solution K_2HPO_4, 5.0 mL stock solution $MgS0_4$. $7H_2O$, 5.0 mL Stock solution Na_2CO_3, 10.0 mL stock solution $CaCl_2 \cdot 2H_2O$, 10.0 mL stock solution $Na_2SiO_3 \cdot 9H_2O$, 1.0 mL stock solution Citric acid and 1.0 mL stock solution PIV metal with enough deionized water to make 1000 mL of medium (Allen, 1968). The pH is adjusted to 7.8 and 5 mL of medium is added to each test tube. These tubes are then sterilized in the autoclave for 20 minutes at 15 psi at 121°C. The test tubes are allowed to cool to 45-50 °C before adding the test compound and the inoculum to the medium.

The inoculum is a cell suspension from a 14-day-old culture *of Chlorella vulgaris* (UTEX 26, University of Texas) that was grown in modified Allen's medium.

Compound incorporation

The concentration of the stock solution in water or acetone of the compound to be tested is dependent on the largest dose required to be tested. Dilutions of the stock solution are made so that 100 *u*L of the stock solution of the corresponding dilution is added per test tube. To the control is added 100 *u*L of the solvent used to prepare the stock solution.

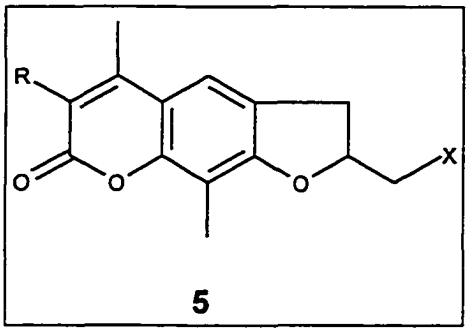
The inoculation consists of the addition of 100 *u*L of inoculum per test tube.

The incubation of the control test tube and the test tubes containing compound and inoculum should occur in an incubator set between 26 to 28° C, with the temperature not varying more than ± 2° C. Light should be provided by plant growth fluorescent tubes and the test tubes should be allowed to stand in the incubator for 14 days.

Rating of the test tubes containing different dosages of the compound will be positive or negative. If the medium in the tubes shows green coloration (algal growth), particularly at the bottom, a positive (contaminated) rating is given. If the medium in the tubes remains colorless, a negative (not contaminated) rating is given. The control is always positive. The minimum inhibitory concentration (MIC) of the compound is determined to be the smallest dosage showing negative algal growth.

Table 4 MIC values against *Chlorella vulgaris* (parts per million, weight/volume)

**NO LIGHT ACTIVATION



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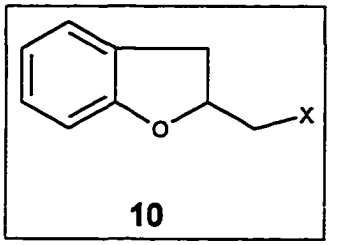
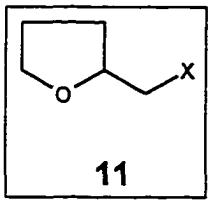
Compound	*Chlorella vulgaris* MIC
R = H, X = HgCl	10
R = H, X = HgI	9
R = H, X = HgOAc	10
R = H, X = Hg-O-$COCF_3$	10



10

Compound	
X = HgCl	10
X = HgI	10
X = HgOAc	10



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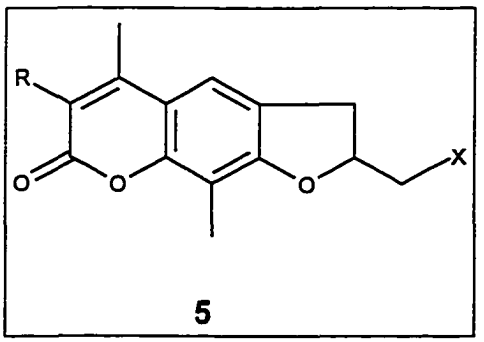
Compound	
X = HgOAc	4
X = Hg-O-$COCF_3$	4

ANTITUBERCULOSIS ASSAY

Compounds were screened in a primary *in vitro* assay by The National Institute of Allergy and Infectious diseases against *Mycobacterium tuberculosis* H37Rv.The primary screen was run using a BACTEC 460 system. Compounds were solubilized in dimethylsulfoxide at 1 mg/mL and sterilized by passage through 0.22 *u*m PFTE filters. Fifty *u*L was added to 4 mL BACTEC 12B medium (Becton Dickinson) to achieve a final concentration of 12.5 *u*g/mL. Approximately 4 x 105 colony forming units of M. tuberculosis H37Rv ATCC 27294 were added and the cultures were incubated at 37°C. Starting on the second day of the incubation, the Growth Index (GI, 1 GI = 0.0025 dpm $1CO_2$) was determined daily until the controls (drug-free) achieved a GI of 999. The percent inhibition was calculated as 1 – (test sample GI ÷ control GI) x 100 (Collins, 1997).

Table 5 Inhibition of *Mycobacterium tuberculosis* (as % inhibition at 12.5 *u*g/ml)



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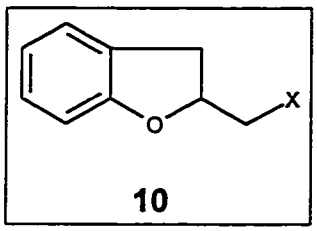
Compound	Anti-tubercular Activity
R = F, X = I	31
R = Br, X = I	25

R = H, X = I 24

R = H, X= -$N(CH_3)_2$ HI salt 18

R = I, X = I 17



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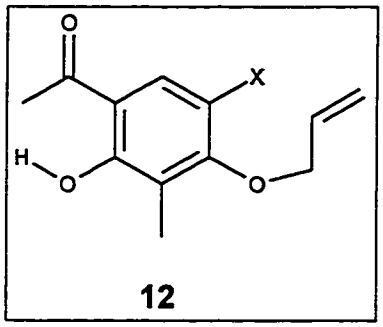
Compound

X = HgI 99



12

Compound

X = I 35

EXPERIMENTAL

GENERAL

Chemicals and solvents were obtained from commercial sources and used without further purification unless otherwise stated. Melting points were determined on a Mettler FP 81 MBC cell with a Mettler FP 80 central processor. Elemental analyses were determined by Oneida Research Services, Whitesboro, New York. Proton NMRs were recorded on a Bruker AC 250 operated at 250.13 MHz in the FT mode using deuterated solvents. Chemical shifts were reported downfield from tetramethylsilane (TMS). Chemical shifts were reported in the following order: (multiplicity, number of protons, coupling constants in Hz). Multiplicity is designated as follows: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet. The decoupled carbon NMR spectra were recorded on the Bruker AC 250 operating at 62.89 MHz in the FT mode. Deuterated solvents employed included $CDCl_3$, DMSO-d_6 and CD_3OD; these served as an internal reference for carbon spectra. Structural determination also included COSY, DEPT and HETCOR experiments. Gas chromatography/mass spectrometry was run on a Varian gas chromatograph 3300 model in series with a Finnegan ITS 40™ Magnum Ion Trap Mass Spectrometer in EI and CI mode. Electrospray mass spectrometry was run by Oneida Research Services on a Finnegan MAT TSQ700™ in the positive ion mode. High performance liquid chromatography (HPLC) analyses were run on a Hewlett Packard HP 1050 Series model.

SYNTHESIS OF 3-BROMOCOUMARINS AND DIHYDROPSORALENS

Preparation of 3-bromo-4,8-dimethyl-6-allyl-7-hydroxycoumarin

A mixture of 4,8-dimethyl-6-allyl-7-hydroxycoumarin (1.25 g, 5.43 mmoles) and N-bromosuccinimide (NBS) (1.12 g, 6.20 mmoles) was added to 50 mL dry tetrahydrofuran with stirring. The mixture was stirred in the dark at room temperature for 1.5 hours. Saturated aqueous potassium sulfite was added and stirring was continued for an additional ten minutes. The THF was evaporated, and solids were taken up in chloroform, washed with brine and dried over $MgSO_4$. The chloroform was evaporated *in vacuo* to give pale yellow crystals. The product was purified on a silica column using 30% ethyl acetate / 70% hexanes, with a yield of 1.29 g (77% yield) 3-bromo-4,8-dimethyl-6-allyl-7-hydroxycoumarin. The product was further purified by column chromatography on silica gel with 5% MeOH / 95% $CDCl_3$ yielding white crystals with a mp of 175.4-175.8°C. TLC on silica gel using 5% MeOH / 95% $CHCL_3$ showed a single spot with an Rf of 0.38.

Anal. Calcd for $C_{14}H_{13}BrO_3$ x 0.2 H_2O: C, 53.76; H, 4.32. Found: C, 53.75; H, 4.36.

^{1}H-nmr ($CDCl_3$): δ 2.32 (s, 3H), 2.57 (s, 3H), 3.47 (d, *J*= 6.2 Hz, 2H), 5.18-5.25 (m, 2H), 5.55 (s, 1H), 5.96-6.04 (m, 1H), 7.24 (s, 1H).

Preparation of 3-bromo-4,8-dimethyl-5'-bromomethyl-4',5'-dihydropsoralen

In a round-bottom flask equipped with stirrer, 4,8-Dimethyl-6-allyl-7-hydroxycoumarin (2.00 g, 8.68 mmoles) was added to 200 mL of anhydrous THF before

the addition of NBS (4.68 g, 26.3 mmoles). The mixture was stirred in the dark at room temperature for 1.5 hours. The GC/MS indicated no starting material remained. Saturated aqueous sodium bisulfite was added, and stirring continued for ten minutes. The THF was evaporated and solids were taken up in chloroform, washed with NaCl solution and water, followed by drying over $MgSO_4$. Evaporation of the chloroform layer yielded mustard yellow crystals. The product was recrystallized from methanol. No evidence was seen of the unwanted pyrano isomer, as was found in the molecular bromination route of the 3-bromo-4,8-dimethyl-6-allyl-7-hydroxycoumarin reported in previous work in this lab. The yield was 2.88 g (85% yield). The compound was purified by column chromatography on silica gel with 5% MeOH / 95% $CHCl_3$ to yield tan crystals. TLC using the same solvent showed one spot with an Rf of 0.72. Further recrystallization from methanol gave white crystals with a mp of 182.3-182.5°C.

Anal. Calcd for $C_{14}H_{12}Br_2O_3$: C, 43.33; H, 3.12. Found: C, 43.76; H, 3.08.

^{1}H-nmr (DMSO-d_6): δ 2.23 (s, 3H), 2.55 (s, 3H), 3.14 (dd, J_1 = 14.3 Hz, J_2 = 9.4 Hz, 1H), 3.46 (dd, J_1 = 14.3 Hz, J_2 = 4.9 Hz, 1H), 3.96 (d, J = 5.6 Hz, 2H), 4.67-4.77 (m, 1H), 7.52 (s, 1H). ^{13}C-nmr (DMSO-d_6): δ 8.77, 8.90, 19.43, 53.31, 53.64, 107.92, 111.26, 111.89, 122.67, 125.07, 149.98, 151.99, 156.58, 156.91.

SYNTHESIS OF 4,8-DIMETHYL-5'-IODOMETHYL-4',5'-DIHYDROPSORALENS

Preparation of 4,8-dimethyl-5'-iodomethyl-4',5'-dihydropsoralen

Route One - To 50 mL methylene chloride was added 4,8-dimethyl-6-allyl-7-hydroxycoumarin (2.00 g, 8.68 mmoles) with stirring to dissolve the solid before the addition of N-iodosuccinimide (NIS) (2.92 g, 13.0 mmoles). The reaction mixture was stirred at room temperature overnight. The GC/MS indicated conversion was complete. Saturated aqueous bisulfite was added with stirring continued for ten minutes. The THF was evaporated, solids were taken up in chloroform, washed with a saturated aqueous NaCl solution, washed with water and dried over $MgSO_4$. Evaporation of the solvent gave 2.84 g (92% yield). Recrystallization from ethanol gave white crystals with mp of 137.4-138.2°C. TLC with 5% MeOH / 95% $CHCl_3$ showed one spot with Rf 0.78.

Anal. Calcd for $C_{14}H_{13}IO_3$: C, 47.21; H, 3.68. Found: C, 47.27; H, 3.77.

^{1}H-nmr ($CDCl_3$): δ 2.19 (s, 3H), 2.28 (s, 3H), 3.04 (dd, J_1 = 15.8 Hz, J_2 = 6.7 Hz, 1H), 3.33 (dd, J_1 = 9.9 Hz, J_2 = 7.6, 1H), 3.40 (dd, J_1 = 15.8 Hz, J_2 = 6.7, 1H), 3.44 (dd, J_1 = 9.9, J_2 = 4.9, 1H), 4.84-4.92 (m, 1H), 6.01 (s, 1H), 7.13 (s, 1H). ^{13}C-nmr ($CDCl_3$): δ 8.48, 8.61, 19.04, 35.77, 82.56, 108.13, 111.24, 113.99, 117.35, 121.98, 152.81, 153.19, 160.91, 161.56.

Route Two - To 50 mL of methylene chloride was added 4,8-dimethyl-6-allyl-7-hydroxycoumarin (5.02 g, 21.8 mmoles), 12 mL 1 M $SnCl_4$ and I_2 (5.50 g, 22.6 mmoles). Stirring was continued at room temperature overnight. Ice water was added and mixture was treated with 0.5 N NaOH, washed with 5% $Na_2S_2O_3$ and water before drying over

$MgSO_4$. Methylene chloride was removed *in vacuo* with a yield of 6.37 g (82% yield). Recrystallization from ethanol or purification on a silica column with 1% acetone / 99% methylene chloride afforded pure product.

Preparation of 3-iodo-4,8-dimethyl-5'-iodomethyl-4',5'-dihydropsoralen

To one mL of glacial acetic acid was added 4,8-dimethyl-5'-iodomethyl-4',5'-dihydropsoralen (100 mg, 28 mmoles) and ICl (72 mg, 42 mmoles) with heating at 50°C and stirring continued overnight. TLC of the reaction mixture on silica gel with 5% MeOH / 95% $CHCl_3$ showed three spots with Rfs of 0.81, 0.57 and 0.27. Tan crystals were collected by filtration and washed with ether. The yield was 114 mg (85% yield) after recrystallization from ethanol. Purification on a silica gel column using 5% MeOH / 95% $CHCl_3$ afforded white crystals with mp 213-214°C.

Anal. Calcd for $C_{14}H_{12}I_2O_3$: C, 34.88; H, 2.51. Found: C, 35.09; H, 2.61.

^{1}H-nmr ($CDCl_3$): δ 2.29 (s, 3H), 2.65 (s, 3H), 3.14 (dd, J_1 = 15.8 Hz, J_2 = 6.7 Hz, 1H), 3.35-3.54 (m, 3H), 4.92-4.99 (m, 1H), 7.31 (s, 1H). ^{13}C-nmr ($CDCl_3$): δ 8.52, 8.61, 25.81, 35.75, 82.79, 88.14, 107.85, 113.61, 117.86, 118.10, 152.25, 156.58, 158.25, 161.21.

Preparation of 3-bromo-4,8-dimethyl-5'-iodomethyl-4',5'-dihydropsoralen

Route one - To 15 mL methylene chloride was added 3-bromo-4,8-dimethyl-6-allyl-7-hydroxycoumarin (0.200 g, 0.645 mmoles) and N-iodosuccinimide (0.217 g, 0.967 mmoles). The flask was stoppered and stirred overnight at room temperature.

Bisulfite was added and stirring continued for an additional ten minutes. The methylene chloride layer was extracted with water. Unreacted starting material was removed by a base extraction. The organic layer was dried over $MgSO_4$, and evaporated to give a pale mustard colored solid. The product may be recrystallized from ethanol or purified by column chromatography using 5% MeOH / 95% $CHCl_3$. The yield of white crystals was 0.271 g (77% yield) with mp of 188.7-189.4°C.

Anal. Calcd for $C_{14}H_{12}BrIO_3$: C, 38.65; H, 2.78. Found: C, 39.37; H, 2.88.

^{1}H-nmr ($CDCl_3$): δ 2.30, (s, 3H), 2.56, (s, 3H), 3.16 (dd, J_1 = 15.9 Hz, J_2 = 6.7 Hz, 1H), 3.40 (dd, J_1 = 9.3 Hz, J_2 = 7.6 Hz, 1H), 3.47 (dd, J_1 = 15.9 Hz, J_2 = 6.7 Hz, 1H), 3.51 (dd, J_1 = 9.3 Hz, J_2 = 4.9, 1H), 4.93-5.30 (m, 1H), 7.28 (s, 1H); ^{13}C-nmr ($CDCl_3$): δ 8.52, 8.61, 19.81, 35.80, 82.74, 108.03, 109.15, 113.84, 117.86, 122.89, 151.29, 151.54, 157.53, 161.06.

The 3-bromo-4,8-dimethyl-5'-iodomethyl-4',5'-dihydropsoralen may also be generated by the iodine/$SnCl_4$ ring closure of bromo-4,8-dimethyl-6-allyl-7-hydroxycoumarin.

Route 2 - To 4,8-dimethyl-5'-iodomethyl-4',5'-dihydropsoralen (0.075 g, 0.210 mmoles) in methylene chloride was added NBS (0.056 g, 0.310 mmoles). Stirring was continued in the dark at room temperature overnight. The solvent was evaporated *in vacuo*, solids were extracted with chloroform and the organic layer was washed with aqueous bisulfite and water. The organic layer was dried and solvent removed *in vacuo* to recover 0.076 g (84% yield) product. Recrystallization from ethanol afforded white crystals with mp 188.9-189.5°C.

Preparation of 3-fluoro-4,8-dimethyl-5'-iodomethyl-4',5'-dihydrofurocoumarin

For three hours at room temperature 3-fluoro-4,8-dimethyl-6-allyl-7-hydroxycoumarin (200 mg, 0.804 mmoles) was stirred with N-iodosuccinimide (271 mg, 1.20 mmoles) in enough methylene chloride to dissolve. Sodium bisulfite was added to decolorize before transferring to a separatory funnel for washing with two 1.5 mL portions of water. Recovered was 277 mg crystals (91% yield). Further purification was achieved by chromatographing on silica gel with 30% ethyl acetate / 70% hexane.

Anal. Calcd for $C_{14}H_{12}FIO_3$: C, 44.94; H, 3.23. Found: C, 45.05; H, 3.26.

^{1}H-nmr ($CDCl_3$): δ 2.30, (s, 3H), 2.56, (s, 3H), 3.16 (dd, J_1 = 15.9 Hz, J_2 = 6.7 Hz, 1H), 3.40 (dd, J_1 = 9.3 Hz, J_2 = 7.6 Hz, 1H), 3.47 (dd, J_1 = 15.9 Hz, J_2 = 6.7 Hz, 1H), 3.51 (dd, J_1 = 9.3 Hz , J_2 = 4.9, 1H), 4.93-5.25 (m, 1H), 7.28 (s, 1H). ^{13}C-nmr ($CDCl_3$): δ 8.94, 9.03, 10.76, 36.27, 82.95, 108.62, 113.46, 117.65, 123.48, 132.03, 142.50, 150.05, 155.73, 160.56.

Preparation of 3-cyano-4,8-dimethyl-5'-iodomethyl-4',5'-dihydropsoralen

3-Cyano-4,8-dimethyl-6-allyl-7-hydroxycoumarin (69.0 mg, 0.270 mmoles) was added with NIS (91.0 mg, 0.405 mmoles) to 2 mL deuterochloroform and stirred at room temperature overnight. Saturated aqueous bisulfite was added and stirring was continued for ten minutes. The organic layer was washed with water and dried over $MgSO_4$. The solvent was evaporated *in vacuo*. Recovered was 70.0 mg (71% yield) bright yellow crystals after column chromatography using 5% MeOH / 95% $CHCl_3$ on silica gel. The mp was 234.7-234.9°C.

Anal. Calcd for $C_{15}H_{12}INO_3$: C, 47.21; H, 3.17. Found: C, 47.08; H, 3.29.

^{1}H-nmr ($CDCl_3$): 2.28 (s, 3H), 2.70 (s, 3H), 3.16 (dd, $J_1 \cong 15$ Hz, $J_2 \cong 6$ Hz, 1H), 3.42-3.56 (m, 3H), 5.00-5.08 (m, 1H), 7.36 (s, 1H). ^{13}C-nmr ($CDCl_3$): δ 8.44, 8.60, 18.50, 35.69, 83.42, 96.52, 108.89, 112.67, 114.53, 119.17, 121.98, 153.65, 157.83, 162.25, 164.14.

SYNTHESIS OF 4,8-DIMETHYL-5'-AMINOMETHYL-4',5'-DIHYDROPSORALENS

Preparation of 4,8-dimethyl-5'-morpholinomethyl-4',5'-dihydropsoralen

4,8-Dimethyl-5'-iodomethyl-4',5'-dihydropsoralen (500 mg, 1.40 mmoles) was added to dry morpholine (4 mL) and heated at reflux for three hours. The mixture was evaporated *in vacuo* to remove excess morpholine and the product was taken up in methylene chloride and washed with water. The organic layer was dried and evaporated *in vacuo* to recover tan crystals, 0.306 g (70% yield). The product was purified by column chromatography on silica gel with 30% ethyl acetate / 70% hexane to give white crystals with mp 159.8-160.9°C. A second portion was purified on a silica gel column with 5% MeOH / 95% chloroform with an Rf 0.43 on a TLC plate with the same solvent. This fraction was submitted for elemental analysis.

Anal. Calcd for $C_{18}H_{21}NO_4$: C, 68.55; H, 6.71; N, 4.44. Found: C, 68.38; H, 6.73; N, 4.34.

^{1}H-nmr ($CDCl_3$): δ 2.17 (s, 3H), 2.27 (s, 3H), 2.40-2.70 (m, 6H), 2.99 (dd, 1H), 3.25 (dd, $J_1 = 15.3$ Hz, $J_2 = 9.2$ Hz, 1H), 3.63 (t, $J = 4.8$ Hz, 4H), 4.87-4.96 (m, 1H), 5.98

(s, 1H), 7.13 (s, 1H). ^{13}C-nmr ($CDCl_3$): δ 8.49, 19.00, 33.60, 54.34 (2 carbons), 62.87, 66.93 (2 carbons), 82.83, 107.66, 110.90, 113.53, 117.23, 122.64, 152.80, 153.05, 160.72, 161.61.

Preparation of 4,8-dimethyl-5'-[N,N-β-(hydroxyethylamino)methyl]-4',5'-dihydropsoralen

4,8-dimethyl-5'-iodomethyl-4',5'-dihydropsoralen (235 mg, 0.660 mmoles) was added to 3 mL diethanolamine and heated at 80°C for eight hours. Recovery of pure product was difficult because of the high boiling point of diethanolamine. The reaction mixture was refluxed in methanol and allowed to stand overnight. Crystals were removed by filtration and were identified by NMR as 4,8,5'-trimethylpsoralen. Methanol was removed under vacuum and the mixture was taken up in chloroform. Diethanolamine separated as the upper layer and was removed. The chloroform layer was washed with dilute HCl and water to remove residual diethanolamine. Purification by column chromatography using 2% methanol / 98% chloroform, yielded pink crystals which weighed 0.050 g (23% yield). Crystals were taken up again into chloroform and washed with dilute aqueous HCl to remove residual diethanolamine which made recrystallization difficult. NMR indicates that no TMP remained as a contaminant in the product.

Anal. Calcd for $C_{18}H_{23}NO_5$ x: 2.9 moles H_2O: C, 63.65; H, 7.02; N, 4.12. Found: C, 63.67; H, 6.91; N, 4.22.

^{1}H-nmr (CD_3COCD_3): δ 2.19 (s, 3H), 2.40 (s, 3H), 2.79 (t, 4H), 2.91 (t, 2H), 3.08 (dd, 1H), 3.37 (dd, 2H), 3.57 (t, 4H), 5.01-5.09 (m, 1H), 6.04 (s, 1H), 7.40 (s, 1H). ^{13}C-

nmr ($CDCl_3$): δ 8.74,19.30, 33.38, 57.64 (2 carbons), 59.98, 60.16 (2 carbons), 83.62, 108.15, 111.07, 114.02, 117.81, 123.13, 153.29, 153.54, 161.21, 162.10.

Preparation of 4,8-dimethyl-5'-(2,6-dimethylmorpholino)methyl-4',5'-dihydropsoralen

4,8-Dimethyl-5'-iodomethyl-4',5'-dihydropsoralen (250 mg, 0.700 mmoles) was added to 2 mL 2,6-dimethylmorpholine and heated at close to reflux overnight. Excess dimethylmorpholine was removed under vacuum. Crystals recovered weighed 0.142 g (73.6% yield). TLC using 5% MeOH / 95% $CHCL_3$ showed two spots with Rf 0.82 and 0.46. Purification on silica gel column using the same solvent afforded white crystals with mp 167.2-169.0°C.

Anal. Calcd for $C_{20}H_{25}NO_4$ x 0.11 H_2O: C, 69.51; H, 7.30; N, 4.05. Found: C, 69.51; H, 7.46; N, 3.87.

^{13}C-nmr (DMSO-d_6): δ 8.27, 18.55, 18.96 (2 carbons), 32.88, 59.30, 59.71, 61.78, 70.94 (2carbons), 82.46, 105.99, 109.92, 113.03, 118.36, 123.27, 152.32, 153.90, 160.32, 160.77.

Preparation of 4,8-dimethyl-5'-[(N,N-dimethylamino)methyl]-4',5'-dihydropsoralen hydroiodide salt

4,8-Dimethyl-5'-iodomethyl-4',5'-dihydropsoralen (216 mg, 0.606 mmoles) was added to a Teflon lined metal reactor with dimethylamine (2 mL dimethylamine in methanol, 2M solution). The reactor was flushed with nitrogen then sealed before heating to 95°C for three hours. ^{13}C NMR indicates conversion to trimethylpsoralen mixed with

the desired 4,8-dimethyl-5'-[(N,N-dimethylamino)methyl]-4',5'-dihydropsoralen (1:1). Recovered weight was 206 mg. The mixture was purified by refluxing in chloroform to solubilize the trimethylpsoralen, and the 4,8-dimethyl-5'-[(N,N-dimethylamino)methyl]-4',5'-dihydropsoralen hydroiodide salt was collected by filtration.

Anal. Calcd for $C_{16}H_{20}NIO_3$: C, 47.90; H, 5.02; N, 3.49. Found: C, 48.27; H, 4.86; N, 3.43.

^{1}H-nmr (DMSO-d_6): δ 2.22 (s, 3H), 2.39 (s, 3H), 2.5 (s, 6H), 3.02 (dd, J_1 = 16 Hz, J_2 = 7 Hz, 1H), 3.42-3.58 (m, 3H), 3.91-4.13 (m, 1H), 5.30-5.46 (m, 1H), 6.21 (s, 1H), 7.54 (s, 1H). ^{13}C-nmr (DMSO-d_6): δ 9.62, 18.54, 32.63, 43.89 (2), 60.54, 81.28, 106.60, 110.32, 118.48, 122.26, 123.54, 152.70, 153.62, 153.78, 160.17.

Preparation of 4,8-dimethyl-5'-(N-pyridiniummethyl)-4',5'-dihydropsoralen iodide salt

4,8-Dimethyl-5'-iodomethyl-4',5'-dihydropsoralen (0.200 g, 56.0 mmoles) was added to 4 mL pyridine and heated at reflux overnight. Crystals were collected by filtration and washed with ether. The yield was 0.196 g (80% yield). Recrystallization from methanol was required to obtain buff yellow crystals that melted between 295-300°C.

Anal. Calcd for $C_{19}H_{18}INO_3$: C, 52.43; H, 4.17; N, 3.22. Found: C, 52.46; H, 4.31; N, 3.14.

^{1}H-nmr (CD_3OD): δ 2.36 (s, 3H), 2.48 (s, 3H), 3.19 (dd, J_1 = 16.5 Hz, J_2 = 5.5 Hz 1H), 3.57-3.63 (m, 1H), 4.82 (dd, J_1 = 14 Hz, J_2 = 10.3 Hz 1H), 5.04 (d, J_1 = 12.2 Hz, 1H), 5.36-5.44 (m, 1H), 6.19 (s, 1H), 7.51 (s, 1H), 8.21 (t, J_1 = 6.1 Hz, 2H), 8.65 (t, J_1 = 8.5 Hz, 1H), 9.10 (d, J = 6.1 Hz, 2H). ^{13}C-nmr (DMSO-d_6): δ 8.36, 18.63, 31.80, 63.24,

82.10, 106.99, 110.58, 113.83, 118.70, 122.30, 128.11 (2 carbons), 145.51 (2 carbons), 146.41, 152.36, 153.90, 159.51, 160.22.

Preparation of 3-bromo-4,8-dimethyl-5'-(N-pyridiniummethyl)-4',5'-dihydropsoralen iodide salt

3-Bromo-4,8-dimethyl-5'-iodomethyl-4',5'-dihydropsoralen (0.200 g, 46.0 mmoles) was added to 3 mL pyridine and heated at reflux overnight. Crystals were collected by filtration and washed with ether. Recrystallization from ethanol was required to obtain pure product, mustard colored crystals, which decomposed above 270°C. The yield was 0.100 g (57% yield).

Anal. Calcd for $C_{19}H_{17}BrINO_3$: C, 44.34; H, 3.30; N, 2.72. Found: C, 44.39; H, 3.24; N, 2.68.

^{1}H-nmr (CD_3OD): δ 2.36 (s, 3H), 2.48 (s, 3H), 3.19 (dd, J_1 = 16.5 Hz, J_2 = 5.5 Hz 1H), 3.57-3.63 (m, 1H), 4.82 (dd, J_1 = 14 Hz, J_2 = 10.3 Hz 1H), 5.04 (d, J = 12.2 Hz, 1H), 5.36-5.44 (m, 1H), 7.51 (s, 1H), 8.21 (t, J = 6.1 Hz, 2H), 8.65 (t, J = 8.5 Hz, 1H), 9.10 (d, J = 6.1 Hz, 2H).

Preparation of 3-cyano-4,8-dimethyl-5'-(N-pyridiniummethyl)-4',5'-dihydropsoralen iodide salt

Pyridine (3 mL) was added with 3-cyano-4,8-dimethyl-5'-iodomethyl-4',5'-dihydropsoralen (175 mg, 0.458 mmoles) and the mixture was heated at reflux for five hours. Crystals were collected by filtration and washed with ether. Recrystallization from ethanol gave pale orange crystals which melted above 300°C. The yield was 168 mg (78% yield) purified product.

Anal. Calcd for $C_{20}H_{17}IN_2O_3 \times 1.2\ H_2O$: C, 49.75; H, 4.24; N, 5.81. Found: C, 49.75; H, 4.15; N, 5.68.

^{1}H-nmr (CD_3OD): δ 2.21 (s, 3H), 2.70 (s, 3H), 3.29-3.34 (m, 1H), 3.70 (dd, J_1 = 16.3 Hz and J_2 = 9.3 Hz, 1H), 4.92-4.98 (m, 1H), 5.09 (dd, J_1 = 13.7 Hz and J_2 = 2.6 Hz, 1H), 5.43-5.53 (m, 1H), 7.69 (s, 1H), 8.21 (dd, J_1 = 7 Hz and J_2 = 7 Hz, 2H), 8.69 (dd, J = 7 Hz, 1H) 9.10 (d, J = 5.5 Hz, 2H). ^{13}C-nmr (DMSO-d_6): δ 8.19, 18.47, 31.43, 63.08, 83.01, 96.52, 107.33, 112.49, 114.79, 120.70, 124.07, 128.09 (2 carbons), 145.41 (2 carbons), 146.40, 152.65, 157.36, 162.43, 163.70.

SYNTHESIS OF 5'-SUBSTITUTED MERCURIMETHYL PSORALEN DERIVATIVES

Preparation of 4,8-dimethyl-5'-acetomercurimethyl-4',5'-dihydropsoralen

4,8-Dimethyl-6-allyl-7-hydroxycoumarin (230 mg, 1.00 mmole) was added to 25 mL methanol and heated to dissolve. Mercury (II) acetate (318 mg, 1.00 mmole) was dissolved in 1 mL water prior to dropwise addition over five minutes into the coumarin / methanol mixture. This was heated slightly for three hours with stirring. After cooling, the crystals were collected by filtration. The crystals were taken up into methylene chloride, extracted with water, then dried with magnesium sulfate. Evaporating *in vacuo* recovered 446 mg (91% yield). Recrystallization from methanol / ether afforded white crystals with mp 194.9-195.5°C.

Anal. Calcd for $C_{16}H_{16}HgO_5$: C, 39.32; H, 3.30. Found: C, 39.26; H, 3.08.

^{1}H-nmr (DMSO-d_6): δ 1.84 (s, 3H), 2.10 (s, 3H), 2.35 (s, 3H), 2.49 (t, J=2.5 Hz, 2H), 2.93 (dd, J_1 = 16 Hz, J_2 = 8 Hz, 1H), 3.43 (dd, J_1 = 16 Hz, J_2 = 8 Hz, 1H), 5.22-5.38 (m, 1H), 6.12 (s, 1H), 7.40 (s, 1H). ^{13}C-nmr (DMSO-d_6): δ 9.60, 18.55, 23.61, 29.26, 37.60, 85.38, 105.95, 109.74, 112.83, 118.30, 123.81, 152.51, 153.91, 160.35, 160.81, 174.68.

Preparation of 4,8-dimethyl-5'-chloromercurimethyl-4',5'-dihydropsoralen

4,8-Dimethyl-6-allyl-7-hydroxycoumarin (0.750g, 3.26 mmoles) was dissolved in 10 mL ethanol with slight heating. Over a five minute period, to this solution was added a solution of mercury (II) chloride (0.991 g, 3.73 mmoles) dissolved in 1 mL ethanol. This was heated at reflux for one hour. The product was recovered as the ring closed product. The yield was 1.09 g (72%). The crystals were taken up in hot ethanol and refluxed for several minutes before cooling. The white crystals were recovered by filtration and washed with ether. The product decomposed between 245-255°C.

Anal. Calcd for $C_{14}H_{13}ClHgO_3$: C, 36.15; H, 2.82. Found: C, 36.39; H, 2.69.

^{1}H-nmr (DMSO-d_6): δ 2.10 (s, 3H), 2.26 (s, 3H), 2.49 (t, J = 2.5 Hz, 2H), 2.92 (dd, J_1 = 16 Hz, J_2 = 8 Hz, 1H), 3.43 (dd, J_1 = 16 Hz, J_2 = 8 Hz, 1H), 5.22-5.38 (m, 1H), 6.13 (s, 1H), 7.42 (s, 1H). ^{13}C-nmr (DMSO-d_6): δ 8.35, 18.50, 29.61, 37.47, 85.31, 105.96, 109.74, 112.86, 118.37, 123.86, 153.91, 160.15, 160.81, 161.30.

Preparation of 4,8-dimethyl-5'-iodomercurimethyl-4',5'-dihydropsoralen

4,8-Dimethyl-5'-acetomercurimethyl-4',5'-dihydropsoralen (130 mg, 266 mmoles) was dissolved in 75 mL ethanol and heated with potassium iodide (145 mg, 880 mmoles) in water. The mixture was heated at reflux for 1.5 hour. Crystals were filtered and washed with water. The yield was nearly quantitative. White crystals were recrystallized from ethanol with mp 137.3-138.2°C.

Anal. Calcd for $C_{14}H_{13}HgIO_3$: C, 30.21; H, 2.35; Hg, 36.02; I, 22.80. Found: C, 32.41; H, 2.52; Hg, 33.58; I, 22.56.

^{1}H-nmr (DMSO-d_6): δ 2.10 (s, 3H), 2.34 (s, 3H), 2.48 (t, 2H), 2.90 (dd, 1 H), 3.40 (dd, 1H), 5.32-5.48 (m, 1H), 6.10 (s, 1H), 7.40 (s, 1H). ^{13}C-nmr (DMSO-d_6): δ 8.60, 18.55, 23.56, 37.58, 85.38, 105.94, 109.70, 112.83, 118.33, 123.80, 151.90, 153.92, 160.34, 160.81.

Preparation of 4,8-dimethyl-5'-trifluoroacetomercurimethyl-4',5'-dihydropsoralen

4,8-Dimethyl-6-allyl-7-hydroxycoumarin (230 mg, 1.00 mmole) was added to 25 mL methanol and dissolved by heating. Mercury (II) trifluoroacetate (443 mg, 1.04 mmoles) in ethanol was added dropwise with stirring. The product was recovered by filtration. The yield was nearly quantitative and white crystals were purified by ethanol / ether recrystallization. Decomposition of crystals began at 185°C.

Anal. Calcd for $C_{16}H_{13}F_3HgO_5$ x 1.2 H_2O: C, 34.02; H, 2.76; Hg, 35.48. Found: C, 34.02; H, 2.53; Hg, 35.53.

^{1}H-nmr (DMSO-d_6): δ 2.19 (s, 3H), 2.35 (s, 3H), 2.49 (t, 2H), 2.85 (dd, 1H), 3.30 (dd, 1H), 5.22-5.38 (m, 1H), 6.10 (s, 1H), 7.37 (s, 1H).

Preparation of 3-fluoro-4,8-dimethyl-5'-acetomercurimethyl-4',5'-dihydropsoralen

Using sufficient ethanol to form a solution, 3-fluoro-4,8-dimethyl-6-allyl-7-hydroxycoumarin (50.0 mg, 0.20 mmoles) was dissolved followed by the dropwise addition of a solution of mercury acetate (64.0 mg, 0.20 mmoles) in 3 mL ethanol. Crystals, 92.0 mg (92% yield), were recovered by filtration and washed with water. Recrystallization from methanol gave white crystals with mp 218-219°C.

Anal. Calcd for $C_{16}H_{15}FHgO_5$: C, 37.76; H, 3.37. Found: C, 37.66; H, 2.75.

^{1}H-nmr (DMSO-d_6): δ 1.84 (s, 3H), 2.10 (s, 3H), 2.35 (s, 3H), 2.49 (t, J=2.5 Hz, 2H), 2.93 (dd, J_1 = 16 Hz, J_2 = 8 Hz, 1H), 3.43 (dd, J_1 = 16 Hz, J_2 = 8 Hz, 1H), 5.22-5.38 (m, 1H), 7.40 (s, 1H).

Preparation of 3-cyano-4,8-dimethyl-5'-acetomercurimethyl-4',5'-dihydropsoralen

3-Cyano-4,8-dimethyl-6-allyl-7-hydroxycoumarin (0.100 g, 0.391 mmoles) was dissolved in 15 mL ethanol with slight heating. After addition of mercury (II) acetate (0.142g, 0.442 mmoles) in 2 mL ethanol, within two minutes a precipitate formed. Heating and stirring continued for two hours. The first crop of crystals recovered weighed 160 mg (80% yield). Methanol was employed to recrystallize pale yellow crystals with mp 214-215°C.

Anal. Calcd for $C_{17}H_{15}HgNO_5$: C, 39.58; H, 3.32; Hg, 38.87; N; 2.72. Found: C, 41.04; H, 3.00; Hg, 39.16; N, 3.76.

^{1}H-nmr (DMSO-d_6): δ 1.85 (s, 3H), 2.12 (s, 3H), 2.48 (s, 3H), 2.64 (t, J=2.5 Hz, 2H), 2.94 (dd, J_1 = 16.5 Hz, J_2 = 6.7 Hz, 1H), 3.47 (dd, J_1 = 16.5 Hz, J_2 = 6.7 Hz, 1H), 5.23-5.39 (m, 1H), 7.67 (s, 1H).

SYNTHESIS OF 4,8-DIMETHYL-5'-IODOMETHYL-4',5'-DIHYDROPSORALEN FROM IODOMERCURIMETHYL-4',5'-DIHYDROPSORALEN

Preparation of 4,8-dimethyl-5'-iodomethyl-4',5'-dihydropsoralen

4,8-Dimethyl-5'-iodomercurimethyl-4',5'-dihydropsoralen (2.60 g, 4.60 mmoles) was finely ground and added to iodine (0.655 g, 2.60 mmoles) and potassium iodide (1.196 g, 7.20 mmoles) in 75 mL water. The mixture was heated to reflux, when heating of the dark red solution was discontinued. Upon evaporation *in vacuo* a red oil resulted which hardened on standing. This was recrystallized from ethanol to give a low yield of pale pink crystals. A second recrystallization afforded white crystals that had physical and spectral properties in agreement with those of the previous synthesis.

SYNTHESIS OF PRECURSORS FOR THE SAMARIUM IODIDE RING CLOSURE

IODO-SUBSTITUTED-4,8-DIMETHYL-7-HYDROXYCOUMARINS AND IODO-SUBSTITUTED-4,8-DIMETHYL-7-ALLYLOXYCOUMARINS

Preparation of 6-iodo-4,8-dimethyl-7-hydroxycoumarin

To a suspension of 4,8-dimethyl-7-hydroxycoumarin (2.50 g, 13.2 mmoles) in 20 mL dioxane was added 50 mL NH_4OH. The turbid coumarin suspension became clear, pale green as a 5% KI / I_2 solution (made by the addition of KI (6.25 g, 37.6 mmoles) and I_2 (3.60 g, 14.0 mmoles) to 125 mL water) was added dropwise over two hours. As the pH was dropped to 3 by addition of HCl, yellow crystals coagulated, were filtered and washed with water followed by an ether wash. The product was taken up in ethyl acetate, washed with water and solvent was removed to recover 2.79 g (68% crude yield). TLC with 5% MeOH / 95% $CHCl_3$ showed two spots with Rf 0.66 and 0.45. Recrystallization from methanol gave white crystals with mp 219-220°C.

Anal. Calcd for $C_{11}H_9IO_3$: C, 41.80; H, 2.87. Found: C, 41.45; H, 2.71.

^{1}H-nmr (DMSO-d_6): δ 2.24 (s, 3H), 2.34 (s, 3H), 6.15 (s, 1H), 7.90 (s, 1H), 10.11 (s, 1H). ^{13}C-nmr (DMSO-d_6): δ 9.65, 18.18, 82.68, 111.07, 112.141, 114.68, 131,76, 152.43, 152.76, 157.11, 159.90.

Preparation of 6-iodo-4,8-dimethyl-7-allyloxycoumarin

6-Iodo-4,8-dimethyl-7-hydroxycoumarin (1.00 g, 3.16 mmoles) was added with K_2CO_3 (1.08 g, 7.89 mmoles) to 25 mL acetone in a three neck flask equipped with a stir bar and condenser. As the mixture was heated to reflux, allyl bromide (1.09 g, 9.08 mmoles) was added dropwise. The reaction mixture was refluxed overnight, and after cooling, the solids were filtered and the acetone evaporated. The solid mixture was taken up in methylene chloride, washed with water, 5% ammonia and dried over $MgSO_4$. The crystals recovered on evaporation of the solvent weighed 1.02 g (91% yield). White crystals were recrystallized from ethanol and had a mp of 154.3-155.5°C.

Anal. Calcd for $C_{14}H_{13}IO_3$: C, 47.21; H, 3.68. Found: C, 47.08; H, 3.55.

^{1}H-nmr (DMSO-d_6): δ 2.24 (s, 3H), 2.34 (s, 3H), 4.68 (d, 2H), 5.33 (d 10.5 Hz, 1H), 5.43 (d, 17 Hz, 1H), 5.92-6.08 (m, 1H), 6.15 (s, 1H), 7.85 (s, 1H).

Preparation of 3-iodo-4,8-dimethyl-7-hydroxycoumarin

4,8-Dimethyl-7-hydroxycoumarin (3.00 g, 15.7 mmoles) was added to 150 mL concentrated acetic acid with ICl (5.35 g, 31.6 mmoles) added dropwise. This mixture was heated at 50°C with stirring overnight. The mixture was cooled and the crystals collected by filtration. The yield was 3.20 g (65% yield). The white crystals were purified by recrystallization from methanol with mp 228-230°C.

Anal. Calcd for $C_{11}H_9IO_3$ x 0.05 H_20: C, 41.68; H, 3.03. Found: C, 41.68; H, 3.04.

^{1}H-nmr (DMSO-d_6): δ 2.14 (s, 3H), 2.58 (s, 3H), 6.84 (d, J = 8.7 Hz, 1H), 7.55 (d, J = 8.7 Hz, 1H), 10.53 (s, 1H).

Preparation of 3,6-diiodo-4,8-dimethyl-7-hydroxycoumarin

4,8-Dimethyl-7-hydroxycoumarin (975 mg, 5.14 mmoles) was added to 50 mL glacial acetic acid with stirring. As ICl solid was added (3.50 g, 20.5 mmoles) the solution was heated to 50°C and maintained at that temperature overnight. The reaction mixture was cooled and crystals collected by filtration. The recovered quantity was 1.58 g (97% yield). The white crystals were recrystallized from methanol and had mp 246.2-247.4°C. TLC on silica gel with 5% MeOH / 95% $CHCl_3$ showed one spot with Rf 0.48.

Anal. Calcd for $C_{11}H_8I_2O_3$: C, 29.89; H, 1.82. Found: C, 30.16; H, 1.88.

^{1}H-nmr (DMSO-d_6): δ 2.24 (s, 3H), 2.58 (s, 3H), 8.01(s, 1H), 10.31(s, 1H). ^{13}C-nmr (DMSO-d_6): δ 9.64, 25.15, 83.29, 88.92, 111.63, 113.98, 132.40, 151.34, 156.01, 157.28, 157.39.

Preparation of 3,6-diiodo-4,8-dimethyl-7-allyloxycoumarin

The 3,6-diiodo-4,8-dimethyl-7-hydroxycoumarin (593 mg, 1.34 mmoles) was added to 70 mL acetone with K_2CO_3 (800 mg, 5.70 mmoles). As the mixture was heated to reflux, the addition of allyl bromide (808 mg, 6.67 mmoles) was begun. Refluxing was continued overnight, and after cooling the solids were removed by filtration. The acetone was evaporated to obtain an additional crop of product. The crystals were taken up in methylene chloride, washed with dilute ammonium hydroxide and dried over $MgSO_4$. Evaporation of the solvent gave 387 mg (60 yield) of pale yellow solid, mp 168.5-170°C. Product was purified on silica gel with 30% ethyl acetate / 70% hexane.

Anal. Calcd for $C_{14}H_{12}I_2O_3$: C, 34.88; H, 2.51. Found: C, 36.5; H, 2.58.

^{1}H-nmr ($CDCl_3$): δ 2.30 (s, 3H), 2.69 (s, 3H), 4.68 (dd, J_1 = 3.5 Hz, J_2 = 1.5 Hz, 2H), 5.33 (d, J = 10.5 Hz, 1H), 5.43 (d, J = 17 Hz, 1H), 5.98-6.13 (m, 1H), 7.85 (s, 1H).

Preparation of 2',4'-dihydroxy-3'-methyl-5-iodoacetophenone

2',4'-Dihydroxy-3'-methylacetophenone (2.79 g, 16.8 mmoles) and a 1 M solution of ICl (21.0 mL, 21.0 mmoles) was added to 200 mL methylene chloride. The reaction was complete after one hour. The reaction mixture was washed with 1 mL saturated aqueous bisulfite solution, twice with 1 mL portions of water and dried over $MgSO_4$. Solvent was removed *in vacuo* and the resulting crystals were twice recrystallized from ethanol. The recovered weight was 4.60 g (94% yield). The white crystals melted at 179-180°C.

Anal. Calcd for $C_9H_9IO_3$: C, 37.01 ; H, 3.11; I, 43.45. Found: C, 37.01; H, 2.89; I, 43.44.

^{1}H-nmr (DMSO-d_6): δ 2.07 (s, 3H), 2.56 (s, 3H), 8.10 (s, 1H), 10.27 (s, 1H), 12.96 (s, 1H).

Preparation of 2'-hydroxy-4'-allyloxy-3'-methyl-5-iodoacetophenone

2',4'-Dihydroxy-3'-methyl-5-iodoacetophenone (2.50 g, 8.60 mmoles) was added with K_2CO_3 (5.35 g, 388 mmoles) to 50 mL acetone and heating begun before the dropwise addition of allyl bromide (5.58 g, 46.0 mmoles). Inorganic solids were removed by filtration and the organic layer washed with water before solvent was removed *in vacuo*. The recovered mass was 1.84 g (65% yield). Crystals were purified on a silica

column with 30% ethyl acetate / 70% hexane to recover pale yellow waxy crystals with mp 55-56°C.

Anal. Calcd for $C_{12}H_{13}IO_3 \times 0.72\ H_2O$: C, 41.13; H, 4.31. Found: C, 41.13; H, 4.06.

^{1}H-nmr ($CDCl_3$): δ 2.13 (s, 3H), 2.48 (s, 3H), 4.29 (d, J_1 = 3.5 Hz, 2H), 5.22 (d, J = 10.5 Hz, 1H), 5.35 (d, J = 17 Hz, 1H) 5.98-6.13 (m, 1H), 7.92 (s, 1H), 12.56 (s, 1H).

SYNTHESIS OF PRECURSORS FOR THE DIAZONIUM TETRAFLUOROBORATE RING CLOSURE

Preparation of 6-nitro-4,8-dimethyl-7-hydroxycoumarin

4,8-Dimethyl-7-hydroxycoumarin (7.50 g, 39.4 mmoles) was dissolved in 75 mL concentrated sulfuric acid at room temperature and chilled to –20^0 C before the addition of chilled nitrating mixture (3 mL concentrated HNO_3 added to 9 mL concentrated H_2SO_4). Stirring was continued for three hours at –20°C with the mixture allowed to warm before pouring into ice. Bright yellow crystals were filtered, washed with water and dried to recover 7.50 g (81% yield). The product was recrystallized from ethanol to give yellow green crystals with mp 229.5-231.5°C

Anal. Calcd for $C_{11}H_9NO_5$: C, 56.18; H, 3.86; N, 5.96. Found: C, 55.94; H, 3.79; N, 5.96.

^{1}H-nmr (DMSO-d_6): δ 2.29 (s, 3H), 2.41 (s, 3H), 6.37 (s, 1 H), 8.19 (s, 1H), 11.30 (s, 1H). ^{13}C-nmr (DMSO-d_6): δ 8.75, 18.00, 112.45, 112.93, 115.12, 119.90, 132.80, 152.91, 154.18, 155.10, 158.96.

Preparation of 6-nitro-4,8-dimethyl-7-allyloxycoumarin

6-Nitro-4,8-dimethyl-7-hydroxycoumarin (5.00 g, 21.2 mmoles) was mixed with dried K_2CO_3 (12.8 g, 93.0 mmoles) in 100 mL DMSO and heated to reflux before the addition of allyl bromide (12.9 g, 106 mmoles) was carried out dropwise with stirring. Refluxing was continued overnight. Solids were removed by filtration and solvent evaporated to isolate the product. The crude product was placed under vacuum to remove residual DMSO. The yield was 4.85 g (83% yield). Recrystallization from isopropanol yielded bright yellow crystals with mp 158.3-158.8°C.

Anal. Calcd for $C_{14}H_{13}NO_5$ x 0.17 H_2O: C, 60.40; H, 4.83; N, 5.03. Found: C, 60.40; H, 4.74; N, 5.02.

^{1}H-nmr (DMSO-d_6): δ 2.31 (s, 3H), 2.41 (s, 3H), 4.54 (d, J = 5.6 Hz, 2H), 5.25 (d, 1H), 5.38 (d, 1H), 5.98-6.10 (m, 1H), 6.48 (s, 1H), 8.20 (s, 1H). ^{13}C-nmr (DMSO-d_6): δ 9.37, 18.14, 75.75, 114.48, 115.82, 119.10, 119.54, 121.97, 132.73, 140.54, 151.63, 152.72, 154.23, 158.92.

Preparation of 6-amino-4,8-dimethyl-7-allyloxycoumarin

To a round bottom flask was added finely ground 6-nitro-4,8-dimethyl-7-allyloxycoumarin (3.00 g, 10.9 mmoles), tin (3.24 g, 27.3 mmoles), $SnCl_2$ (3.04 g, 16.0 mmoles) and 2 mL concentrated HCl in 200 mL ethanol. The mixture was stirred overnight during which all tin dissolved. Most of the ethanol was removed *in vacuo*. As the solution was allowed to cool, a gel formed which was filtered to recover a small amount of pearlized crystals. Ether was added to the filtrate and a precipitate formed

which was filtered and rinsed with ether to remove $SnCl_2$. The solids were dried and extracted with hot ethanol on the funnel of a filtration flask. Evaporation of the ethanol gave a crude product that was recrystallized from ethanol to provide 2.20 grams (82% yield) of product. The bright mustard yellow crystals had a mp of 145.6-147.4^{0}C.

Anal. Calcd for $C_{14}H_{15}NO_3$: C, 68.56; H, 6.93; N, 5.66. Found: C, 68.24; H, 6.08; N, 5.52.

^{1}H-nmr (DMSO-d_6): δ 2.28 (s, 3H), 2.49 (s, 3H), 4.54 (d, 2H), 5.28 (d, 1 H), 5.48 (d, 1H), 5.98-6.32 (m, 1H), 6.39 (s, 1H), 7.58 (s, 1H). ^{13}C-nmr (DMSO-d_6): δ 9.43, 18.14, 74.57, 113.64, 115.13, 116.00, 118.69, 120.09, 125.52, 133.48, 147.0, 151.49, 152.50, 159.62.

Preparation of 6-diazoniumtetrafluoroborate-4,8-dimethyl-7-allyloxycoumarin

6-Amino-4,8-dimethyl-7-allyloxycoumarin (1.00 g, 4.08 mmoles) was added to 3.36 mL tetrafluoroboric acid (24% aqueous) and chilled in an ice / acetone bath before the addition of a 40% aqueous sodium nitrite solution (0.714 g) in 1 mL water. A precipitate formed instantly with much frothing. After fifteen minutes, the solids were collected by filtration, rinsed with 5% cold HBF_4, ice cold methanol, and rinsed again with ether. The solids were used without further drying. The weight recovered was 0.685 g (82% yield). Recrystallization of solids from acetone / ether afforded tan crystals with mp 139-141°C.

Anal. Calcd for $C_{14}H_{13}BF_4N_2O_3$: C, 48.89; H, 3.81; N, 8.14. Found: C, 48.51; H, 3.23; N, 7.93.

^{1}H-nmr (CD_3OD): δ 2.50 (s, 6H), 5.02 (d, 2H), 5.48 (d, 1H), 5.61 (d, 1H), 6.14-6.35 (m, 1H), 6.57 (s, 1H), 8.91 (s, 1H).

Preparation of 4,8-dimethyl-4'-iodomethyl-4',5'-dihydropsoralen

Sodium iodide (0.680 g, 4.50 mmoles) was added to 15 mL acetone with a small crystal of I_2 added. Separately, the tetrafluoroborate salt (0.775 g, 2.23 mmoles) was dissolved in acetone and added dropwise to the sodium iodide / I_2 solution. After fifteen minutes, the solvent was removed *in vacuo*. The solids were taken up in chloroform, washed with water and aqueous bisulfite and dried over $MgSO_4$. Evaporation of the solvent recovered pink tinged crystals that weighed 0.750 g (95% crude yield). Recrystallization form ethanol yielded white crystals, with mp 179-180°C, with structural confirmation by NMR.

Anal. Calcd for $C_{14}H_{13}IO_3$: C, 47.21; H, 3.68. Found: C, 47.32; H, 3.44.

^{1}H-nmr ($CDCl_3$): δ 2.16 (s, 3H), 2.42 (s, 3H), 3.19 (t , J = 9.5 Hz, 1H), 3.35 (dd, J_1 = 10 Hz, J_2 = 5 Hz, 1 H), 3.78-3.92 (m, 1H), 4.38 (dd, J_1 = 10 Hz, J_2 = 5 Hz, 1H), 4.65 (t, J = 9.1 Hz, 1H), 6.10 (s, 1H), 7.20 (s, 1H). ^{13}C-nmr (DMSO-d_6): δ 8.67, 11.84, 19.50, 44.70, 79.70, 108.14, 111.61, 114.81, 119.72, 126.94, 154.55, 154.80, 161.47, 163.05.

Preparation of 3-bromo-4,8-dimethyl-4'-iodomethyl-4',5'-dihydropsoralen

4,8-Dimethyl-4'-iodomethyl-4',5'-dihydropsoralen (135 mg, 0.379 mmoles) was taken up in 15 mL methylene chloride, to which was added N-Bromosuccinimide (68 mg, 0.382 mmoles) and reaction mixture was stirred overnight. The solvent was evaporated

and the resulting solids were taken up in chloroform, washed with saturated aqueous bisulfite, two 1 mL portions of water, and then dried over $MgSO_4$ before the solvent was evaporated. The tan crystals had a mp of 205.7-205.9°C after recrystallization from ethanol. The weight recovered was 165 mg (92% yield).

Anal. Calcd for $C_{14}H_{12}BrIO_3$: C, 38.65; H, 2.78. Found: C, 38.66; H, 2.61.

^{1}H-nmr ($CDCl_3$): δ 2.29 (s, 3H), 2.51 (s, 3H), 3.19 (t, J = 9.5 Hz, 1H), 3.39 (dd, 1 H), 3.75-3.87 (m, 1H), 4.40 (t, 1H), 4.67 (t, 1H), 7.16 (s, 1H).

Preparation of 4,8-dimethyl-4'-pyridiniummethyl-4',5'-dihydropsoralen iodide salt

Two ml pyridine was added to 4,8-dimethyl-4'-iodomethyl-4',5'-dihydropsoralen (0.150 g, 0.421 mmoles), and the mixture was heated at reflux for 2 hours. Solids formed upon cooling and pyridine was evaporated *in vacuo*. Residual pyridine was removed by placing the flask on a vacuum pump overnight. Solids were refluxed in chloroform for one hour to solubilize 4,8,4'-trimethylpsoralen which formed as the major product. Undissolved quaternary compound was recovered by filtration and washed with acetone to remove the slight red coloration and then with ether. The 4,8-dimethyl-4'-pyridiniummethyl-4',5'-dihydropsoralen iodide salt was recrystallized from ethanol slowly and 22 mg (12% yield) was recovered. The bright tan crystals melted between 290-292°C.

Anal. Calcd for $C_{19}H_{18}INO_3$: C, 52.43; H, 4.17; N, 3.22. Found: C, 52.26; H, 4.02, N, 3.21.

^{1}H-nmr (CD_3OD): δ 2.21 (s, 3H), 2.33 (s, 3H), 4.20-4.28 (m, 1H), 4.73 (d, 2H), 4.87-4.97 (m, 2H), 6.16 (s, 1H), 7.15 (s, 1H), 8.13 (t, 2H), 8.63 (t, 1H), 8.93 (d, 2H).

The major product, the 4,8,4'-trimethylpsoralen, was recovered from the chloroform layer by removal of solvent *in vacuo* and recrystallization from ethanol. ^{1}H-nmr ($CDCl_3$): δ 2.19 (s, 3H), 2.43 (s, 3H), 2.46 (s, 3H), 6.16 (s, 1H), 7.44 (s, 1H), 7.51 (s, 1H).

Preparation of 3-bromo-4,8-dimethyl-4'-pyridiniummethyl-4',5'-dihydropsoralen iodide salt

One ml pyridine was added to the 3-bromo-4,8-dimethyl-4'-iodomethyl-4',5'-dihydropsoralen (0.130 g, 0.308 mmoles) and the mixture was heated at reflux for 2 hours. Solids formed upon cooling, and pyridine was evaporated *in vacuo*. Residual pyridine was removed by placing the flask on a vacuum pump overnight. Solids were refluxed in chloroform for one hour to solubilize 3-bromo-4,8,4'-trimethylpsoralen which formed as the major product (0.065 g recovered). Undissolved quaternary compound was recovered by filtration and washed with acetone to remove the slight red coloration and was rinsed again with ether. The 4,8-dimethyl-4'-pyridiniummethyl-4',5'-dihydropsoralen iodide salt was recrystallized from ethanol slowly, and 18 mg (11% yield) was recovered. The dark tan crystals melted above 270°C.

Anal. Calcd for $C_{19}H_{17}BrINO_3$: C, 44.39; H, 3.33, N, 2.72. Found: C, 44.39; H, 3.24, N, 2.68.

^{1}H-nmr (CD_3OD): δ 2.28 (s, 3H), 2.52 (s, 3H), 4.22-4.30 (m, 1H), 4.73 (d, 2H), 4.87-4.97 (m, 2H), 7.22 (s, 1H), 8.13 (t, 2H), 8.66 (t, 1H), 8.91 (d, 2H).

SYNTHESIS OF MERCURY-CONTAINING MODEL COMPOUNDS

Preparation of 2-chloromercurimethyl-2,3-dihydrobenzofuran

Mercury (II) chloride (1.31 g, 4.82 mmoles) was dissolved in 50 mL water before the dropwise addition of o-allyl phenol (0.625 g, 4.65 mmoles). A precipitate formed immediately. The product was recovered by filtration and washed with water to give 1.49 g (89% crude yield). Recrystallization from ethanol afforded white crystals with mp 136.1-136.3°C.

Anal. Calcd for C_9H_9HgClO: C, 29.29; H, 2.46. Found: C, 29.62; H, 2.40.

^{1}H-nmr (DMSO-d_6): δ 2.09 (d, J =2.5 Hz, 2H), 2.83 (dd, J_1 = 16 Hz, J_2 = 8 Hz, 1H), 3.25 (dd, J_1 = 16 Hz, J_2 = 8 Hz, 1H), 4.99-5.31 (m, 1H), 6.68-6.74 (m, 2H), 7.03-7.07 (m, 2H). ^{13}C-nmr ($CDCL_3$): δ 32.39, 39.33, 82.26, 110.33, 121.24, 125.79, 126.68, 128.80, 177.75.

Preparation of 2-acetomercurimethyl-2,3-dihydrobenzofuran

Mercury (II) acetate (2.37 g, 7.43 mmoles) was dissolved in 10 mL water and added dropwise with vigorous stirring to the a suspension of 1.00 g o-allyl phenol (7.45 mmoles) in 10 mL water. Evaporation and filtration yielded 2.64 g (90% crude yield). White crystals were recrystallized from ethanol with mp 80-81°C

Anal. Calcd for $C_{11}H_{12}HgO_3$: C, 33.64; H, 3.08. Found: C, 33.43; H, 2.89.

^{1}H-nmr (DMSO-d_6): δ 1.84 (s, 3H), 2.09 (d, J =2.5 Hz, 2H), 2.83 (dd, J_1 = 16 Hz, J_2 = 7 Hz, 1H), 3.29 (dd, J_1 = 16 Hz, J_2 = 8 Hz, 1H), 4.98-5.13 (m, 1H), 6.68-6.74 (m,

2H), 7.02 (m, 2H). ^{13}C-nmr ($CDCL_3$): δ 23.83, 32.39, 39.33, 82.26, 110.33, 121.24, 125.79, 126.68, 128.80, 158.83, 177.75.

Preparation of 2-iodomercurimethyl-2,3-dihydrobenzofuran

The 2-acetomercurimethyl-2,3-dihydrobenzofuran (0.300 g, 0.763 mmoles) was taken up in 20 mL water with heating to bring about solution. Potassium iodide (0.415 g, 2.50 mmoles) was added to the reaction mixture. Heating was continued for two hours and the product was recovered by filtration and recrystallized from ethanol to yield 0.175 g (50% yield). The mp was 115.8-117.2°C.

Anal. Calcd for C_9H_9HgIO: C, 23.47; H, 1.97; Hg, 43.53; I; 27.55. Found: C, 23.11; H, 1.76; Hg, 43.84; I, 27.32.

^{1}H-nmr ($CDCL_3$) δ 2.21 (dd, J_1 = 11.9 Hz, J_2 = 4.6 Hz, 1H), 2.40 (dd, J_1 = 11.6 Hz, J_2 = 5.5 Hz, 1H), 2.75 (dd, J_1 = 15.9 Hz, J_2 = 5.5 Hz, 1H), 3.38 (dd, J_1 = 15.9 Hz, J_2 = 8.5 Hz, 1H), 5.40-5.56 (m, J_1 = 8.5 Hz, J_2 = 5.5 Hz, 1H), 6.80-6.90 (m, 2H), 7.11-7.21 (m, 2H). ^{13}C-nmr (DMSO-d_6): δ 39.17, 47.04, 82.80, 110.74, 121.61, 125.93, 126.50, 129.18, 158.83

Preparation of 2-acetomercurimethyl tetrahydrofuran

Mercury (II) acetate (407 mg, 1.27 mmoles) was added to 5 mL THF, partially dissolved and chilled to 0° C before the dropwise addition to 4-penten-1-ol (100 mg, 1.16 mmoles) in 5 mL THF at 0°C. The reaction mixture was stirred three hours before the

solvent was removed *in vacuo*. The crystals thus isolated were washed with ether to remove unreacted mercury (II) acetate. The recovered yield was 343 mg (86% yield) waxy pale tan crystals with mp 45-46.5°C.

Anal. Calcd for $C_7H_{12}HgO_3$: C, 24.39; H, 3.51. Found: C, 24.48; H, 3.31.

^{1}H-nmr ($CDCl_3$): δ 1.28 (m, 1H), 1.85 (m, 2H), 1.98 (s, 3H), 2.04 (m, 1H), 2.12 (dd, J_1 = 12 Hz, J_2 = 5.5 Hz , 1H), 2.27 (dd, J_1 = 12 Hz, J_2 = 5.5 Hz, 1H), 3.62 (q, J_1 = 15 Hz, J_2 = 7.5 Hz, 1H), 3.84 (q, J_1 = 15 Hz, J_2 = 7.5 Hz , 1H), 4.03 (m, 1H). ^{13}C-nmr ($CDCl_3$): δ 22.76, 26.47, 32.45, 35.71, 68.22, 78.32, 176.88.

Preparation of 2-trifluoroacetomercurimethyl tetrahydrofuran

A solution of mercury (II) trifluoroacetate (817 mg, 1.90 mmoles) was added to 5 mL THF and with stirring was dissolved. This mixture was chilled before addition to 4-penten-1-ol (150 mg, 1.74 mmoles) in 5 mL THF that was chilled to 0°C. The reaction mixture was stirred overnight and the solvent was removed *in vacuo*. The crystals were placed on the funnel of a filtration flask and washed with ether to remove unreacted mercury trifluoroacetate. Waxy tan crystals were recrystallized from methanol with mp 55-56°C.

Anal. Calcd for $C_7H_9F_3HgO_3$: C, 21.09; H, 2.28; F, 14.30; Hg, 50.30. Found: C, 20.31; H, 2.04, F, 15.18; Hg, 50.11.

^{1}H-nmr ($CDCl_3$): δ 1.28 (m, 1H), 1.89 (m, 2H), 2.04 (m, 1H), 2.26 (dd, J_1 = 12 Hz, J_2 = 5.5 Hz , 1H), 2.46 (dd, J_1 = 12 Hz, J_2 = 5.5 Hz, 1H), 3.65 (q, J_1 = 15 Hz, J_2 = 7.5 Hz, 1H), 3.85 (q, J_1 = 15 Hz, J_2 = 7.5 Hz , 1H), 4.00 (m, 1H).

SYNTHESIS OF ANGELICINS

Preparation of 4-methyl-5'-acetomercurimethyl-4',5'-dihydroangelicin

8-Allyl-4-methyl-7-hydroxycoumarin (200 mg, 0.938 mmoles) was added to 10 mL ethanol with warming of the solvent to effect solution. Mercury (II) acetate (325 mg, 1.00 mmole) in 1 mL water was added dropwise to the 8-allyl-4-methyl-7-hydroxycoumarin solution. The formation of a precipitate was almost immediate, and stirring was continued for three hours. White crystals (383 mg) were recovered by filtration and washed with ether. The yield was 86% after recrystallization from ethanol with mp 175.0-175.4°C.

Anal. Calcd for $C_{15}H_{14}HgO_5$: C, 37.95; H, 2.97. Found: C, 38.00; H, 2.80.

^1H (DMSO-d_6): δ 1.85 (s, 3H) 2.36 (s, 3H) 2.48 (t, 2H) 3.00 (dd, J_1 = 15.7 Hz, J_2 = 7 Hz, 1H) 3.48 (dd, J_1 = 15.8, J_2 = 9 Hz, 1H) 5.22-5.38 (m, 1H) 6.14 (s, 1H) 6.78 (d, 8.5 Hz, 1H) 7.53 (d, 8.5 Hz, 1H). ^{13}C (DMSO-d_6): δ 18.40, 23.59, 29.59, 34.57, 85.97, 106.41, 110.24, 113.30; 113.45, 126.05, 150.39, 153.85, 159.93, 162.84, 174.67.

Preparation of 4-methyl-5'-iodomercurimethyl-4',5'-dihydroangelicin

4-Methyl-5'-acetomercurimethyl-4',5'-dihydroangelicin (183 mg, 0.386 mmoles) was suspended in 200 mL ethanol before the addition of potassium iodide (247 mg, 1.49 mmoles) in 2 mL ethanol / water (1:1). The mixture was heated at reflux for several

hours. The solvent was removed by evaporation and the recovered crystals were washed with water to remove the unreacted potassium iodide. The yellow crystals were dried and recrystallized from methanol to recover 173 mg (83% yield) with a mp 195.5-197.5 °C.

Anal. Calcd for $C_{13}H_{11}HgIO_3$: C, 28.78; H, 2.04; Hg, 36.95. Found: C, 28.69; H, 2.08; Hg, 36.96.

^{1}H (DMSO-d_6): δ 2.36 (s, 3H) 2.48 (t, 2H) 2.90 (dd, J_1 = 15.7 Hz, J_2 = 7 Hz, 1H) 3.40 (dd, J_1 = 15.8, J_2 = 9 Hz, 1H) 5.22-5.38 (m, 1H), 6.14 (s, 1H), 6.78 (d, 8.5 Hz, 1H), 7.53 (d, 8.5 Hz, 1H).^{13}C (DMSO-d_6): δ 18.20, 18.49, 34.3, 86.20, 106.51, 110.20, 113.45, 123.75, 126.20, 153.84, 159.92, 160.55, 162.70.

Preparation of 4-methyl-5'-iodomethyl-4',5'-dihydroangelicin

4-Methyl-8-allyl-7-hydroxycoumarin (300 mg, 1.38 mmoles) was added to 20 mL methylene chloride and treated to the dropwise addition of tin (IV) chloride (0.759 mL 1 M solution in methylene chloride, 0.759 mmoles). Iodine (353 mg, 1.38 mmoles) was dissolved in methylene chloride and was added with stirring continuing overnight. The methylene chloride layer was washed with aqueous bisulfite, brine and water. The solvent was evaporated and 413 mg crystals (88% crude yield) were recovered from the organic layer. Purification on silica gel with 5% MeOH / 95% chloroform gave white crystals with mp 141.3-141.6°C.

Anal. Calcd for $C_{13}H_{11}IO_3$: C, 45.64; H, 3.24. Found: C, 45.92; H, 3.23.

^{1}H (DMSO-d_6): δ 2.39 (s, 3H), 2.50 (d, J = 2.2 Hz, 2H), 3.01 (dd, J_1 = 15.3 Hz, J_2 = 6 Hz, 1H), 3.62 (dd, J_1 = 15.3 Hz, J_2 = 6 Hz, 1H), 4.98-5.11 (m, 1H), 6.19 (s, 1H), 6.87

(d, J= 8.5 Hz, 1H), 7.59 (d, 8.5 Hz, 1H). ^{13}C (DMSO-d_6): δ 8.70, 19.32, 33.61, 83.86, 107.03, 111.84, 112.11, 124.77, 126.14, 153.84, 159.93, 160.55, 162.70.

Preparation of 4-methyl-5'-pyridiniummethyl-4',5'-dihydroangelicin iodide salt

The 4-methyl-5'-iodomethyl-4',5'-dihydroangelicin (100 mg, 0.292 mmoles) was added to 2 mL dry pyridine and the mixture was heated at reflux for two hours. Precipitation formed after thirty minutes. The pyridine was removed by evaporation *in vacuo* and residual pyridine was removed on a vacuum pump overnight. Eight mL chloroform was added to solubilize the 4, 5'-dimethylangelicin which was formed as the major product. Crystals were recovered by filtration and washed with ether. Tan crystals recovered weighed 22 mg (17% yield). NMR showed product contains approximately 3:1 desired product with pyridinium iodide.

Anal. Calcd for $C_{18}H_{16}INO_3$: C, 51.33; H, 3.83; N, 3.33. Found: C, 46.75; H, 2.88; N, 3.60.

^{1}H-nmr (CD_3OD): δ 2.44 (s, 3H), 3.70 (dd, J= 16.5, 10 Hz, 1H), 4.90-4.98 (m, 2H), 5.14 (dd, 1H), 5.43-5.57 (m, 1H), 6.16 (s, 1H), 6.84 (d, J= 8.5 Hz, 1H), 7.62 (d, 8.5 Hz, 1H), 8.18 (t, 2H), 8.68 (t, 1H), 9.07 (d, 2H).

Preparation of 4-methyl-5'-morpholiummethyl-4',5'-dihydroangelicin

The 4-methyl-5'-iodomethyl-4',5'-dihydroangelicin (40.0 mg, 0.117 mmoles) was added to 2 mL dry morpholine and the mixture was heated at reflux for three hours. Within thirty minutes, a white precipitate began to form. The solids were filtered while the solution was still warm and the morpholine layer was evaporated to dryness under vacuum. The recovered glassy solid was taken up in chloroform and washed with dilute

HCl. After evaporation of solvent, solids were taken up in methanol / ether with a drop of water added. After four days, crystals formed which were filtered and washed with ether to dry. The recovered weight was 15 mg (43% yield) tan sharp crystals with a mp of 115-116°C.

Anal. Calcd for $C_{17}H_{19}NO_4 \times 0.1\ H_2O$: C, 67.48; H, 6.73; N, 4.63. Found: C, 67.48; H, 6.73; N, 4.37.

^{1}H-nmr ($CDCl_3$): δ 2.30 (s, 3H), 2.52 (s, 4H), 2.68 (dd, J_1 = 13 Hz, J_2 = 8 Hz, 2H), 3.02 (dd, J_1 = 15 Hz, J_2 = 8 Hz, 1H), 3.36 (dd, J_1 = 15 Hz, J_2 = 8 Hz, 1H), 3.66 (s, 4 H), 4.98-5.13 (m, 1H), 6.01 (s, 1H), 6.67 (d, 8.5 Hz,1H), 7.31 (d, 8.5 Hz,1H).

MOLAR ABSORPTIVITIES OF X-MERCURIMETHYLDIHYDROPSORALENS AND MODEL COMPOUNDS

COMPOUND	ε (methanol)	λ_{max} nm	Concentration ($L/mol^{-1}cm^{-1}$)
[Structural variation of (5)			
R = H, T = $HgOCOCH_3$	$1.6 \cdot 10^4$	332.1	0.041 mM
R = H, T = HgI	$1.6 \cdot 10^4$	330.2	0.032 mM
[Structural variation of (10)]			
R = H, T = $HgOCOCH_3$	$3.0 \cdot 10^3$	279.4	0.27 mM
R = H, T = HgCl	$3.1 \cdot 10^3$	279.6	0.27 mM
R = H, T = HgI	$3.4 \cdot 10^3$	279.6	0.056 mM
[Structural variation of (7)]			
R = R_1 = H, T = $HgOCOCH_3$	$1.4 \cdot 10^4$	323.7	
[Structural variation of (11)]			
T = $HgOCOCH_3$	no absorbance evidenced		
T = HgI	no absorbance evidenced		

ELECTROSPRAY MASS SPECTROMETRY OF ORGANOMERCURY – CYSTEINE MODELS

Positive ion electrospray mass spectrometry (ES-MS) is an important tool to investigate the interaction of organomercury (II) compounds with peptides or amino acids containing cysteinyl ligands. Addition by mercury occurs primarily at the sulfhydryl group. ES-MS transfers analytes from a solution state into the gas phase with little decomposition (D'Agostino, 1996). As a model system for mercurated psoralen – protein interaction, cysteine or glutathione was stirred overnight with a mercurated psoralen in methanol / water (99:1). The product was recovered by filtration or removal of solvent by evaporation followed by a water wash and drying. Samples were submitted to Oneida Research Services, Whitesboro, NY for analysis. A molecular ion peak of 552 for 5'-cysteinylmercurimethyl-4,8-dimethyl-4',5'-dihydropsoralen was present, with the characteristic pattern for $RHg^{+\cdot}$ ions based on the relative abundance of mercury isotopes, but fragmentation patterns did not permit interpretation of which cysteine ion was linked covalently to mercury. A molecular ion peak for the 3-cyano-5'-glutathionylmercurimethyl-4,8-dimethyl-4',5'-dihydropsoralen was not detected by mass spectrometry. A cation peak at m/z 642 may have arisen from loss of H_2O and $HOOCH(NH_2)NHCH_3$ from the protonated molecular ion, but no other mercury containing ions could be identified in the mass spectrum.

UV STUDY OF ORGANOMERCURY / THIOL INTERACTION

Methanol stock solutions of p-methoxybenzenethiol and acetomercurimethyltetrahydrofuran (0.019mM) were generated. Solutions were made to contain constant concentration of methoxybenzenethiol with/without the presence of mercurated tetrahydrofuran. A change in absorbance and a change in absorbance maxima were noted as the sulfhydryl group becomes bound to mercury (Figure 34). Titration of proteins by mercury compounds has been used to determine the number of thiols per protein fragment (Boyer, 1954). This method was applied to determine that, of the three possible binding sites on cysteine, it was the sulfur which had the greatest affinity for mercury in methanol solution. The dark activity of the mercurated psoralens probably arises from covalent binding to thiols in proteins.

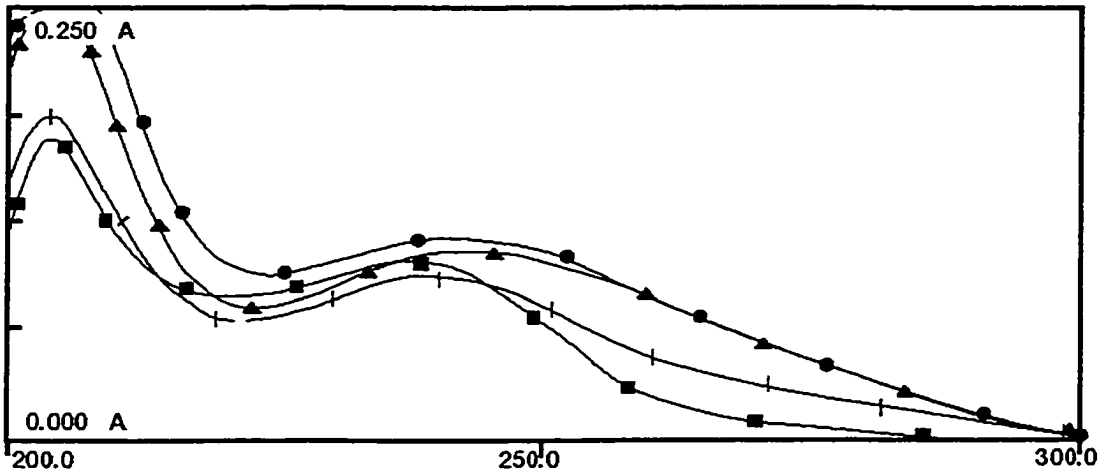


Figure 34 UV study of cysteine/mercury binding

Square represents o-methoxybenzenethiol
Dash represents first addition of acetomercurimethyltetrahydrofuran
Triangle represents second addition of acetomercurimethyltetrahydrofuran
Circle represents third addition of acetomercurimethyltetrahydrofuran

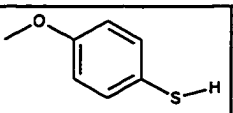
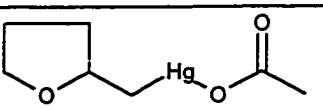
		Methanol		Absorbance maximum	Absorbance
1	5 mL	5 mL	0 mL	238.2	0.101
2	5 mL	4 mL	1 mL	240.2	0.093
3	5 mL	3 mL	2 mL	244.2	0.109
4	5 mL	0 mL	5 mL	244.2	0.117

Figure 33 UV study of cysteine / mercury interaction

LIGHT ACTIVATION OF 2-ACETOMERURIMETHYLTETRAHYDROFURAN IN THE PRESENCE OF PEROXIDES

To study the effects of light activation of organomercurial compounds in the presence of peroxides, stock solutions of 2-acetomercurimethyltetrahydrofuran (0.320g in 10 mL D_2O) and hydrogen peroxide (0.5 mL [35% in H_2O] diluted to 10 mL in D_2O) were generated. In four pyrex test tubes was added 1 mL of 2-acetomercurimethyltetrahydrofuran solution; to two was added 100 *u*L hydrogen peroxide. One pair of test tubes, with and without peroxide, was placed in the Rayonet reactor and irradiated for fourteen hours. The other pair remained on the bench top. Later, an additional set of samples was irradiated for 45 minutes to determine shorter term effects of irradiation.

Results of this study correlate with previous work (Abel, 1995 and Russel, 1982) in which elemental mercury was extruded from iodomercuriorganometallics or from simple alkylmercurihalides upon photolysis at 350 nm, even when the organic mercury compound lacked a chromophore in that region. NMR spectra of filtered solutions showed no change in solutions which were not irradiated or those which were irradiated without peroxide. The increased biological activity of the mercurated psoralens in the presence of light activation may be the result of formation of free radicals, after the extrusion of elemental mercury, that attack cellular components.

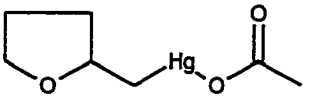
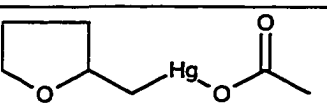
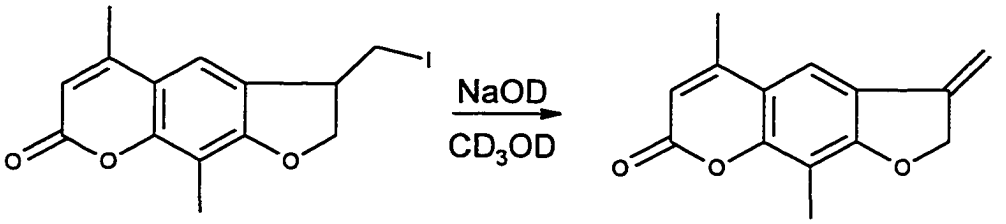
		 + H_2O_2
Light - 14 hours	No change	Dark precipitate
No light - 14 hours	No change	No change
Light - 0.75 hours	No change	White preciptate

Figure 34 UV Study of acetomercurimethyltetrahydrofuran with peroxide

BASE CATALYZED DEHYDROHALOGENATION OF 4'-IODOMETHYL-4,8-DIMETHYL-4',5'-DIHYDROPSORALEN



The base catalyzed dehydrohalogenation of the 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen to 4'-methylene-4,8-dimethyl-4',5'-dihydropsoralen presented evidence that the mechanism of the pyridine substitution / elimination reaction with 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen proceeded through an exocyclic methylene intermediate. The 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen (10 mg) was taken up in CD_3OD (0.5 mL) in an NMR tube and initial spectrum was acquired. One drop of NaOD solution (0.05 mL NaOD in 1 mL CD_3OD) was added to the NMR tube and a spectrum was acquired immediately, after five miutes, after ten minutes and after two weeks (Figure 35). The exocyclic methylene remained stable in base, for no rearrangement to 4,8,4'-trimethylpsoralen was detected by NMR.

^{1}H-nmr (CD_3OD): δ 2.25 (s, 3H), 2.47 (s, 3H), 5.15 (t, 1H), 5.25 (t, 2H), 5.62 (t, 1H), 6.18 (s, 1H), 7.78 (s, 1H).

Four weeks after the initial experiment, excess deuterated trifluoroacetic acid was added to the NMR tube to determine if acid would catalyze the rearrangement of the exocyclic methylene to 4,8,4'-trimethylpsoralen. Rearrangement was not detected by

NMR, though Bhandal noted treatment of the 2,3-dihydro-3-methylene benzofuran with a catalytic amount of trifluoroacetic acid in dry chloroform did rearrange (Bhandal, 1990).

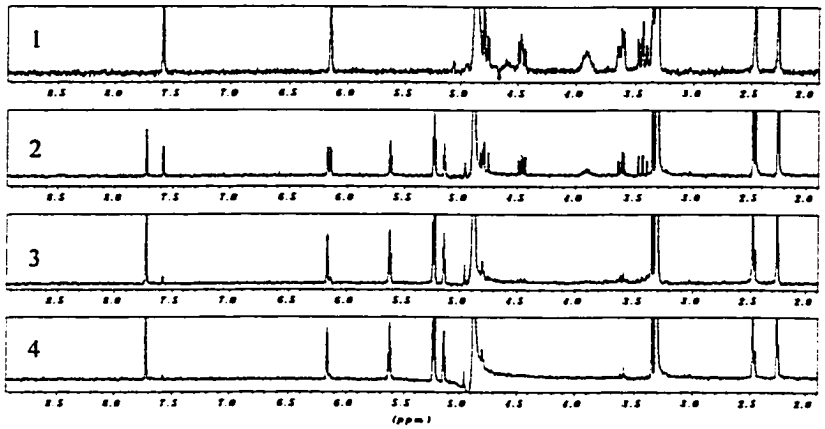


Figure 35 NMR study of base catalyzed dehydrohalogenation

Spectrum 1: 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen
Spectrum 2: 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen with NaOD/CD_3OD after one minute
Spectrum 3: 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen with NaOD/CD_3OD after five minutes
Spectrum 4: 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen with NaOD/CD_3OD after ten minutes

Resonances at 3.30 and 4.89 represent the CD_3OD and peaks at 2.16 and 2.42 are psoralen methyl groups, which shift only slightly and are ignored in this analysis. Peaks which disappear are: the iodomethyl protons at 3.42 and 3.62; the furan methylene at 4.45 and 4.85; and the furan 4' methine at 3.78-3.92. As the exocyclic methylene forms, resonances appear at 5.15 (=CH*H*), 5.25 (OCH_2) and 5.62 (=C*H*H). The 3CH at 6.15 and the 5CH at 7.58 are slightly shifted downfield as the system becomes more conjugated.

MECHANISM STUDY

Three parallel reactions were set up to compare the rate of reaction and product composition between pyridine with 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen, 5'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen and 5'-iodomethyl-4-methyl-4',5'-dihydroangelicin. The compounds (10 mg) were added to three 5 mL vials equiped with magnetic stirrers and air condensers before pyridine (0.115 mg) was added. During the first hour of heating above 100°C, most of the unreacted pyridine had evaporated. Initial NMR spectra in CD_3OD were acquired. Solutions were returned to the vials and methanol evaporated before additional pyridine (0.5 mL) was added and heating continued. After four hours most of the pyridine was removed under vacuum and additional spectra were acquired. The 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen reaction had gone to completion with approximately 62% conversion to 4,8,4'-trimethylpsoralen and 31% conversion to the desired pyridinium iodide salt. The NMR spectrum was acquired in a mixture of $CDCl_3$ and CD_3OD to solubilize both the pyridinium iodide salt and the TMP. Both the 5'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen and 5'-iodomethyl-4-methyl-4',5'-dihydroangelicin were slower to react and additional pyridine was added to each of these when the solutions were returned to the vials for an additional four hours of heating. The removal of pyridine before rerunning of the spectra was done *in vacuo* and the 5'-iodomethylangelicin was found to react more slowly than the 5'-iodomethylpsoralen. Neither showed evidence of dehydrohalogenation. For the 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen and the

5'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen reactions, the fully aromatized trimethyl psoralens are also plotted for comparison (Figure 36, Figure 37).

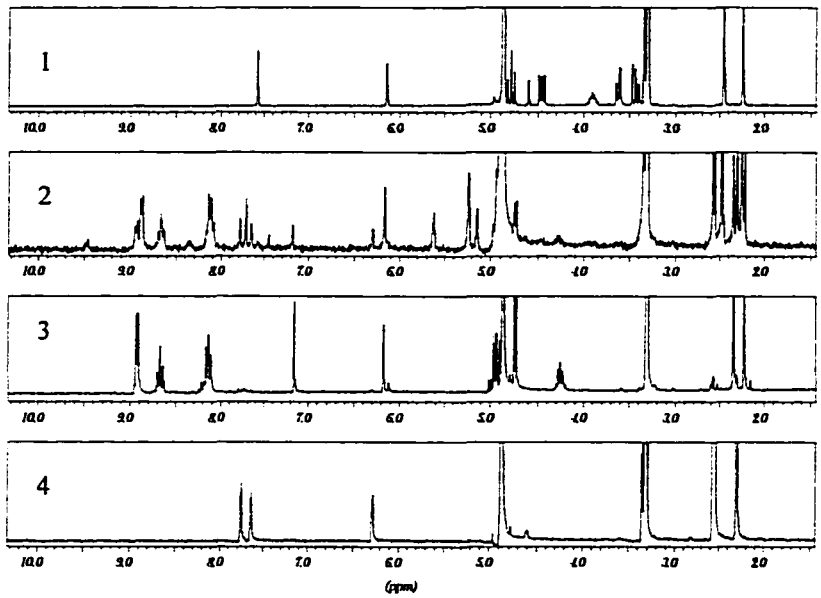


Figure 36 Formation 4'-pyridiniummethyl-4,8-dimethyl-4',5'-dihydropsoralen iodide salt

Spectrum 1: 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen
Spectrum 2: 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen with pyridine after one hour
Spectrum 3: 4'-pyridiniummethyl-4,8-dimethyl-4',5'-dihydropsoralen iodide salt purified
Spectrum 4: 4,8,4'-trimethylpsoralen

In spectrum 2, after one hour in the reaction of pyridine with 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen many compounds are present: starting material (spectrum 1), 4'-pyridiniummethyl-4,8-dimethyl-4',5'-dihydropsoralen iodide salt (spectrum 3), the dehydrohalogenation product 4,8,4'-trimethylpsoralen (spectrum 4), and the exocyclic methylenepsoralen seen in the base dehydrohalogenation of 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen (Figure 34, spectra 2,3,4). Elimination predominates over substitution to form 4,8,4'-trimethylpsoralen as the major product (spectrum 2).

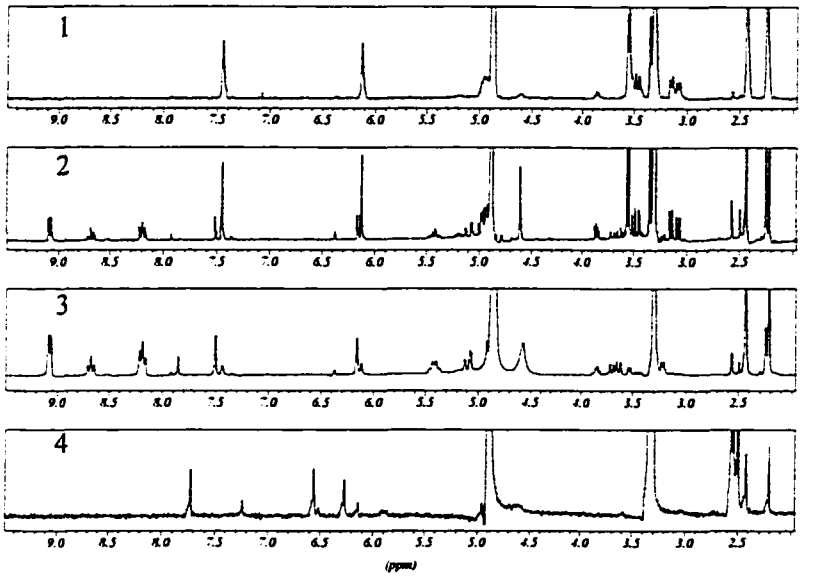


Figure 37 Formation of 5'-pyridiniummethyl-4,8-dimethyl-4',5'-dihydropsoralen iodide salt

Spectrum 1: 5'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen
Spectrum 2: 5'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen with pyridine after four hours
Spectrum 3: 5'- pyridiniummethyl-4,8-dimethyl-4',5'-dihydropsoralen iodide salt after eight hours
Spectrum 4: 4,8,5'-trimethylpsoralen

After four hours the reaction of 5'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen with pyridine (spectrum 2) shows some conversion to the 5'- pyridiniummethyl-4,8-dimethyl-4',5'-dihydropsoralen iodide salt, remaining starting material and no conversion to the fully aromatized 4,8,5'-trimethylpsoralen. There is less tendency for dehydrohalogenation with weak bases than is seen with the 4'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralens.

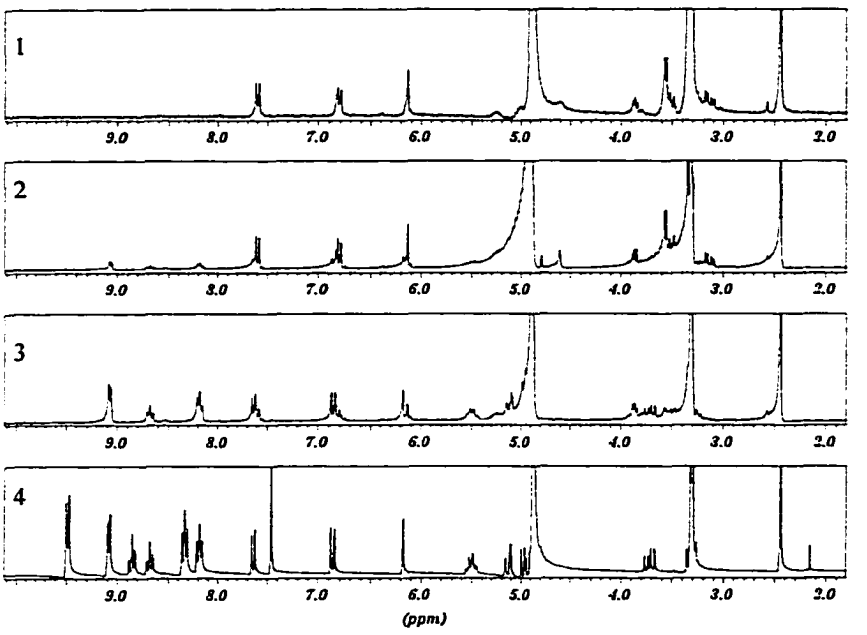


Figure 38 Formation of 4-methyl-5'-pyridiniummethyl-4',5'-dihydroangelicin iodide salt

Spectrum 1: 5'-iodomethyl-4-methyl-4',5'-dihydroangelicin
Spectrum 2: 5'-iodomethyl-4-methyl-4',5'-dihydroangelicin with pyridine after four hours
Spectrum 3: 5'- pyridiniummethyl-4-methyl-4',5'-dihydroangelicin iodide salt after eight hours
Spectrum 4: 5'- pyridiniummethyl-4-methyl-4',5'-dihydroangelicin iodide salt with pyridinium hydroiodide

The 5'-iodomethyl-4-methyl-4',5'-dihydroangelicin (spectrum 1) reactivity with pyridine (spectrum 2) parallels the reaction of 5'-iodomethyl-4,8-dimethyl-4',5'-dihydropsoralen, although the reaction rate is slower. After eight hours the presence of 5'-pyridiniummethyl-4-methyl-4',5'-dihydroangelicin iodide salt and starting material are seen (spectrum 3). There is no tendency to dehydrohalogenate in weak bases and pyridine substitution readily occurs.

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VITA

Marilyn Schulte Whittemore was born in Jamestown, New York on June 3, 1950 to Russell and Rachel Schulte. She graduated from The University of South Alabama in 1982 with a B.S. in chemistry. She earned a Masters Degree in chemistry from The University of Memphis in 1994. She is a member of the American Chemical Society. By coordinating a volunteer program at The Neighborhood School to show the children that science can be fun, she has involved many coworkers at Buckman.

Publications include:

Master's thesis: "^{113}Cd Nuclear Magnetic Resonance of the Zinc Binding Region of 92 kDa Gelatinase" submitted to The Journal of Biological Chemistry
"12% of the Gelatinase B Protein Sequence is Required for Proteolytic Activity and TIMP-1 Binding" Pourmotabbed, T; Kaur, K.; Whittemore, M.; Petersen, R.; Lichte, A.; and Tschesche, A.

patents issued to Buckman Labs:

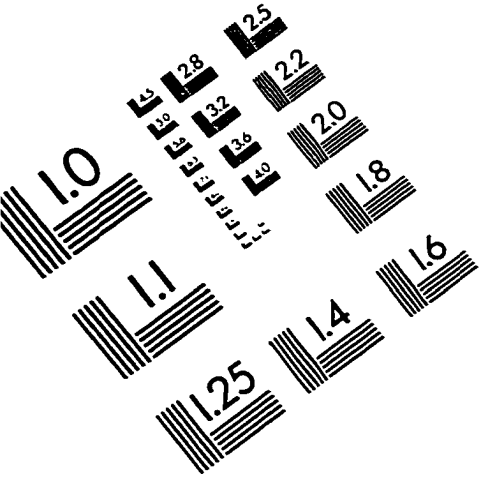
5,250,194 N-Dodecyl Heterocyclic Compounds useful as Industrial Microbicides and Preservatives Hollis, G.; Rayudu R. and Whittemore, M.

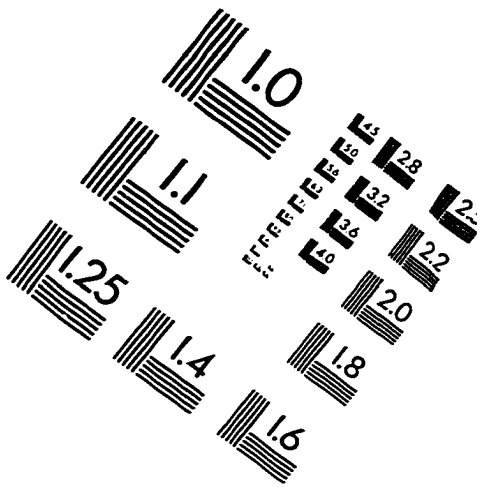
5,162,354 Novel 3-Halo-5-Halomethyl-2-Oxazolidinones and their use as Microbicides del Corral, F.; Rayudu, R. and Whittemore, M.

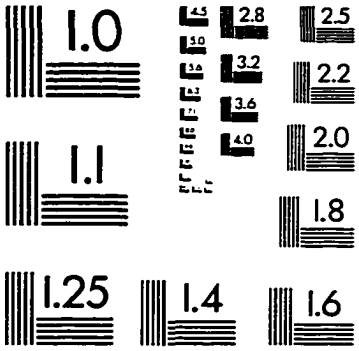
5,693,631 Potentiation of the Microbicide 2-(Thiocyanomethyl)benzothiazole using N-alkyl Heterocyclic Compounds Whittemore, M.; Glover, D.; Didato D. and Rayudu R.

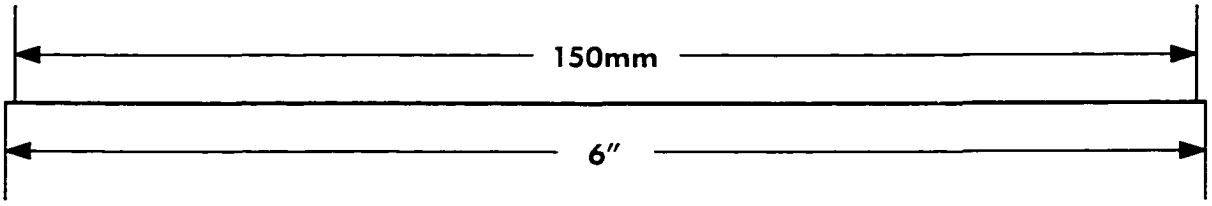
5,814,668 Methods and Compositions for Controlling Biofouling using Amides Whittemore, M.; Glover, D.; Zollinger, M. and Bryant, S.

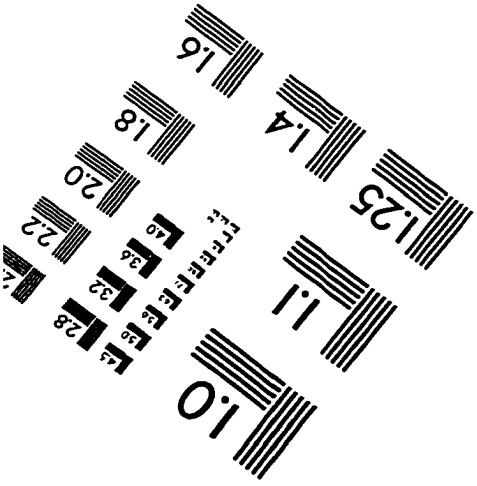
IMAGE EVALUATION TEST TARGET (QA-3)

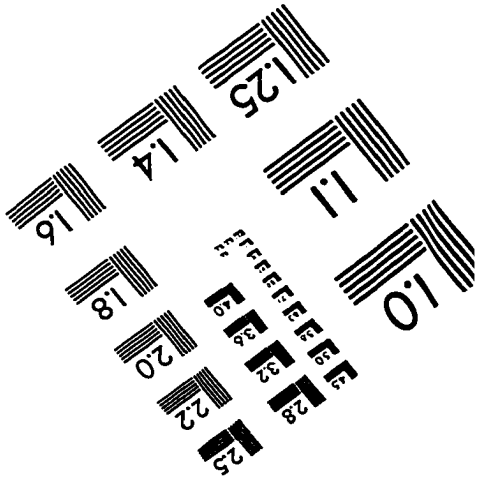












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