Psoralens And Coumarins For Receptor Targeting On Epidermal Cells.

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Psoralens and coumarins for receptor targeting on epidermal cells

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Psoralens and Coumarins for Receptor Targeting on Epidermal Cells

by

Michele Morris Jetter

A Dissertation
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in
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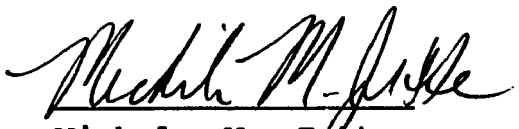
CERTIFICATE OF PRESENTATION

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.



Michele M. Jotter

A CERTIFICATE OF APPROVAL

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

July 25, 1989

Date

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DEDICATION

This dissertation is dedicated to the author's father, Herbert A. Morris Jr., whose continous support and encouragement made this research possible. This work is also dedicated to the author's husband, Jim, whose love and support has made it all worthwhile.

TABLE OF CONTENTS

Abstract

Psoralens, also known as linear furocoumarins, are among the most well characterized of the photosensitizing agents. They are used, associated with UVA irradiation, for the photochemotherapy of psoriasis. Psoralens form a noncovalent complex with DNA then photobind with the pyrimidine bases of the DNA forming mono- and bifunctional adducts.

Dr. Jeffrey Laskin has identified specific binding sites for the psoralens, discrete from DNA, in different epidermal cell lines. These receptors are saturable and are alkylated by the action of psoralens + UVA light. A psoralen receptor has been partially purified and established to be a protein of approximately 20,000 daltons. Inhibition of the binding of epidermal growth factor to its reeptor and inhibition of the tyrosine kinase activity of the EGF receptor has been associated with PUVA treatment. These findings conflict with the general assumption that the biological effects of psoralens as photoactive compounds are associated with their ability to covalently bind to and crosslink DNA.

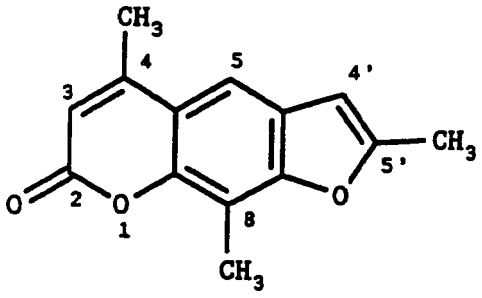
In collaboration with Laskin's laboratory, several classes of psoralen agonists were synthesized. These compounds include coumarins, furocoumarin and benzodipyran-2-one derivatives. The methods of preparation were varied and include variants of the Claisen rearrangement, acid and base-catalyzed condensations.

The synthesized compounds were tested for their potential inhibition of ^{125}I-EGF receptor binding. It was discovered that many of these agents showed potent inhibition activity similar to the psoralens. This data offers the possibility that sites of action, other than DNA, are involved in the mechanism by which photoactivated psoralens modulate epidermal cell lines.

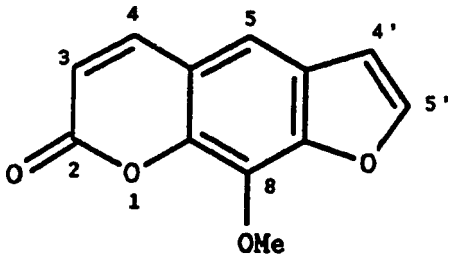
HISTORICAL

Psoralens are linear furocoumarins which were first recognized as potent dermal photosensitizing agents by the ancient Egyptians who used plant and seed extracts containing them in the treatment of vitiligo, a skin depigmentational disorder. These compounds occur naturally in more than two dozen plant species including Rutaceae, Umbelliferae, and Psoralea corylifolia.[1]

The two most widely used psoralens are 8-methoxypsoralen, 8-MOP or methoxsalen and 4, 5', 8-trimethyl psoralen, TMP or trioxsalen. They are available by prescription, are used clinically and are manufactured in the United States by ICN Pharmaceuticals. Figure 1 shows the structures of these two psoralens and the currently used numbering system.



4,5',8-trimethylpsoralen



8-methoxypsoralen

Figure 1

These compounds along with UVA light (wavelength of 320-400 nm) are used in the treatment of skin diseases such as psoriasis, a disease of accelerated keratinocyte proliferation, vitiligo and mycosis fungoides.[2] Typically a patient is administered trioxsalen topically or methoxsalen orally and then treated with a measured dose of UVA irradiation. This is known as PUVA therapy, Psoralens + Ultra Violet A light.[3] Use of these drugs is associated with two opposing effects on different epidermal cell populations. Photoactivated psoralens stimulate melanocyte proliferation and at the same time inhibit keratinocyte division.[4]

Traditionally, the biological activity of psoralens has been correlated with their photoreactivity toward the pyrimidine bases of DNA.[5] The formation of a weak molecular reversible complex plays an important role in the subsequent irreversible photoreaction between psoralens and DNA. The molecular complexes between nucleic acids and psoralens are formed by the intercalation of the planar furocoumarins into the base pairs of double stranded DNA in a dark reaction. While it is likely that this intercalation is somewhat sequence specific, this has not yet been determined.

Following UVA irradiation, the DNA-intercalated psoralens form mono and difunctional adducts with the pyrimidine bases in DNA. Adduct formation occurs by a 2 + 2 photocycloaddition reaction involving the 5,6-double bond of the pyrimidine base (primarily thymine) and either the 3,4 or 4′,5′-double bond of the furocoumarin. The 4′,5′-monoadducts can absorb the 365 nm radiation and undergo a further photoreaction with another pyrimidine base to give a diadduct; in double-stranded DNA a crosslink results.[6] These psoralen-DNA monoadducts and diadducts have been isolated and characterized by high resolution mass spectroscopy and proton NMR.[7] Figure 2 illustrates the intercalation of the psoralens into DNA and Figure 3 gives the structures of the DNA-psoralen adducts.

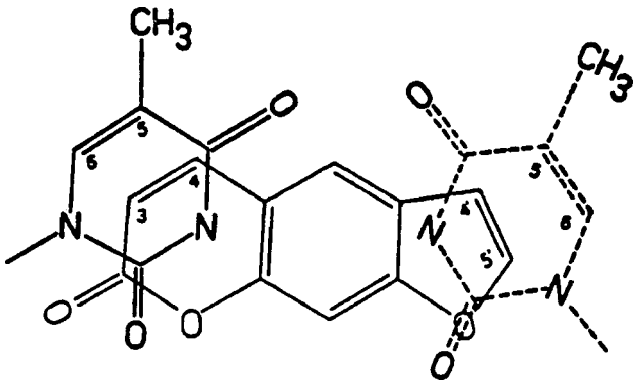
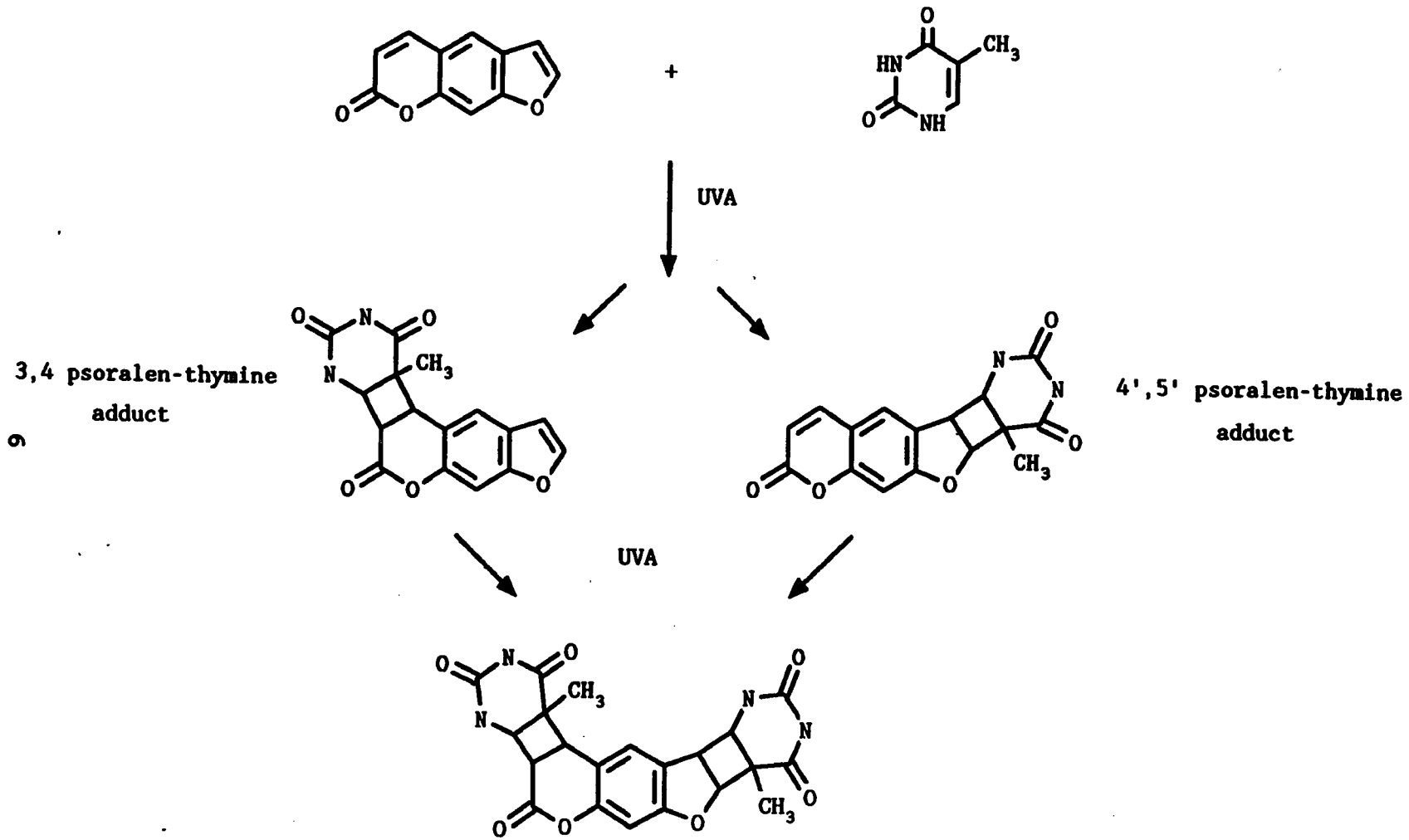


Figure 2

Figure 3



Formation of Monofunctional and Difunctional Adducts of Psoralen and Thymine

Dr. Jeffrey Laskin of Robert Wood Johnson Medical School has provided evidence that in mammalian cells, a site other than DNA is a major target for the psoralens.[8] Specific, saturable high-affinity binding sites for the psoralens have been identified in a number of different cell types in culture (Table 1). These binding sites are distinct from the DNA, are associated with the cytoplasm and cell membrane and become covalently modified by the psoralen following ultraviolet light exposure. Binding of a number of psoralen analogs to these sites paralleled their biological activity as skin photosensitizing agents.

Partial purification of the psoralen receptor was described by Laskin and coworkers in 1987.[9] HeLa (human cervical carcinoma) cells were incubated with ^{3}H-8-methoxypsoralen and then pulsed with UVA light. The nuclear and nonnuclear fractions of the treated cells were analyzed by SDS-polyacrylamide gel electrophoresis. The ^{3}H-labelled psoralen receptor was visualized in the cytoplasmic and plasma membrane fractions of the cells. It was established that the radioligand was bound to a protein of apparent molecular mass of approximately 22,000 daltons.

Table 1[8]

Specific binding of [^{3}H]8-MOP to human cell lines

Cell line	Origin	Specific binding cpm per 10^6 cells
HeLa	Epithelial carcinoma	4072
KB	Epithelial carcinoma	3064
G-361	Melanoma	2267
KM	Fibroblast	617
HF-A	Fibroblast	290

Table 2[10]

Inhibition of ^{125}I-EGF-specific binding after exposure to different cell lines in culture to UVA light and psoralens

	^{125}-EGF-specific binding, cpm per 10^6 cells			
Cell line	Control	TMP	UVA	UVA + TMP
HeLa	2390	2356	2223 (7)	581 (76)
KB	9303	9390	5396 (42)	3456 (63)
Hep-2	2302	2271	1888 (18)	907 (61)

Numbers in parentheses represent the percentage inhibition of specific ^{125}I-EGF binding compared to controls.

Recently, Laskin demonstrated that the binding of psoralens to their specific binding sites followed by UVA light activation is associated with the inhibition of epidermal growth factor (EGF) receptor binding.[10] Laskin found that UVA light alone inhibited the binding of ^{125}I-EGF to its receptor in different cell lines. However, psoralens and UVA light significantly potentiated this effect (Table 2). Inhibition of epidermal growth factor binding by psoralens and UVA radiation is rapid, irreversible and occurs by an indirect mechanism. In competitive binding assays, it was discovered that ^{125}I-EGF receptor binding was not inhibited by psoralens alone and furthermore, EGF did not compete with psoralens for receptor binding. This information suggests that psoralens do not compete directly with EGF for its receptor. The precise mechanism by which the psoralens + UVA light inhibit EGF binding is not yet known but is currently being investigated.

The epidermal growth factor receptor is a 170 kD transmembrane glycoprotein with intrinsic protein tyrosine kinase activity.[11] The nucleotide sequence of the EGF receptor predicts a 1186 amino acid protein consisting of an extracellular domain and an

intracellular domain separated by a single hydrophobic transmembrane region.[12] The cysteine-rich extracellular region forms the binding domain and the intracellular domain contains the tyrosine kinase activity and autophosphorylation sites. The receptor can utilize either ATP or GTP as the phosphate source, although ATP is the preferred substrate.

EGF binding to the extracellular receptor domain stimulates its tyrosine kinase activity and leads to increased intracellular phosphorylation as well as self-phosphorylation.[13] Upon binding EGF, a conformational change in the tyrosine kinase domain occurs which maximizes its catalytic activity. Although it is known that ligand binding is the major regulator of the enzyme activity of the EGF receptor, comparatively little is known of the molecular mechanisms involved in EGF receptor activation.[12]

The human epidermoid carcinoma cell line A-431 contains an extraordinarily high concentration of membrane receptors for EGF, approximately 2 to 3 x 10^6 receptors per cell. In these cells, EGF receptor binding rapidly leads to activation of the intrinsic EGF receptor tyrosine kinase activity. Laskin and coworkers have found that in addition to inhibition of EGF

binding, treatment of A-431 cells with psoralens, 4',5'-dihydropsoralens and benzopyranones succeeded by UVA irradiation inhibited EGF activated tyrosine kinase activity.[14]

INTRODUCTION

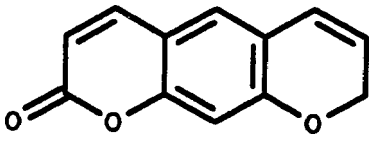
This dissertation research was conducted with the aim of developing new psoralen analogs as dermatological therapeutic agents and to integrate the synthesis of these compounds with the supporting biology provided by Dr. Laskin's group. The overall research target is elaboration of the cellular mechanisms of action of psoralens. Specifically, the aims of this research were:

1. To produce modified psoralens as carriers for toxins.
2. To produce biologically active analogs incapable of crosslinking DNA.
3. To deduce structural requirements for psoralen binding to its receptor.

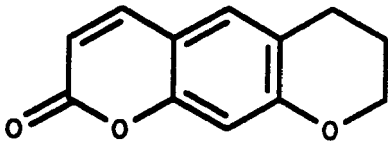
Toward that end we have generated photoactive psoralen-mimics from several diverse chemical families including coumarins, psoralens, dihydropsoralens, benzodipyranones and dihydrobenzodipyranones. These analogs have been designed and prepared with variations in methyl, methoxy, nitro, carboxyl, pendant olefinic and pendant acetylenic functionalities. Their generalized structures are shown in Figure 4.

Figure 4

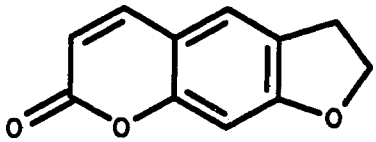
General Structures



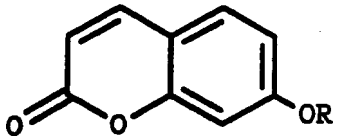
8H-Benzodipyran-2-ones



Dihydrobenzodipyran-2-ones



Dihydropsoralens



7-Alkoxycoumarins

As we have noted before herein, psoralens are well-known to intercalate and to covalently bind by photoinduced cyclobutane formation to nuclear DNA. We have also noted the work of Laskin and coworkers in which a specific membrane receptor for psoralens has been identified and in which binding to that receptor has been shown to inhibit the binding to epidermal growth factor (EGF) receptor.[10]

It is significant that agents which inhibit the binding and action of EGF would, in themselves, be expected to exert potent anticancer effects. EGF receptors have been detected on a broad range of human tumors including bladder, ovarian, thyroid, lung, skin and breast. Harris reported that the presence of elevated levels of EGF receptors has a prognostic value in human breast cancer. The observation was made that, especially in breast cancer, the increased expression of EGF receptors is related to the tumor stage and differentiation of the malignancy.[15] In addition, Murphy has disclosed evidence for the expression of the epidermal growth factor gene in some human breast cell lines. His data suggests that the presence of EGF receptors in breast cancer cells may have a role in regulating growth of tumors of the human breast.[16]

The biological evaluation (inhibition of EGF binding) of the compounds generated during the course of this work provides activity measurements whose precision appears to have reproducibility within ± 10%. Since the absolute numbers obtained in such measurements vary over 10^2, it would seem feasible to seek physical measurements for the candidate phototoxins which may correlate with the activities. In search of such a structure-activity relationship we have considered: (1) electronic effects, (2) steric-structure effects and (3) solubility/partition effects. This document details the synthetic methods involved in the preparation of the target analogs and also addresses the considerations mentioned above.

Electronic effects in these compounds are not likely to be found in traditional Hammett sigma effects, although many cases are known in which these correlate with relative drug potency [17], because the range of sigmas is not sufficiently diverse. In a photoactivated drug, potency might be expected to correlate with some not readily apparent composite function of available light quanta, concentration of drug, light absorption or extinction coefficient and quantum efficiency of the particular bond-forming

reaction.[18,19]

The precise mechanism by which the psoralens and coumarins alkylate their protein binding site is not yet known although the effect of psoralens on proteins in the presence of UVA irradiation has been studied.[20] Veronese and Rodighiero reported that covalent photobinding occurred between the psoralens and a number of different proteins including bovine serum albumin and chymotrypsin.[21] This photobinding was observed both in the presence and absence of oxygen suggesting two different pathways for the photoaddition. The excited furocoumarin could bind directly to the protein or could form, via a singlet oxygen mechanism, photodecomposition products that covalently bind to the protein.

Supporting evidence for the former pathway may be found in a photo-CIDNP study conducted by Marko and coworkers.[22] The photoreactions of several furocoumarins with different aromatic amino acids were studied by means of the photo-CIDNP technique. Nuclear spin polarizations were observed on both reactants when the furocoumarins were irradiated in acetonitrile solutions containing N-acetyltyrosine. In one case the formation of a new photoproduct was observed but this

photoproduct was not identified. The results of this study confirmed that the interaction between certain furocoumarins and amino acids proceeded through a radical pathway. It is, therefore, probable that the covalent modification of the psoralen receptor is brought about by a radical event.

Steric-structure effects are one of the most obvious chemical properties expected to display correlations with biological potency. Systematic alterations in chemical architecture within a class of compounds have long been successful in deducing information about the shape and properties of the binding site by correlation of biology with drug structure.[23] We have synthesized coumarins with structural variations at C-3, C-4, C-6, C-7 and C-8 and studied their relative biological activity. We have also explored a host of pendant variants of psoralens, dihydropsoralens, benzodipyranones and dihydrobenzodipyranones.

Solubility/partition effects have, in many cases, been found to possess a high degree of correlation with biological activity.[24] The relative distribution between an aqueous phase (e.g. blood or a nutrient test medium) and lipid phase (e.g. cell membrane) can be

approximated by the partition of the compound in question between octyl alcohol and water (or buffer). Furthermore, such an equilibrium would be expected to play a role in the bioactivity of our psoralen agonists since the physical presence of the drug on the cell membrane is an obvious prerequisite to its binding to a receptor located on the membrane.

Another quantity which must be considered in the search for an a priori structure-activity correlation is the ability of the candidate drug to non-covalently associate with DNA. While the work of Laskin provides evidence for a non-nucleic acid target for these agents [8,9,10] there is considerable prior work in the literature which claims that psoralen-like behavior depends on the ability of the drug to photobind to DNA.[6]

RESULTS and DISCUSSION

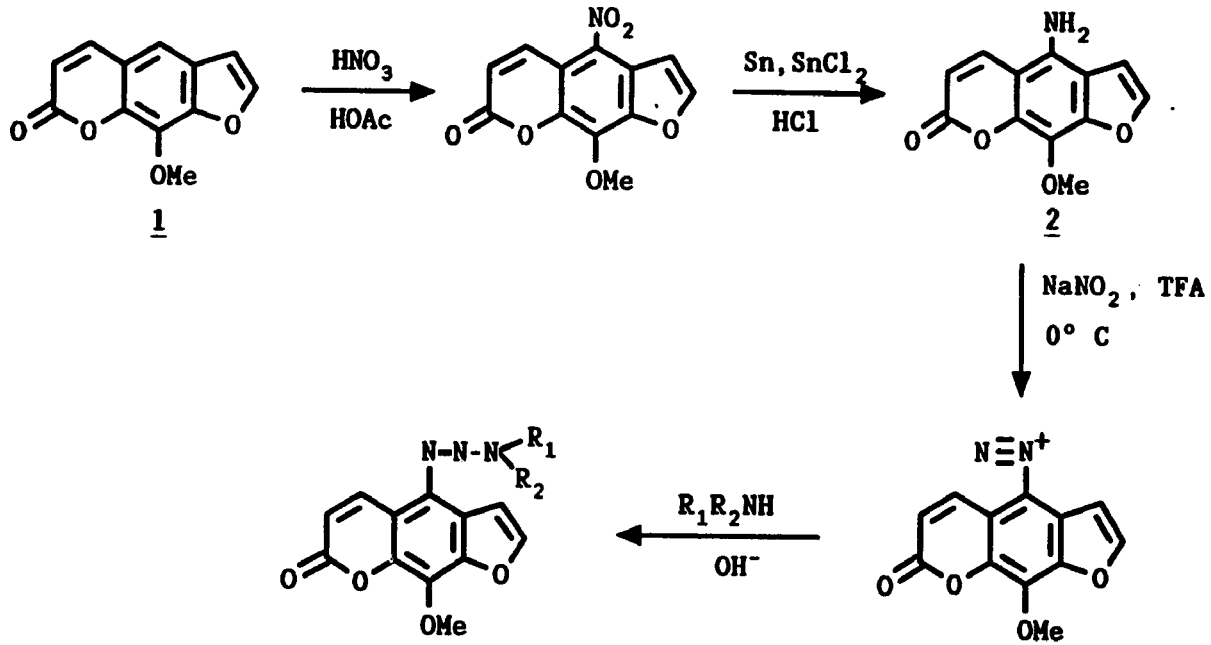
Chemotherapy Using Psoralens As Cytotoxics

Seiber and Adamson suggested that psoralens be chemically modified and used as carriers for typical chemotherapeutic cytotoxic functions such as nitrogen mustards for treatment of malignant melanoma.[25] Their logic was that since it was well established that psoralens show a biological effect in human skin, perhaps they fit a receptor and could thereby transport or fix a generic cytotoxin to a malignant dermal target. That idea is now seen to have merit since Laskin's work seems to imply that regulation of the epidermal growth factor receptor can be brought about by psoralens and psoralen analogs.

In this study we have prepared several psoralens modified with antineoplastic attachments. Using various alkylating agents such as Dacarbazine (DTIC) and Estramustine as models, a number of psoralen analogs with cytotoxic side chains were synthesized. These include the bischloroethylamine derivative of trioxsalen and four methoxsalen derivatives with a triazene "warhead" attached. Outlined in Schemes 1 and 2 and 3 are the synthetic routes. These compounds are currently undergoing screening against B16 melanoma at the National Cancer Institute.

Scheme 1

Synthesis of 8-MOP triazenes



3 R_1, R_2- CH_3

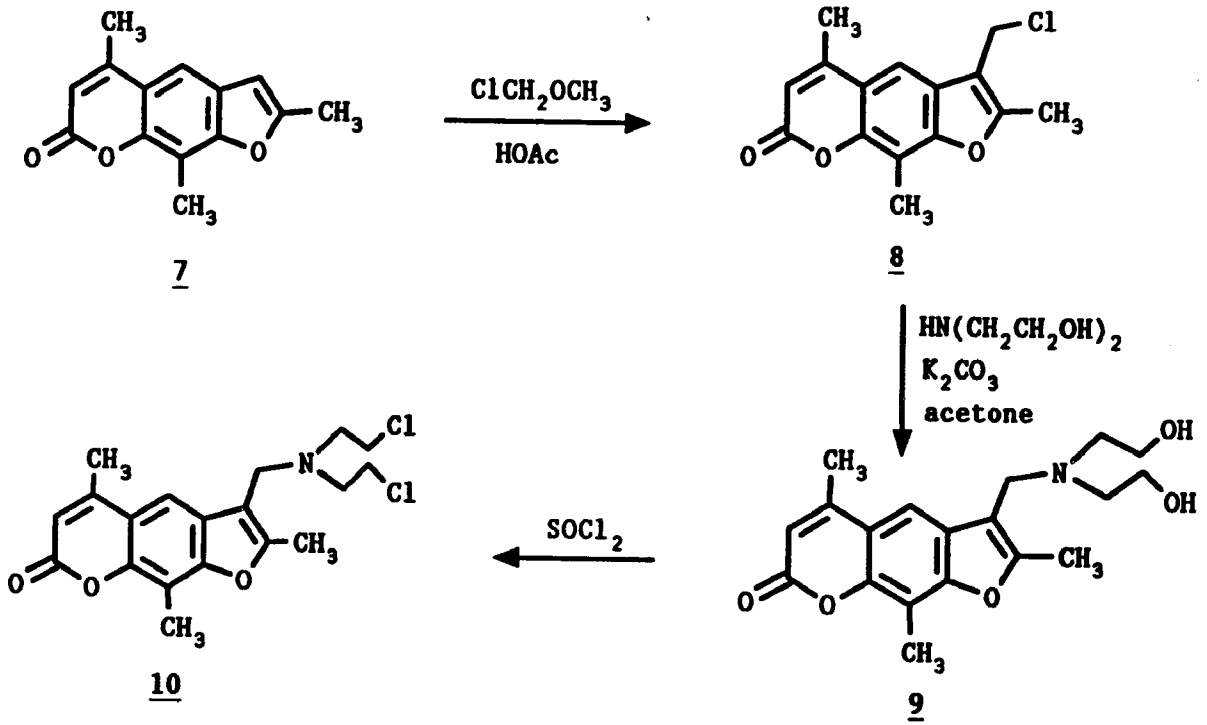
4 R_1, R_2- CH_2CH_3

5 R_1- CH_3, R_2- CH_2CH_3

6 R_1- $(CH_2)_3CH_3$, R_2- CH_3

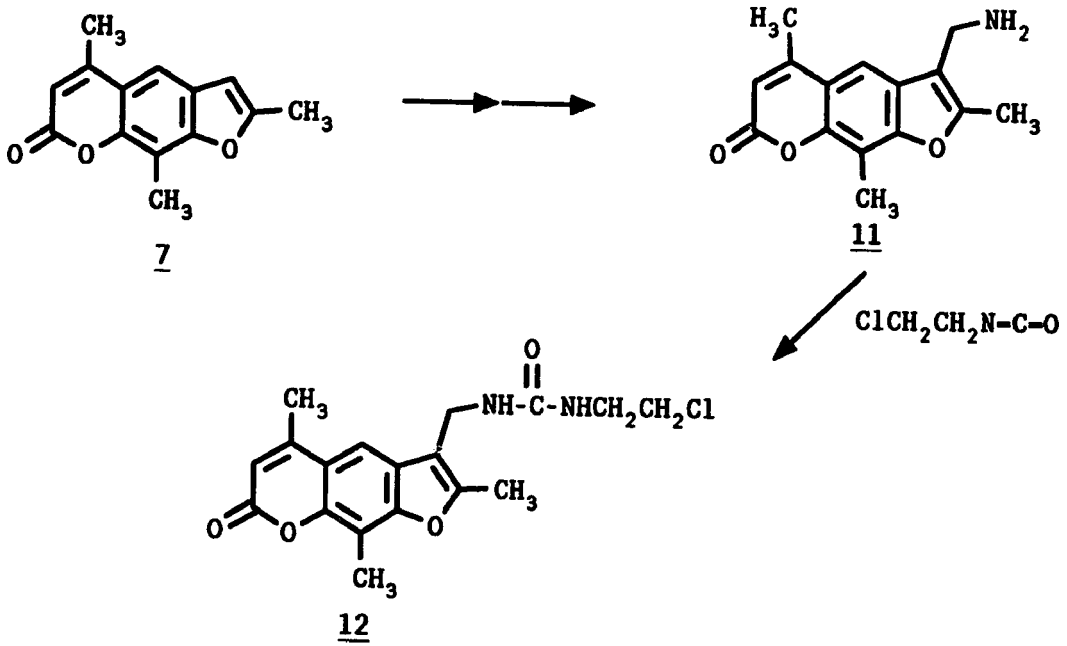
Scheme 2

Synthesis of TMP nitrogen mustard



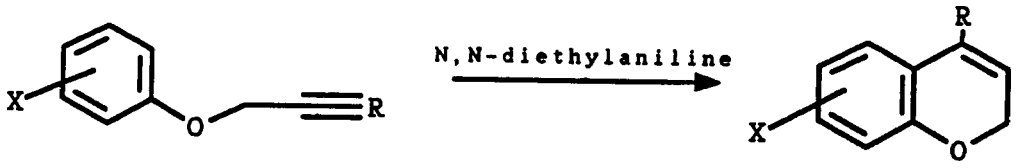
Scheme 3

Synthesis of TMP urea



Syntheses of Benzodipyran-2-ones

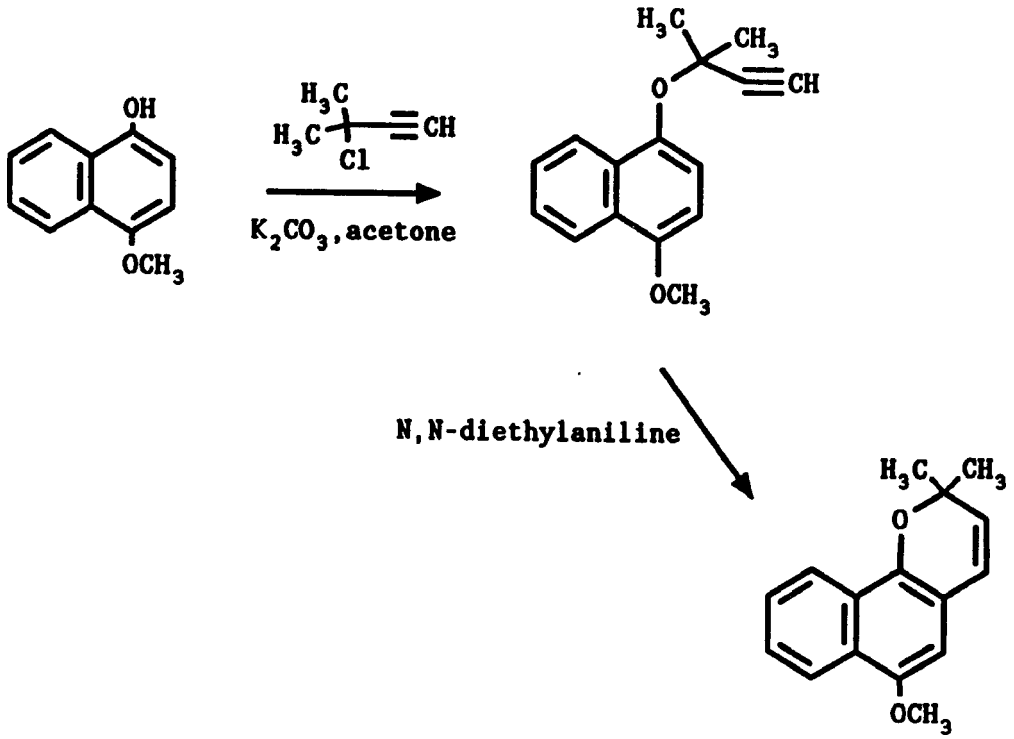
Iwai and Ide found that rearrangement of some simple aryl propargyl ethers in boiling N,N-diethylaniline yielded chromenes.[26] The reaction is a variant of the Claisen allylic rearrangement.



Hlubeck and coworkers exploited this reaction to prepare several natural products such as seselin and lapechenole by thermal rearrangement of the corresponding α, α-dimethylpropargyl ethers.[27] (Schemes 4 and 5).

Scheme 4[27]

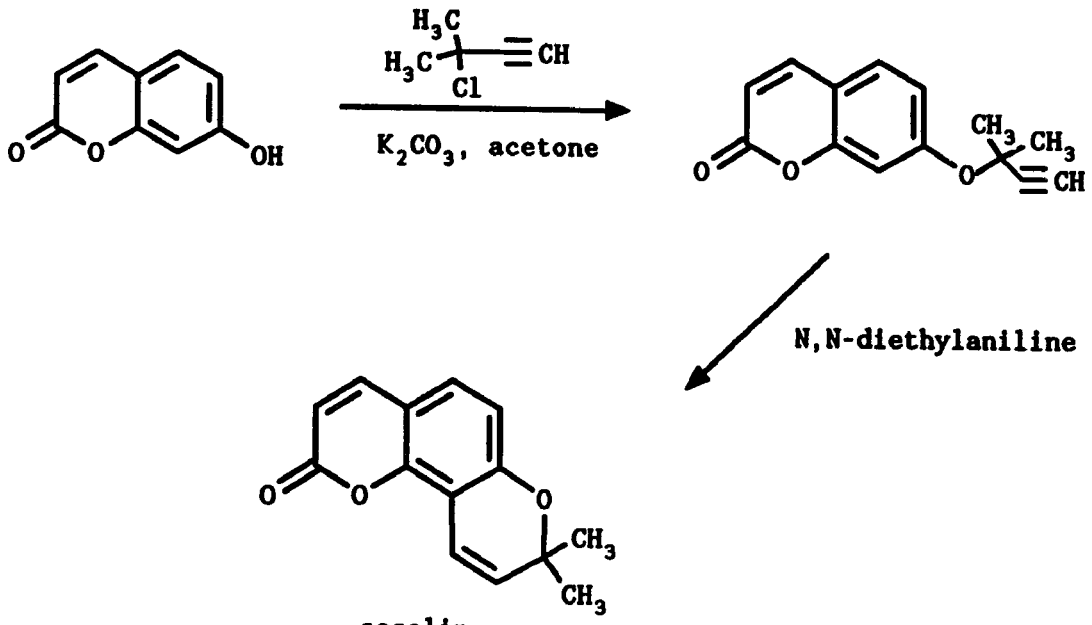
Hlubucek synthesis of lapechenole



lapechenole

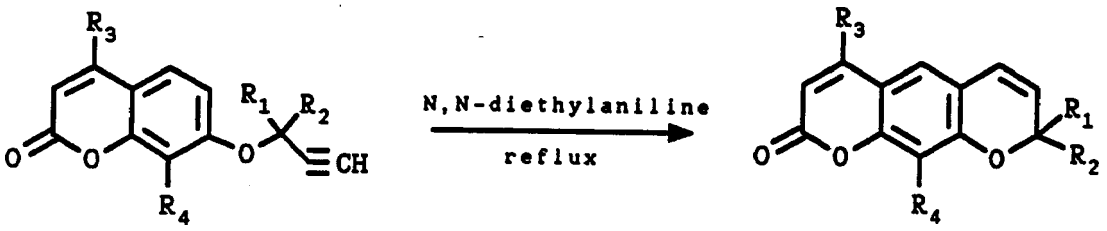
Scheme 5[27]

Hlubucek synthesis of seselin



seselin

Recently, Rodighiero reported the syntheses of methyl derivatives of 8-methylxanthyletin by direct cyclization in boiling N,N-diethylaniline of the propargyl or methyl-substituted propargyl ethers of the appropriate 7-hydroxycoumarins.[28] (Scheme 6) The preferred position in the cyclization is the #8 position of the coumarin nucleus and , therefore, this position must be blocked with either a methyl group or an iodine atom. The iodine atom is removed during the cyclization process.

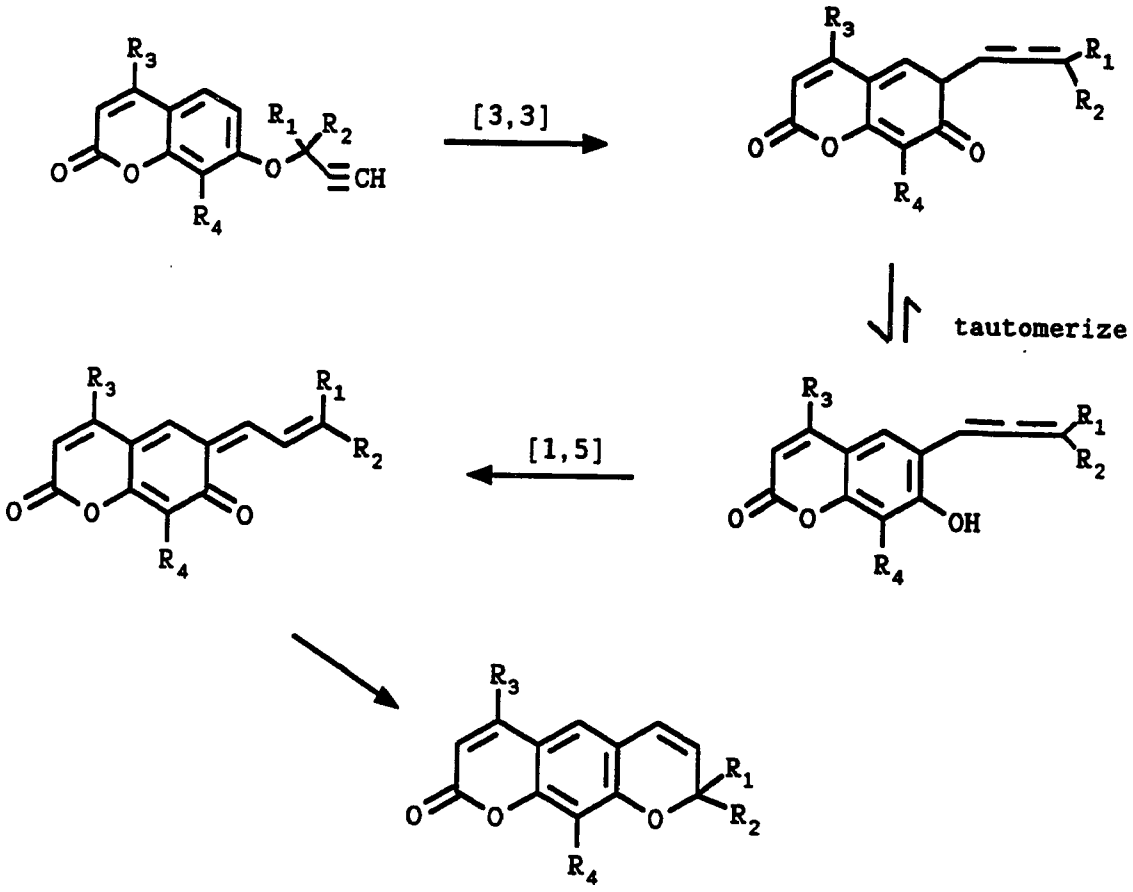


13 R_1,R_2- H, R_3- CH_3, R_4- CH_3
14 R_1,R_2- H, R_3- CH_3, R_4- I
15 R_1,R_2- H, R_3- H, R_4- I

16 R_1,R_2- H, R_3- CH_3 R_4- CH_3
17 R_1,R_2- H, R_3- CH_3 R_4- H
18 R_1,R_2,R_3,R_4- H

Scheme 6[28]

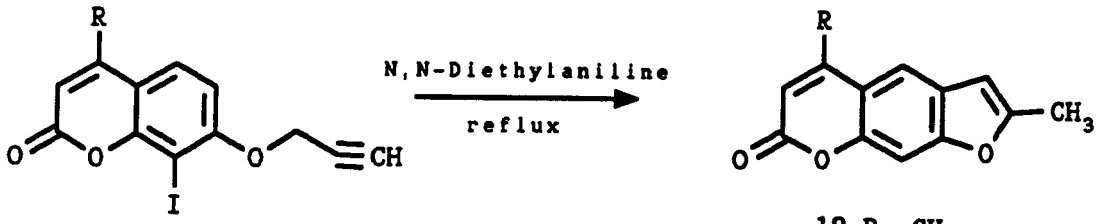
One commonly advanced mechanism of the Claisen rearrangement of aryl propargyl ethers is as follows (Scheme 7):



Scheme 7 [26,27]

The mechanism involves an initial [3,3] rearrangement to the allene followed by tautomerization to the phenol. This then undergoes a [1,5] sigmatropic rearrangement which leads by electrocyclic reaction to the unsaturated cyclized product.

In order to obtain 8H-benzodipyran-2-ones for biological testing, various linear isomers were prepared by Rodighiero's method. An interesting side-product obtained by this author but not reported in the literature was the furocoumarin derivative (Scheme 8).



14 R= CH_3
15 R= H

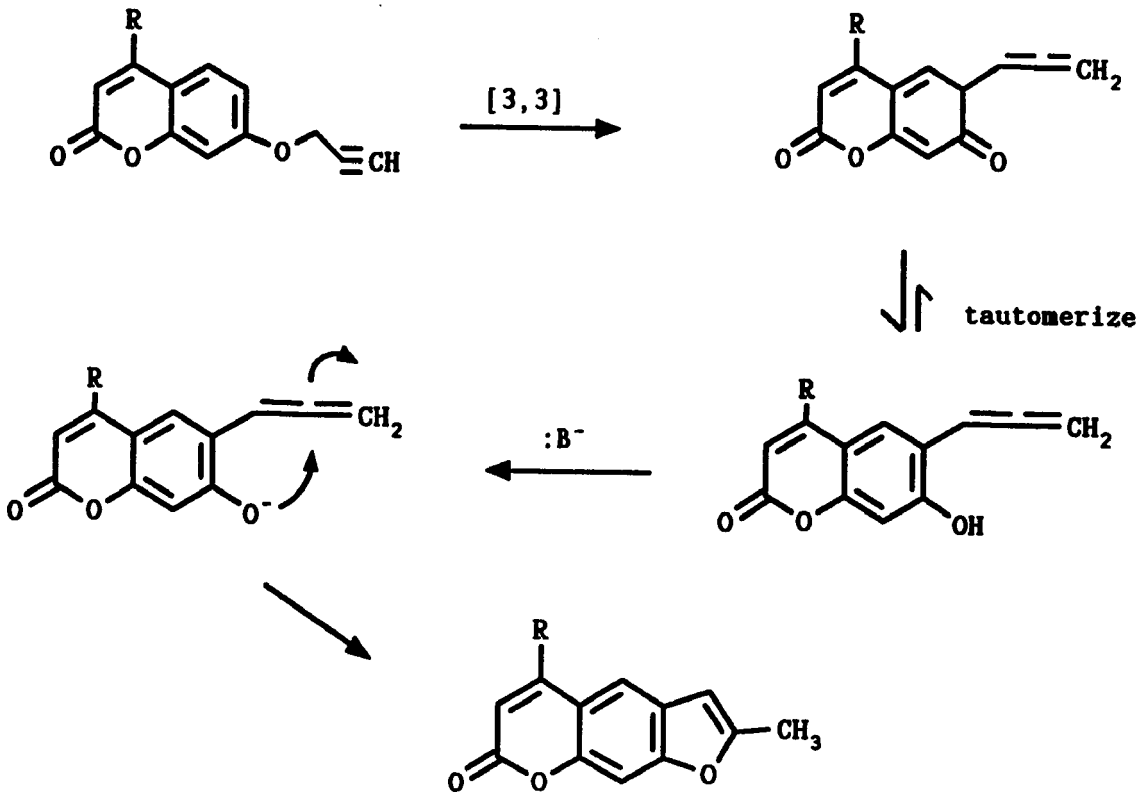
19 R= CH_3
20 R= H

Scheme 8

Recalling the mechanism of the rearrangement, one can see how the 5-membered ring product might be formed (Scheme 9). This product was identified by ^1H NMR and was not further purified.

Scheme 9

Possible mechanism of 5 MR formation

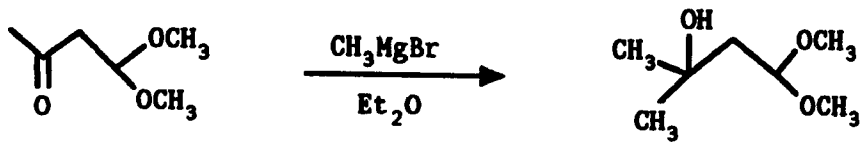


Although Rodighiero reported yields of up to 49%, we isolated pure compound in yields of only 20-25 %. The high temperature (> 200 ^{o}C) brought about the formation of tarry material which necessitated the purification of the desired product by tedious chromatography.

Pyridine-catalyzed condensation of α-β-unsaturated carbonyl compounds with phenols is a viable route to chromenes.[69] Use of masked forms of the alkenals, particularly the derived acetals, largely overcomes the problems associated with this reaction owing to the instability of these reagents. In particular, 4,4-dimethoxy-2-methyl-butan-2-ol(3-hydroxyisovaleraldehyde dimethyl acetal) has been developed as an easily available reagent to replace 3-methylcrotonaldehyde in dimethylchromene formation.[70] It is simply prepared by the addition of methyl magnesium bromide to acetoacetaldehyde dimethylacetal. Accordingly, 4,8-dimethyl-7-hydroxycoumarin was reacted with 4,4-dimethoxy-2-methyl-butan-2-ol and pyridine to afford 4,8,8,10-tetramethyl-2H,8H-benzo[1,2-b;5.4-b']dipyran-2-one in 49% yield after purification by preparative thin layer chromatography (Scheme 10).

Scheme 10 [70]

Pyridine-catalyzed condensation



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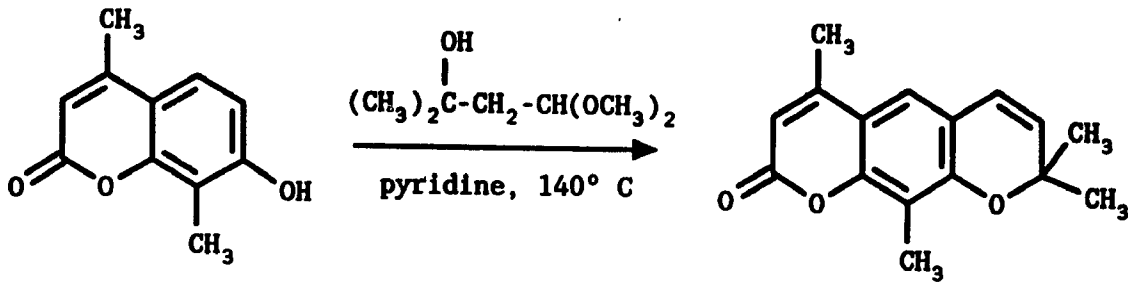
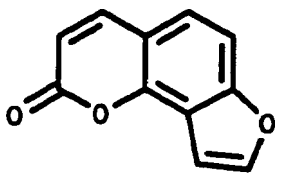


22 23

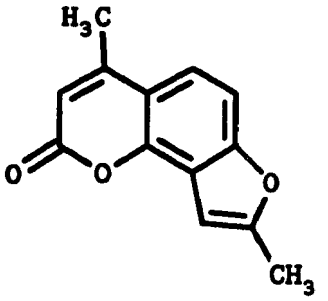
Syntheses of Dihydro Derivatives

Furocoumarins of the monofunctional type do exist which are capable, for steric reasons as in the angular angelicins, or as a consequence of chemically blocking one of the reactive sites of the psoralen molecule as in 3-carbethoxypsoralen, of forming only monoadditions to DNA. These monoadducts appear to be less involved in the undesired side effects of PUVA therapy such as skin phototoxicity and risk of skin cancer.[29]

Angular furocoumarins, known as angelicins, while possessing two reactive sites are able to photoinduce only monofunctional adducts in DNA. For geometric reasons, intercalated angelicins are capable of photoreacting with the pyrimidine bases of DNA at only one of the two reactive sites.[30]

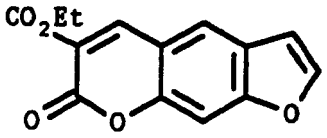


angelicin



4,5'-dimethylangelicin

Other monofunctional agents are derivatives of psoralens substituted at the 4',5' or 3,4 reaction site such as 3-carbethoxypsoralen.[31] Chemically blocking these reactive sites renders these analogs incapable of inducing DNA cross-links.



3-carbethoxypsoralen

These monofunctional furocoumarins have been proven to possess antiproliferative and therapeutic activity similar to the psoralens.[32] Even simple mono-unsaturated coumarins such as 5,7-dimethoxycoumarin[33] have shown parallel biological behavior to the classic psoralens, 8-methoxypsoralen and 5-methoxypsoralen. Given these observations, hemi-saturated furocoumarins, hemi-saturated pyranocoumarins and certain coumarins were synthesized in an effort to investigate their activity in our biological system.

From the results to be presented later in this thesis it will be noted that in inhibition of the binding of EGF to its receptor, and in competition with labeled 8-methoxypsoralen for the extra-nuclear psoralen receptor, key members of these chemical classes are all highly active agents.

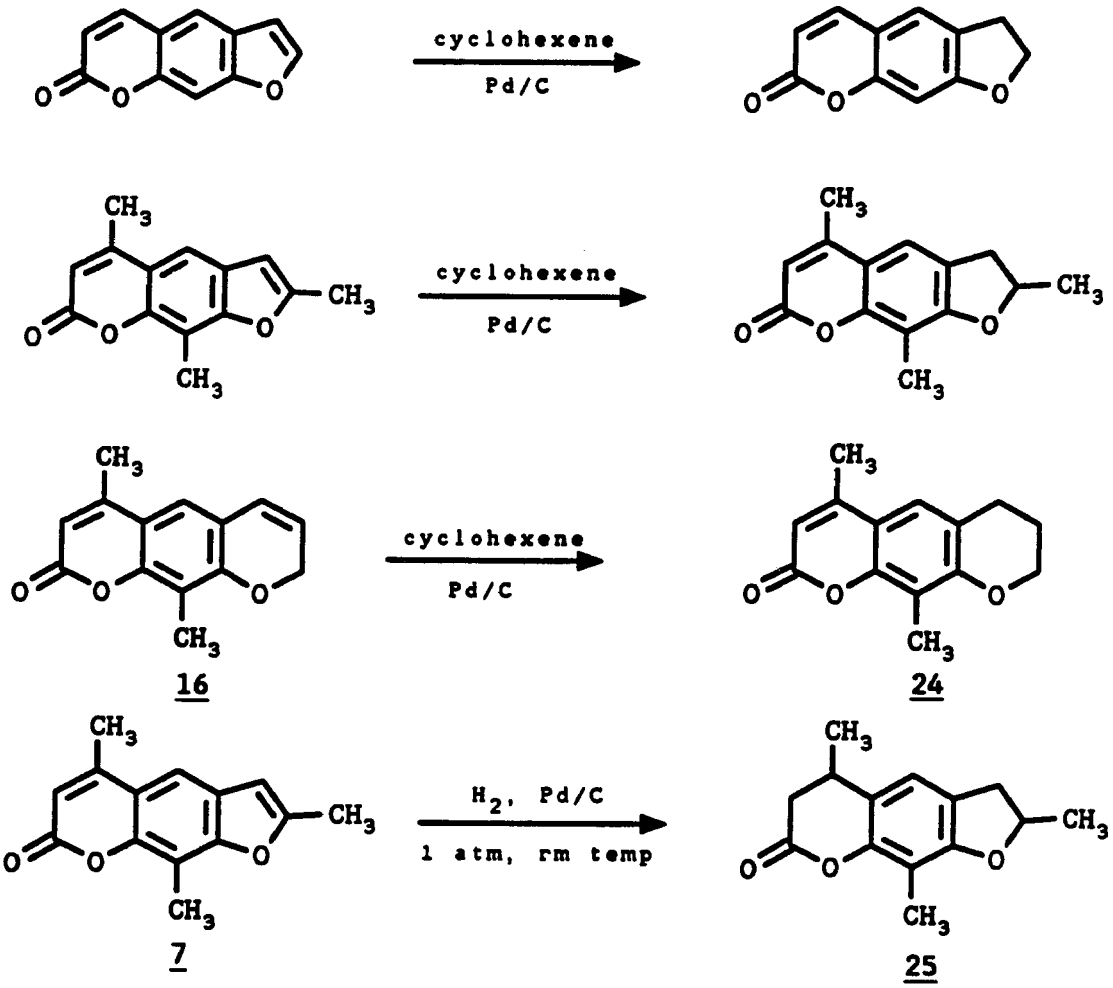
In recent years, our laboratories have developed a unique controlled reduction reaction as a route to dihydrofurocoumarin derivatives.[34] The method is a transfer-hydrogenation process utilizing cyclohexene in refluxing ethanol as the hydrogen donor and palladium supported on carbon as the catalyst. Entwistle reported these mild conditions as successful in the selective reduction of aromatic nitro compounds but the reaction was not extended to include the reduction of heterocyclic unsaturation.[35]

We found that under the conditions described, hydrogenation of the furano bond could be effected rapidly, selectively and in high yield (64-77%). By this method several furanocoumarins were converted to their dihydro derivatives. To produce the tetrahydro derivative of trioxsalen, it was necessary to utilize more traditional hydrogenation conditions (Scheme 11).

We have now applied this selective rapid transfer-hydrogenation to benzodipyran-2-ones. The process is facile, and exclusively reduces the 6,7 unsaturation of 8-H-benzodipyran-2-one derivatives without reduction of the 3,4 unsaturation or the carbonyl unsaturation. The reaction conditions employed were as related above.

Scheme 11 [34,63]

Synthesis of saturated psoralen derivatives



16 24

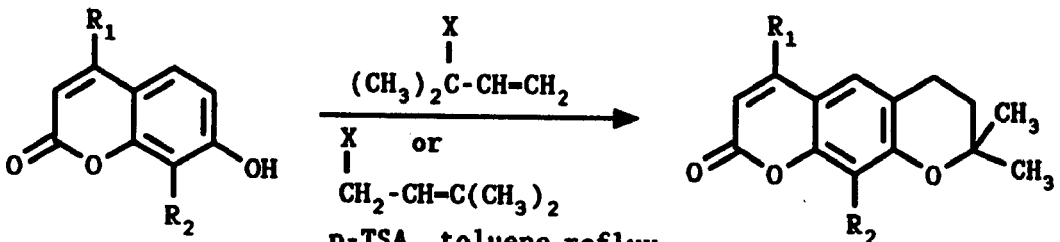
7 25

The transfer-hydrogenation provided us with a splendid route to the dihydropyranocoumarins. However, it required that we have the unsaturated derivatives on hand. We have discovered a more efficient one-step synthesis of selected geminally-substituted dihydro analogs starting from the preformed 7-hydroxycoumarins. The 7-hydroxycoumarin was reacted with an appropriate alkylating agent in the presence of p-toluenesulfonic acid in refluxing toluene. This reaction affords directly the desired cyclized product. The alkylating agent may be the allylic halide or alcohol or even the homoallylic alcohol. The acid catalyst may be p-toluenesulfonic acid or benzenesulfonic acid. The reflux times were between 3-5 hours and the products were purified by flash chromatography (Scheme 12).

Presumably the mechanism involves initial C-alkenylation followed by cyclization. Intermediates were not isolated from this reaction but in a case where the alkylating agent used was 3-methyl-3-buten-1-ol, a small amount of the C-3 alkylation product, 4,8-dimethyl-3(3-methyl-2-buten-1-yl)-7-hydroxycoumarin, was, in fact, isolated (Scheme 13).

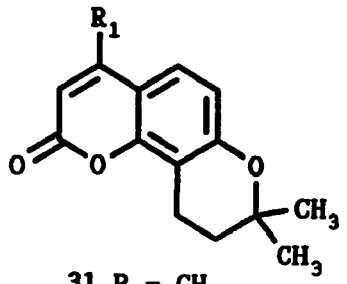
Scheme 12

Acid-catalyzed cyclization route to dihydropyran-2-ones



22 R_1, R_2- CH_3
26 R_1- CH_3, R_2- H
27 R_1, R_2- H
X - Cl, Br, OH

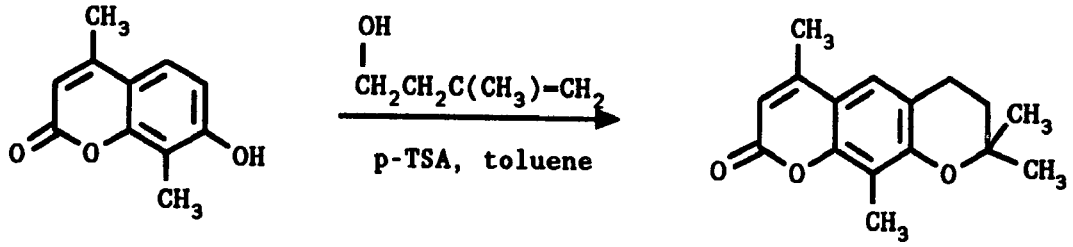
28 R_1, R_2- CH_3
29 R_1- CH_3, R_2- H
30 R_1, R_2- H



31 R_1- CH_3
32 R_2- H

Scheme 13

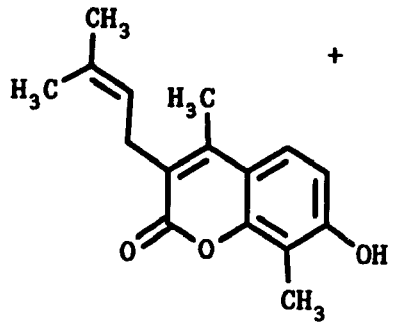
Acid-catalyzed cyclization utilizing homoallylic alcohol



22

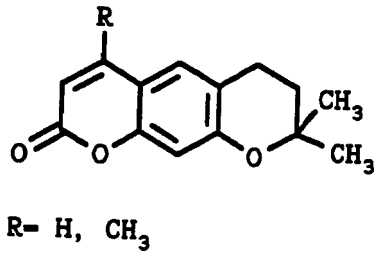
28 MAJOR

+

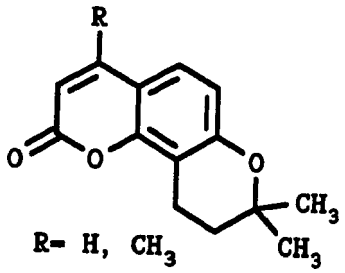


33 MINOR

When the original 7-hydroxycoumarin employed is unsubstituted in both carbon #6 and carbon #8, mixtures of the linear isomer (dihydrobenzo[1,2-b;5,4-b']dipyran-2-ones) and the angular (6,7-dihydrobenzo[1,2-b;3,4-b']dipyran-2-ones) result. The angular isomers can be recognized by their ^1H NMR spectra. A coupled A-B doublet of doublets (J = ca. 9 Hz) is present for the protons on carbons #5 and #6 on the benzenoid ring.



LINEAR ISOMER



ANGULAR ISOMER

If linear isomers are desired this may be achieved in three ways:

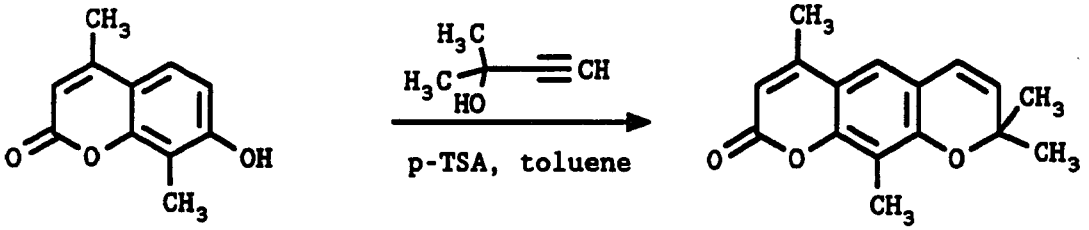
(1) selection of a coumarin bearing an alkyl substituent in position #8 to preclude closure to the angular isomer,

(2) introducing an iodide atom onto the carbon #8 position which is subsequently removed in the cyclization process to force closure in one direction.

This method has been employed successfully in the Claisen rearrangement of propargyl ethers.[26,27,28]

(3) chromatographic separation of the mixture of the linear and angular isomers when they do form in the reaction. This technique is illustrated in the Experimental section.

Additionally it was found that acid-catalyzed cyclization of a propargyl alcohol with 4,8-dimethyl-7-hydroxycoumarin yielded an 8-H-benzodipyran-2-one, without isolation of any intermediate species and without the use of elevated temperatures which induce char and tar formation. The yield was lower than in the dihydro instances (32%), but was higher than those generally obtained by the route of Rodighiero.(Scheme 14).



22 23

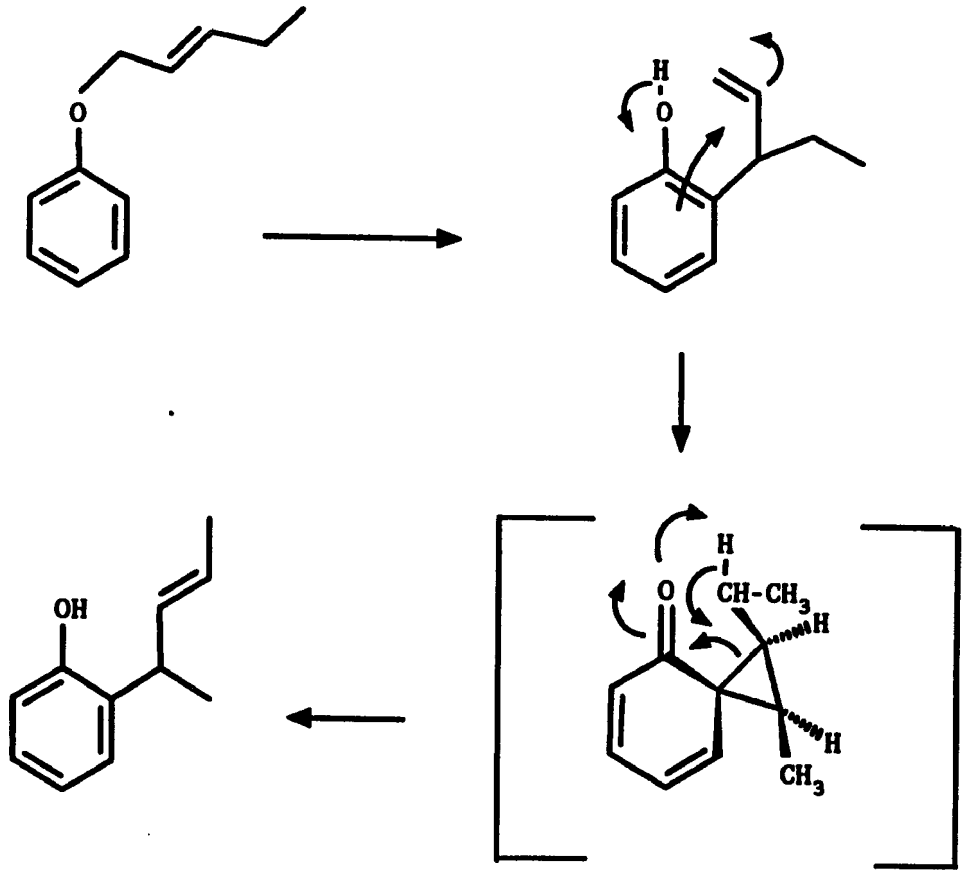
Scheme 14

Coumarin Syntheses by Claisen Rearrangement

Isoprenyl and other alkylated allyl ethers often undergo an abnormal Claisen rearrangement in which the initially formed ortho product isomerizes, probably via a spirodienone intermediate. Conclusive evidence of this was first reported by Marvell and coworkers in the rearrangement of γ-ethylallyl phenyl ether to o-(α,γ-dimethylallyl)phenol.[36] They reported that the α,γ-dimethylallyl ether was not an intermediate in the reaction, that this ether was stable when heated for 21 hours at 215 ^{o}C, and that the methyl ether of o-(α, ethylallyl)phenol did not rearrange even in the presence of added phenol. Their experiments showed that (a) the reaction depends on the phenolic hydroxyl, (b) the reaction is intramolecular, and (c) the geometric relation between the hydroxyl and the side chain is critical. The mechanism of this abnormal Claisen reaction was proposed by Marvell and coworkers (Scheme 15).

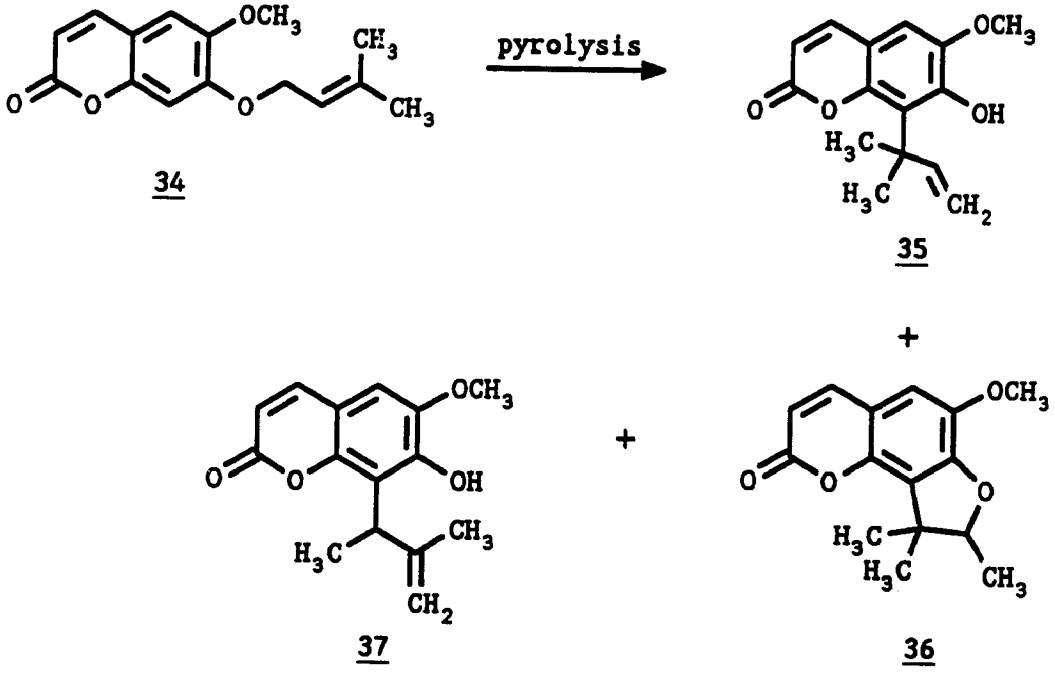
Scheme 15 [36]

Mechanism of abnormal Claisen as proposed by Marvell



spirodienone intermediate

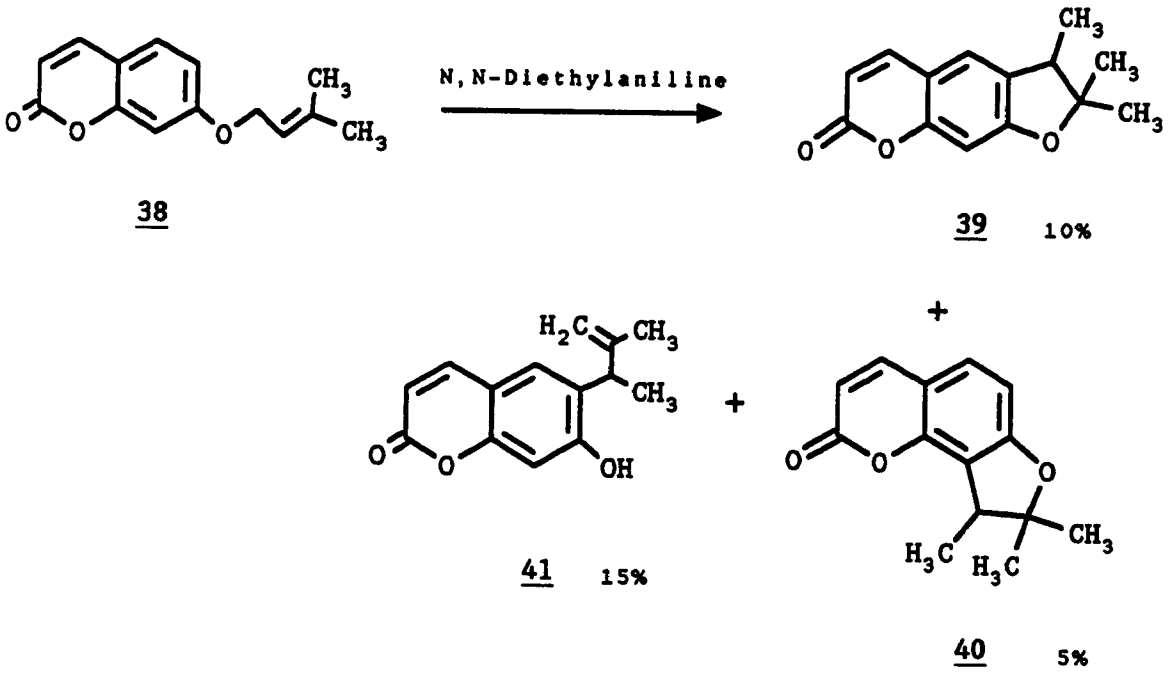
Evidence of this type of abnormal Claisen rearrangement occurring in allyloxy coumarins can be found in the literature. Ballantyne, Murray and Penrose reported the formation of an abnormal Claisen product in the pyrolysis at 195 $^{o}C/0.03mm$ of the naturally occurring 7-O-(3,3-dimethylallyl) scopoletin.[37](Scheme 16)



34 35 37 36

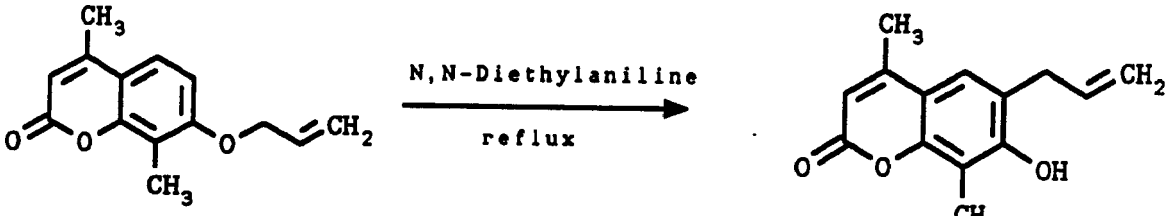
Scheme 16[37]

Raj, Kapil and Popli isolated, in low yield, the product of abnormal Claisen reaction of 7-(3,3-dimethylallyl)oxycoumarin.[38] Additional components of the complex mixture of products were the cyclized compounds derived from both the normal and abnormal Claisen rearrangement products. The formation of a coumaran in a Claisen rearrangement is a simple isomerization of the normal or abnormal Claisen products probably acid-catalyzed by the phenol present (Scheme 17).

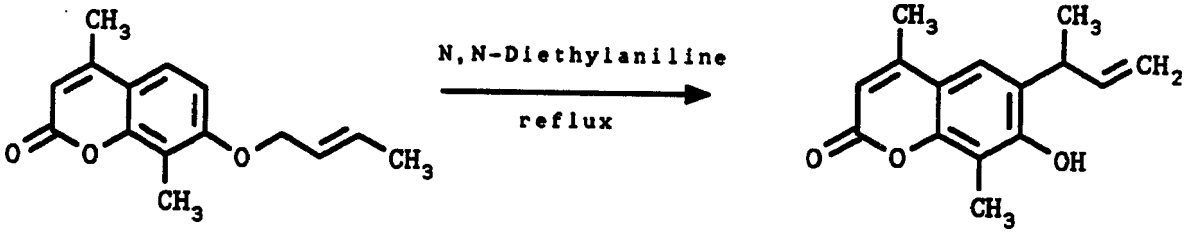


Scheme 17[38]

In the course of this thesis work, Claisen rearrangements of a number of substituted and unsubstituted 7-allyloxy coumarins in refluxing N,N-diethylaniline (DEA) were performed. In cases where the allyloxy group was unsubstituted or monomethyl-substituted, the product obtained was that expected from the normal Claisen rearrangement. Thus, 4,8-dimethyl-7-allyloxycoumarin rearranged in DEA to give 4,8-dimethyl-6-allyl-7-hydroxycoumarin. 4,8-Dimethyl-7-(2-butenyl)oxycoumarin in refluxing diethylaniline also underwent normal Claisen rearrangement to yield 4,8-dimethyl-6(2-buten-3-yl)-7-hydroxycoumarin (Scheme 18).



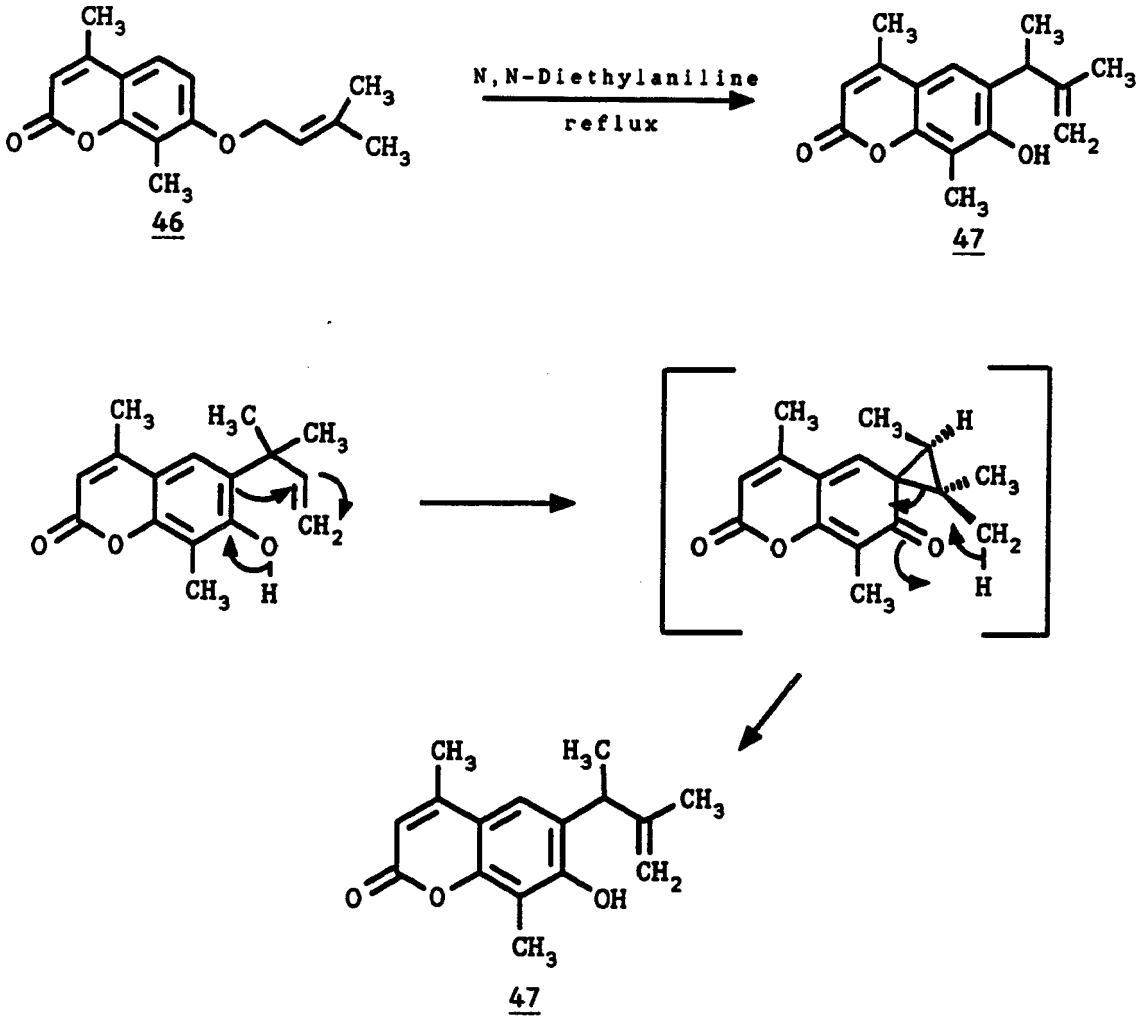
42 43



44 45

Scheme 18

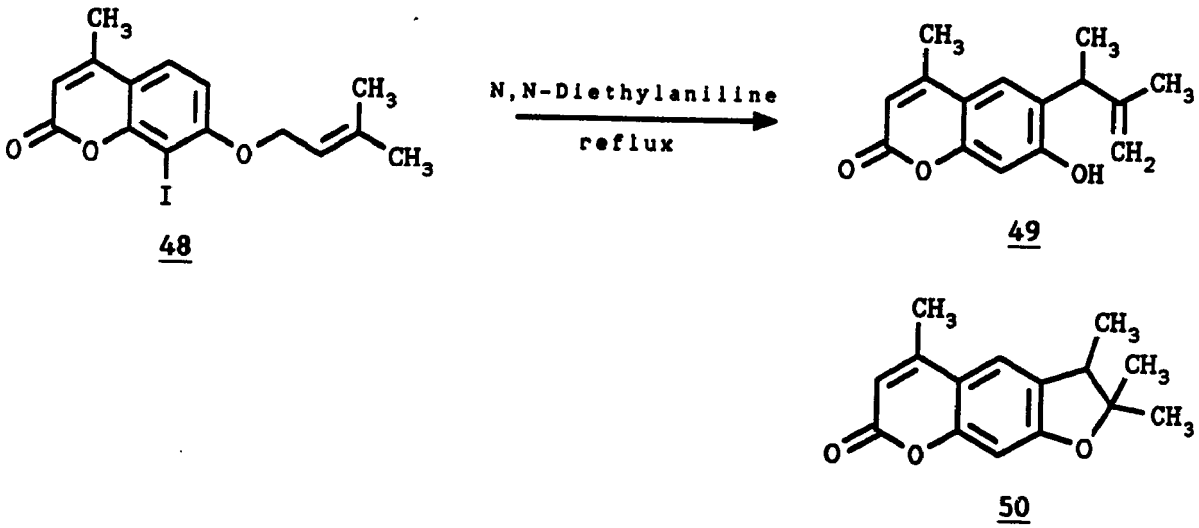
However, thermal rearrangement of 4,8-dimethyl-7-(3,3-dimethylallyl)oxycoumarin in DEA yielded only the abnormal Claisen product, 4,8-dimethyl-6-(2-methyl-1-buten-3-yl)-7-hydroxycoumarin, in 77% yield. The structure of the product was assigned on the basis of ^{1}H and ^{13}C NMR. A likely mechanism of its formation from the normal Claisen product is shown in Scheme 19.



46 47 47

Scheme 19

When the Claisen rearrangement was carried out on 4-methyl-8-iodo-7-(3,3-dimethyallyl)oxycoumarin, two products were isolated. The first was identified as the product of abnormal Claisen rearrangement. The other product was identified as a dihydrobenzofuran. This compound corresponds to the cyclization product arising from the closure of the abnormal Claisen rearrangement product (Scheme 20).



48 49 + 50

Scheme 20

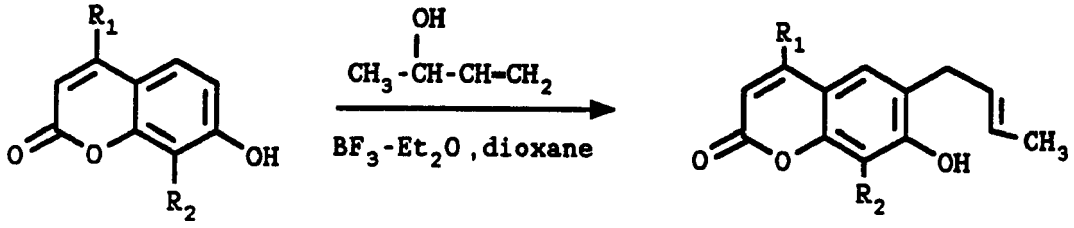
Syntheses of Alkoxy and Alkenyl Coumarins

Included in the set of target compounds are coumarins that contain an olefinic side chain at carbon 6. Compounds of this class permit structure-activity deductions relative to open-chain analogs nearly isostructural with psoralens and benzodipyranones. One commonly used method for the synthesis of such allylic residues on coumarins derived from the model reaction of a substituted salicylaldehyde with an allylic halide followed by elaboration of the salicylaldehyde side chain by Perkin condensation to the desired coumarin.[39]

The difficulty of specifically introducing alkylated allylic groups into the 6 position of a preformed 7-hydroxycoumarin is well documented.[40] Direct nuclear allylation of a 7-hydroxycoumarin has been reported in the literature but was only successful when a methoxy group in position 5 was also present.[41]

In this study, a Lewis acid-catalyzed alkylation reaction was utilized to prepare 7-hydroxycoumarins with a 2-butenyl residue in the 6 position. 4,8-Dimethyl-7-hydroxycoumarin and 4-methyl-7-hydroxycoumarin were reacted with 3-butene-2-ol in the presence of BF_3-etherate to yield the C-alkylated products. The yields of these reactions were low (15-20%) but the starting materials were easily recovered

and could, in fact, be recycled for another reaction (Scheme 21).



22 R_1, R_2= CH_3

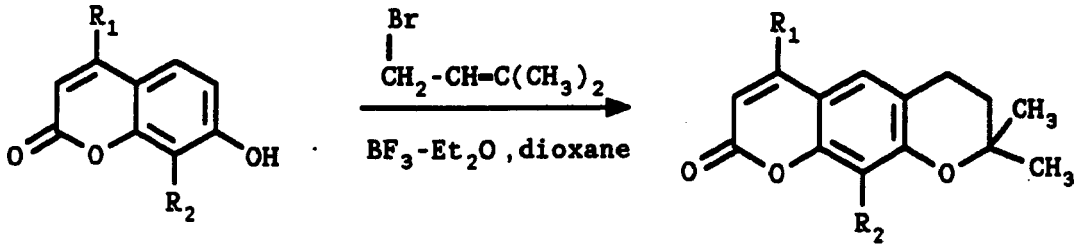
51 R_1= CH_3, R_2= H

52 R_1, R_2= CH_3

53 R_1= CH_3, R_2=H

Scheme 21

However, when this alkylation reaction was attempted in order to introduce an isoprenyl residue into the 6 position of the 7-hydroxycoumarins, the only products obtained, also in low yield were the dimethylchromans resulting from subsequent cyclization of the intermediate phenols. A possible explanation for the difference between the butenyl-substituted coumarins and the isoprenyl-substituted coumarins could be the increased stability of the tertiary carbocation formed in the latter case. This would increase the propensity of the isoprenyl coumarins toward cyclization (Scheme 22).



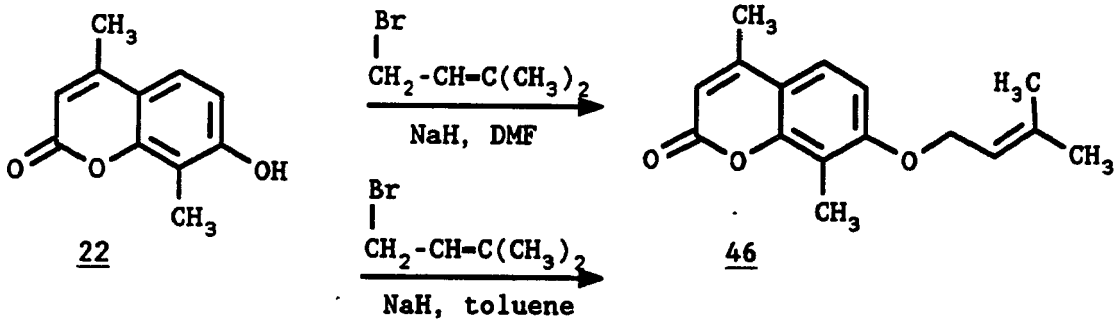
22 R_1, R_2- CH_3
51 R_1- CH_3 R_2- H

28 R_1, R_2- CH_3
29 R_1- CH_3, R_2-H

Scheme 22

Another approach to direct C-dimethylallylation was to alkylate under basic conditions, selecting those conditions which would be most favorable for maximum C-alkylation. These conditions, as described by Kornblum include the use of a nonpolar solvent, heterogeneous medium and maximum coordinating power of the cation.[42]

Treatment of 4,8-dimethyl-7-hydroxycoumarin with NaH in dimethylformamide, followed by reaction with the alkylating agent gave as expected a 95% yield of the O-alkylation product. Changing the solvent from a polar aprotic solvent (DMF) to a nonpolar solvent (toluene) produced a lower yield (82%) of the O-alkylation product but no C-alkylation product was isolated (Scheme 23).

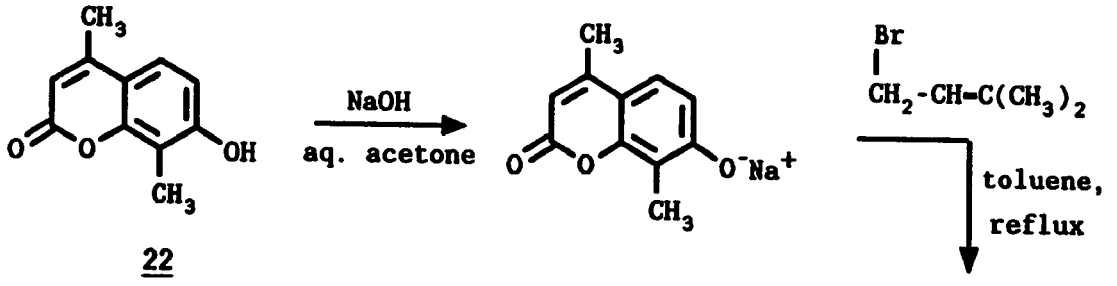


Scheme 23

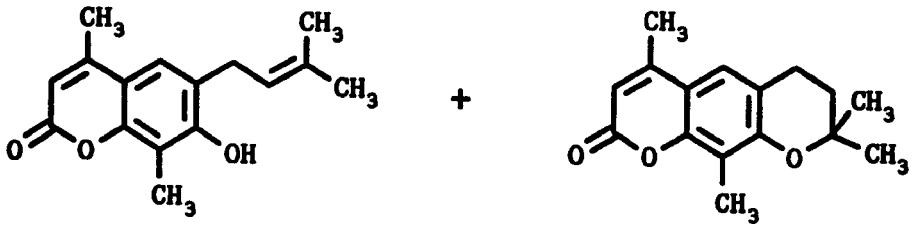
The alkylation reaction was attempted with the preformed sodium salt of the hydroxycoumarin in toluene and in this case, C-alkylation product was obtained but in very low yield. The bulk of the reaction product was the dimethyldihydropyranocoumarin (Scheme 24).

Scheme 24

Alkylation reaction
with preformed sodium salt



22



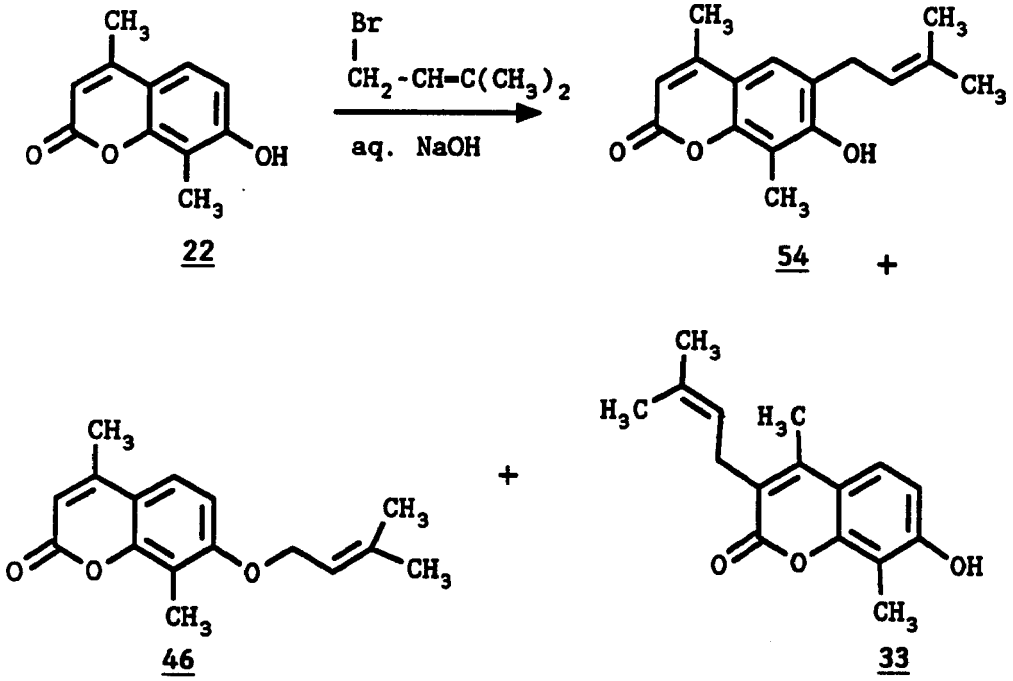
54 MINOR

28 MAJOR

Kornblum et al[43] have described in great detail studies of the influence of the solvent on the alkylation of ambident anions. In particular, the importance of the hydrogen bonding capacity of the solvents has been investigated. In protic solvents such as water, fluorinated alcohols and phenol, solutions of phenolic salts may react to give a large percentage of carbon alkylation. When an anion such as a phenoxide anion is dissolved in a solvent which is unusually effective at forming hydrogen bonds, the oxygen of the phenoxide ion is extensively solvated and therefore is not as available for nucleophilic displacement. In addition, protic solvents such as water, fluorinated alcohols and phenol solvate the leaving group of the alkylating agent to a large extent. These two effects, operating jointly have been offered as a possible explanation for the observed course of the reaction, namely C-alkylation.

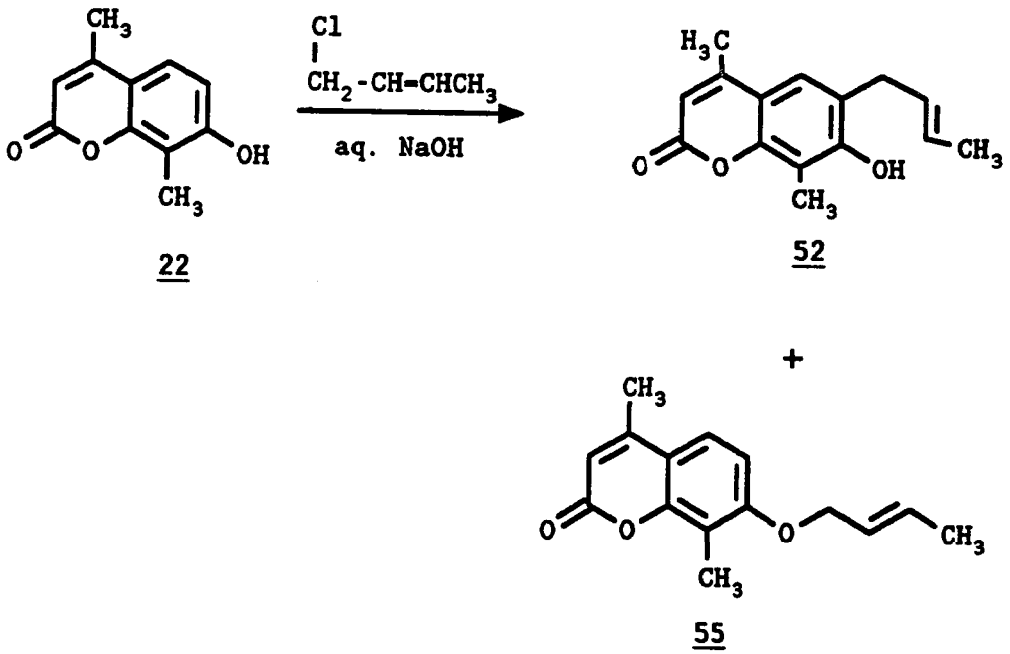
In light of this, the alkylation of 4,8-dimethyl-7-hydroxycoumarin was carried out in aqueous sodium hydroxide solution at room temperature with 1-bromo-3-methyl-2-butene as the alkylating agent. The main component of the product mixture was determined to be 4,8-dimethyl-3-(3,3-dimethylallyl)-7-hydroxycoumarin (Scheme 25). The key identifying feature in the ^{1}H NMR

spectrum was the peak at ca. 6 ppm. Originally mistaken for the C_3 proton, this peak disappeared after shaking the sample with D_2O, indicating that it was actually the phenolic OH peak. The product structure was assigned as shown below from the assembled spectral data.



Scheme 25

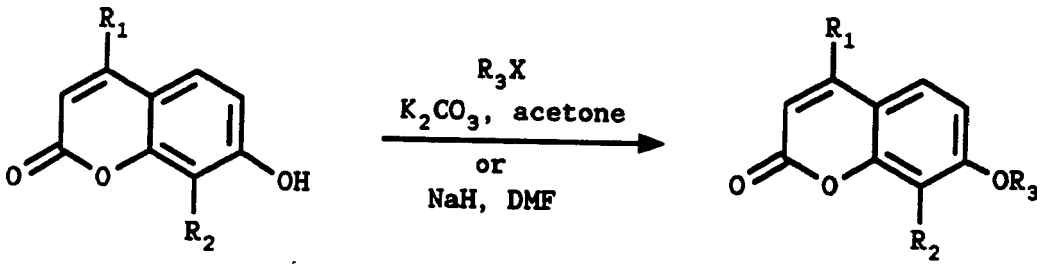
Reaction of 4,8-dimethyl-7-hydroxycoumarin with 1-chloro-2-butene in aqueous sodium hydroxide solution at room temperature produced three main components which were separated by flash chromatography (Scheme 26). The product with the highest R_f value was identified as the product of O-alkylation. The main product was the C-alkylation product and the third was found to be unchanged starting material.



22 52 55

Scheme 26

An extended set of coumarin analogs have been prepared which are etherified at carbon # 7 with alkyl groups of varying chain length, olefinic and acetylenic functionalities. The route to these compounds was a Williamson-type ether synthesis utilizing either a K_2CO_3/acetone system or NaH in DMF (Scheme 27).



As can be readily seen, the previously described classes of compounds lack the second site of unsaturation found in the model parent, the psoralens. This renders these analogs incapable of forming DNA crosslinks and they lack the mutagenic/carcinogenic potential of the psoralens. The 4′, 5′-dihydropsoralens, 6,7-dihydrobenzodipyran-2-ones and substituted coumarins may represent new psoralen-like dermatological agents.

Scheme 27

Compound	R_1	R_2	R_3
56	CH_3	CH_3	CH_3
57	CH_3	CH_3	$(CH_2)_2CH_3$
58	CH_3	CH_3	$(CH_2)_3CH_3$
59	CH_3	CH_3	$(CH_2)_5CH_3$
60	CH_3	CH_3	$(CH_2)_7CH_3$
42	CH_3	CH_3	$CH_2CH=CH_2$
44	CH_3	CH_3	$CH_2CH=CHCH_3$
46	CH_3	CH_3	$CH_2CH=C(CH_3)_2$
48	CH_3	I	$CH_2CH=C(CH_3)_2$
61	H	H	$CH_2C\equiv C(CH_2)_4CH_3$
62	CH_3	CH_3	$CH_2C\equiv(CH_2)_4CH_3$
63	CH_3	I	$C(CH_3)_2C\equiv CH$
64	CH_3	CH_3	$(CH_2)_7COOH$
13	CH_3	CH_3	$CH_2C\equiv CH$
65	CH_3	CH_3	$C(CH_3)_2C\equiv CH$

DNA BINDING STUDY

As stated previously, psoralens form noncovalent complexes with DNA deriving from intercalation of their planar molecules between two base pairs.

Furocoumarin + DNA non-covalent complex covalent combination

The formation of the preliminary intercalated complex between furocoumarins and DNA is an important step which markedly affects the successive covalently photobinding to DNA.[44] Only complexed molecules photobind covalently with DNA, and the affinity of the various furocoumarins toward the macromolecule influences the extent of the photolesions that can be produced in DNA upon subsequent irradiation.[45] Although our evidence supports a site other than DNA for psoralen binding, we investigated the association of a set of representative compounds with DNA for its possible pertinence to EGF binding inhibition.

The dark interaction between furocoumarins and nucleic acids was first investigated by Rodighiero and Dall'Acqua in 1966.[46] They studied the solubilization and modification of the optical properties of several psoralens in DNA and RNA solutions.

In general, the increase in the solubility of the psoralens in the presence of DNA is assumed to be due to complex formation and the amount of substance solubilized in DNA more than in water is considered bound to DNA.[47] Likewise, when a small molecule forms a complex with DNA involving its chromophoric moiety, a modification of its spectrophotometric properties can be observed. These changes include a bathochromic (red) shift of the λ_{max} of the molecule and a decrease in its extinction coefficient (hypochromic effect).

The enhancement of the melting temperature of DNA can also be used as an indication of DNA intercalation. When double-stranded DNA molecules are subjected to extremes in temperature or pH, the H bonds of the double helix are ruptured and the two strands are no longer held together. The DNA is said to denature and changes from a double helix to a random coil. When heat is used as the denaturant the DNA is said to melt and the temperature at which the strands separate is the melting or transition temperature, T_m.[48] Intercalation of a molecule into DNA would cause stabilization of the double helix and subsequently increase the melting temperature.

The formation in the dark of a complex between the furocoumarins and native DNA was clearly shown by

their increased solubility and the variation of their spectrophotometric properties in DNA solution. There was an evident shift of the λ_{max} of the psoralens to a longer wavelength (bathochromic shift) and a marked decrease of their absorbing properties (hypochromicity). Additionally, a slightly higher T_m value was found in the DNA in the presence of the psoralens.

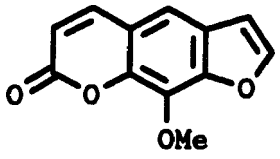
The ability of some coumarins and 4'5'-dihydropsoralens to form complexes with DNA was examined by Dall'Acqua et al.[49] It was found that these compounds were solubilized more in DNA than in water, similar to the psoralens. However, Dall'Acqua reported that although these coumarins and dihydrofurocoumarins showed a slight decrease in their extinction coefficients in DNA solution, there was practically no shift in the λ_{max} of any of the compounds tested. They hypothesized that the complexes formed by coumarins and 4',5'-dihydrofurocoumarins with DNA are of different kinds than that formed by psoralens. That is, they are not due to an intercalation of the molecules between two base pairs of DNA. Antonello and coworkers have also reported experimental evidence that 7-hydroxycoumarin derivatives have a low ability to form complexes with DNA.[50]

Rodighiero evaluated the dark interaction between pyranocoumarins and DNA.[51] It was discovered that pyranocoumarins possessing gem-dimethyl groups showed a much lower ability to form a molecular complex with DNA than psoralens. Their findings suggested that the behavior of such compounds toward DNA, relative to the furocoumarins, was quite different. It was plausible that a very weak complex, if formed, was not of the intercalative type due to the steric hindrance exerted by the methyl groups.[52]

For our purposes we studied the DNA binding behavior of compounds **1**, **13**, **28**, **16** and **56** employing various experimental methods. The solubilities and the uv absorbance properties in water and in 0.1% DNA solution of a number of representative compounds were measured (Figure 5). The melting temperature (T_m) of DNA was also measured in the absence and presence of the test compounds. The compiled results are listed in Tables 3 and 4. Figure 6 shows a typical absorption spectrum of one of the test compounds which does not exhibit intercalative behavior in the absence and presence of DNA. Figure 7 shows the absorption spectra of a typical compound which does exhibit intercalative behavior. Figure 8 is the ultraviolet spectrum obtained in a typical DNA melting experiment.

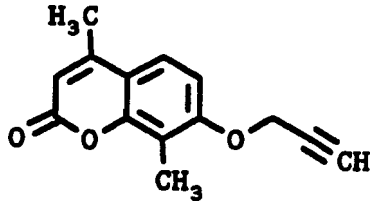
Figure 5

Compounds used for DNA Study



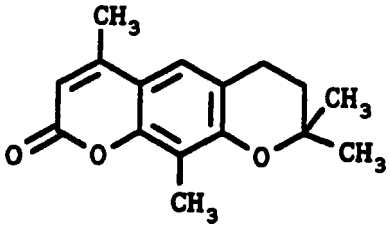
Compound A

1



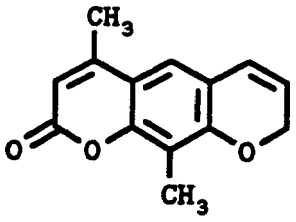
Compound B

13



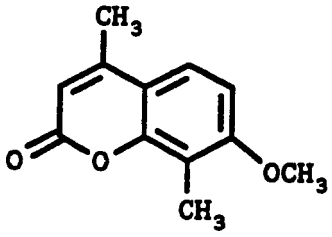
Compound C

28



Compound D

16



Compound E

56

Table 3

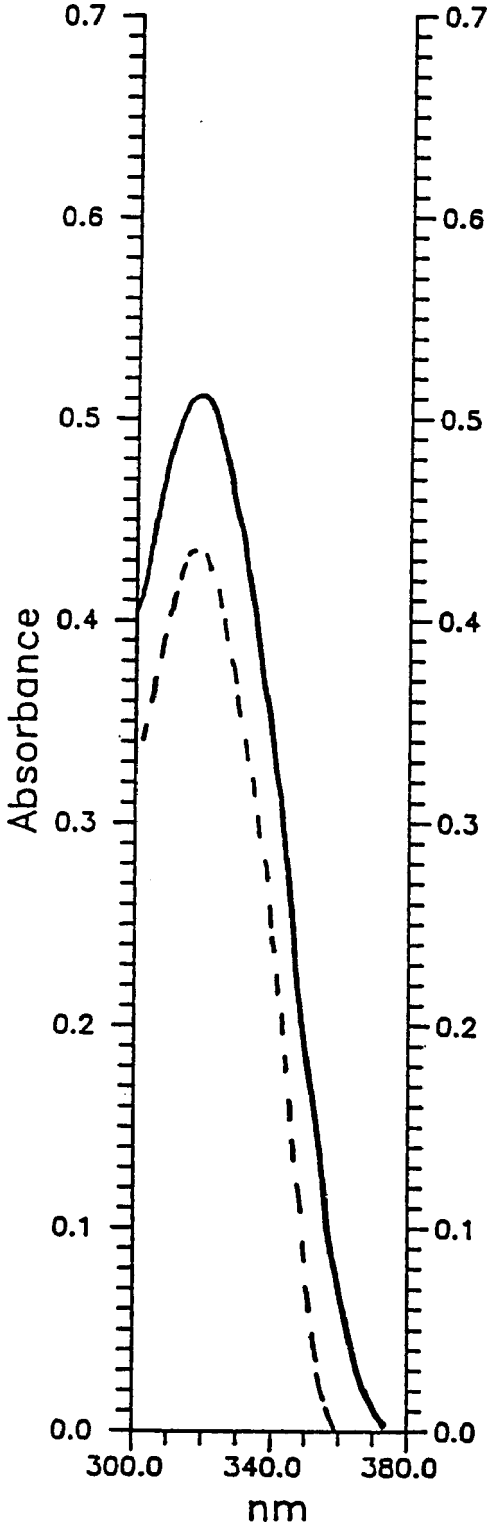
Ultraviolet Absorption Properties in the Presence and Absence of DNA

Compound	λmax aq.	λmax 0.1% DNA	extinction coefficient aq.	extinction coefficient 0.1% DNA
A	312	321	11890	8156
B	318	318	6720	6654
C	330	330	14062	13892
D	347	356	5838	1385
E	320	320	11302	9548

Table 4

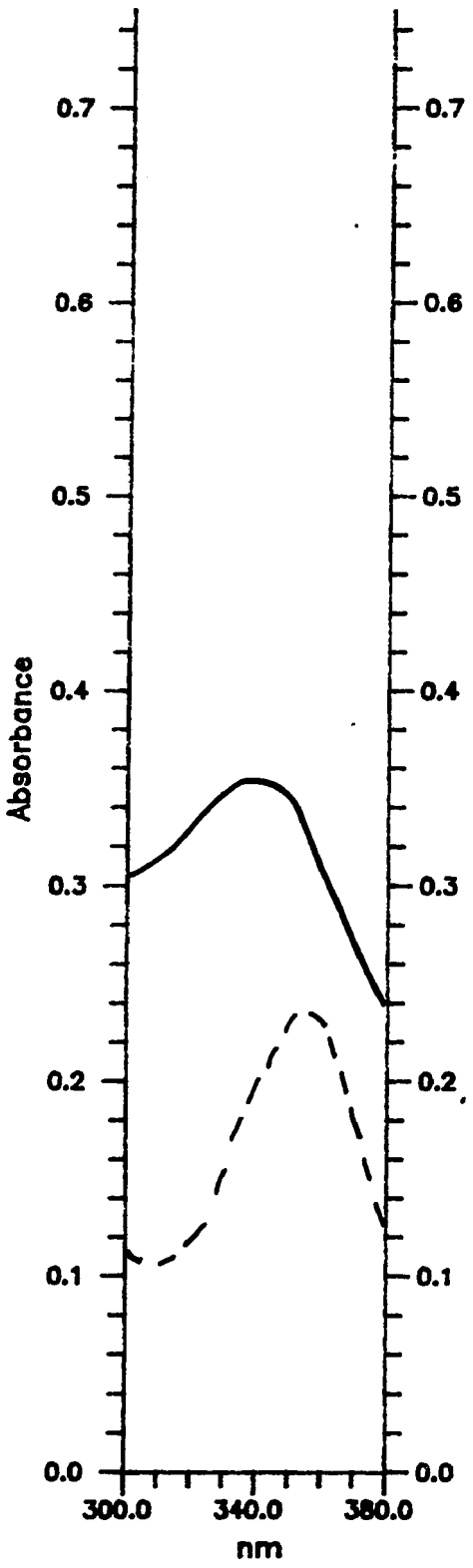
Solubility in water and 0.1% DNA, DNA melting temperature in presence and absence of compounds

Compound	Solubility (μg/ml) aq.	Solubility (μg/ml) 0.1% DNA	T_m (0.1% DNA) °C
A	35.6	85.2	64 °C
B	10.4	11.3	59 °C
C	15.6	15.9	59 °C
D	12.6	42.8	77 °C
E	41.2	80.9	66 °C
DNA alone			60 °C



Absorption spectra of compound E (8.12 ug/ml) in water ——— and in 0.1% aq. DNA ------

Figure 6



Absorption spectra
of compound D (10.8 ug/ml)
in water ———
and 0.1% aq. DNA - - - - - -

Figure 7

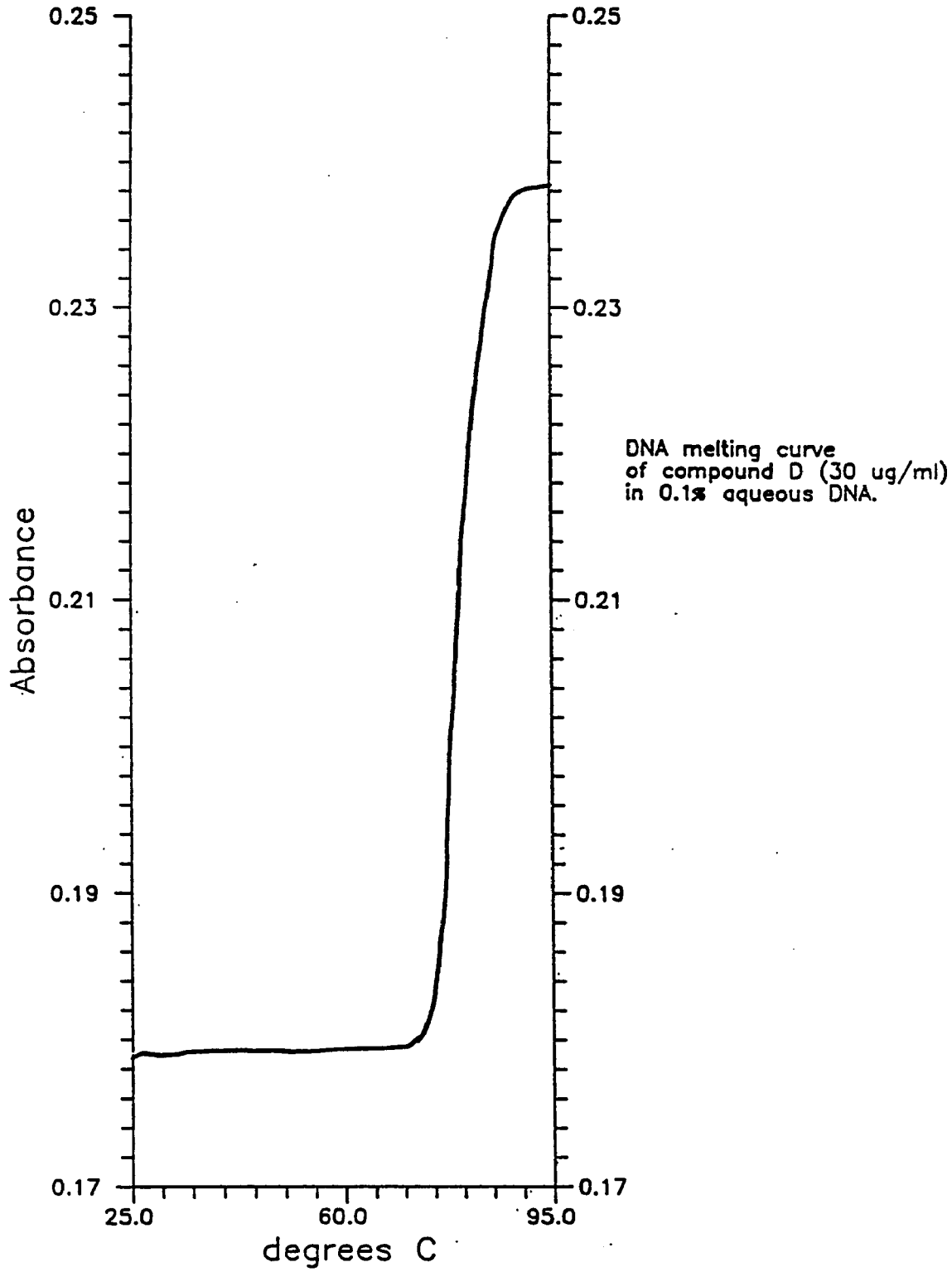

0.25
0.23
0.21
0.19
0.17
Absorbance
25.0
60.0
95.0
degrees C
DNA melting curve
of compound D (30 ug/ml)
in 0.1% aqueous DNA.

Figure 8

Compound A is 8-methoxypsoralen which is known to form an intercalation complex with DNA. As can be seen from the data, only one of the other compounds tested, compound D, exhibits behavior suggestive of intercalative binding with DNA. Its solubility in DNA solution increased by a factor of 3 relative to water and the λ_{max} of absorbance of the compound showed a bathochromic shift of 9 nm. Furthermore, in the presence of compound D the melting temperature of the DNA increased by 17 ^{o}C.

The methoxycoumarin, compound E, showed an increased solubility in DNA solution by a factor of 2 and an increased T_m of the DNA of 6 ^{o}C. However, the absorbing properties of the compound in DNA solution were essentially unchanged from its properties in water. This information is in accordance with Dall'Acqua's hypothesis that any interaction of alkoxycoumarins with DNA probably does not involve intercalation.

The other compounds tested apparently have no binding interactions with DNA. In the case of the dihydropyranocoumarin, compound C, the gem-dimethyl groups of the pyran ring could be providing a steric barrier to complex formation with DNA.

INTERPRETATION OF BIOLOGICAL RESULTS

Epidermal growth factor is a low molecular weight polypeptide which binds to cell surface receptors and which is known to be an important regulator of growth in those cells which possess these particular cell surface receptors. Psoriasis, mycosis fungoides and similar proliferative diseases are often characterized by abnormal cell growth regulation. This may be related to the action of EGF on these cells. The use of the EGF inhibition assay is based on the observation that psoralen phototherapeutics are extremely potent inhibitors of binding of epidermal growth factor to cell surface receptors in mammalian cells including humans.

Inhibition of EGF binding was rapid, dependent on concentration, and required light activation. Employing this inhibition as a quantitative bioassay, we have been able to generate dose-response curves such as the one shown in Figure 9. From these curves we are able to calculate IC_{50} values for our candidate agents. The IC_{50} value is the concentration (μg/ml) of the drug that produces 50% inhibition of the binding of ^{125}I-EGF to its receptor. Tables 5, 6, 7 and 8 list compound structures and their corresponding IC_{50} values.

Figure 9

Biological Evaluation of

4,8-Dimethyl-7-propargyloxycoumarin

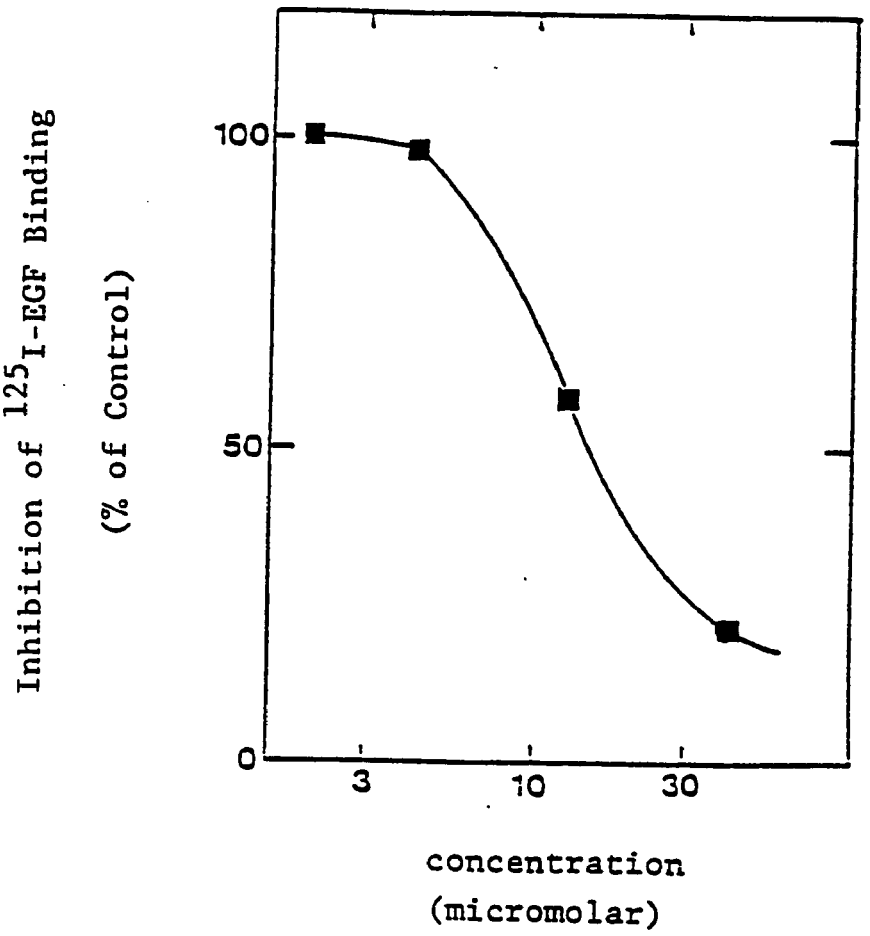
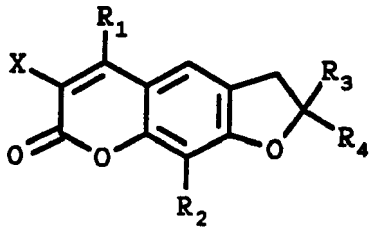


Table 5

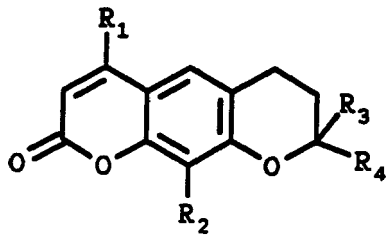
Inhibition of ^{125}I-EGF specific binding by furocoumarins



Compound	R_1	R_2	R_3	R_4	IC_{50} (μM)
TMP (1)	---	---	---	---	6.6
69	H	H	H	H	10.8
70	CH_3	CH_3	CH_3	H	7.0
71	CH_3	CH_3	CH_3	CH_3	23.8
67	CH_3	CH_3	CH_3	H	24.0
	X = NO_2				
68	H	H	H	H	4.9
	X = CO_2Et				
25	CH_3	CH_3	CH_3	H	>215
	(saturated 3,4-bond)				

Table 6

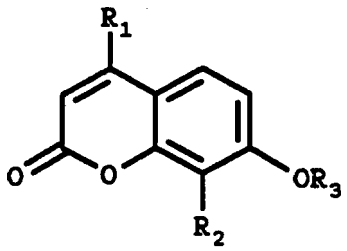
Inhibition of ^{125}I-EGF specific binding by pyranocoumarins



Compound	R_1	R_2	R_3	R_4	IC_{50} (μM)
24	CH_3	CH_3	H	H	7.4
28	CH_3	CH_3	CH_3	CH_3	40.0
29	CH_3	H	CH_3	CH_3	2.1
30	H	H	CH_3	CH_3	152.0
66	CH_3	CH_3	H	CH_3	43.0
31	CH_3	---	CH_3	CH_3	3.3
	(angular isomer)				
16	CH_3	CH_3	H	H	17.5
	(unsaturated 6,7-bond)				

Table 7

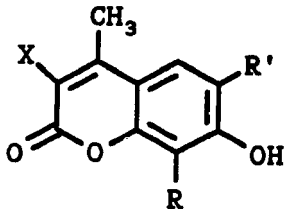
Inhibition of ^{125}I-EGF specific binding by 7-alkoxycoumarins



Compound	R_1	R_2	R_3	IC_{50} (μM)
57	CH_3	CH_3	$(CH_2)_2CH_3$	2.2
58	CH_3	CH_3	$(CH_2)_3CH_3$	25.2
59	CH_3	CH_3	$(CH_2)_5CH_3$	>109
60	CH_3	CH_3	$(CH_2)_7CH_3$	>99
42	CH_3	CH_3	$CH_2CH=CH_2$	4.6
44	CH_3	CH_3	$CH_2CH=CHCH_3$	6.8
46	CH_3	CH_3	$CH_2CH=C(CH_3)_2$	5.0
48	CH_3	I	$CH_2CH\equiv C(CH_3)_2$	1.5
61	H	H	$CH_2C\equiv C(CH_2)_4CH_3$	>130
62	CH_3	CH_3	$CH_2C\equiv C(CH_2)_4CH_3$	>99
63	CH_3	I	$C(CH_3)_2C\equiv CH$	7.9
64	CH_3	CH_3	$(CH_2)_7COOH$	24.1
13	CH_3	CH_3	$CH_2C\equiv CH$	15.0
65	CH_3	CH_3	$C(CH_3)_2C\equiv CH$	5.5
72	CH_3	CH_3	H	60.5

Table 8

Inhibition of ^{125}I-EGF specific binding by 6-alkenylcoumarins



Compound	R	R'	IC_{50} (μM)
43	CH_3	$CH_2CH=CH_2$	1.3
45	CH_3	$CH(CH_3)CH=CH_2$	45.1
47	CH_3	$CH(CH_3)C(CH_3)=CH_2$	7.0
52	CH_3	$CH_2CH=CHCH_3$	2.7
53	CH_3	$CH_2CH=CHCH_3$	14.8
54	CH_3	$CH_2CH=C(CH_3)_2$	5.4
33	CH_3	H	9.1

X = $CH_2CH=C(CH_3)_2$

As we have noted, the range of structural variance is not high in the compounds studied because, once active analogs had been discovered, it was thought advisable to stay close to those structural types. This thesis represents an initial report of the synthesis and photobiology of these extended coumarins. Subsequent workers must probe the limits of phototherapeutic action. Cationic or anionic moieties directly attached to the heteronucleus and bioisosteric substitutions of the ring-oxygens for other heteroatoms must be explored. Nevertheless, within the range of structural variations that were synthesized, some structure-activity relationships (SAR) can be deduced.

1. There is clearly no requirement that the third ring, fused upon the coumarin, be a five-membered (furano) moiety to retain activity. Systems with a six-membered pyrano ring, the benzodipyranones, had equivalent potency.

2. Moreover, the actual presence or absence of the third ring appears unrelated to potency since many of the simple coumarins (ex: 4,8-dimethyl-7-([3-methyl-2-butenyl)]oxycoumarin were among the most potent compounds screened.

3. Since the third ring (furano or pyrano) does

not appear essential for activity, it is probably not surprising that the absence of unsaturation does not significantly alter photobiological behavior. Thus the 6,7-dihydrobenzodipyranones compared to the 2H,8H-benzodipyranones and the 4',5'-dihydropsoralens versus the psoralens display comparable activities. In fact, the reduced angular benzodipyranone (compound **31**) is one of the most active compounds tested, possessing activity similar to that of the parent compound, trioxsalen. Furthermore, the dihydropsoralens tested which have a substituent in the C-3 position (compounds **67** and **68**) and the 3-substituted coumarin tested (compound **33**) are all active agents. These observations point to the difference between the biological mechanism which must operate in the EGF inhibition system versus that traditionally thought to be operable in the classic psoralens; that is, the photoinduced DNA cross-linking leading to bis-cyclobutane analogs.

4. Although the third ring is a structural option for activity, it appears to have a steric requirement for virtual planarity if it is indeed present in the linear three-ring systems. For example, gem-dimethyl substitution on C-5' of the reduced psoralens and on C-

8 of the linear benzodipyranones, leads to less active analogs. It is interesting to note that in the reduced angular benzodipyranone (compound **31**) the gem-dimethyl compound exhibits very potent activity. It appears that steric bulk below the linear axis constituted by the three rings does not diminish activity. Whatever the spatial requirements of the receptor, it has less tolerance for steric bulk in the linear three ring systems.

5. If, in fact, unsaturation in the third ring is not necessary for activity, the double bond of the pyrone does seem to be important. The completely reduced tetrahydrotrioxsalen (compound **25**) showed no activity in our inhibition assay.

6. The simplest compound, coumarin itself, does not exhibit activity in the inhibition assay up to a dose of 200 μM. The parent 4,8-dimethyl-7-hydroxycoumarin exhibits modest activity but this activity increases when the 7-OH is capped with alkyl, olefinic, or acetylenic substituents. However, the alkoxy group on C-7 should not be longer than 4 carbons. Longer chains drastically reduce activity to little or none at all, both when R=alkyl (ex: compound **60**) and when R=acetylenic (compound **62**).

7. Unsaturation in the ether linkage at C-7 on the coumarins seems to slightly increase the activity but not significantly.

8. Alkenyl substitution at C-6 or C-3 of the coumarins retains activity even if the hydroxyl on C-7 is not etherified. One possible explanation might be that each coumarin analog is fitting a lipophilic pocket on the receptor and that the <u>increased</u> lipophilicity by substitutions at C-3 and C-6 overcomes the necessity for lipophilic side-chains at the C-7 hydroxyl. It is interesting that the -I- for -H- substitution at C-8 does not alter the photobiological activity. In most systems, attachment of an iodine for a hydrogen will add approximately 14 units of lipophilicity.[53] Obviously a simple lipophilic correlation does not exist.

9. In support of the above statement, the measured partition coefficients were almost identical across the set, with the exception of the phenolic parent compound, 4,8-dimethyl-7-hydroxycoumarin. (Table 9) These results further indicate that overall molecular lipophilicity is not a strong correlate of biological potency.

10. In a theoretical approach to SAR, molecular

Table 9

Compound	Partition coefficient (P)	log P
4,5',8-trimethylpsoralen	12.9	1.11
4,8-dimethyl-7-propargyl oxycoumarin (13)	15.4	1.19
4,8-dimethyl-7-omega-carboxyl heptyloxycoumarin (64)	16.1	1.21
4,8-Dimethyl-7-[(2-butenyl) oxy coumarin (44)	38.1	1.59
6,7-Dihydro-4,8,8,10-tetra-methyl-2H,8H-benzo-[1,2-b;5,4-b'] dipyran-2-one (28)	12.1	1.08
4,8-Dimethyl-7-n-butyloxy coumarin (58)	25.7	1.41
4,10-Dimethyl-2H,8H-benzo-[1,2-b;5,4-b']-dipyran-2-one (16)	15.1	1.18
4,8-Dimethyl-7-[(3,3-dimethyl-propargyl)oxy]coumarin (65)	20.8	1.32
4,8-Dimethyl-6-(2-methyl-1-buten-3-yl)-7-hydroxycoumarin (47)	11.7	1.07
4,8-Dimethyl-3-[(3-methyl-2-buten-1-yl)]-7-hydroxycoumarin (33)	13.1	1.12

orbital calculations were used to predict a correlation with the measured IC_{50} values. Professor Thomas S. Lillie of Lafayette College, applied the Clark Still software, Model, as well as the Michael Dewar software package, Ampac , to the biological activities generated by the Laskin laboratory. Dr. Lillie specifically tested for possible correlations with electron density (both total electron density and pi-electron density), triplet state energies, polar surface areas and total surface areas for each of the candidate phototherapeutics. No statistically valid correlation was found for the first two named parameters. Additionally, it was found experimentally that there was not a wide variation in the extinction coefficients across a large set of compounds. Table 10 lists the measured extinction coefficients for a number of the compounds tested in the biological assay. In the case of the polar surface area (PSA) an apparent qualitative correlation was found. The percent polar surface area does show a qualitative parabolic correlation with inhibition activity in that those molecules with approximately 15-20 percent polar surface had the highest activity but molecules with higher (and lower) percent polar surface area

Table 10

Compound	λ max	extinction coefficient
4,8-Dimethyl-7-hydroxycoumarin	326	15500
4,8-Dimethyl-7-octynyloxycoumarin (62)	319	11300
4,8-Dimethyl-7-n-propoxycoumarin (57)	322	9690
4,8-Dimethyl-7-propargyloxycoumarin (13)	319	6730
4,8-Dimethyl-7-[(2-butenyl)oxy] coumarin (44)	321	8780
4,8-Dimethyl-7-allyloxycoumarin (42)	321	8850
4,8-Dimethyl-8-iodo-7-[(3-methyl-2-butenyl)oxy]coumarin (48)	320	10600
4,8-Dimethyl-7-(omega-carboxyl heptyloxy)coumarin (64)	322	14500
4,8-Dimethyl-6-allyloxycoumarin (43)	329	7480
4,8-Dimethyl-3-(3-methyl-2-buten-1-yl)coumarin (33)	327	11050
4,8-Dimethyl-6-[(2-buten-1-yl)] coumarin (52)	330	17300
6,7-Dihydro-4,8,8,10-tetramethyl-2H,8H-benzo-[1,2-b;5,4-b']dipyran-2-one (28)	331	14200
6,7-Dihydro-4,8,8-trimethyl-2H,8H-benzo-[1,2-b;5,4-b']dipyran-2-one (29)	330	11100
4,10-Dimethyl-2H,8H-benzo-[1,2-b;5,4-b']dipyran-2-one (16)	345	5840

demonstrated lower photobiological efficacy. As has often been noted in discussions of Hansch partition coefficients, a parabolic relationship to pharmacological activity is common.[71] In a simple sense, this behavior means that for any given drug class some optimum solubility ratio (lipid/blood) exists which favors active-site incorporation of the agent.

Percent polar surface may be viewed in a similar light for it represents the total exposed heteroatom surface area expressed as a percentage of the total surface area in square Angstoms. The surface area is represented by the sum of the van der Waals radii of the individual atoms.

It has been recognized that a high correlation exists between the logarithm of the capacity factor, log k′, and molecular surface area.[73] The capacity factor is generally measured by reverse-phase liquid chromatography and is considered a promising method for the determination of the logarithm of the partition coefficient (log P). For a number of different organic systems, workers have found that molecular surface area could be used as a predictor of retention times (capacity factor) in reverse-phase liquid chromatography.[72]

While not one of the most commonly employed

theoretical parameters used in SAR correlations, in one recent study, polar surface area was found to correlate well with the cytotoxicity (against E. coli and a common algae) of aryl and alkyl organotin and lead compounds.[54] Admittedly, the true meaning on a molecular level of a correlation between polar surface area and biological activity is unclear at this time. Nevertheless, with the data collected herein it seems that there is a window requirement that exists for an optimum proportion of polar surface necessary to insure a positive photo effect. Too high a polar surface seems to thwart activity and a similar result is seen at low percentage polar surface area. In effect, to the degree that polar surface can be viewed as a regional probe of lipid/blood solubility, an optimum requirement for lipophilicity seems to exist in these photo-active coumarin analogs. As such, the polar surface area does seem to be a valid predictor for EGF binding inhibition. Table 11 gives the values calculated for the polar surface area for a number of the test compounds and Figure 10 shows the plot obtained in the correlation between IC_{50} values and PSA.

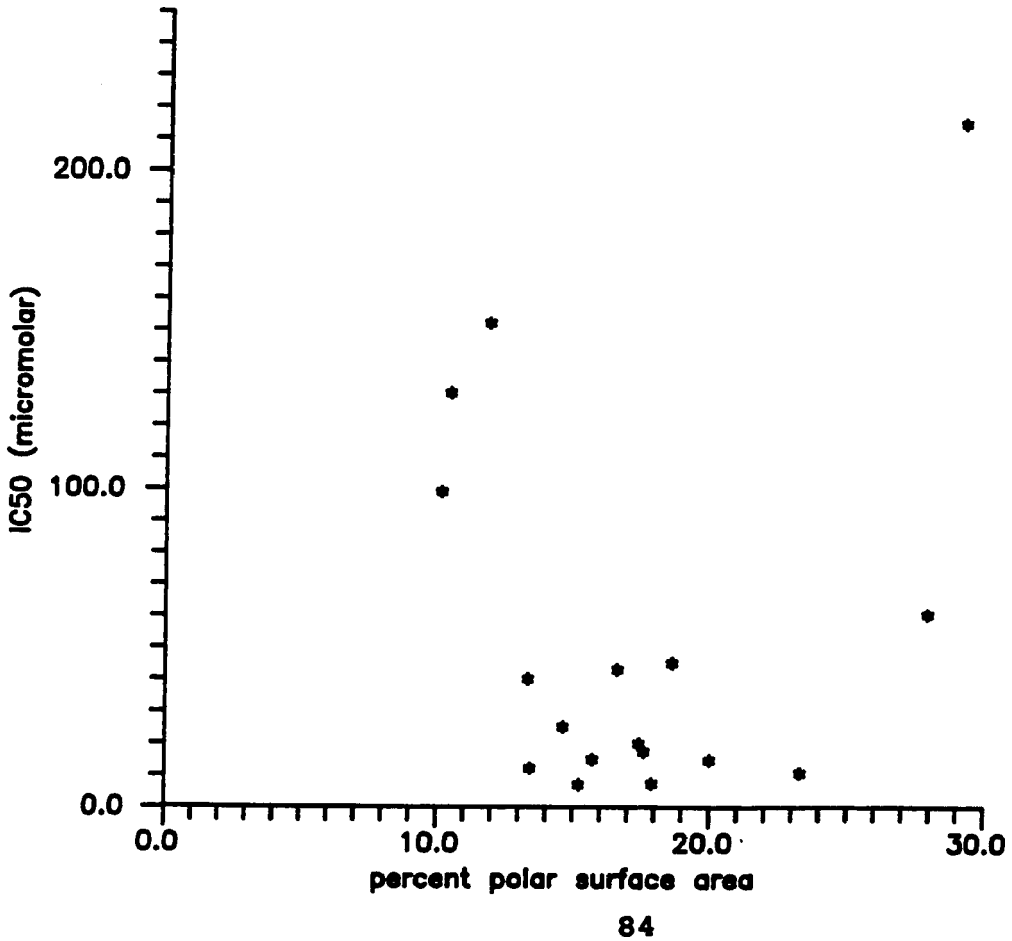
Table 11

Calculated values for the polar surface area of psoralen and coumarin derivatives

Compound	(PSA) Polar surface area (sq.Å)	% PSA
13	35.2	15.7
16	33.0	17.6
24	43.8	17.9
25	64.5	29.1
28	37.4	13.3
30	29.3	11.8
45	45.9	18.6
53	45.7	20.0
56	31.6	17.4
60	34.1	10.1
61	32.9	10.4
63	39.5	13.4
66	43.6	16.6
69	40.8	14.6
70	30.8	15.2
72	42.4	27.9

Figure 10

Inhibition data plotted
versus % polar surface area



EXPERIMENTAL

General

Infrared spectra were recorded on a Perkin Elmer 283 spectrometer as 2.5-5% concentration KBr pellets. Ultraviolet absorption spectra were recorded on a Perkin-Elmer Lambda 5 UV/VIS Spectrophotometer using quartz cuvettes with a path length of 1 cm. Melting points were determined on a Thomas-Hoover melting point apparatus and are reported uncorrected. Elemental analyses were performed by Robertson Laboratories, Inc., Madison, N,J. The ^{1}H NMR spectra were recorded on a JEOL FX-90Q spectrometer operated at 89.55 MHz using (unless otherwise indicated) deuterochloroform ($CDCl_3$) as the solvent. Chemical shifts are reported in parts per million downfield from tetramethylsilane (TMS). ^{1}H NMR data are reported as follows: chemical shift (multiplicity, number of protons, coupling constants in Hz, assignment). Multiplicity is designated as follows: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, c=complex, br=broad.

The decoupled ^{13}C NMR spectra were recorded on either a JEOL FX-90Q spectrometer operated at 22.49 MHz or a Bruker spectrometer operated at 360 MHz. Deuterochloroform was used as the solvent and the

internal standard. Chemical shifts are reported in ppm downfield from TMS. ^{13}C NMR assignments were made with the assistance of HETCOR and APT spectra which were recorded on a Bruker spectrometer operated at 360 MHz.[55]

4,8-Dimethyl-7-methoxycoumarin (56)

A solution of 4,8-dimethyl-7-hydroxycoumarin (0.190 g, 1.00 mmol) and methyl iodide (0.180 g, 1.30 mmol) in 50 ml of dry acetone was heated at reflux in the presence of 0.5 g of potassium carbonate for 14 hours. The solution was then cooled and filtered. The filtrate was evaporated under reduced pressure to yield a beige solid. Phenolic impurities were removed by washing the solid with 20% aqueous sodium hydroxide. The resulting tan solid was collected by vacuum filtration. The crude product was recrystallized from methanol to yield shiny white crystals, 0.135 g (66%), m.p. 162-164 ^{o}C. ^{1}H NMR ($CDCl_3$): δ 2.27 (s, 3H, C_8-CH_3), 2.41 (d, 3H, J = 1.2 Hz, C_4- CH_3), 3.92 (s, 3H, OCH_3), 6.11 (q, 1H, J = 1.2 Hz, C_3-H), 6.85 (d, 1H, J = 8.8 Hz, C_6-H), 7.45 (d, 1H, J = 8.8 Hz, C_5-H). IR(KBr): 1720 cm^{-1}, unsaturated γ -lactone.

Anal. Calcd. for $C_{12}H_{12}O_3$: C 70.59 H 5.88

Found : C 70.34 H 5.92

4,8-Dimethyl-7-n-propoxycoumarin (57)

The reaction was carried out similarly to the preparation of 4,8-dimethyl-7-methoxycoumarin using 4,8-dimethyl-7-hydroxycoumarin (0.19 g, 1.0 mmol), 1-chloropropane (0.11 g, 1.5 mmol) and 3 g of potassium carbonate in 50 ml of acetone. The reaction mixture was heated for 16 hours. The crude product was recrystallized from methanol to yield an off-white cotton-like solid, 0.185 g (80%), m.p. 103-104 ^{o}C. ^{1}H NMR ($CDCl_3$): δ 1.15 (t, 3H, J = 1.8 Hz, $CH_2\underline{CH}_3$), 1.83 (m, 2H, $CH_2\underline{CH}_2CH_3$), 2.31 (s, 3H, C_8-CH_3), 2.40 (d, 3H, J = 0.97 Hz, C_4-CH_3), 4.05 (t, 2H, J = 6.3 Hz, OCH_2), 6.11 (q, 1H, J = 0.97 Hz, C_3-H), 6.82 (d, 1H, J = 8.7 Hz, C_6-H)), 7.32 (d, 1H, J = 8.7 Hz, C_5-H). ^{13}C NMR: δ 8.19 C_8-CH_3, 10.58 CH_2CH_3, 18.7 C_4-CH_3, 22.6 $CH_2CH_2CH_3$, 70.14 OCH_2, 107.5 C_6, 111.6 C_3, 122.3 C_5. IR(KBr): 1725 cm^{-1}, unsaturated γ -lactone. UV(EtOH), λ max, log ϵ: 322 nm, 3.99; 255 nm, 3.43; 207 nm, 4.27.

Anal. calcd. for $C_{14}H_{16}O_3$: C 72.41 H 6.90

Found: C 72.25 H 7.00

4,8-Dimethyl-7-butyloxycoumarin (58)

The reaction was carried out similarly to preparation of 4,8-dimethyl-7-methoxycoumarin using 4,8-dimethyl-7-hydroxycoumarin (1.9 g, 0.010 mol), n-butyl bromide (2.06 g, 0.010 mol), and heating under reflux for 7 hours. Recrystallization of the product from aqueous methanol yielded 2.02 g (82%) of off-white needles, m.p. 106-108 ^{o}C. ^{1}H NMR ($CDCl_3$): δ 1.06 (t, 3H, J = 6.8 Hz, $CH_2\underline{CH_3}$), 1.63-1.91 (c, 4H, CH_2CH_2), 2.33 (s, 3H, C_8-CH_3), 2.41 (d, 3H, J = 1.2 Hz, C_4-CH_3), 4.08 (t, 2H, J = 6.7 Hz, OCH_2), 6.12 (q, 1H, J = 1.2 Hz, C_3-H), 6.83 (d, 1H, J = 8.7 Hz, C_6-H), 7.25 (d, 1H, J = 8.7 Hz, C_5-H).

Anal. Calcd. for $C_{15}H_{18}O_3$: C 73.17 H 7.30

Found : C 73.04 H 7.40

4,8-Dimethyl-7-hexyloxycoumarin (59)

The procedure was similar to the preparation of the above compound using 4,8-dimethyl-7-hydroxycoumarin (0.95 g, 5.0 mmol), 1-bromohexane (1.2 g, 7.5 mmol), and heating under reflux for 5 hours. Recrystallization of the product from aqueous methanol gave 1.21 g (88%) of fluffy white needles, m.p. 83-84 ^{o}C. ^{1}H NMR ($CDCl_3$): δ 0.97 (t, 3H, $-(CH_2)_5\underline{CH_3}$), 1.31-1.52 (c, 6H, $-(\underline{CH_2})_3CH_3$), 1.84 (m, 2H, $-\underline{CH_2}(CH_2)_3CH_3$),

2.30 (s, 3H, C_8-CH_3), 2.42 (d, 3H, J = 1.2 Hz, C_4-CH_3), 4.04 (t, 2H, J = 6.4 Hz, OCH_2), 6.12 (q, 1H, J = 1.2 Hz, C_3-H), 6.84 (d, 1H, J = 8.8 Hz, C_6-H), 7.31 (d, 1H, J = 8.8 Hz, C_5-H). ^{13}C NMR: δ 7.9 C_8 CH_3, 13.8 $(CH_2)_5\underline{CH}_3$, 18.4 C_4 CH_3, 22.4 $(CH_2)_4\underline{CH}_2CH_3$, 25.5 $(CH_2)_3\underline{CH}_2CH_2CH_3$, 28.9 $OCH_2CH_2\underline{CH}_2$, 31.3 $OCH_2\underline{CH}_2$, 68.5 OCH_2, 107.3 C_6, 111.2 C_3, 122.2 C_5, 159.6 C_7, 161.3 C_2.

Anal. Calcd. for $C_{17}H_{22}O_3$: C 74.5 H 8.03

Found: C 74.3 H 8.19

4,8-Dimethyl-7-octyloxycoumarin (60)

The reaction was similar to the above examples using 4,8-dimethyl-7-hydroxycoumarin (1.9 g, 0.010 mol), 1-bromooctane (2.9 g, 0.015 mol) and heating under reflux for 4 hours. The product was recrystallized twice from methanol to give 2.17 g (72%) of white crystalline product, m.p. 80-82 ^oC. ^1H NMR: δ 0.85-1.91 (c, 15H, $(CH_2)_6CH_3$)), 2.29 (s, 3H, C_8-CH_3), 2.35 (d, 3H, J = 1.2 Hz, C_4-CH_3), 4.04 (t, 2H, OCH_2), 6.12 (q, 1H, J = 1.2 Hz, C_3-H), 6.83 (d, 1H, J = 8.6 Hz, C_6-H), 7.41 (d, 1H, J = 8.6 Hz, C_5-H).

Anal. Calcd. for $C_{19}H_{26}O_3$: C 75.49 H 8.61

Found : C 75.49 H 8.56

4,8-Dimethyl-7-allyloxycoumarin (42)

A solution of 4,8-dimethyl-7-hydroxycoumarin (4.85 g, 25.5 mmol) in 200 ml of acetone was reacted with 3-bromopropene (4.62 g, 38.2 mmol) in the presence of anhydrous potassium carbonate (10 g). The reaction mixture was heated under reflux for 4 hours, cooled and filtered. The collected solid was washed with fresh acetone. The filtrate and washings were combined and the solvent evaporated under reduced pressure. The crude product was recrystallized from methanol to yield the title compound as fluffy, white needles, m.p. 107-108 ^{o}C,[56] 3.5 g, 60% yield. ^{1}H NMR ($CDCl_3$): δ 2.32 (s, 3H, C_8-CH_3), 2.43 (d, 3H, C_4-CH_3 J = 1.2 Hz), 4.62 (dt, 2H, O-CH_2 J = 1.5, 4.8 Hz), 5.45 (m, 2H, =CH_2, J = 1.5, 4.8 Hz), 5.96 (m, 1H, -CH=CH_2), 6.11 (d, 1H, C_3-H, J = 1.2 Hz), 6.83 (d, 1H, C_6-H, J = 9.0 Hz), 7.34 (d, 1H, C_5-H, J = 9.0 Hz). ^{13}C NMR: δ 8.26 C_8-CH_3, 18.70 C_4-CH_3, 69.19 OCH_2, 107.74 C_6, 117.50 CH=$\underline{CH}_2$, 122.33 C_5, 132.61 $\underline{CH}$=CH_2. IR(KBr): 1720 cm^{-1}, 1640 cm^{-1}.

Anal calcd for $C_{14}H_{14}O_3$: C 73.03 H 6.13.

Found: C 73.02 H 6.20.

4,8-Dimethyl-7-[(2-buten-1-yl)oxy]coumarin (44)

Prepared in a similar manner to the above using 4,8-dimethyl-7-hydroxycoumarin (0.57 g, 3.0 mmol), 1-bromo-2-butene (0.61 g, 4.5 mmol) and heating under reflux for 5 hours. Pure product was obtained after recrystallization from aqueous methanol to yield off-white needles, 0.63 g (87%), m.p. 114-116 ^{o}C. ^{1}H NMR ($CDCl^3$): δ 1.82 (d, 3H, J = 5.1 Hz, =CH$\underline{CH}_3$), 2.32 (s, 3H, C_8 -CH_3), 2.41 (d, 3H, J = 0.98 Hz, C_4- CH_3), 4.62 (d, 2H, OCH_2), 5.83 (m, 2H, CH=CH), 6.13 (q, 1H, J = 0.98 Hz, C_3-H), 6.85 (1H, J = 8.55 Hz, C_6-H), 7.32 (d, 1H, J = 8.55 Hz, C_5-H). ^{13}C NMR: δ 8.11 C_8-CH_3, 17.7 =CH$\underline{CH}_3$, 18.5 C_4-CH_3, 69.1 OCH_2, 107.7 C_6, 111.5 C_3, 122.2 C_5, 125.4 CH=$\underline{CH}$$CH_3$, 130.2 $\underline{CH}$=$CHCH_3$, 159.3 C_7, 161.4 C_2. UV(EtOH), λ max, log ϵ : 321 nm, 3.94; 254 nm, 3.40; 207 nm, 4.28.

Anal. Calcd. for $C_{15}H_{16}O_3$: C 73.75 H 6.55

Found: C 73.56 H 6.49

4,8-Dimethyl-7-[(3-methyl-2-buten-1-yl)oxy]coumarin (46)

A. The reaction was carried out as above using 4,8-dimethyl-7-hydroxycoumarin (0.57 g, 3.0 mmol), 1-

chloro-3-methyl-2-butene (0.47 g, 4.5 mmol) and heating under reflux for 4 hours. Recrystallization from aqueous methanol yielded the title compound as cream colored crystals, 0.641 g (83%), m.p. 94-95 °C. ^{1}H NMR ($CDCl_3$): δ 1.86 (bs, 6H, 2 CH_3's), 2.34 (s, 3H, C_8-CH_3), 2.41 (d, 3H, J = 1.2 Hz, C_4-CH_3), 4.62 (bd, 2H, OCH_2), 5.55 (bt, 1H, =CH), 6.13 (q, 1H, J = 1.2 Hz, C_3-H), 6.82 (d, 1H, J = 8.8 Hz, C_6-H), 7.34 (d, 1H, J = 8.8 Hz, C_5-H). ^{13}C NMR: δ 8.08 C_8-CH_3, 18.11 CH_3, 18.48 C_4-CH_3, 25.5 CH_3, 65.45 OCH_2, 107.74 C_6, 111.39 C_3, 119.22 =CH, 122.17 C_5, 137.95 =$\underline{C}(CH_3)_2$, 159.48 C_7, 161.39 C_2.

Anal. Calcd. for $C_{16}H_{18}O_3$: C 74.39 H 7.02

Found : C 74.48 H 6.89

B. To pentane-washed (20 ml) sodium hydride (0.19 g, 8.0 mmol) in 100 ml of dimethylformamide was added 4,8-dimethyl-7-hydroxycoumarin (1.52 g, 8.0 mmol) and the solution was allowed to stir under a nitrogen atmosphere for 15 minutes. To this stirred solution was added 1-bromo-3-methyl-2-butene (2.16 g, 16.0 mmol) and the reaction mixture was heated to 60 °C for 2 hours. The reaction mixture was poured into 200 ml of water and a precipitate formed. The solid was

collected by vacuum filtration and recrystallized from methanol to yield the product as a white solid, 1.96 g (95%), m.p. 94-95^{o} C. Spectral data was in agreement with that described in (A).

C. To pentane-washed (20 ml) sodium hydride (0.096 g, 4.0 mmol) in 45 ml of toluene was added 4,8-dimethyl-7-hydroxycoumarin (0.76 g, 4.0 mmol) and the solution was allowed to stir under a nitrogen atmosphere for 20 minutes. To this stirred solution was added 1-bromo-3-methyl-2-butene (1.08 g, 8.0 mmol) and the reaction mixture was heated under reflux for 24 hours. After cooling to room temperature , the reaction mixture was washed with 10% Na_2CO_3 (3 x50 ml), brine and then water. The organic extract was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. Recrystallization from methanol yielded the product as a white solid, 0.74 g (72%), m.p. 94-95 ^{o}C. Spectral data was in agreement with that described in (A).

8-iodo-7-hydroxycoumarin

To a solution of 7-hydroxycoumarin (5.0 g, 31.0 mmol) and iodine (3.95 g, 15.5 mmol) in 100 ml of ethanol was added a solution of periodic acid (1.5 g, 7.8 mmol) in 15 ml of water. The mixture was stirred

at room temperature for 2 hours and then was poured into 500 ml of water. The solid precipitate was collected by vacuum filtration, washed with water and recrystallized from ethanol to yield the product as pale yellow crystals, 3.9 g (44%), m.p. 219-220 ^{o}C (lit.[28] m.p. 220 ^{o}C). ^{1}H NMR (DMSO-d_6): δ 6.23 (d, 1H, J = 9.5 Hz, C_3H), 7.02 (d, 1H, J = 8.55 Hz, C_6H), 7.55 (d, 1H, J = 8.55 Hz, C_5H), 7.91 (d, 1H, J = 9.5 Hz, C_4H).

4-Methyl-8-iodo-7-hydroxycoumarin

To a solution of 4-methyl-7-hydroxycoumarin (10.86 g, 61.7 mmol) in 200 ml of ethyl alcohol were added iodine (7.90 g, 31.0 mmol) and periodic acid (3.00g, 15.6 mmol) in 20 ml of water. The reaction mixture was stirred at room temperature for 2 hours and then poured into 1000 ml of water. The precipitate was collected by vacuum filtration, washed with water and recrystallized from ethanol to yield an off-white crystalline solid, 9.20 g (49.5%), m.p. 219-220 ^{o}C (lit.[28] m.p. 220 ^{o}C). ^{1}H NMR (DMSO-d_6): δ 2.47 (d, 3H, J = 1.2 Hz, C_4-CH_3), 2.9 (bs, 1H, C_7-OH), 6.01 (q, 1H, J = 1.2 Hz, C_3-H), 6.84 (d, 1H, J = 8.55 Hz, C_6-H), 7.41 (d, 1H, J = 8.55 Hz, C_5-H).

4-methyl-8-iodo-7-[(3-methyl-2-buten-1-yl)oxy]coumarin (48)

A solution of 4-methyl-8-iodo-7-hydroxycoumarin (3.86 g, 12.8 mmoles) and 1-chloro-3-methyl-2-butene (1.96 g, 19.1 mmoles) in 200 ml of acetone was heated under reflux in the presence of 8 g of anhydrous potassium carbonate for 4 hours. After it was cooled to room temperature the reaction mixture was filtered and the solid residue was washed with fresh acetone. The filtrate and washings were combined and the solvent was evaporated under reduced pressure. The solid yellow residue was recrystallized from ethyl acetate to yield the title compound as a microcrystalline white solid, 2.56 g (55%), m.p. 151.5-153 ^{o}C. ^{1}H-NMR ($CDCl_3$): δ 1.78 (s, 6H, CH_3's), 2.42 (d, 3H, J = 1.3 Hz, C_4-CH_3), 4.65 (d, 2H, J = 6.8 Hz, =CH_2), 5.56 (bt, 1H, J = 6.8 Hz, =CH), 6.13 (d, 1H, J = 1.2 Hz, C_3-H), 6.83 (d, 1H, J = 8.8 Hz, C_6-H), 7.51 (d, 1H, J = 8.8 Hz, C_5-H). ^{13}C NMR: δ 18.48 CH_3, 18.67 C_4-CH_3, 25.79 CH_3, 66.92 OCH_2, 108.46 C_6, 112.41 C_3, 118.70 CH=, 125.59 C_5. IR(KBr): 1720 cm^{-1}, 1630 cm^{-1}. UV(EtOH), λmax, log ϵ : 321 nm, 4.03, 263 nm, 3.69; 208, 4.41.

Anal calcd for $C_{15}H_{15}O_3I$: C 48.67 H 4.08 I 34.28

Found: C 48.48 H 3.86 I 34.41

7-[(2-Octyn-1-yl)oxy]coumarin (61)

A solution of 7-hydroxycoumarin (0.324 g, 1.99 mmol) and 1-bromo-2-octyne[57] (0.378 g, 1.99 mmol) in 200 ml of acetone was heated under reflux in the presence of anhydrous potassium carbonate (3 g) for 24 hours. The reaction mixture was cooled, filtered and the collected solid was washed with fresh acetone. The acetone washings and the filtrate were combined and the solvent evaporated under reduced pressure to yield a pale yellow viscous liquid. The crude product was dissolved in a minimum amount of ethyl acetate and petroleum ether added until the solution became cloudy. The cloudy solution was concentrated under reduced pressure and a white precipitate formed. This precipitate was collected by vacuum filtration and recrystallized from aqueous methanol to yield the title compound as shiny, colorless plates, m.p. 74-75 °C, 0.245 g, 50%. ^{1}H-NMR ($CDCl_3$): δ 0.85-2.21 (m, 11H, $(CH_2)_4CH_3$), 4.69 (t, 2H, $O\underline{CH_2}C{\equiv}C$), 6.22 (d, 1H, J = 9.3 Hz, C_3-H), 6.82 (dd, 1H, J = 8.1 Hz, C_6-H), 6.93 (d, 1H, J = 2.1 Hz, C_8-H), 7.4 (dd, 1H, C_5-H, J = 8.1 Hz),

7.6 (d, 1H, C_4-H, J = 9.3 Hz). IR(KBr): 1715 cm^{-1}, 2190 cm^{-1}, C≡C.

Anal calcd for $C_{17}H_{18}O_3$: C 75.53 H 6.66

Found: C 75.45 H 6.76

4,8-Dimethyl-7-[(2-octyn-1-yl)oxy]coumarin (62)

The reaction was carried out similarly to the preparation of 7-[(2-octyn-1-yl)oxy]coumarin using 4,8-dimethyl-7-hydroxycoumarin (0.57 g, 3.0 mmol), 1-bromo-2-octyne (0.57 g, 3.0 mmol) and heating under reflux for 18 hours. The crude product was a yellow oil which was crystallized from ethyl acetate:petroleum ether. Recrystallization from methanol yielded the product as a white powdery solid, 0.47 g (53%), m.p. 87-88.5 ^{o} c. ^{1}H NMR ($CDCl_3$): δ 0.81-2.14 (c, 11H, $(CH_2)_4CH_3$), 2.33 (s, 3H, C_8-CH_3), 2.42 (d, 3H, J = 0.98 Hz, C_4-CH_3), 6.12 (q, 1H, J = 0.98 Hz, C_3-H), 6.82 (d, 1H, J = 8.8 Hz, C_6-H), 7.41 (d, 1H, J = 8.8 Hz, C_5-H). ^{13}C NMR: δ 8.18 C_8-CH_3, 13.82 $(CH_2)_4\underline{CH_3}$, 18.56 C_4-CH_3, 18.58 $(CH_2)_3\underline{CH_2}CH_3$, 22.01 $(CH_2)_2\underline{CH_2}CH_2CH_3$, 27.92 $\underline{CH_2}(CH_2)_2CH_3$, 30.83 ≡C-$\underline{CH_2}(CH_2)_3CH_3$, 58.99 OCH_2, 108.22 C_6, 111.81 C_3, 122.08 C_5. IR(KBr): 1715 cm^{-1}, 2190 cm^{-1}. UV(EtOH), λ max, log ϵ : 319 nm, 4.05; 204 nm, 4.47.

Anal. Calcd. for $C_{19}H_{22}O_3$: C 76.50 H 7.38

Found : C 76.90 H 7.30

4-Methyl-8-iodo-7-[(2-methyl-3-butyn-2-yl)oxy]coumarin (63)

The reaction was carried out similarly to the preparation of 4-methyl-8-iodo-7-[(2-methyl-3-buten-2-yl)oxy]coumarin using 4-methyl-8-iodo-7-hydroxycoumarin (7.71 g, 25.0 mmol), 3-chloro-3-methyl-butene (3.92 g, 38.0 mmol) and heated under reflux for 4 hours. Recrystallization twice from methanol yielded the title compound as pale yellow crystals, 5.2 g (56%), m.p. 175-177 ^{o} C. ^{1}H NMR ($CDCl_3$): δ 1.85 (s, 6H, 2 CH_3), 2.41 (d, 3H, J = 1.1 Hz, C_4-CH_3), 2.74 (s, 1H, =CH), 6.19 (q, 1H, J = 1.1 Hz, C_3-H), ^{13}C NMR: δ 18.72 C_4-CH_3, 29.65 C_1,-CH_3's, 75.06 $\equiv$CH, 113.21 C_3, 114.23 C_6, 124.79 C_5.

Anal. Calcd. for $C_{15}H_{13}O_3I$: C 48.94 H 3.56 I 34.47

Found : C 48.83 H 3.50 I 34.53

4,8-Dimethyl-7-(omega-carboxylheptyloxy)coumarin (64)

To pentane-washed sodium hydride (0.641 g) in 20 ml of N,N-dimethylformamide was added 4,8-dimethyl-7-hydroxycoumarin, (1.52 g, 8.00 mmol) and then a solution of 8-bromooctanoic acid, (1.78 g, 7.97 mmol)

in 20 ml of DMF dropwise with stirring. The reaction mixture was diluted with DMF to a final volume of 200 ml and heated at 80°C (oil bath) for 16 hours. Progress of the reaction was monitored by TLC (silica, 99:1 $CHCl_3$: isopropanol). The reaction mixture was cooled, diluted with distilled H_2O, and acidified with 6N HCl until pH = 2. A tan precipitate formed and was collected by vacuum filtration. The crude product was treated with decolorizing carbon and recrystallized from methanol to yield the title compound as an off-white crystalline solid, m.p. 128-130 °C, 1.7 g, 68%. ^{1}H-NMR ($CDCl_3$): δ 1.21-1.82 (m, 12H, $(CH_2)_6$), 2.29 (s, 3H, C_8-CH_3), 2.38 (d, 3H, J = 0.98 Hz, C_4-CH_3), 4.01 (t, 2H, O-CH_2), 6.17 (d, 1H, J = 0.98 Hz, C_3-H), 6.82 (d, 1H, C_6-H, J = 8.8 Hz), 7.39 (d, 1H, C_5-H, J = 8.8 Hz). IR(KBr): 2950 cm^{-1}, 1725 cm^{-1}. UV(EtOH), λmax, log ϵ : 322 nm, 4.16; 205 nm, 4.55.

Anal calcd for $C_{19}H_{24}O_5$: C 68.66 H 7.28

Found: C 68.43 H 7.30

4-Methyl-6-(2-buten-1-yl)-7-hydroxycoumarin (53)

To a cooled (10 °C) solution of 4-methyl-7-hydroxycoumarin (1.76 g, 10.0 mmol) and 3-buten-2-ol (0.72 g, 10.0 mmol) in 30 ml of dioxane was added

dropwise BF_3-etherate (1.42 g, 10.0 mmol). Reaction progress was followed by thin layer chromatography (silica, 7:1 chloroform:ethyl acetate). The reaction mixture was heated to a temperature of 60 ^oC for 36 hours. After cooling, the reaction mixture was poured into water and extracted with ethyl acetate. The organic extract was washed with water and dried over sodium sulfate. The dried solution was evaporated under reduced pressure to leave a dark brown oily residue. This residue was subjected to flash chromatography on silica using 8:1 chloroform:ethyl acetate as eluent. Fractions were pooled, the solvent evaporated under reduced pressure and the crude product recrystallized from methanol/ethyl acetate to yield the title compound as white fluffy crystals, 0.48 g (22%), m.p. 186-188 ^oC. ^1H NMR (d_6-acetone): δ 1.58 (dt, 3H, CH_3), 2.37 (d, 3H, J = 1.2 Hz, C_4-CH_3), 3.42 (d, 2H, CH_2), 5.57 (m, 2H, CH=CH), 6.10 (q, 1H, J = 1.2 Hz, C_3-H), 6.73 (s, 1H, C_8-H), 7.5 (s, 1H, C_5-H).

Anal. Calcd. for $C_{14}H_{15}O_3$: C 72.73 H 6.49

Found : C 72.79 H 6.57

4,8-Dimethyl-6-(2-buten-1-yl)-7-hydroxycoumarin (52)

A. The reaction was carried out in a similar

manner to the above compound using 4,8-dimethyl-7-hydroxycoumarin (1.9 g, 10.0 mmol) and 3-buten-2-ol (0.72 g, 10.0 mmol). The reaction mixture was heated at 60 °C for 28 hours. The crude product was purified by flash chromatography (silica, 99:1 $CHCl_3$:ethyl acetate) and recrystallized from methanol/ethyl acetate to yield the title compound as a white chalky powder, 0.64 g (26%), m.p. 158-160 °C. ^1H NMR (d_6-acetone): δ 1.65 (dt, 3H, $=CHCH_3$), 2.29 (s, 3H, C_8-CH_3), 2.42 (d, 3H, J = 1.2 Hz, C_4-CH_3), 3.43 (bd, 2H, CH_2), 5.61 (m, 2H, CH=CH), 6.13 (q, 1H, J = 1.2 Hz, C_3-H), 7.37 (s, 1H, C_5-H). ^{13}C NMR: δ 8.26 C_8-CH_3, 17.89 C_4-CH_3, 18.81 C_3,-CH_3's, 34.54 $\underline{CH_2}=C(CH_3)_2$, 111.54 C_3, 120.68 $CH_2\underline{CH}=$ 122.59 C_5, 128.32 $=\underline{CH}CH_3$. UV(EtOH), λ max, log ϵ : 330 nm, 4.26; 205 nm, 4.71.

Anal. Calcd. for $C_{15}H_{17}O_3$: C 73.38 H 6.93

Found : C 73.20 H 6.68

B. To a solution of 4,8-dimethyl-7-hydroxycoumarin (0.95 g, 5.0 mmol) in 30 ml of aqueous NaOH (0.2 g, 5.0 mmol) was added 1-chloro-2-butene (0.68 g, 7.5 mmol) and the mixture was stirred at room temperature for 18 hours. At the end of this period, the reaction mixture was acidified with 10% HCl and the precipitate formed was collected by vacuum filtration.

Thin layer chromatography (silica, methylene chloride: isopropanol 95:5) of the filtered solid revealed 3 components, one of which corresponded to unchanged statring material. The crude product was subjected to flash chromatography (silica, methylene chloride: isopropanol 99:1). The first component to elute was identified as the product of O-alkylation, 4,8-dimethyl-7-[2-buten-1-yl)oxy]coumarin, 0.44 g (36%), m.p. 115-116 ^{o}C. The main component of the product mixture was identified as 4,8-dimethyl-6-(2-buten-1-yl)-7-hydroxycoumarin, 0.47 g (38%), m.p. 158-160 ^{o}C. Spectral data was in accordance with that described in (A).

4,8-Dimethyl-6-allyloxycoumarin (43)

A solution of 4,8-dimethyl-7-hydroxycoumarin (1.15 g, 5.0 mmol) in 40 ml of N,N-diethylaniline was heated under a nitrogen atmosphere at reflux temperature for 4 hours. After cooling to room temperature, the reaction mixture was diluted with 30 ml of petroleum ether (30-60 ^{o}C). The solid product precipitated and was collected by vacuum filtration. The crude product was recrystallized from 95% ethanol to yield the title compound as short white needles, 0.93 g (81%), m.p.

170-171 °C (lit.[56] m.p. 168-170 °C). ^{1}H NMR ($CDCl_3$): δ 2.32 (s, 3H, C_8-CH_3), 2.38 (d, 3H, J = 0.98 Hz, C_4-CH_3), 3.42 (bd, 2H, C_1,-CH_2), 5.15-5.28 (c, 2H, CH=$\underline{CH_2}$), 5.78 (bs, 1H, phenolic OH), 5.81-5.98 (m, 1H, $\underline{CH}$=CH_2), 6.11(q, 1H, J = 0.98 Hz, C_3H), 7.19 (s, 1H, C5H). ^{13}C NMR: δ 8.25 C_8-CH_3, 18.80 C_4-CH_3, 35.34 C_1,-CH_2, 111.69 C_3, 117.39 CH=$\underline{CH_2}$, 122.81 C_5, 135.76 $\underline{CH}$=CH_2, 161.79 C_2 (pyrone quat C).

4,8-Dimethyl-6-(1-buten-3-yl)-7-hydroxycoumarin (45)

A solution of 4,8-dimethyl-7-[(2-buten-1-yl)oxy]coumarin (0.40 g, 1.6 mmol) in 30 ml of N,N-diethylaniline was heated under a nitrogen atmosphere at reflux temperature for 8 hours. After cooling, the reaction mixture was poured into 40 ml of chloroform and extracted with 200 ml of 25% aq. HCl. The chloroform extract was then washed with water and dried over sodium sulfate. The solvent was evaporated from the dried solution under reduced pressure to yield a yellow-brown solid. This solid was recrystallized from methanol to yield the title compound as a fluffy beige solid, 0.31 g (79%), m.p. 175-176 °C. ^{1}H NMR ($CDCl_3$): δ 1.41 (d, 3H, -CH$\underline{CH_3}$), 2.32 (s, 3H, C_8-CH_3), 2.42 (d, 3H, J = 0.98 Hz, C_4-CH_3), 3.80 (m, 1H, -$\underline{CH}CH_3$), 5.15-5.35 (m, 2H, =CH_2), 5.93-6.23 (m, 1H, CH=), 6.12 (q,

1H, J = 0.98 Hz, C_3-H), 7.24 (s, 1H, C_5-H). ^{13}C NMR: δ 8.18 C_8CH_3, 18.68 C_4CH_3, 37.52 CH, 111.30 C_3, 114.9 $\underline{CH}=CH_2$, 120.4 $CH=\underline{CH_2}$, 127.1 C_5, 161.4 C_2. IR(KBr): 3300, 1715, 1640 cm^{-1}.

Anal. Calcd. for $C_{15}H_{16}O_3$: C 73.77 H 6.56

Found : C 73.83 H 6.65

4,8-Dimethyl-6-(2-methyl-1-buten-3-yl)-7-hydroxycoumarin (47)

The rearrangement was carried out under similar conditions to that above using 4,8-dimethyl-7[(3-methyl-2-buten-1-yl)oxy]coumarin (0.50 g, 1.9 mmol) in 30 ml of N,N-diethylaniline and heating at reflux temperature for 12 hours. The crude product was recrystallized from methanol to yield a beige crystalline solid, 0.38 g (77%), m.p. 174-175 ^oC. ^1H NMR ($CDCl_3$): δ 1.54 (d, 3H, J = 7.08 Hz, $CH\underline{CH_3}$), 1.73 (s, 3H, $C(\underline{CH_3})=CH_2$), 2.33 (s, 3H, C_8-CH_3), 2.42 (d, 3H, J = 1.2 Hz, C_4-CH_3), 3.63 (q, 1H, J = 7.08 Hz, $\underline{CH}CH_3$), 5.12 (m, 2H, $=CH_2$), 6.11 (q, 1H, J = 1.2 Hz, C_3-H), 6.20 (bs, 1H, C_7-OH), 7.28 (s, 1H, C_5H).

Anal. calcd. for $C_{16}H_{18}O_3$: C 74.39 H 6.98

Found: C 74.31 H 6.92

4-Methyl-6-(2-methyl-1-buten-3-yl)-7-hydroxycoumarin (49)

The rearrangement was carried out as described above using 4-methyl-8-iodo-7-[(3-methyl-2-buten-1-yl)oxy]coumarin (0.39 g, 1.0 mmol) in 20 ml of N,N-diethylaniline and heating at reflux temperature for 16 hours. The crude product was purified by preparative thin layer chromatography on silica gel plates (Analtech GF; 500 um) developing with 70:30 methylene chloride: ethyl acetate. The title compound was obtained after recrystallization from methanol as beige crystals, 0.15 g (62%), m.p. 168-169 ^{o}C. ^{1}H NMR ($CDCl_3$): δ 1.39 (d, 3H, J = 7.1 Hz, C_1,-CH_3), 1.68 (bs, 3H, C_2,-CH_3), 2.41 (d, 3H, J = 1.2 Hz, C_4-CH_3), 3.72 (q, 1H, J = 7.1 Hz, C_1,H), 5.03 (m, 2H, J = 0.98 Hz, =CH_2), 6.13 (q, 1H, J = 1.2 Hz, C_3H), 7.03 (s, 1H, C_8H), 7.33 (s, 1H, C_5H).

Anal. calcd. for $C_{15}H_{16}O_3$: C 73.77 H 6.56

Found : C 73.59 H 6.41

An additional product was isolated by preparative TLC in trace quantity and identified as 4'5'-dihydro-4,4',5',5'-tetramethylpsoralen **(50)**. This oil was not

further purified and no yield or m.p. was obtained. ^1H NMR ($CDCl_3$): δ 1.42 (d, 3H, J = 6.9 Hz, C_4,-CH_3), 1.54 (s, 6H, C_5,-CH_3's), 2.38 (d, 3H, J = 1.2 Hz, C_4-CH_3), 3.38(q, 1H, J = 6.9 Hz, C_4,H), 6.10 (q, 1H, J = 1.2 Hz, C_3H), 6.86 (s, 1H, C_8H), 7.27 (s, 1H, C_5H).

4,8-Dimethyl-3-(3-methyl-2-buten-1-yl)-7-hydroxycoumarin (33)

To a solution of 4,8-dimethyl-7-hydroxycoumarin (1.9 g, 10.0 mmol) in 50 ml of aqueous NaOH (0.40 g, 10.0 mmol) was added 1-bromo-3-methyl-2-butene (1.5 g, 10.0 mmol) and the reaction mixture was stirred at room temperature for 14 hours. After this period, a solid was evident in the reaction mixture which was collected by vacuum filtration. This solid was washed with 20% aq. NaOH to remove any phenolic products. The NaOH-insoluble material was identified as 4,8-dimethyl-7-[3-methyl-2-buten-1-yl)oxy]coumarin, 0.44 g (18%), m.p. 94-95 ^oC. The NaOH-soluble fraction was acidified with 6 N HCl and the precipitate formed was collected by vacuum fitration. Thin layer chromatography (silica, chloroform of the filtered solid revealed the presence of 3 components, one of which corresponded to the starting material. Much of the starting material was

removed by dissolving the filtered solid in ethyl acetate and collecting by vacuum filtration the undissolved material. The ethyl acetate filtrate was evaporated under reduced pressure to yield an orange oil which was subjected to flash chromatography on a silica gel column with a mixture of chloroform: ethyl acetate (95:5) as eluent. The main product was identified as 4,8-dimethyl-3-(3-methyl-2-buten-1-yl)-7-hydroxycoumarin, colorless prisms, 0.55 g (22.4%), m.p. 199-200 °C. ^{1}H NMR ($CDCl_3$): δ 1.68 (d, 3H, J = 1.2 Hz, C_3'-CH_3,), 1.78 (s, 3H, C_3,-CH_3), 2.32 (s, 3H, C_8-CH_3), 2.36 (s, 3H, C_4-CH_3), 3.35 (d, 2H, J = 6.5 Hz, C_1,-CH_2), 5.08 (t, 1H, J = 6.5, 1.2 Hz,C_2,H), 5.86 (s, 1H, phenolic OH-disappears with D_2O), 6.88 (d, 1H, J = 8.5 Hz, C_6H), 7.27 (d, 1H, J = 8.5 Hz, C_5H). ^{13}C NMR: δ 8.09 C_4-CH_3, 17.85 C_3,-CH_3's, 18.75 C_8-CH_3, 34.56 C_1,-CH_2, 111.63 C_6, 122.63 C_5, 128.67 C_2,-CH. UV (EtOH), λ max, log ϵ: 327 nm, 4.04; 207 nm, 4.34.

Anal. calcd. for $C_{16}H_{18}O_3$: C 74.39 H 7.02

Found: C 74.79 H 6.89

The product of C-alkylation, **4,8-Dimethyl-6-(3-methyl-2-buten-1-yl)-7-hydroxycoumarin (54)**, was the second component to elute and was isolated as off-white short needles, 0.31 g, (12.2%), m.p. 166.5-168 °C. ^{1}H NMR

($CDCl_3$): δ 1.81 (bs, 6H, C_3,-CH_3's), 2.36 (s, 3H, C_8-CH_3), 2.39 (d, 3H, J = 1.2 Hz, C_4-CH_3), 3.42 (d, 2H, J = 6.8 Hz, C_1,-CH_2), 5.30 (bt, 1H, J = 6.8, 1.2 Hz, C_2,-CH), 5.95 (s, 1H, phenolic H), 6.12 (q, 1H, J = 1.2 Hz, C_3H), 7.18 (s, 1H, C_5H). ^{13}C NMR: δ 8.16 C_4-CH_3, 17.93 C_3,-CH_3, 18.75 C_8-CH_3, 25.79 C_3,-CH_3, 30.20 C_1,-CH_2, 111.55 C_3, 121.06 C_2,-CH, 122.22 C_5, 161.83 C_2 (pyrone quat C).

Anal. calcd. for $C_{16}H_{18}O_3$: C 74.39 H 7.02

Found : C 74.13 H 7.09

4,8-Dimethyl-7-propargyloxycoumarin (13)

According to the method of Rodighiero[28], a solution of 4,8-dimethyl-7-hydroxycoumarin (4.85 g, 25.5 mmol) in 200 ml of acetone was reacted with 3-bromo-1-propyne (4.6 g, 38.2 mmol) in the presence of 10 g of anhydrous potassium carbonate by refluxing the mixture for 4 hours. After cooling, the solid material was filtered off and washed with fresh acetone. From the pooled filtrate and washings, the solvent was removed under reduced pressure. The yellow residue was recrystallized from methanol to yield the title compound as white needles, 3.72 g (64%), m.p. 139-141 ^oC (lit. m.p. 139-140 ^oC). ^1H NMR ($CDCl_3$): δ 2.32 (s,

3H, C_8-CH_3), 2.66 (t, 1H, J = 2.4 Hz, acetylenic H), 4.82 (d, 2H, J = 2.4 Hz, OCH_2), 6.12 (q, 1H, J = 1.2 Hz, C_3-H), 6.94 (d, 1H, J = 8.8 Hz, C_6-H), 7.44 (d, 1H, J = 8.8 Hz, C_5-H). ^{13}C NMR: δ 8.17 C_8-CH_3, 18.56 C_4CH_3, 56.24 OCH_2, 75.9 $\equiv CH$, 77.9 $CH_2C\equiv$, 107.9 C_6, 112.1 C_3, 122.1 C_5, 157.9 C_7, 161.1 C_2. IR(KBr): 1725 cm^{-1}, 2200 cm^{-1}. UV(EtOH), λ max, log ϵ : 319 nm, 3.83; 206 nm, 4.19.

Anal. calcd. for $C_{14}H_{12}O_3$: C 73.67 H 5.29

Found : C 73.44 H 5.49

4,8-Dimethyl-7(3,3-dimethylpropargyl)oxycoumarin (65)

A solution of 4,8-dimethyl-7-hydroxycoumarin (0.38 g, 2.0 mmol) and 2 g of potassium carbonate in 30 ml of aqueous acetone (5% v/v) was stirred at room temperature for 30 minutes. To this stirred solution was added 3-chloro-3-methyl-1-butyne (0.40 g, 4.0 mmol) and 0.50 g of potassium iodide and the reaction mixture was heated under reflux for 16 hours. The reaction mixture was then cooled, filtered and the solvent removed under reduced pressure. The crude product was recrystallized from methanol to yield a pale yellow crystalline solid, 0.36g (72%), m.p. 143-145 ^oC. ^1H NMR ($CDCl_3$): δ 1.77 (s, 6H, CH_3's), 2.32 (s, 3H, C_8-

CH_3), 2.43 (d, 3H, J = 1.2 Hz, C_4-CH_3), 2.61 (s, 1H, acetylenic H), 6.09 (q, 1H, J = 1.2 Hz, C_3-H), 7.40 (d, 1H, J = 8.8 Hz, C_6-H), 7.55 (d, 1H, J = 8.8 Hz, C_5-H). ^{13}C NMR: δ 9.21 C_8-CH_3, 18.75 C_4-CH_3, 29.73 C_1,-CH_3's, 73.82 $\equiv CH$, 112.51 C_3, 114.80 C_5, 121.44 C_6. IR(KBr): 1720 cm^{-1}, 2190 cm^{-1}.

Anal. calcd. for $C_{16}H_{16}O_3$: C 74.98 H 6.25

Found: C 74.38 H 6.30

4,10-Dimethyl-2H,8H-benzo[1,2-b;5,4-b']dipyran-2-one (16)

According to the method of Rodighiero[28], a solution of 4,8-dimethyl-7-propargyloxycoumarin (0.71 g, 3.1 mmol) in 15 ml of N,N-diethylaniline was heated under reflux in a stream of nitrogen for 4 hours. After cooling, the mixture was diluted with 25 ml of ethyl acetate and washed (2 x 30 ml) with 6N hydrochloric acid and then with water (2 x 25 ml). The organic phase was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The dark brown tarry residue was subjected to flash chromatography on a silica gel column eluting with chloroform. As this procedure did not produce sufficiently pure material, the crude product was then purified by preparative thin layer chromatography on

silica gel plates (Analtech GF; 500 um) developing with 35:65 ethyl acetate : cyclohexane. The title compound was obtained after recrystallization from methanol as white crystals, 0.15 g (21%), m.p. 178-180 ^{o}C (lit. m.p. 176-179 ^{o}C. ^{1}H NMR ($CDCl_3$): δ 2.25 (d, 3H, J = 0.45 Hz, C_{10}-CH_3), 2.34 (d, 3H, J = 1.2 Hz, C_4-CH_3), 4.94 (dd, 2H, J = 1.9, 3.5 Hz, C_8-CH_2), 5.80 (dt, 1H, J = 9.9, 3.4 Hz, C_7H), 6.11 (q, 1H, J = 1.2 Hz, C_3H), 6.43 (dt, 1H, J = 9.9, 1.9 Hz, C_6H)., 6.97 (bs, 1H, C_5H).

4-Methyl-2H,8H-benzo[1,2-b;5,4-b']dipyran-2-one (17)

This compound was prepared as outlined above from 4-methyl-8-iodo-7-propargyloxycoumarin (1.05 g, 3.1 mmol). The title compound was obtained after preparative thin layer chromatography as a white powder, 0.18 g (17%), m.p. 175-177 ^{o}C (lit.[28] m.p. 176-177 ^{o}C). ^{1}H NMR ($CDCl_3$): δ 2.35 (d, 3H, J = 1.2 Hz, C_4-CH_3), 4.92 (dd, 2H, J = 3.5, 2.0 Hz, C_8-CH_2), 5.81 (dt, 1H, J = 10.0, 3.5 Hz, C_7H), 6.07 (q, 1H, J = 1.2 Hz, C_3H), 6.45 (dt, 1H, J = 10.0, 2.0 Hz, C_6H), 6.69 (s, 1H, C_{10}H), 7.11 (s, 1H, C_5H).

A component of the product mixture was identified as the furocoumarin, 4,5'-dimethylpsoralen (19), white

crystals, m.p. 163-164 °C (lit.[58] m.p. 161-162 °C). ^{1}H NMR ($CDCl_3$): δ 2.45 (d, 3H, J = 1.2 Hz, C_4-CH_3), 2.55 (d, 3H, J = 0.98 Hz, C_5,-CH_3), 6.21 (q, 1H, J = 1.2 Hz, C_3H), 6.41 (q, 1H, J = 0.98 Hz, C_4,H), 7.19 (s, 1H, C_8H), 7.51 (s, 1H, C_5H).

2H,8H-Benzo[1,2-b;5,4-b']dipyran-2-one (18)

This compound was prepared as outlined above from 8-iodo-7-hydroxycoumarin (1.01 g, 3.1 mmol). The title compound was obtained after preparative thin layer chromatography as a white powder, 0.19 g (19%), m.p. 173-174 °C (lit.[28] m.p. 173-174 °C. ^{1}H NMR ($CDCl_3$): δ 4.86 (dd, 2H, J = 3.5, 1.9 Hz, C_8-CH_2), 5.73 (dt, 1H, J = 10.0, 3.5 Hz, C_7H), 6.12 (d, 1H, J = 9.4 Hz, C_3H), 6.35 (dt, 1H, J = 10.0, 1.9 Hz, C_6H), 6.60 (s, 1H, C_{10}H), 6.92 (s, 1H, C_5H), 7.49 (d, 1H, J = 9.4 Hz, C_4H).

A component of the product mixture was identified as the furocoumarin, 5'-methylpsoralen **(20)**. This product was not further purified and neither yield nor melting point were obtained. ^{1}H NMR ($CDCl_3$): δ 2.54 (d, 3H, J = 0.98 Hz, C_5,-CH_3), 6.32 (d, 1H, J = 9.5 Hz, C_3H), 6.57 (q, 1H, J = 0.98 Hz, C_4,H), 7.22 (s, 1H, C_8H), 7.48 (s, 1H, C_5H), 7.66 (d, 1H, J = 9.5 Hz, C_4H).

4,4-Dimethoxy-2-methyl-butan-2-ol (21)

Acetoacetaldehyde dimethylacetal (0.99 g, 75.0 mmol) was added dropwise, with stirring, to a solution of methyl magnesium bromide (25 ml of 3.0 M solution in ethyl ether) in 25 ml of dry ether at such a rate as to maintain a gentle reflux. The reaction mixture was kept under a nitrogen atmosphere during the addition. Ammonium acetate (40%, 50 ml) was then added to the reaction mixture and the ether layer was separated. The aqueous layer was further extracted with 30 ml of ether. The combined ether extracts were dried over anhydrous magnesium sulfate and the solvent was removed under reduced pressure. The residue was vacuum distilled to yield the title compound as a clear, colorless liquid, 7.3 g (65%), b.p. 66-69 ^{o}C/9 mm (lit.[70] b.p. 70-80 ^{o}C/14 mm). ^{1}H NMR: δ 1.18 (s, 6H, 2 CH_3's), 1.75 (d, 2H, J = 6.0 Hz, CH_2), 3.04 (s, 1H, OH), 3.31 (s, 6H, 2 OCH_3's), 4.7 (t, 1H, J = 6.0 Hz, CH).

4,8,8,10-Tetramethyl-2H,8H-benzo[1,2-b;5,4-b']dipyran-2-one (23)

A. The 4,8-dimethyl-7-hydroxycoumarin (0.19 g, 1.0 mmol) and 4,4-dimethoxy-2-methyl-butan-2-ol (0.15

g, 1.0 mmol) and anhydrous pyridine (0.10 g, 1.5 mmol) were heated together at 140 °C for 11 hours under a stream of nitrogen. The cooled mixture was evaporated to dryness under reduced pressure. The crude brown residue was chromatographed on silica gel plates (Analtech GF; 500 um) developing with chloroform. The product was isolated after recrystallization from ethyl acetate as a white solid, 0.12 g (49%), m.p. 112-113 °C. ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 6H, C_8-CH_3's), 2.24 (s, 3H, C_{10}-CH_3), 2.37 (d, 3H, J = 1.2 Hz, C_4-CH_3), 5.71 (d, 1H, J = 10.0 Hz, C_7H), 6.08 (q, 1H, J = 1.2 Hz, C_3H), 7.08 (bs, 1H, C_5H). IR (KBr): 1740, 1654 cm^{-1}.

B. The 4,8-dimethyl-7-hydroxycoumarin (0.19 g, 1,0 mmol), 2-methyl-3-butyn-2-ol (0.13 g, 1.5 mmol) and p-toluenesulfonic acid (0.02 g, 0.1 mmol) in 15 ml of toluene were heated at reflux with stirring for 6 hours. The reaction mixture was cooled to room temperature and the solvent removed under reduced pressure. The dark brown oily residue was chromatographed on a silica gel column eluting with methylene chloride. The product was isolated after recrystallization from methanol as a white solid, 0.08 g (32%), m.p. 112-113 °C. Spectral data was in agreement with that described in (A).

6,7-Dihydro-4,10-dimethyl-2H,8H-benzo[1,2-b;5,4-b']dipyran-2-one (24)

A mixture of 4,10-dimethyl-2H,8H-benzo[1,2-b;5,4-b']dipyran-2-one (0.010 g, 4.94 mmol), cyclohexene (0.5 ml, 4.94 mmol) and palladium on activated carbon (10%, 0.050 g) in 25 ml of ethanol was heated under reflux for 5 hours. The reaction mixture was cooled, filtered and the solvent removed under reduced pressure. The residue was recrystallized from benzene/cyclohexene to afford the title compound as colorless needles, 0.040 g (40%) m.p. 162-163 ^{o}C. ^{1}H NMR ($CDCl_3$): δ 1.93 (m, 2H, C_7H), 2.11 (s, 3H, C_{10}-CH_3), 2.24 (d, 3H, J = 1.0 Hz, C_4-CH_3), 2.75 (t, 2H, J = 6.5 Hz, C_6 H), 4.20 (t, 2H, J = 5.2 Hz, C_8 H), 5.94 (q, 1H, J = 1.0 Hz, C_3 H), 6.99 (s, 1H, C_5 H). ^{13}C NMR: δ 7.88 C_{10}-CH_3, 18.52 C_4-CH_3, 21.85, 24.86 C_6 and C_7, 66.90 C_8, 111.29 C_3, 112.73, 112.84 C_{5a} and C_{10}, 118.31 C_{4a}, 122.08 C_5, 150.92 C_{9a}, 152.55 C_4, 155.74 C_{10a}, 161.54 C_2 (pyrone quat C).

Anal. calcd. for $C_{14}H_{14}O_3$: C 73.02 H 6.13

Found : C 72.84 H 6.24

6,7-Dihydro-4,8,8,10-tetramethyl-2H,8H-benzo[1,2-b;5,4-b']dipyran-2-one (28)

The 4,8-dimethyl-7-hydroxycoumarin (0.95 g, 5.0 mmol), 1-chloro-3-methyl-2-butene (1.4 g, 7.5 mmol) and p-toluenesulfonic acid (0.095 g, 0.50 mmol) in 25 ml of toluene were heated at reflux with stirring for 3 hours. The reaction mixture (dark brown in color) was cooled to room temperature and the solvent was removed under reduced pressure. The residue, a dark brown oil, was flash chromatographed on silica gel using methylene chloride as eluent. Fractions were analyzed by TLC (silica:CH_2Cl_2), pooled and the solvent evaporated under reduced pressure. The crude product was recrystallized from aqueous methanol to yield white crystals , 0.80 g (62%), mp 149-150 ^oC. ^1H NMR ($CDCl_3$): δ 1.38 (s, 6H, C_8-CH_3's), 1.8 (t, J = 6.8 Hz, 2H, C_6-CH_2), 2.21 (s, 3H, C_{10}-CH_3), 2.42 (d, J = 1.2 Hz, 3H, C_4-CH_3), 2.76 (t, J = 6.8 Hz, 2H, C_7-CH_2), 6.12 (q, J = 1.2 Hz, 1H, C_3-H), 7.19 (s, 1H, C_5-H). ^{13}C NMR: δ 8.12 C_{10} CH_3, 18.71 C_4CH_3, 22.42 C_6 CH_2, 27.04 2 CH_3's, 32.45 C_7 CH_2, 75.35 C_8, 111.36 C_3, 121.87 C_5, 161.93 C_2 (pyrone quat C). UV(EtOH), λ max, log ϵ : 331 nm, 4.15; 258, 3.53; 248 nm, 3.55; 210 nm, 4.40.

Anal. calcd. for $C_{15}H_{16}O_3$: C 74.39 H 7.02

Found: C 74.02 H 6.85

Note: Other alkylating agents suitable for use in this reaction include 1-bromo-3-methyl-butene, 3-methyl-2-buten-1-ol, 2-methyl-3-buten-2-ol, 3-methyl-3-buten-1-ol and 2-chloro-3-butene. The yields of the product were comparable in all cases.

6,7-Dihydro-4,8,10-trimethyl-2H,8H-benzo[1,2-b;5,4-b']dipyran-2-one (66)

The reaction was carried out as described above using 4,8-dimethyl-7-hydroxycoumarin (0.95 g, 7.5 mmol), 1-chloro-2-butene (0.68 g, 7.5 mmol), and benzenesulfonic acid (0.089 g, 0.5 mmol). The residue was flash chromatographed on silica gel using methlyene chloride as eluent. The fractions were analyzed by thin layer chromatography (silica:CH_2Cl_2), pooled and the solvent evaporated. Two products were obtained and recrystallized from methanol. The first product to elute was a white crystalline solid identified as 4,8-dimethyl-7[(2-buten-1-yl)oxy]coumarin, 0.10 g, m.p. 165-167 ^oC. ^1H NMR ($CDCl_3$): δ 1.81 (d, 3H, J = 5.1 Hz, $=CHCH_3$), 2.32 (s, 3H, C_8-CH_3), 2.41 (d, 3H, J = 0.98 Hz, C_4-CH_3), 4.61 (d, 2H, OCH_2), 5.85 (m, 2H, CH=CH), 6.11 (q, 1H, J=0.98 Hz, C_3-H), 6.86 (d, 1H, J = 8.55 Hz, C_6-H), 7.26 (d, 1H, J = 8.55 Hz, C_5-H).

Anal. Calcd. for $C_{15}H_{16}O_3$: C 73.77 H 6.55

Found : C 73.30 H 6.31

The second product was isolated as shiny white crystals, 0.18 g m.p. 159-160 °C. and identified as the dihydropyranone. ^1H NMR ($CDCl_3$): δ 1.55 (d, 3H, C_8-CH_3), 1.82-2.11 (m, 2H, C_7-CH_2), 2.32 (s, 3H, C_{10}-CH_3), 2.42 (d, 3H, J = 1.2 Hz, C_4-CH_3), 2.85 (t, 2H, C_6-CH_2), 4.17 (m, 1H, C_8-H), 6.12 (q, 1H, J = 1.2 Hz, C_3-H), 7.23 (s, 1H, C_5H).

Anal. Calcd. for $C_{15}H_{16}O_3$: C 73.77 H 6.55

Found : C 73.23 H 6.60

Synthesis and Purification of 6,7-Dihydro-4,8,8-trimethyl-2H,8H-benzo[1,2-b;5,4-b']dipyran-2-one (29) (linear isomer) and 9,10-Dihydro-4,8,8-trimethyl-2H,8H-benzo[1,2-b;3,4-b']dipyran-2-one (angular isomer). (31)

A mixture prepared from 4-methyl-7-hydroxycoumarin (0.88 g, 5.0 mmol), 2-methyl-3-buten-2-ol (0.65 g, 7.5 mmol), and p-toluenesulfonic acid (0.095 g, 0.5 mmol) in 25 ml of toluene was heated with stirring at reflux for 4 hours and the solvent removed by distillation in vacuo. The crude tan solids were dissolved in a minimum amount of methylene chloride and charged to a silica gel column. With methylene chloride eluent the

angular isomer elutes first (29% yield, white needles, m.p. 160-162 °C after methanol recrystallization) followed by the linear isomer (36% yield, white needles, m.p. 174-175 °C after methanol recrystallization). ^{1}H-NMR for angular isomer in $CDCl_3$ with key features for identification underlined, δ 1.36 (s, 6H, C_8-CH_3's), 1.80 (t, 2H, C_9-CH_2, J = 6.5 Hz), 2.40 (d, 3H, C_4-CH_3, J = 0.97 Hz), 2.79 (t, 2H, C_{10}-CH_2, J = 6.5 Hz), 6.12 (q, 1H, C_3-H, J= 0.97 Hz), 6.80 (d, 1H, C_6-H, J = 8.8 Hz), and 7.29 (d, 1H, C_5-H, J = 8.8 Hz).

Anal. calcd. for $C_{15}H_{16}O_3$: C 73.75 H 6.60
Found: C 73.82 H 6.56

^{1}H NMR ($CDCl_3$) for the linear isomer: δ 1.30 (s, 6H, C_8-CH_3's), 1.85 (t, 2H, J = 6.6 Hz, C_6CH_2), 2.30 (d, 3H, J = 1.2 Hz,C_4-CH_3), 2.77 (t, 2H, J = 6.6 Hz, C_7CH_2), 6.02 (q, 1H, J = 1.2 Hz, C_3H), 6.65 (s, 1H, C_{10}H), 7.19 (s, 1H, C_5H). ^{13}C NMR: δ 18.62 C_4-CH_3, 22.09 C_6 CH_2, 26.84 C_8-CH_3's, 34.48 C_7 CH_2, 75.62 C_8, 104.59 C_{10}, 111.75 C_3, 125.06 C_5, 161.57 C_2 (pyrone quat C). UV(EtOH), λ max, log ϵ : 330 nm, 3.95; 207 nm, 4.32.

Anal. calcd. for $C_{15}H_{16}O_3$: C 73.75 H 6.60

Found : C 73.66 H 6.77

Synthesis and Purification of 6,7-Dihydro-8,8-dimethyl-2H,8H-benzo[1,2-b;5,4-b']dipyran-2-one (30) (linear isomer) and 9,10-Dihydro-8,8-dimethyl-2H,8H-benzo[1,2-b;3,4-b']dipyran-2-one (angular isomer) (32)

The 7-hydroxycoumarin (0.81 g, 5.0 mmol), 1-chloro-3-methyl-2-butene (1.4 g, 7.5 mmol) and p-toluenesulfonic acid (0.095 g, 0.50 mmol) in 25 ml of toluene were heated, with stirring, at reflux temperature for 4 hours. The reaction mixture (dark brown) was cooled to room temperature and the solvent removed under reduced pressure. The crude product, a dark brown gummy solid, was subjected to flash chromatography on silica gel with methylene chloride as eluent. Fractions were analyzed by TLC, pooled and the solvent evaporated under reduced pressure. The angular isomer (dihydroseselin) was the first product to elute (white crystals, 19%, m.p. 103-104 ^{o}C after recrystallization from methanol,lit.[59] m.p. 103-104 ^{o}C) followed by the linear isomer (dihydroxanthyletin, white crystals, 22%, m.p. 123-124 ^{o}C after recrystallization from methanol, lit.[59] m.p. 124-125 ^{o}C). ^{1}H NMR ($CDCl_3$) of the angular isomer with key

features for identification underlined: δ 1.25 (s, 6H, C_8 CH_3's), 1.84 (t, 2H, J = 6.9 Hz, C_9 CH_2), 2.91 (t, 2H, J = 6.9 Hz, C_{10} CH_2), 6.20 (d, 1H, J = 9.4 Hz, C_3H), 6.73 (d, 1H, J = 8.9 Hz, C_6H), 7.18 (d, 1H, J = 8.9 Hz, C_5H), 7.56 (d, 1H, J = 9.4 Hz, C_4H).

^1H NMR ($CDCl_3$) of the linear isomer: δ 1.36 (s, 6H, C_8 CH_3's), 1.83 (t, 2H, J = 6.7 Hz, C_6 CH_2), 2.84 (t, 2H, J = 6.7 Hz, C_7 CH_2), 6.24 (d, 1H, J = 9.35 Hz, C_3H), 6.72 (s, 1H, $C_{10}H$), 7.15 (s, 1H, C_5H), 7.65 (d, 1H, J = 9.35 Hz, C_4H).

PSORALEN DERIVATIVES

5-Amino-8-methoxypsoralen (2)

Prepared according to the method of Brokke and Christensen.[60]

5-(N,N-3,3-Dimethyltriazeno)-8-methoxypsoralen (3)

A suspension of 5-amino-8-methoxypsoralen (0.23 g, 1.0 mmol), sodium nitrite (0.076 g, 1.1 mmol), and 10 ml of ice-water was stirred vigorously while 1.0 ml of trifluoroacetic acid was added dropwise. The addition took 5 minutes during which the temperature of the reaction mixture was kept between 0 ^{o}C and 5 ^{o}C with an ice bath. The cooling bath was removed, the reaction mixture was stirred for 5 minutes, and then added slowly, with stirring to an ice-cold solution of dimethylamine (0.064 g, 1.4 mmol) in 15 ml of 1M KOH. Stirring was continued at 0 ^{o}C for 10 additional minutes. The pH of the solution was adjusted to slightly basic and the solid material filtered. The filtered solid was flash chromatograghed on silica using CH_2Cl_2/ethyl acetate (10:1) as eluent. Fractions were analyzed by thin layer chromatography, pooled and the solvent evaporated under reduced pressure to yield a yellow solid. The pure product was obtained after

recrystallization from 95% ethanol as fluffy bright yellow needles, 0.21 g (70%), m.p. 187-189 ^{o}C. ^{1}H NMR ($CDCl_3$): δ 3.46 (bs, 6H, $N(CH_3)_2$), 4.23 (s, 3H, OCH_3), 6.28 (d, 1H, J = 9.6 Hz, C_3H), 7.29 (d, 1H, J = 1.9 Hz, C_4,H), 7.63 (d, 1H, J = 1.9 Hz, C_5,H), 8.64 (d, 1H, J = 9.6 Hz, C_4H). ^{13}C NMR: δ 60.10 OCH_3, 106.72 C_5,, 11.85 C_3, 140.92 C_4, 144.67 C_4,, 160.44 C_2 (pyrone quat C).

Anal. Calcd. for $C_{14}H_{13}N_3O_4$: C 58.54 H 4.56 N 14.63

Found : C 58.27 H 4.56 N 14.51

5-(N,N-3,3-Diethyltriazeno)-8-methoxypsoralen (4)

The reaction was carried out as in the above example using 5-amino-8-methoxypsoralen (0.23 g, 1.0 mmol), sodium nitrite (0.76 g, 1.1 mmol) and 1.0 ml of trifluoroacetic acid. The product was purified by flash chromatography on silica using chloroform as eluent followed by recrystallization from 95% ethanol. The title compound was collected as an orange crystalline solid, 0.24 g (76%), m.p. 123-124 ^{o}C. ^{1}H NMR ($CDCl_3$): δ 1.36 (t, 6H, J = 6.8 Hz, $CH_2\underline{CH_3}$), 3.77 (q, 4H, J = 6.8 Hz, $\underline{CH_2}CH_3$), 4.23 (s, 3H, OCH_3), 6.37 (d, 1H, J = 9.6 Hz, C_3H), 7.27 (d, 1H, J = 2.1 Hz, C_4,H), 7.65 (d, 1H, J = 2.1 Hz, C_5,H), 8.65 (d, 1H, J =

9.6 Hz, C_4H). ^{13}C NMR: δ 63.05 OCH_3, 109.69 $C_{5'}$, 113.86 C_3, 143.04 C_4, 147.13 $C_{4'}$, 162.32 C_2.

Anal. Calcd. for $C_{16}H_{17}N_3O_4$: C 60.94 H 5.43 N 13.33
Found : C 60.64 H 5.57 N 13.60

5-(N,N-3-Ethyl-3-methyltriazeno)-8-methoxypsoralen (5)

The reaction was carried out in a similar manner to the above examples using 5-amino-8-methoxypsoralen (0.23 g, 1.0 mmol), N-ethyl methylamine (0.089 g, 1.4 mmol), sodium nitrite (0.076 g, 1.1 mmol), and 1.0 ml of trifluoroacetic acid. The product was purified by flash chromatography (silica, chloroform) followed by recrystallization from 95% ethanol to yield yellow needles, 0.20 g (66%), m.p. 145-147 ^oC. ^1H NMR ($CDCl_3$): δ 1.38 (t, 3H, J = 7.1 Hz, $CH_2\underline{CH}_3$), 3.34 (bs, 3H, CH_3), 3.88 (q, 2H, J = 7.1 Hz, $\underline{CH}_2CH_3$), 4.23 (s, 3H, OCH_3), 6.26 (d, 1H, J = 9.8 Hz, C_3H), 7.27 (d, 1H, J = 2.0 Hz, C_4,H), 7.64 (d, 1H, J = 2.0 Hz, C_5,H), 8.6 (d, 1H, J = 9.8 Hz, C_{4H}). ^{13}C NMR: δ 13.61 N-$CH_2\underline{CH}_3$, 33.85 N-CH_3, 50.89 N-$\underline{CH}_2CH_3$, 61.19 OCH_3, 107.87 $C_{5'}$, 111.85 C_3, 141.70 C_4, 145.78 $C_{4'}$.

Anal. Calcd. for $C_{15}H_{15}N_3O_4$: C 59.80 H 5.02 N 13.95

Found : C 59.51 H 4.97 N 13.72

5-(N,N-3-n-Butyl-3-methyltriazeno)-8-methoxypsoralen (6)

The reaction was carried out as in the above examples using 5-amino-8-methoxypsoralen (0.46 g, 2.0 mmol), n-butyl methylamine (0.24 g, 2.8mmol), sodium nitrite (0.19 g, 2.8 mmol) and 2.0 ml of trifluoroacetic acid. The product was purified by flash chromatography (silica, chloroform) followed by recrystallization from 95% ethanol. The product was collected as dark yellow crystals, 0.322 g (49%), m.p. 66-67 ^{o}C. ^{1}H NMR ($CDCl_3$): δ 0.92 (t, 3H, $CH_2\underline{CH_3}$), 1.51 (m, 4H, $\underline{CH_2}CH_2\underline{CH_3}$), 3.32 (bs, 3H, CH_3), 3.87 (t, 2H, $\underline{CH_2}CH_2CH_2CH_3$), 4.22 (s, 3H, OCH_3), 6.36 (d, 1H, J = 10.0 Hz, C_3H), 7.28 (d, 1H, J = 2.4 Hz, C_4,H), 7.62 (d, 1H, J = 2.4 Hz, C_5'H), 8.66 (d, 1H, J = 10.0 Hz, C_4H). ^{13}C NMR: δ 12.32 $(CH_2)_3\underline{CH_3}$, 18.52 $(CH_2)_2\underline{CH_2}CH_3$, 33.39 N-$CH_3$, 51.62 N-$\underline{CH_2}(CH_2)_2CH_3$, 60.23 OCH_3, 106.83 C_5,, 110.99 C_3, 140.22 C_4, 144.33 C_4,, 159.48 C_2.

Anal. Calcd. for $C_{17}H_{19}N_3O_4$: C 62.01 H 5.81 N 12.76

Found : C 61.80 H 5.52 N 12.55

4'-Chloromethyl-4,5'-8-trimethylpsoralen (8)

Prepared according to the method of Isaacs.[61]

4'-N-Phthalimidomethyl-4,5',8-trimethylpsoralen

Prepared by the procedure described by Heindel.[62]

4'-Aminomethyl-4,5',8-trimethylpsoralen (11)

Prepared by the procedure described by Heindel.[62]

4'-N-(2-Chloroethyl)-N'-4,5',8-trimethylpsoralen urea (12)

The 4'-aminomethyl-4,5',8-trimethylpsoralen (0.26 g, 1.0 mmol) was dissolved in 25 ml of chloroform by gentle warming followed by addition of 2-chloroethylisocyanate (0.11 g, 1.0 mmol). The reaction mixture was heated at reflux for 15 hours under a nitrogen atmosphere. After the reflux period the reaction mixture was filtered to yield a white chalky powder, 0.25 g (66%), m.p. 249-252 ^{o}C. ^{1}H NMR (DMSO-d_6): δ 2.45-2.53 (m, 9H, J = 1.95 Hz, $C_{4'}$, C_5, C_8 methyls), 3.54 (m, 4H, CH_2CH_2Cl), 4.30 (d, 2H, J = 5.6 Hz, CH_2NH), 6.13 (bt, 1H, NH), 6.34 (q, 1H, C_3H), 6.62 (bt, 1H, NH), 7.88 (s, 1H, C_5H).

Anal. calcd. for $C_{18}H_{19}N_2O_4Cl$: C 59.50 H 5.28 N 7.72
Cl 9.77

Found : C 59.90 H 5.18 N 7.43
Cl 9.80

4'-Aminomethyl-Bis[N-(2-hydroxyethyl)]-4,5',8-trimethylpsoralen (9)

A solution of 4'-chloromethyl-4,5',8-trimethylpsoralen (0.56 g, 2.0 mmol) and diethanolamine (0.25 g, 2.4 mmol) in 25 ml of acetone was heated under reflux in the presence of 0.4 g of anhydrous potassium carbonate for 7 hours. The reaction mixture was then poured onto 30 g of ice and extracted with 50 ml of chloroform. The chloroform extract was dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure to yield a yellow solid. This solid was purified by flash chromatography on silica gel using methylene chloride with increasing percentages of isopropyl alcohol (1-10%) as eluent. The product was obtained as an off-white powdery solid, 0.43 g, (62%), m.p. 189-191 ^{o}C. ^{1}H NMR ($CDCl_3$): δ 2.24 (bs, 2H, OH's), 2.46-2.52 (m, 9H, C_4, C_5, C_8 methyl's), 2.72 (t, 4H, J = 5.1 Hz, $N\underline{CH_2}CH_2OH$), 3.71 (t, 4H, J = 5.1 Hz, $NCH_2\underline{CH_2}OH$), 6.16 (q, 1H, C_3H), 7.74 (s, 1H, C_5H).

Anal.calcd. for $C_{19}H_{23}NO_5$ .5 H_2O : C 64.37 H 6.82 N 3.95

Found : C 64.96 H 6.97 N 3.78

3,4,4′,5′-Tetrahydro-4,5′,8-trimethylpsoralen (25)

A solution of 4,5′,8-trimethylpsoralen (0.60 g, 2.2 mmol) in 80 ml of glacial acetic acid was stirred vigorously at 25 °C with H_2 at atmospheric pressure in the presence of 150 mg of 10% Pd/charcoal until hydrogen uptake had ceased. Filtration of the reaction mixture and evaporation of the solvent gave a crude product. Recrystallization of the crude product from 95% ethanol gave the title compound as colorless crystals, 0.169 g (34%), m.p. 81-82 °C (lit.[63] m.p. 79-80 °C). ^1H NMR ($CDCl_3$): δ 1.24 (d, 3H, J = 7.0 Hz, C_4CH_3), 1.43 (d, 3H, J = 6.3 Hz, C_5,CH_3), 2.15 (s, 3H, C_8CH_3), 2.46 (dd, 2H, J = 7.0 Hz, $C_3H's$), 2.81 (dd, 1H, J = 6.3 Hz, C_4,H), 3.21 (m, 1H, J = 7.0 Hz, C_4H), 3.33 (dd, 1H, J = 6.3 Hz, C_4,H), 4.92 (m, 1H, J = 6.3 Hz, C_5,H), 6.85 (s, 3H, C_5H). ^{13}C NMR: δ 8.56 C_8-CH_3, 20.20 C_4-CH_3, 21.74 $C_5,$-CH_3, 29.36 C_4, 37.01 $C_4,$, 80.07 $C_5,$, 108.47 C_3, 118.86 119.19 121.78, 149.08, 157.76, aromatic protons, 168.80 C_2 (pyrone quat C). UV (EtOH), λ max, log ϵ: 286 nm, 3.47; 291 nm, 3.50.

3-Nitro-4',5'-dihydro-4,5',8-trimethylpsoralen[64] **(67)**

To a solution of 4',5-dihydropsoralen[34] (0.23 g, 1.0 mmol) in 4.5 ml of glacial acetic acid was added 4.0 ml of a 75:25 glacial acetic acid:concentrated nitric acid solution over a period of 10 minutes. The reaction was stirred at room temperature for 72 hours then diluted with 250 ml of water. The precipitated solids were collected by vacuum filtration and the product was isolated by recrystallization from 2:1 petroleum ether:chloroform, m.p. 241-243 ^{o}C. ^{1}H NMR ($CDCl_3$): δ 1.50 (d, 3H, C_5,-CH_3), 2.25 (s, 3H, C_8-CH_3), 2.45 (s, 3H, C_4-CH_3), 2.89 (dd, 1H, C_4,-H), 3.41 (dd, 1H, C_4,-H), 5.07 (m, 1H, C_5,-H), 7.35 (s, 1H, C_5-H).

Anal. calcd. for $C_{14}H_{13}NO_3$: C 61.10 H 4.76 N 5.09

Found : C 61.19 H 4.60 N 5.09

EXPERIMENTAL PROTOCOL FOR DNA BINDING STUDY

MATERIALS AND METHODS

DNA. Calf Thymus, highly polymerized (Sigma); GC=40%, T_m [65]= 87 °C.

Test compounds. The compounds used were synthesized by the author as detailed in the Experimental section. The 8-methoxypsoralen was provided by ICN Pharmaceuticals.

Solubilization procedure. Measured amounts (0.5-1 mg) of the test compounds were finely ground and suspended in 50-100 ml of water or 0.1% aqueous DNA solution. The suspensions were shaken for 12 hours at ambient temperature and then filtered through Millipore Millex SV 5 micron membranes. As small amounts of the dissolved substances may be adsorbed by the filter membrane, the first 5 ml of the filtrate of each suspension were discarded. The remaining 5 ml were then filtered through the same saturated membrane.

Spectrophotometric measurements. Ultraviolet spectra of the solutions obtained as described above were registered on a Perkin-Elmer Lambda 5 UV/VIS spectrophotometer. For the determination of the DNA thermal denaturation curve the spectrophotometer was

equipped with an attachment for the determination of the absorbance at various controlled temperatures. Quartz cuvettes with an optical path length of 1 cm were used.

Solubility determination. The concentration at the maximum solubility for each of the test compounds was determined graphically. The uv absorbance of increasing concentrations of the compounds was measured. This absorbance was plotted versus the concentration of the solutions and the maximum solubility was taken as the point at which the absorbance stabilized with no further increase in value.

Measurement of T_m. The melting temperature of the DNA was determined in a way analogous to the method described by Marmur and Doty.[66] Each of the test compounds was solubilized in 0.1% DNA solution as outlined above. The temperature profiles of the solutions were determined spectrophotometrically. The thermal denaturation profile of the DNA in the absence of the compounds was determined in aqueous solution and also in the presence of 0.2 M Na^+. As it has been found that the presence of salts affects the complexation, the drug-DNA solutions were completely without salts.

DETERMINATION OF PARTITION COEFFICIENTS

The octanol-water partition coefficients (P) were determined by the general method of Hansch.[67]

The compound to be partitioned was precisely weighed (0.5-1 mg) and dissolved in 100 ml of octanol-saturated phosphate buffer, pH=7.4. The solution was then placed in a 30 ml glass centrifuge bottle. Phosphate buffer-saturated octanol (5 ml) was then added to the centrifuge bottle and the bottle was stoppered with a ground glass stopper. The bottle was inverted 100 times within 5 minutes, after which the octanol and aqueous layers were allowed to separate by standing for one week. The phases were separated and their UV absorbances were measured at the λ max of the compound. These measurements were made against blanks of phosphate buffer-saturated octanol and octanol-saturated phosphate buffer which had been shaken in the same manner as the test compound. The ratio of the absorbance of the octanol phase to the absorbance of the aqueous phase was used as a measure of the ratio of the concentration in each phase. This ratio was given as the partition coefficient (P). Alternatively, the uv absorbance of the original aqueous solution was compared to the absorbance of the aqueous phase at the

same wavelength to give the relative concentration of the compound in the aqueous phase. Both methods gave similar results.

^{125}I-EGF INHIBITION ASSAY[68]

To assay compounds for inhibition of EGF binding, human cells (HeLa) grown in vitro were used. Cells (1.8 x 104/cm^2) were inoculated into 5 cm culture dishes in growth medium consisting of Dulbecco's modified Eagle's medium supplemented with 10% newborn calf serum. After 4-5 days at 37 ^{o}C in a humidified CO_2 incubator, the cells were washed three times with 2 ml of phosphate-buffered saline and then incubated with the different test compounds in 2 ml of Eagle's salt solution supplemented with 5.2 mM D-glucose/25 nM Hepes buffer, pH 7.2.

Control cultures were incubated in 2 ml of Earle's salt solution in the absence of the test compounds. After 30 minutes, the cells were then exposed to ultraviolet light (UVA, 320-400 nm) emitted from a bank of four BLB fluorescent light tubes (F40BL/Sylvania) placed approximately 10 cm above the cell culture plates. The incident light delivered onto the culture plates was 3.4 mW per cm^2 as measured with an International Light UV-Radiometer and the cells received 2.1 J/cm^2 of UVA light. After this light exposure, the cells were rinsed with phosphate-buffered saline and submitted for assay of epidermal growth

factor binding. Cells which had been treated with test compounds were then incubated for 2 hours at 4 ^{o}C with 2 ml of binding buffer (Dulbecco's modified Eagle's medium/25 mM Hepes buffer, pH 7.2) containing 2 mM labeled epidermal growth factor. (^{125}I-EGF, specific activity 200 Ci/g).

Nonspecific binding was determined by incubating separate plates of cells with buffer containing the radioligand and excess unlabeled epidermal growth factor (1 microgram/ml). The binding reaction of the radioligand to the cells was terminated by aspirating the binding buffer from the culture dishes and washing the cells four times with ice-cold phosphate-buffered saline. The cells were then solubilized with 2 ml of 0.2 M NaOH and duplicate 0.5 ml aliquots were taken for gamma counting. Specific binding of epidermal growth factor to its receptor was calculated by subtracting nonspecifically-bound material from the total. Under the conditions of the assay, specific epidermal growth factor receptor binding represented 80% of the total bound to the cells. The assay may be performed on a variety of cells which possess EGF receptors.

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