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Synthesis and Structural Studies of Radiopharmaceutical Precursors

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SYNTHESIS AND STRUCTURAL STUDIES OF RADIOPHARMACEUTICAL PRECURSORS

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Natalie Foster

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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.

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ABSTRACT

The design and synthesis of tumor-seeking radiopharmaceuticals for detecting malignancy requires the solution to two problems: 1) selection of a "carrier group" with demonstrated specificity for the target to be imaged, and 2) selection of a way to attach the nuclide to that carrier. The psoralens, natural products well-established as malanizing agents, are known to be selectively incorporated into functioning melanocytes. Psoralens are the candidate agents for conversion into melanoma-localizing radiopharmaceuticals.

This dissertation reports the development of several pre-labeling candidates for melanoma imaging. A facile new labeling method, the triazene decomposition, has been studied, its generality probed, its sensitivity to molecular substituent effects evaluated, and its applicability to other pharmaceutical classes tested. The triazene intermediates required for this labeling method have been examined for biological activity, for chemical stability, and for sensitivity of ring positions to transmission of electronic effects as measured by C-13 NMR.

The triazene labeling method requires amino-bearing precursors. As a means to simple, non-pressurized reduction of nitro groups to amines to generate these necessary precursors, a palladium-catalyzed hydrogen transfer reaction with cyclohexene as the donor has proven extraordinarily useful.

HISTORICAL

The successful clinical management of cancer has long been directly associated with the early and accurate detection of malignancy. One of the great challenges facing the synthetic chemist involved in medicinally oriented research remains the design of imaging agents able to aid in the diagnostic process by virtue of their selective localization in areas of dysfunction within the human body. Classically, candidate agents for this task have been sought through the application of a variety of rationales for compound selection, and among the fruitful areas explored have been the synthetic modification and subsequent evaluation of the biodistribution of substances with demonstrated physiological activity. Hence, mercury compounds, known diuretics, have been developed as radioisotopic probes of kidney function,[1] derivatives of neuroleptic drugs have been used as imagers of the brain,[2] and modified steroids have been utilized as indicators of the state of the adrenal glands.[3] Not only has the examination of these materials occasionally lead to useful diagnostic imaging agents, but even more importantly studies involving derivatives of such compounds have often provided valuable insights into the molecular nature of normal as well as dysfunctioning physiological states.

Malignant melanoma is one type of cancer that is relatively easy to detect if it involves the skin, but is particularly insidious and difficult in cases where melanotic tumor growth occurs in the eye (ocular melanoma) or is associated with solid tumors of the internal organs. Rapid metasthesis of melanomas also contributes to the danger of the disease and the extreme necessity for early detection of the primary growth.[4] This disease derives its name from the presence in the tumors of a higher than normal amount of melanin, the as yet incompletely characterized biopolymer responsible for pigmentation.

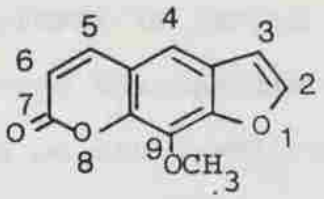
The main focus of this work is the development of a synthetically clean, rapid and efficient means of incorporating radionuclides into molecules to be screened as potential melanoma delineating agents. The evaluative tools for both in vivo and in vitro testing of the candidate agents require radiolabeled materials. The molecular system of ultimate interest in this study is the general class of compounds known as psoralens, of which 9-methoxy-7H-furo[3,2-g][1]benzopyran-7-one (hereafter: methoxsalen) is the primary compound selected for synthetic modification for labeling.

Selection of Candidate Compounds

The compounds selected for modification to enable labeling are the psoralens, linear furocoumarins that have been for centuries recognized as potent dermal photosensitizing agents.[5] The ancient Egyptians were the first to use natural plant extracts containing members of this class of compounds to encourage sun tanning and prevent sun burning. As early as 1400 B.C. Indian literature describes "the plant that produces an even color," and classical clinical work was done in the middle of the 13th Century in Egypt by Abou-el-Bitar, who used an extract of the plant Ammi majus and sunlight to treat vitiligo.[6] More recently, psoralens have been observed to concentrate in and stimulate melanin production by melanocytes, the pigment producing cells in human tissue, and it has been suggested that this selectivity helps account for their physiological activity.[7,8] The observed biodistribution pattern provides a logical rationale for evaluating the potential of the psoralens as melanoma delineating radiopharmaceuticals.

Currently, methoxsalen (1) is being used clinically in the treatment of psoriasis and other skin disorders. The beneficial effects of PUVA therapy (the combination of topically applied or orally administered methoxsalen and successive irradiation of affected skin with 365 nm

light) have provided considerable incentive for synthesis and evaluation of modified psoralens. The undesirable



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side effects of skin phototoxicity and risk of induction of skin cancer that are unfortunately associated with this therapeutic application of methoxsalen have also spurred increasing interest in the molecular behavior of psoralens with cellular components.

On the molecular level, psoralens are capable of three distinct interactions with DNA:

(1) intercalation in the DNA helix, a necessary precondition for

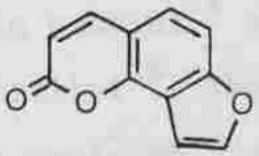
(2) photoinduced formation of mono-adducts with pyrimidine bases by reaction of the 2,3- or more typically the 5,6- double bond of the psoralen with the 5,6-double bond of the base, and

(3) the subsequent formation of a diadduct which results in interstrand cross-linking in the macromolecule.[9]

It is currently thought that the undesirable side-effects associated with psoralen therapy of skin disorders are connected with the formation of bifunctional

lesions of the type described above.[10] At least regarding the risk of skin cancer induction, the mono-adducts seem to be much less involved.[5, 11]

In an effort to reduce or eliminate these side effects while retaining therapeutic efficacy, research effort has been focused on the development of agents capable of forming only mono-adducts with DNA. Most prevalently, derivatives of non-linear furocoumarins (the so-called angelicins, (2)), which seem to behave as mono-functional reagents in spite of the presence of two potentially photoreactive sites, have been evaluated.[10]



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Another apparent route to materials that could still intercalate but only form mono-adducts would be the selective reduction of either the 2,3- or 5,6- double bond in the psoralen to yield an agent that contains only one photoactive bond. The comparison of the behavior of a series of compounds differing only in their degree of saturation would provide an interesting possibility for structure-activity correlations. In the context of using this class of molecules for imaging melanotic tumors, the observed affinity of psoralens for functioning melanocytes may also reflect a specificity of the compounds for melanin. The biopolymer melanin is thought to possess

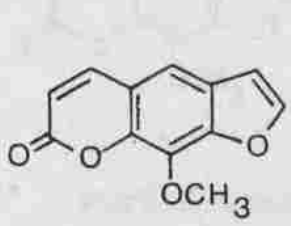
several different types of binding sites and the deviations in planarity and electronic character of loci on the psoralen molecule occasioned by this selective reduction may prove helpful in more precisely defining the architecture of the melanin biopolymer.

Previous Synthetic Studies on Methoxsalen

Methoxsalen, earlier known as xanthotoxin, was isolated from the skin of the fruits of the plant Fagara zanthoxyloides by Thomas[12] and Preiss.[13] Thomas was the first to assign a correct structure to this substance. Methoxsalen is known to be toxic to fish, frogs and cold-blooded animals in general[14] and also has considerable molluscicidal activity.[15] This and other psoralens have subsequently been isolated from Ammi majus,[16] domestic cold-pressed lemon oil,[17] oil of bergamot[18] and a variety of other natural sources.[19]

Literature reports on the psoralens may be classified into three different areas on the basis of the nature of the work being reported: (1) isolation of psoralens from plant extracts and proof of structure of the constituents; (2) synthesis of the furocoumarin system, and (3) synthetic modifications yielding derivatives of the naturally occurring materials. Chief among the natural psoralens whose modification has been studied is methoxsalen.

Stanley and Vannier,[17] Abu-Mustafa and co-workers (in footnotes 20-22 and references cited therein) and Chatterjee et al.[23] have separated and characterized no fewer than twelve furocoumarin derivatives from natural sources. A few representative structures of the more abundant isolates are shown below:



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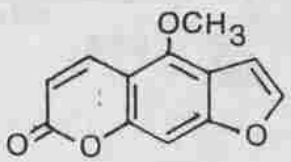

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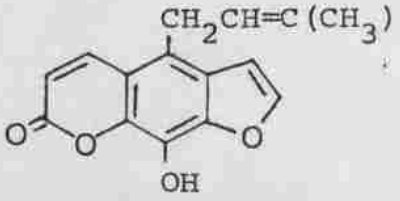

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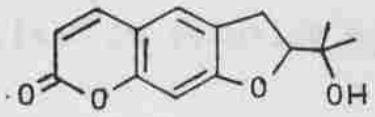

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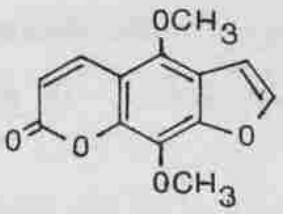

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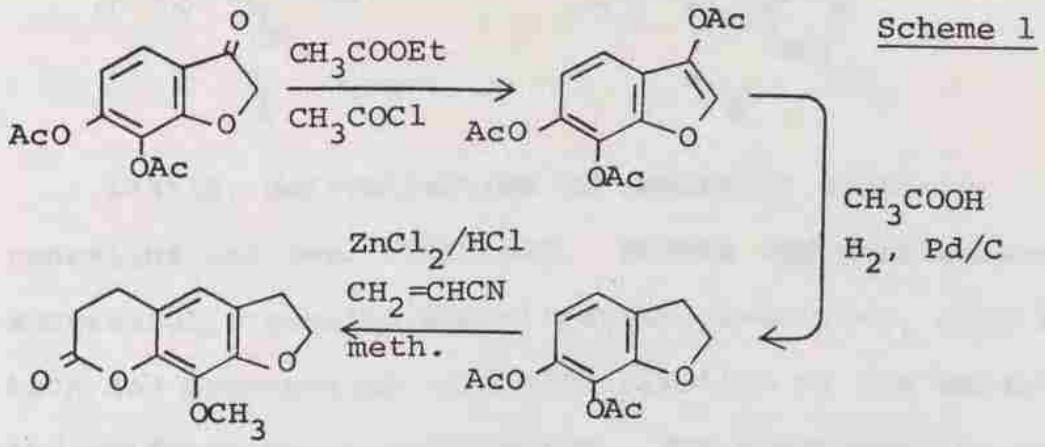
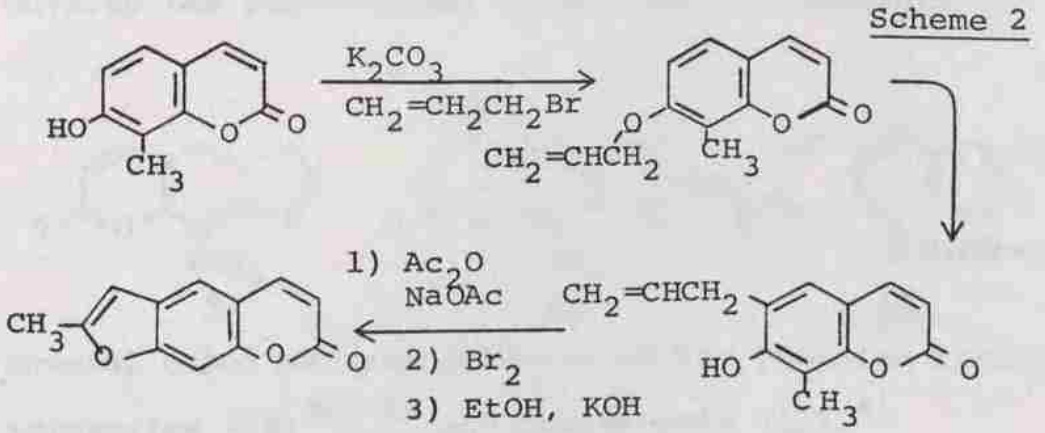
Early synthetic work on the psoralen system was accomplished by Späth and Pailer, who began with systems already containing the furan system on to which they closed the coumarin ring. In this fashion they accomplished the first total synthesis of methoxsalen[19] and allobergaptol.[24] A similar approach was taken by Davies and Deegan,[25] Lagercrantz[26] and Chatterjee and

Kalyanmay.[27] (Summarized in reaction scheme 1).



Kurt Kaufman[28] and co-workers[29-33] synthesized numerous psoralens by the related approach of closing the furan ring on to an already established coumarin system. (Summarized in reaction scheme 2). Additionally, recent



reports[34,35] have described the synthesis of psoralen isosteres of the thienobenzopyranone class (7, 8).

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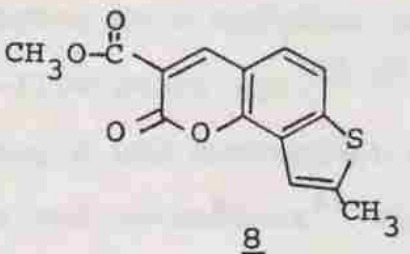

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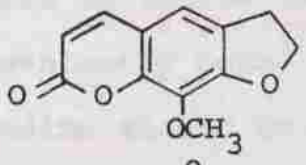
Lastly, derivatization of naturally occurring psoralens has been attempted. Brokke and Christensen[36,37] successfully studied the nitration, reduction, chlorination and bromination (via diazotization of the amine) and sulfonation of methoxsalen. The electrophilic reactions were all reported to proceed at the 4-position of the ring, para to the methoxy group. Reduction of the furan ring, however, altered this pattern, and 2,3-dihydromethoxsalen (9) has been shown to nitrate and brominate at the 6-position.[38] Additional reports have covered the reactivities toward similar reagents of



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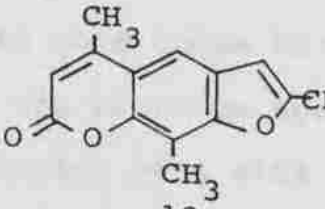

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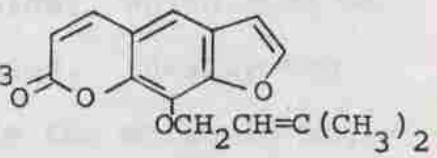

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several other natural products in the psoralen family, trioxsalen (10)[39, 40] and imperatorin (11).[41]

A main route to the reduced methoxsalen derivatives applied in this study is the selective rapid transfer reduction. The reduction of both aromatic nitro and ethylenic compounds by transfer hydrogenation using

Pd/C catalyst and cyclohexene as a hydrogen donor was demonstrated over twenty-five years ago.[42,43] That the reduction is selective, rapid and convenient was shown subsequently by Entwistle and co-workers[44] who evaluated the mono-reduction of polynitrated benzenes by this method. This technique provides easy accessibility to the reduced methoxsalen system as well as to amines needed in the labeling method for these compounds. The application of transfer reductions to members of the methoxsalen family has yielded a facile new route to known methoxsalen derivatives as well as some unexpected products not previously reported in the literature.

Radiolabeling Methodology

To be suitable for evaluation and ultimate clinical use, the molecules of interest must bear a gamma-emitting nuclide as a radiolabel. The radiolabel of choice in this work is iodine (or in some cases bromine), which must be covalently bound to the psoralen species. Ideally the iodine should be attached at a site in the molecule where it will not markedly decrease the specific binding of the molecule to the target tissue or alter unfavorably its biodistribution. Because the mechanism of psoralen binding to melanocytes and the mechanism of psoralen activity are both unknown, a necessary part of the study will require a method of radiolabeling that will place

the iodine on the various accessible positions of the molecule. The influence of the iodine's presence can thereby be determined and some information about the nature of the binding site may be inferred.

Simply incorporating a radiolabel into a molecule is insufficient in nuclear medicine. "Successful labeling" connotes a reaction that proceeds quickly, giving a high yield of only one product, or at least a desired product that is readily separable from any residual reactants or by-products. The imaging agent itself may well be the result of a multi-step synthetic pathway, as long as the incorporation of the radiolabel takes place at the end of the sequence. Because an area of concern is also the integrity of the label *in vivo*, the halogen is best attached to an aromatic moiety in the agent. Aliphatically bound halogens have long been known to be susceptible to rapid *in vivo* dehalogenation.

Numerous radioiodinated compounds have already found considerable utility as *in vivo* imaging agents. Enzyme inhibitors for monitoring the adrenal medulla,[45] steroids for estrogen receptor binding,[46] molecular probes for muscarinic cholinergic receptor studies,[47] derivatives of beta-adrenergic blockers for myocardial imaging[48] and a wealth of other chemical species have been successfully radiohalogenated and evaluated for use as imaging tools in clinically significant situations.

Several methods are popular for rapidly radioiodinating aromatic portions of molecules, but each is fraught with its own characteristic difficulties and limitations. Photocatalyzed exchange[49] and thermal or melt exchange techniques[50] have been used to prepare aryl bound iodides. In the first method, cold iodocompound is incubated with iodine and hot radioiodide; the exchange is effected by photoreaction. In the latter, cold compound is fused with radio sodium iodide. Both methods suffer from the fact that the exchanges are equilibrium reactions and the product is a mixture of hot and cold materials not ammenable to physical or chemical separation. This may not be problematical in some applications, but in cases where the goal is imaging tissues containing low density, high affinity drug receptors, the presence of a significant portion of cold compound that is isostructural with labeled material would greatly decrease the quality of the clinical image. Hence the requirement of <u>high specific activity</u> -- here meaning a sample in which almost every molecule bears a radiolabel and consequently very few cold isomers capable of occupying specific receptors in the target tissue are present -- demands alternative synthetic procedures to avoid this inherent difficulty.

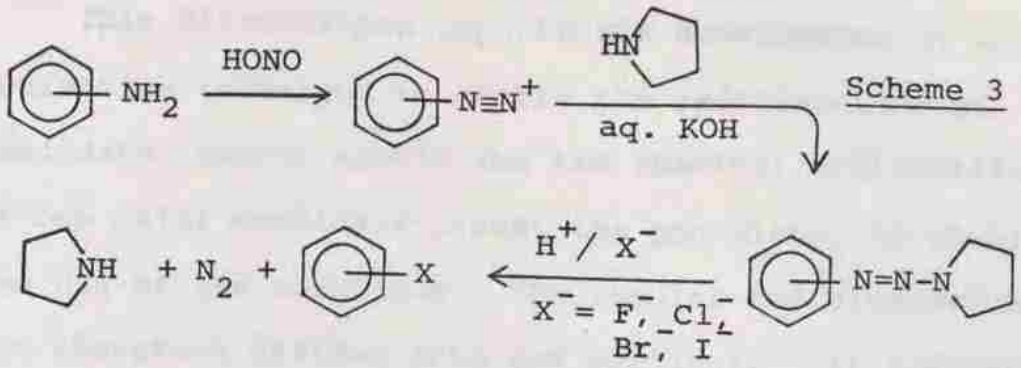
The exchange methods have been supplemented by electrophilic iodinations, which tend to be most

successful on activated aryl moieties: iodine monochloride, iodine and silver trifluoroacetate, molecular iodine, and the generation of iodonium with chloramine-T have all been successful at tracer level.[51]

Diazotization of an amine via the classical Sandmeyer method also provides an alternative labeling procedure, but the preparation of a radiopharmaceutical in this fashion requires considerable chemical expertise and would therefore not be viable in the clinical laboratory. The diazonium species must be prepared in situ every time a given agent is to be labeled, conditions must be precisely controlled, and traces of nitrous acid left in the medium from the diazotization can remove radioiodine via a redox reaction and thereby effect lower radiochemical yields. In addition, several important drug analogs with complex structures have been shown to resist Sandmeyer iodination at tracer level concentrations at which the reactions are carried out.[52]

Several researchers[53] have applied the Wallach reaction[54,55] to the problem of incorporating F-18 into radiopharmaceuticals. Modifications of the Wallach reaction provide the method of choice for the radiolabeling studies undertaken herein. In this reaction, a nascent diazonium ion formed from an aromatic amine is trapped by reaction in basic medium with a secondary

amine to form a triazene (reaction scheme 3).



Scheme 3

The triazene species is readily isolable from the reaction medium, is recrystallizable and is shelf stable. In a subsequent acid-catalyzed reaction in the presence of halide ion, the triazene decomposes, yielding aryl iodide, dinitrogen and secondary amine (reaction scheme 3). The obvious advantage of the triazene decomposition reaction is the ease with which the starting material in the radiolabeling step (the triazene) may be separated chromatographically from the final product (the radiohalogenated species of interest). In theory, therefore, this new modification of the Wallach reaction enables the swift and clean radioiodination of amine bearing aryl rings in candidate molecules.

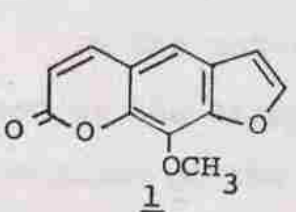
The main thrust of the work reported herein is: (1) the development of the triazene decomposition technique for halogenation of candidate compounds, and (2) the evaluation of the methoxsalen system for its reactivity and adaptability to the triazene labeling method.

RESULTS AND DISCUSSION

This dissertation reports the development of an iodination technique to enable the radiolabeling of candidate imaging agents and the chemical modification of one major candidate class, the psoralens, to enable the use of the technique. The results and discussion are therefore divided into two sections: (1) iodinations, and (2) derivatization involving methoxsalen.

I. Iodination Technique

Common methods of incorporating iodine into aromatic moieties within molecules have been reviewed.[51] None of these readily applicable techniques has resulted in successful, clinically feasible iodination methods for methoxsalen (1) or two other drug classes on which they were attempted -- benzodiazepines (12, X = H or CH_3, of interest for myocardial imaging) and diazoxide (13, a potential pancreas imaging agent). A new modification of the Wallach reaction was evaluated in this study in a series of simple aromatic compounds to develop a useful labeling



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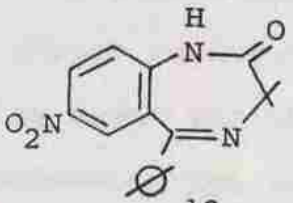

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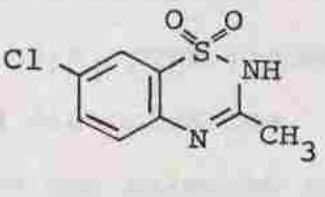

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methodology for these functionally more complex drug molecules. (See also footnotes 54, 55 for early examples).

Each of the drug molecules in question can easily be nitrated and reduced to form an amine. This route has been applied to attempts at radiolabeling these species via the Sandmeyer diazotization reaction. Methoxsalen has proven resistant to iodination in this fashion and benzodiazepine and diazoxide, although both yield iodinated products at mass level via the Sandmeyer reaction, undergo competing reactions at tracer level at the other nitrogen loci in their structures and yield either no useful radioiodinated product (as is the case with benzodiazepine) or miniscule amounts (as with diazoxide).

As Wallach first noted in 1886,[54] it is possible to trap a nascent diazonium ion as a stable, recrystallizable entity which can later be decomposed in the presence of a protic acid and a nucleophile to yield a substituted aromatic compound. Yields in the Wallach reaction for the formation of aromatic diazopiperidines (hereafter: triazenes) were described by Wallach as "quantitative," while yields from the chlorination and iodination reactions of the triazenes ranged from 40 to 60%.

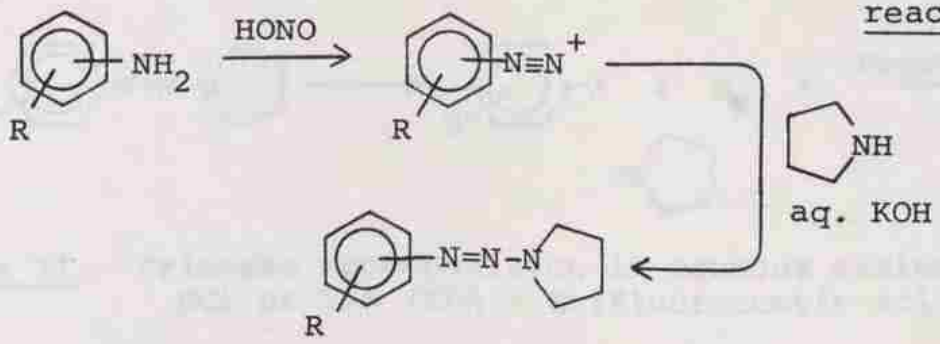
In the model series reported here, pyrrolidine was used as the trapping agent for the diazonium ion. The formation of the diazonium ion from the selected amines and its trapping with pyrrolidine was in every case facile. Results are summarized on Table I (p. 19).

Two aromatic amines yielded products which were not solids at room temperature and which were not characterized further: 2,4-dimethoxyaniline formed a deep red oil and m-toluidine a very low melting orange solid.

A. General reactivities of the triazenes

While functionality on the aryl ring did not seem to alter chemical yields of the triazenes, such functionality is crucial in determining the course of the acid-induced halogenations. The decomposition reaction of these model triazenes proved to be amazingly variable and dependent not only upon substituents on the triazene itself (Table II, p. 20) and solvent (Table III, p. 21), (Table IV, p. 22), but also on the acid used in the decomposition reaction (Table II, p.20, Table IV, p.22). Furthermore, conditions providing maximum yields at mass level did not necessarily provide highest yields when reactions were run at tracer level. In addition to iodinations and because of the clinical availability of suitable radiobromine and fluorine sources, brominations and fluorinations were also explored.

GENERAL REACTION FOR THE FORMATION OF TRIAZENES

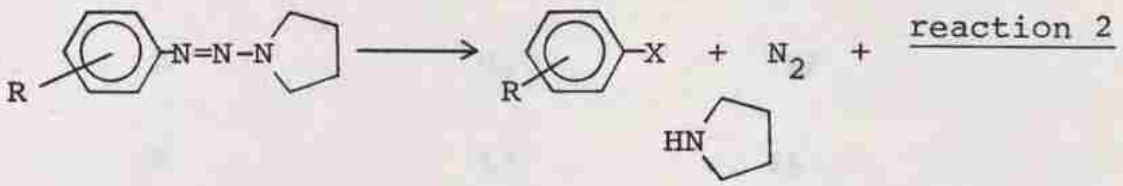


reaction 1

Table I: Yield of triazenes as a function of phenyl substituents

R	Yield (%)	m. p. (°C)
H	78	51-53
4-OCH_3	62	56-57
4-CH_3	67	78.5-80
3-NO_2	78	82.5-83
3-Cl-4-OCH_3	61	84.5-86
2-OCH_3-4-NO_2	64	111-112.5
2-OCH_3-5-NO_2	67	137.5-139
4-NO_2	65	161.5-163

GENERAL REACTION FOR THE DECOMPOSITION OF TRIAZENES IN THE PRESENCE OF NUCLEOPHILE (X = I^-, Br^-, F^-)



reaction 2

Table II: Triazene decomposition in aqueous medium with HCl or TFA (TFA = trifluoroacetic acid)

R	HCl	TFA
H	52	49
4-CH_3	19	51
4-OCH_3	39	63
4-NO_2	no rxn.	46
3-Cl-4-OCH_3	48	66

Conditions: aqueous medium at 5°C; time of reaction 0.5-1.0 hr; numbers recorded are percent iodinated product isolated; I^- source is NaI. The reaction of the 4-NO_2-phenyltriazene could not be effected with HCl even at reflux temperatures.

Table III: Radioiodinations run at tracer level as a function of solvent

R	H_2O	THF
H	13	96
4-CH_3	--	94
4-NO_2	44	86
4-OCH_3	12	92

Conditions: solvent and triazene reacted in vial charged with 50μCi of radio-NaI and heated at reflux for 48 hr; product identification by counting hot spot with same R_f as known cold material on TLC.

Table IV: Radioiodinations run at tracer level, variable acid and solvent (MS=methane sulfonic acid)

R	THF		70:30 THF:ØBr		ØBr		MeOH	
	TFA	MS	TFA	MS	TFA	MS	TFA	MS
H	---	---	---	---	---	---	---	---
4-CH_3	84	3	92	1	---	---	10	6
4-OCH_3	10	0	72	0	0*	0*	5**	2**

Conditions: solvent, triazene and acid sonicated for 0.5 hr; total reaction time 2 hr at room temperature; product identified by HPLC with radioactivity and UV-visible spectrophotometric detectors.

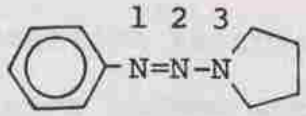
*unreacted triazene left in the mixture plus unreacted I-123 NaI.

**nearly all triazene reacted; I-123 NaI remains.

Regarding the observations indicated on Table IV (above), the total disappearance of the 4-OCH_3 triazene in methanol is not surprising in light of the observed half-lives toward hydrolytic decomposition for similar 1-aryl-3,3-dialkyltriazenes. Kolar and Preussman[56] determined the half-life of 1-(4-methoxyphenyl)-3,3-dimethyl triazene to be 12 minutes in aqueous medium at

37°C buffered at pH=7.0 in a phosphate buffer. That the 4-NO_2 compound remains unreacted corresponds to the observed half-life of its analogous dimethyl triazene of 160 days in the same buffered medium. The authors determined that the nature of the meta or para substituent on the aromatic moiety directly effected the rate of hydrolytic decomposition of the triazenes studied. They also observed a linear correlation between log k/k_H and the Hammett sigma substituent constant for the group meta or para to the triazene linkage.

The reaction constant (rho) thereby determined bore a negative sign, indicating that electron withdrawing groups retard the decomposition reaction. This information correlates with the proposed mechanism of triazene decomposition in which the first step of the decomposition is protonation at the nitrogen in the 3 position of the triazene linkage. The presence of an electron withdrawing group lowers the basicity of the triazene chain



and thereby lowers the rate of protonation.[56] In general, the relative reactivities of the triazene species observed at mass level with trifluoracetic acid paralleled this trend.

A C-13 chemical shift assignment for four model

triazenes prepared in this study (Tables V, VI, VII, p. 26, 27, 28 ; spectra in Appendix) further substantiates the relationship between the rate of hydrolytic decomposition and the net electron donating or withdrawing effects of the groups para to the triazene linkage. Literature calculations have indicated that of the three terms influencing the C-13 chemical shift the most--a paramagnetic shielding term, a diamagnetic shielding term, and an anisotropic term--the paramagnetic term normally dominates C-13 shifts.[57] The paramagnetic term arises from contributions to the ground state from higher electronic states (i.e. low level excited states) of a particular carbon atom within a molecule. Because of this term, the C-13 shift observed for a series of carbons in analogous structures may provide an index of reactivity for those carbons. In addition, C-13 shifts also show a particular sensitivity to hybridization and substituent electronegativity. Hence, in the case of the triazene decomposition reaction, where the electron density at the ring carbon attached to the triazene markedly influences the basicity of the linkage and therefore its reactivity, some correlation to C-13 chemical shift is expected. It has also been noted that effects of substituents on C-13 chemical shifts are generally additive and that a good correlation exists

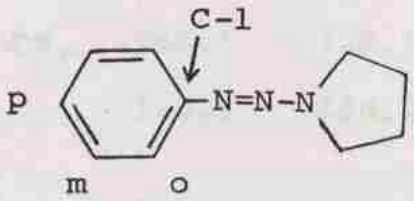
between the shifts of ring carbons para to substituent groups and calculated electron densities of those same carbons.[58]

Indeed, a comparison of the C-1 chemical shifts for the series of model triazenes shows an increasing downfield shift with decreasing electron donating ability on the part of the para substituent on the phenyl ring (Table VI, p. 27). It should also be noted that the triazene linkage itself has a substituent effect approximately like that of the methoxy group, causing a considerable downfield shift in the C-1 carbon, a larger upfield shift for the ortho carbon than for the para, and a slight downfield shift for the meta carbon (Table V,p. 26).

Assignment of the substituent chemical shifts due to the pyrrolidyl triazene moiety have been approximated by observing the deviation in shift shown in the phenyl triazene system when compared to that of benzene (Table V, p. 26). Observed shifts caused by the methyl, methoxy and nitro groups are available in the literature.[58] The calculation of chemical shifts from this data merely involves the summation of the shift effects for each substituent on the ring. In all cases, the agreement between the calculated shift in the triazenes and the experimentally observed shift is reasonable (Table VI, p. 27). Spin-spin coupling constants are also assigned

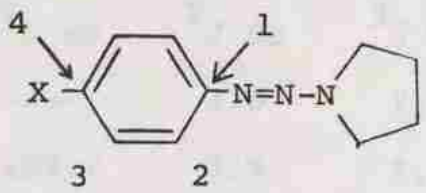
where possible on the basis of observed splittings in NOE spectra and literature precedent for mono- and di-substituted benzenes (Table VII, p. 28).

Table V: Calculated substituent chemical shifts for triazene moiety on phenyl ring.
$\Delta\delta$ in ppm from benzene; δ_c = 128.5 ppm



C-1	ortho	meta	para
+ 22.8	-8.3	+0.2	-3.8

Table VI: Calculated vs. observed chemical shifts for disubstituted triazenes; all numbers in ppm.



	X	C-1	C-2	C-3	C-4
calculated	$-CH_3$	148.4	120.1	129.4	133.6
observed		148.9	120.1	129.1	134.5
calculated	$-OCH_3$	143.6	121.2	114.3	156.1
observed		145.3	121.2	113.9	157.3
calculated	$-NO_2$	157.1	121.1	123.9	144.7
observed		156.3	120.3	124.9	144.3
observed	-H	151.3	120.2	128.7	124.7

Table VII: C-H coupling constants for triazenes

Phenyltriazene

CS	ppm	^{1}J Hz	^{2}J Hz	^{3}J Hz
C-1	151.2	----	2.0	7.1
C-2	120.2	159.7	0.9	7.1(J_{24}), 4.4(J_{26})
C-3	128.7	159.7	1.8	7.1
C-4	124.7	159.9	1.8	7.1

p-Methyoxyphenyltriazene

C-1	145.3	----	---complex multiplet---	
C-2	121.2	159.8	nm	6.2
C-3	113.9	159.8	nm	5.3
C-4	157.3	----	---complex multiplet---	

p-Methylphenyltriazene

C-1	148.9	----	1.4	8.5
C-2	120.1	158.4	nm	5.6
C-3	129.1	156.2	4.2	7.1
C-4	134.5	----	nm	7.1

p-Nitrophenyltriazene

C-1	156.3	----	---complex multiplet---	
C-2	120.3	166.0	4.9	---
C-3	124.9	168.5	4.9	---
C-4	144.3	----	---complex multiplet---	

"nm" = not measurable; insufficient resolution.

Triazenes as a general class of compounds are also of interest for their biological reactivity as well as for their synthetic utility. The 3,3-dimethyltriazenes have long been known to possess certain mutagenic, carcinogenic, antitumor and toxic properties,[59-62] but no clear mechanism is presently accepted to explain these activities. To assess the antibacterial activity of the four model compounds listed in Table IV and to see if their behavior in this assay yielded any insight into the chemical nature of their biological potency, radiometric susceptibility testing was applied to evaluate the sensitivity of six bacteria strains to the compounds.

The method applied is the so-called "bactec" technique, in which quantitative detection of C-14 CO_2 produced by the bacterial metabolism of C-14 labeled glucose indexes the changes in bacterial growth due to the presence of a substance to which the organism is susceptible. This assay has been successfully applied to the rapid screening of bacterial cultures for their resistance to antitubercular drugs,[63] as well as to the initial screening of antibacterial agents for efficacy[64] and bacterial strains for drug susceptibility.[64,66] The radiometric technique was applied in this case to evaluate structure-activity correlations between the electronic nature of the para-substituted moiety on the phenyl ring

and the ability of the triazenes to inhibit bacterial growth.

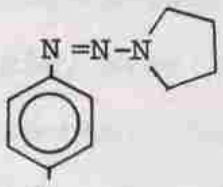
In a related study of biological activity not utilizing radiometric methods, Kolar, Fahrig and Vogel[67] evaluated the activity of substituted 3,3-dimethyl-1-phenyltriazenes to induce mutations in Drosphila melanogaster and in Saccharomyces cerevisiae. Triazenes rapidly hydrolyzed at physiological conditions were the most active mutagens in the yeast, whereas the hydrolytically stable triazenes were the most active in Drosphila. From this it was interpreted that the phenyldiazonium cation was most probably responsible for the mutagenic activity in the yeast, but the biological metabolites from the hydroxylation of the $-NCH_3$ were essential for the action in Drosphila. This points to the possibility of two different mechanisms governing the in vivo reactivity of the triazne class: the cleavage of the 2N-3N bond in labile compounds to liberate a reactive aryldiazonium cation (a step accelerated by electron donating groups and analogous to the proposed decomposition mechanism in vitro), or enzymatic activation of the more stable compounds involving oxidative dealkylation which yields alkylating agents upon heterolysis of the metabolite (a process accelerated by the presence of electron withdrawing groups).

Hathaway et al.[60] demonstrated that quantitative

structure-activity relationships derived from triazene behavior with L 1210 leukemia in mice showed electron donating groups on the aryl ring increasing the antitumor potency of the compounds. The authors noted that this type of alteration also increased the instability of the triazene.

The four model arylpyrrolidyl triazenes were tested for their ability to inhibit the bacterial growth of three gram-positive and three gram-negative strains of bacteria (listed on Table VIII). Percent inhibition of bacterial growth as a function of triazene concentration

Table VIII: Triazenes and bacteria strains evaluated

compounds	GRAM-POSITIVE BACTERIA
	Staphylococcus aureus Streptococcus group D Enterobacter Micrococcus luteus
	GRAM-NEGATIVE BACTERIA
X= $-NO_2$, -H $-CH_3$, $-OCH_3$	Escherichia coli Pseudomonas aeruginosa Klebsiella pneumonia

in culture medium was plotted as a function of time. Those triazenes which yielded growth inhibition curves that demonstrated susceptibility of the organism to the compound were then further examined for linear correlations of inhibition with Hammett σ_{ρ}^{+} values.

An interesting qualitative observation follows from simple visual inspection of the growth inhibition curves (Graphs I-VI; p. 45-50). For the p-methoxyphenyltriazene all of the bacteria (except M. luteus) show very similar distribution of data points indicating dose-dependent responses (higher dose corresponding to greater percent inhibition), and all curves for a single strain peak at approximately the same time: E. coli (Graph I, p. 45) and K. pneumonia (Graph III, p. 47) at three hours after inoculation; P. aeruginosa (Graph II, p. 46) and S. aureus (Graph VI, p. 50) at 8 hr; S. group D Entero. (Graph V, p. 49) between 1 and 3 hr. As one progresses through the series of compounds from the p-methoxy- to the p-nitrophenyltriazene, the curves lose the regularity of their appearance and, in the case of the gram-negative bacteria, even lose the dose-dependency and consistency of response. Only S. aureus (Graphs IV, VII--IX, p. 48, 51-53) demonstrates logical dose-dependency and consistency of response for the compound series tested, and even in this case the p-nitrophenyltriazene yielded a growth inhibition curve of somewhat erratic nature (Graph IX, p. 53).

This pattern can be interpreted as reflecting the rate of decomposition of the triazenes in solution and the susceptibility of the exposed strain to the phenyl-

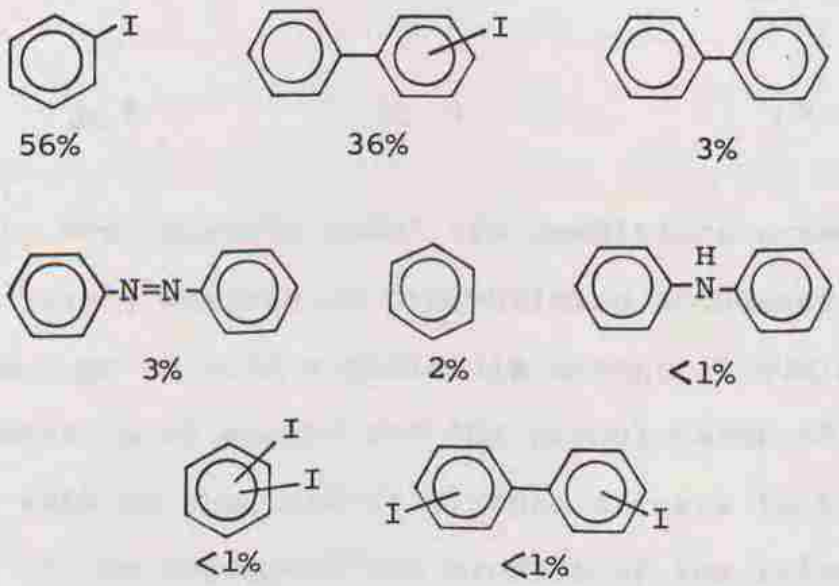
diazonium cation. In addition, gram-negative bacteria have been reported[68] to show poorer correlations in other structure-activity studies done in bacteria cultures due to the more complex nature of their cell walls. This factor may also be expressed here in the more erratic behavior of the gram-negative in response to exposure to the triazenes. Linear correlations are observed for percent inhibition and Hammett σ_{ρ}^{+} values in S. aureus cultures at four hours at the 100 µg/ml dose level and at six hours for both the 25 µg/ml and 50 µg/ml doses (Graphs X--XII, p.54-56). The slope of the lines indicates that electron withdrawing groups result in decreasing inhibition of growth. This additional factor substantiates the idea that it is the presence of the phenyldiazonium cation that is accounting for the observed inhibition. This is similar to the activity of the triazenes in yeast, where the phenyldiazonium cation was implicated due to the greater reactivity of the hydrolytically unstable triazenes, as opposed to the situation in Drosphila, in which the triazenes needed enzymatic modification to exert their biological effect.

B. Specific evaluations of halogenation reactions

In an effort to gain further insight into the nature of the triazene decomposition reaction, the iodination of the phenyltriazene was carried out in aqueous medium and

and the crude product mixture analyzed by GC/MS. Several interesting products in addition to the expected iodobenzene were noted (see Table IX below). In light of the appearance of products, possibly the result of coupling

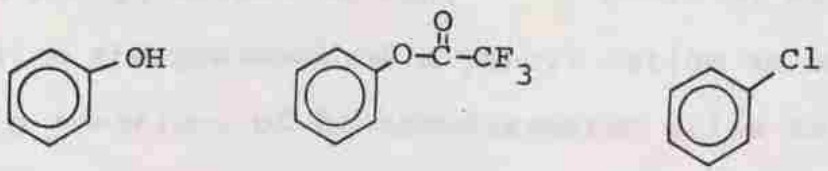
Table IX: GC/MS analysis of crude product mixture from iodination reaction in aqueous medium



reactions or other dimeric processes involving phenylcarbonium ion reactions (such as biphenyl, iodobiphenyl, benzene), as well as products obviously involving cleavage of the triazene linkage (N-phenylaniline, azobenzene), a second GC/MS experiment was run. To probe the reactivity of a triazene without any intentional nucleophile present, the pyrrolidyl triazene of benzene was suspended in water and treated with trifluoroacetic acid. After ten minutes, the organic fraction was extracted with chloroform and

analyzed by GC/MS (Table X below). This protocol may

Table X: GC/MS analysis of triazene decomposition in aqueous medium with TFA in the absence of a nucleophile



86 % 12 % 2 %

actually most closely model the conditions present at tracer level, wherein an overwhelming abundance of triazene is reacted with a miniscule amount of nucleophile. The abundance of phenol and the phenol ester of trifluoroacetic acid in the product mixture attests to the interaction of the decomposition product of the triazene with the water solvent in the absence of an intentional nucleophile. This type of solvent-triazene interaction would be much less feasible in THF; therefore, one would expect the higher yields of iodinated product at tracer level in THF that are shown on Table III (p. 21).

The presence of coupling products in Table IX (p. 34) and phenol and phenol derivatives in Table X (above) is in accordance with the proposed hydrolytic (non-enzymatic, in vitro) mechanism for triazene decomposition. After

the protonation of the nitrogen in the triazene linkage in aqueous or alcoholic medium, the mechanism is believed to proceed through an α-hydroxylation followed by subsequent breakdown of the molecule resulting in the generation of a phenyl carbonium ion.[56,69] Swain et al.[69] have demonstrated the presence of a phenyl cation as an intermediate in reactions of benzenediazonium salts in solutions in the absence of the strong bases and reducing agents but in the presence of weakly basic nucleophiles like the halides and water. Phenyl carbonium ions, if generated in the triazene decompositions, could easily account for the coupling products as well as the phenols.

Brominations of triazenes were also attempted at mass level. The reactions were performed with trifluoroacetic acid and monitored by TLC until all starting triazene had disappeared or until no further qualitative changes were noted. The resultant mixtures of reaction products were analyzed by GLPC with retention times for known compounds matched with those of peaks in the chromatograms. Results for mass level brominations are summarized on Table XI (p. 37). Water is a more suitable solvent than either THF or bromobenzene both in terms of absolute yield of brominated product and in number of by-products produced. The variation in yield as a function of bromide ion source (KBr giving

consistently higher yields than t-butyl ammonium bromide) may perhaps reflect the different dissociations of both species in each solvent.

Table XI: Brominations of triazenes at mass level:
acid = TFA
% brominated product (number of other peaks--not solvent)

	Br^- (KBr)		Br^- (Bu_4NBr)	
triazene	Ø-T	p-CH_3-Ø-T	Ø-T	p-CH_3-Ø-T
solvent				
THF	12% (5)	5% (13)	2% (4)	4% (5)
ØBr	------	29% (3)	------	5% (5)
H_2O	83% (1)	94% (2)	49% (6)	22% (3)

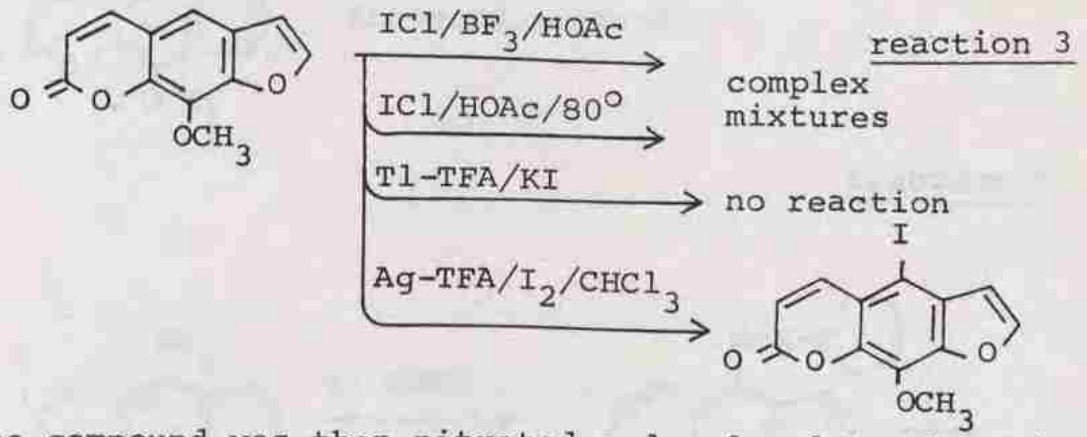
Fluorinations of triazenes were also attempted but without success. Triazene decompositions with TFA and F^- (both from anhydrous KF and KF·HF), aqueous HF, TFA/NaF or KF/18-crown-6, all were unsuccessful, yielding extraordinarily complex product mixtures. Three solvent systems were evaluated: water, THF and benzene/18-crown-6; evidence of the desired aryl fluoride product could only be found in the THF product mixture. Water and the benzene/18-crown-6 system showed no trace of fluorinated product. Because of this, the fluorinations were not attempted at tracer level, the general feeling being that if one does not observe any product at mass level with an

abundance of fluoride ion present, there is little hope for producing any product at tracer level.

No satisfying rationale may be developed at present to account for the sensitivity of this reaction to the variables examined. One generality is that the reactivity differences among the triazenes parallel the electrophilicity differences at the aryl carbon at the site of the reaction. Undoubtedly the molecular environment of the triazene in a medium where the concentration of nucleophile is miniscule, as it is in tracer experiments, is decidedly different from those reactions run at mass level. The requirements for the transition state for an acid-induced iodination may be simply statistically less likely to occur on a molecular level when the concentration of nucleophile is low. Further a unique balance must be struck for each molecule regarding the proper polarity of the solvent for maximum yield in halogenations. The medium must be polar enough to allow the product-directed pathway to be favored, but not so polar that the solvent participates in the decomposition as a nucleophile or solvates the intentional nucleophile so extensively that the halide ion is not free to react with the intermediates in the triazene decomposition. Tracer experiments clearly show that trifluoroacetic acid is superior to methane sulfonic acid in effecting iodinations at that level.

C. Iodinations of Drug Molecules

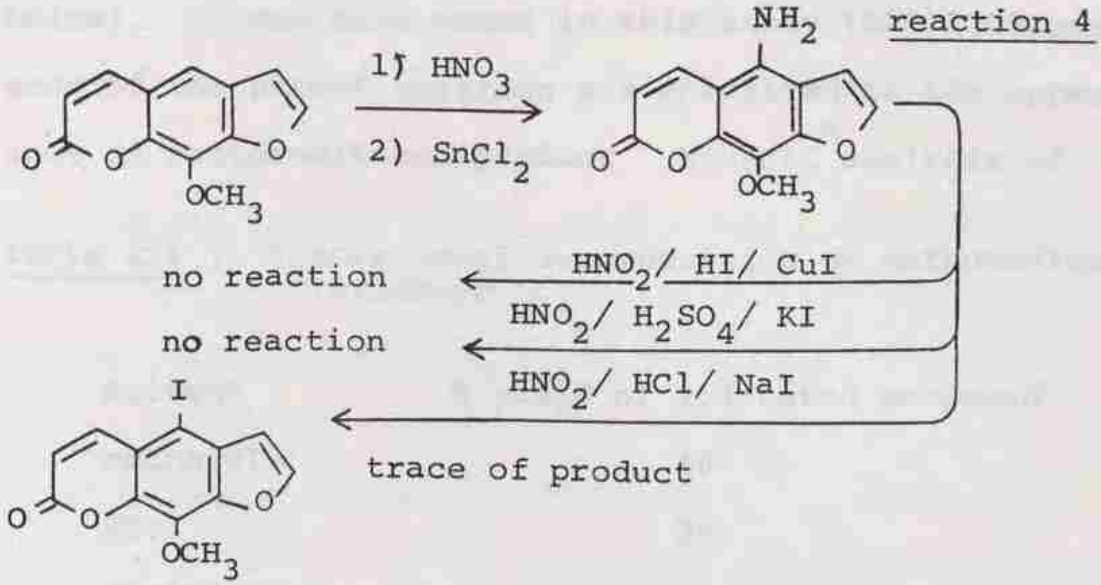
When the iodination via the triazene decomposition method was applied to the drug molecules of interest, variability of yields with conditions was again noted. In the first case, methoxsalen (1) had previously resisted attempts at iodinations by direct methods with $ICl/BF_3/HOAc$, with $ICl/HOAc/80^oC$, and by Tl-TFA/KI [70] (summarized in reaction 3).



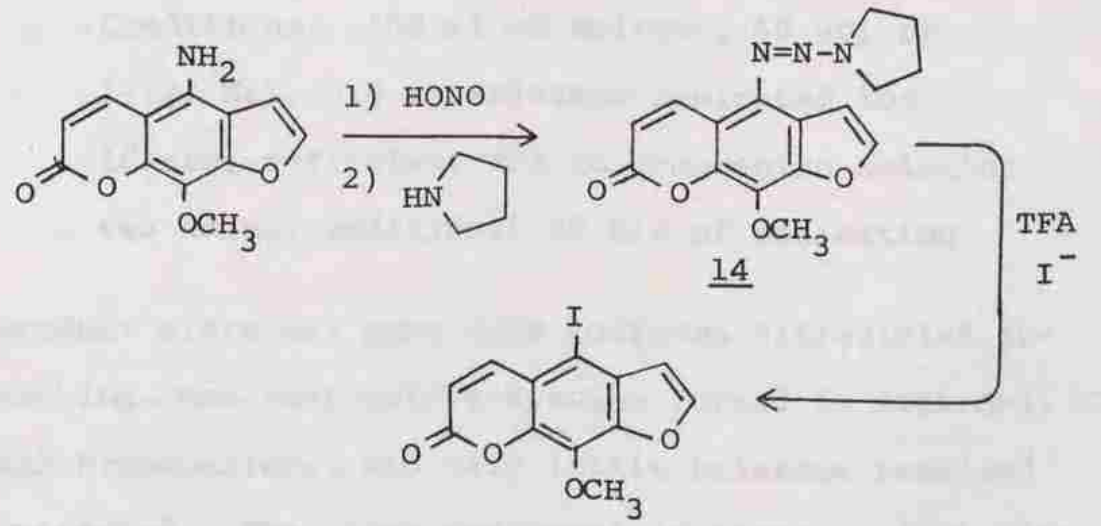
reaction 3

The compound was then nitrated and reduced to the amine;[36] the resultant 4-aminomethoxsalen resisted indirect iodination attempts with $HNO_2/HI/CuI$ and $HNO_2H_2SO_4/KI$, and only a trace of product was detected with $HNO_2/HCl/NaI$ (summarized in reaction 4). The compound 4-iodomethoxsalen was finally synthesized by treatment of methoxsalen with iodine and silver trifluoroacetate in chloroform;[58] (reaction 3). On the other hand, the triazene of 4-aminomethoxsalen (14) was easily prepared and the decomposition reaction run at mass level in water resulted in an

isolated yield of 24% of desired product (reaction 5).



reaction 5



A related compound, 2,3-dihydromethoxsalen, also formed a pyrrolidyl triazene with ease. Its decomposition with TFA in the presence of iodide ion in aqueous medium resulted in 50% yield of iodinated product.

The tracer level decomposition of methoxsalen

triazene in a variety of solvents showed a different variability than did the model compounds (Table XII below). It was also noted in this study that disappearance of the parent triazene was unrelated to the appearance of radioiodinated product. By HPLC analysis of

Table XII : Tracer level decomposition of methoxsalen triazene

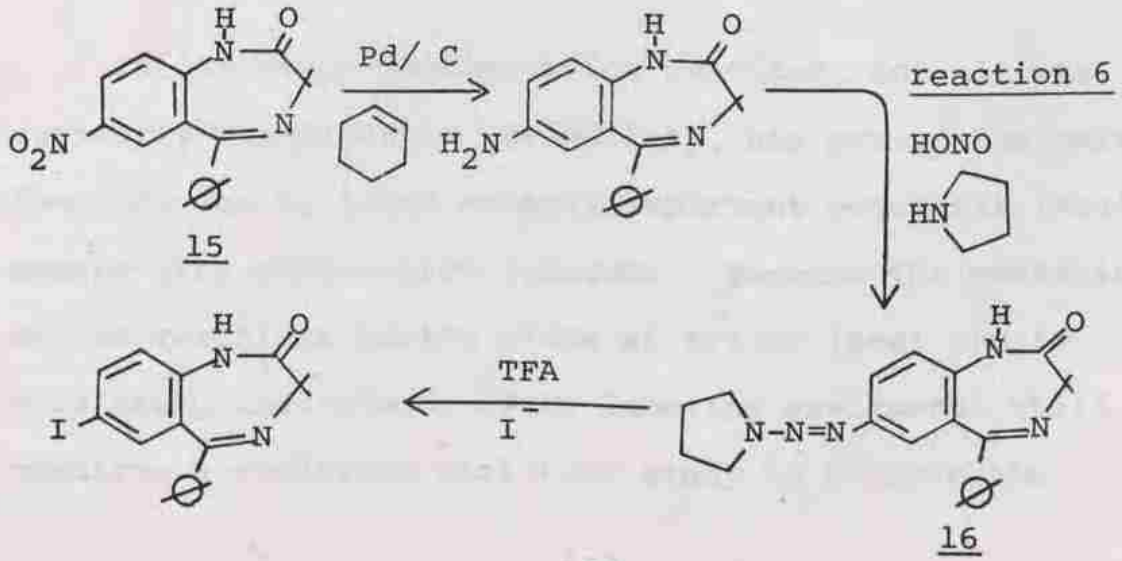
solvent	% yield of iodinated compound
methanol	40
ØBr	36
pyridine	12
THF	2

Conditions: 100 µl of solvent, 50 µCi of I-123 NaI, 5.0 mg triazene sonicated for 15 min; sufficient TFA to homogenize solution was added; additional 30 min of sonication.

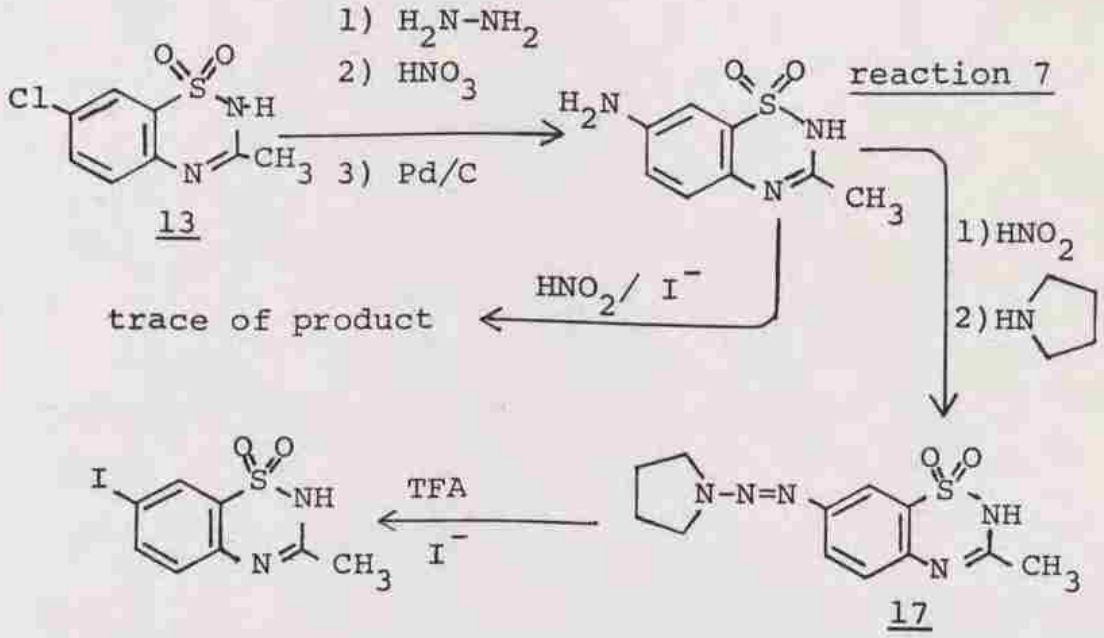
product mixtures, more than fourteen ultraviolet absorbing, non-radioactive species formed in methanol, THF and bromobenzene, and very little triazene remained unreacted. The major component in these product mixtures was methoxsalen itself. In the basic solvent pyridine, the small amount of acid added did not render the medium acidic, most of the triazene remained unreacted, little I-123 iodomethoxsalen was formed, and

only a trace of methoxsalen itself was generated.[52] Despite all of these disadvantages, pyridine was still a better solvent in terms of yield of desired product than was THF, which oddly enough was one of the better solvents for the model compounds.

In the case of the attempted radioiodinations of the benzodiazepine derivatives, nitrazepam (15)was reduced by the selective rapid transfer reduction (explained in detail in the next section) to the amine. The material reacted in the Sandmeyer iodination procedure at mass level to yield 51% of the desired 7-iodobenzodiazepine (reaction 6). However, no yield at all of iodinated compound was realized at tracer level when a Sandmeyer reaction was attempted. The amino-benzodiazepine reacted smoothly to form a triazene (16) which could then be decomposed under tracer level conditions to yield 42% of desired radiolabeled product.

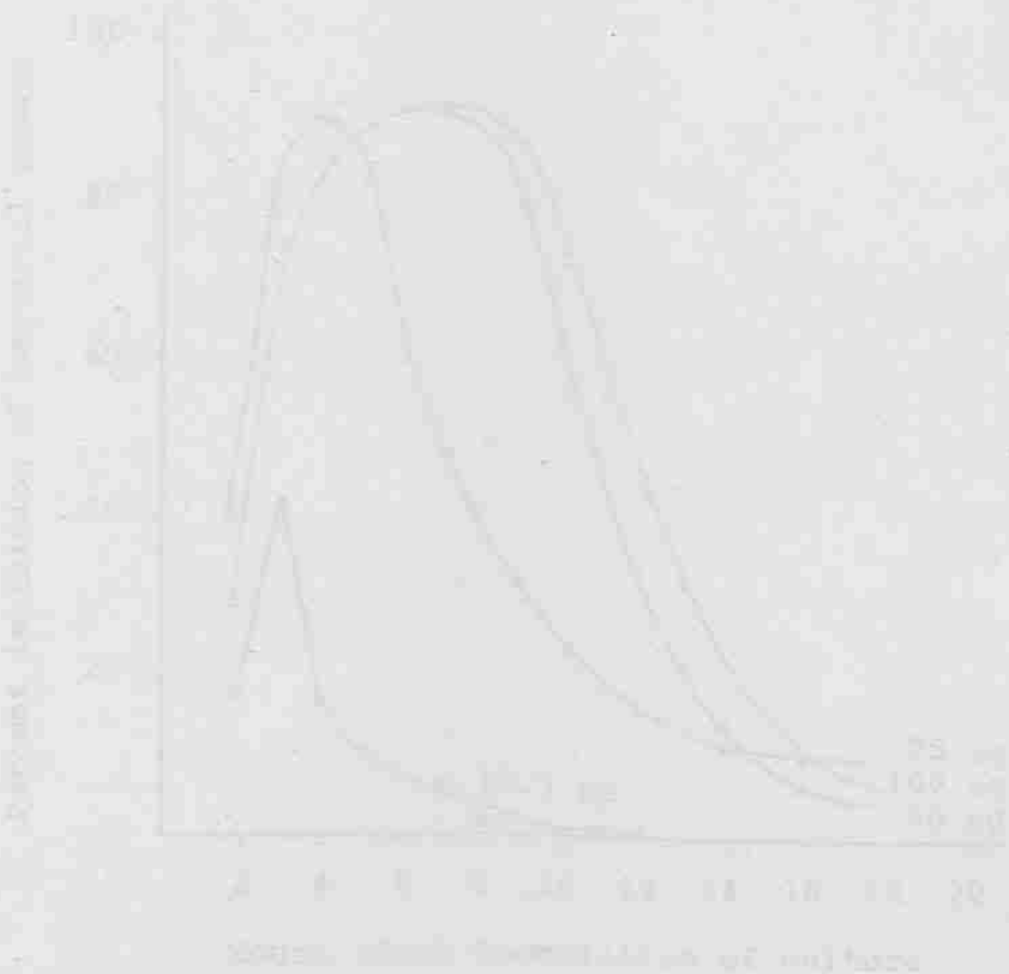


Similarly, after dechlorination, nitration and reduction, diazoxide (13) gave excellent yields of its 7-iodo-analog at mass level via the Sandmeyer reaction[71] but resulted in a miniscule quantity of radiolabeled product at tracer level. The triazene (17), nevertheless, could be decomposed at tracer level to yield 16% of the desired radioiodinated diazoxide (reaction 7, below).

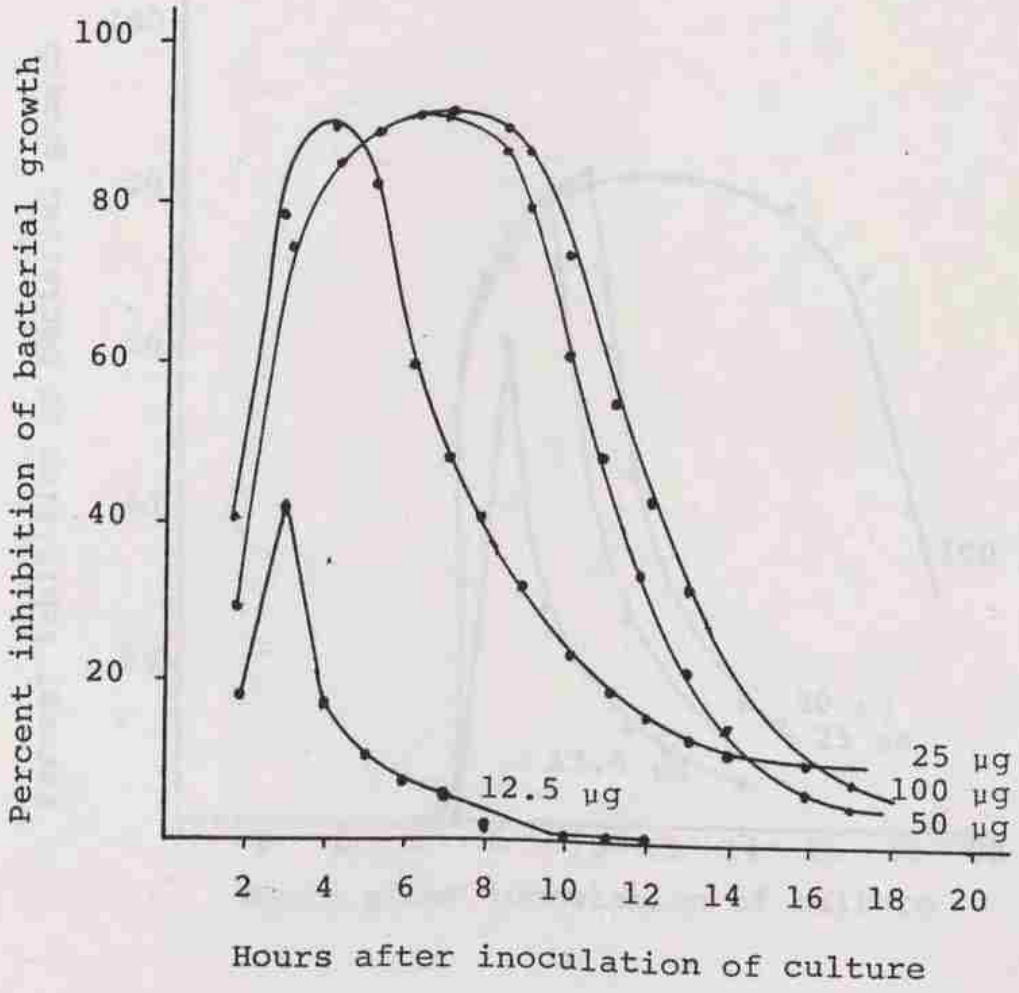


The triazene decomposition reaction, for all its currently inexplicable variability, has proved the only feasible way to label several important potential imaging agents with radioactive halogens. Because the mechanism of the reactions taking place at tracer level are incompletely understood, every labeling assignment still requires a condition variation study to achieve the

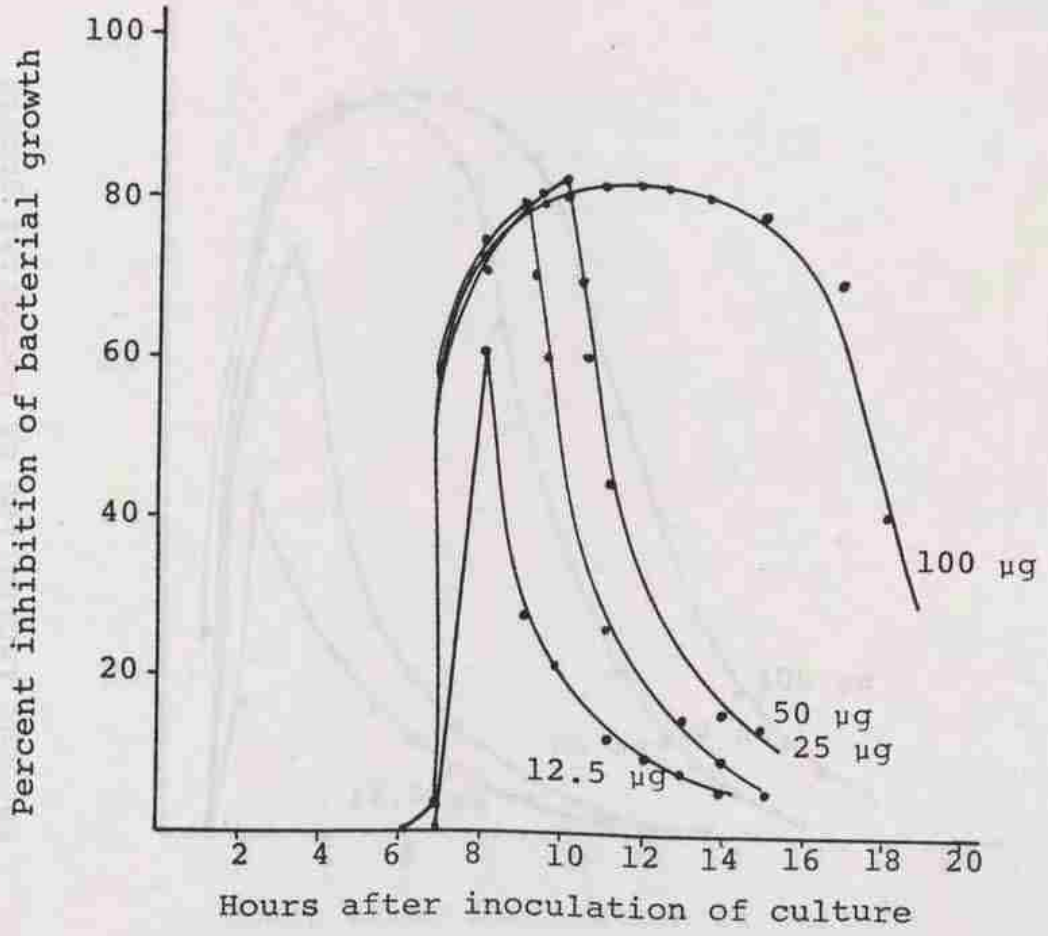
proper set of variables to insure maximum yield of labeled product. Despite this, the method has proved useful and reliable.



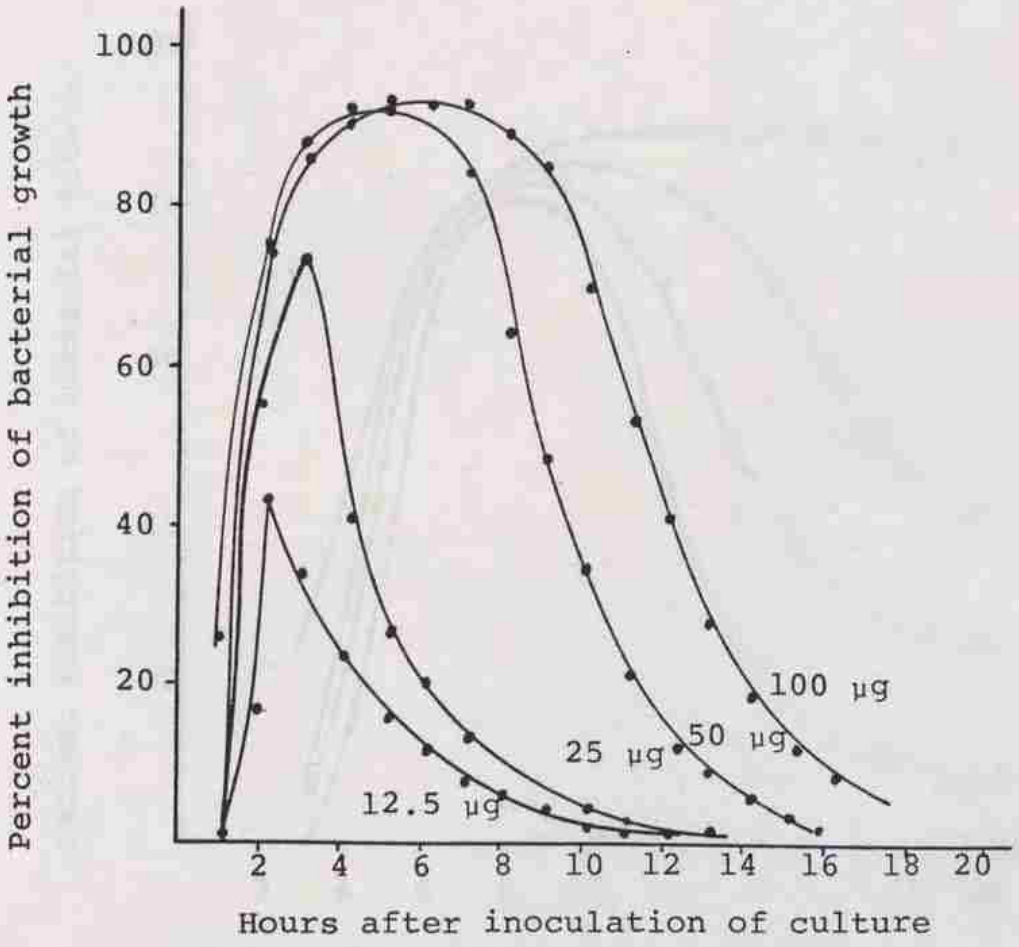
Graph I: Growth inhibition curves
E. coli by p-methoxyphenyltriazene



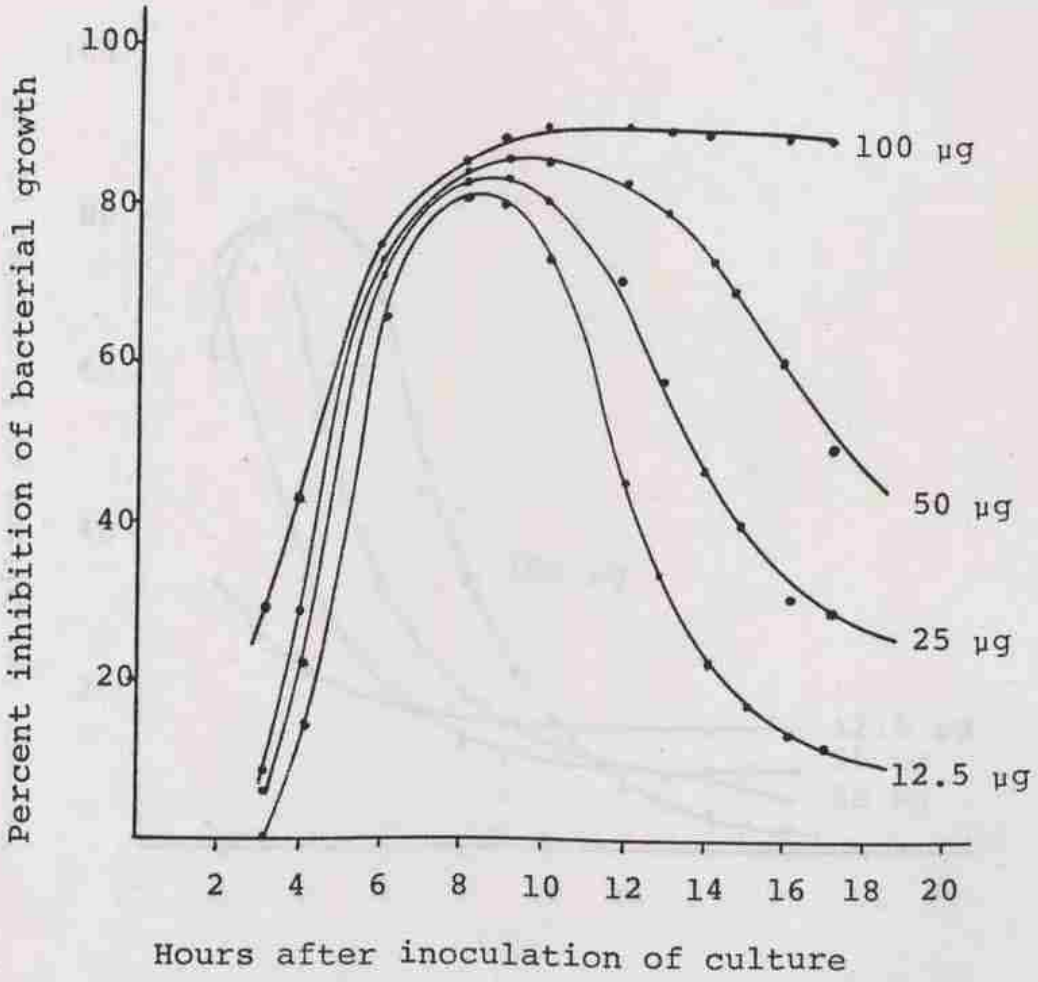
Graph II: Growth inhibition curves
P. aeruginosa by p-methoxyphenyltriazene



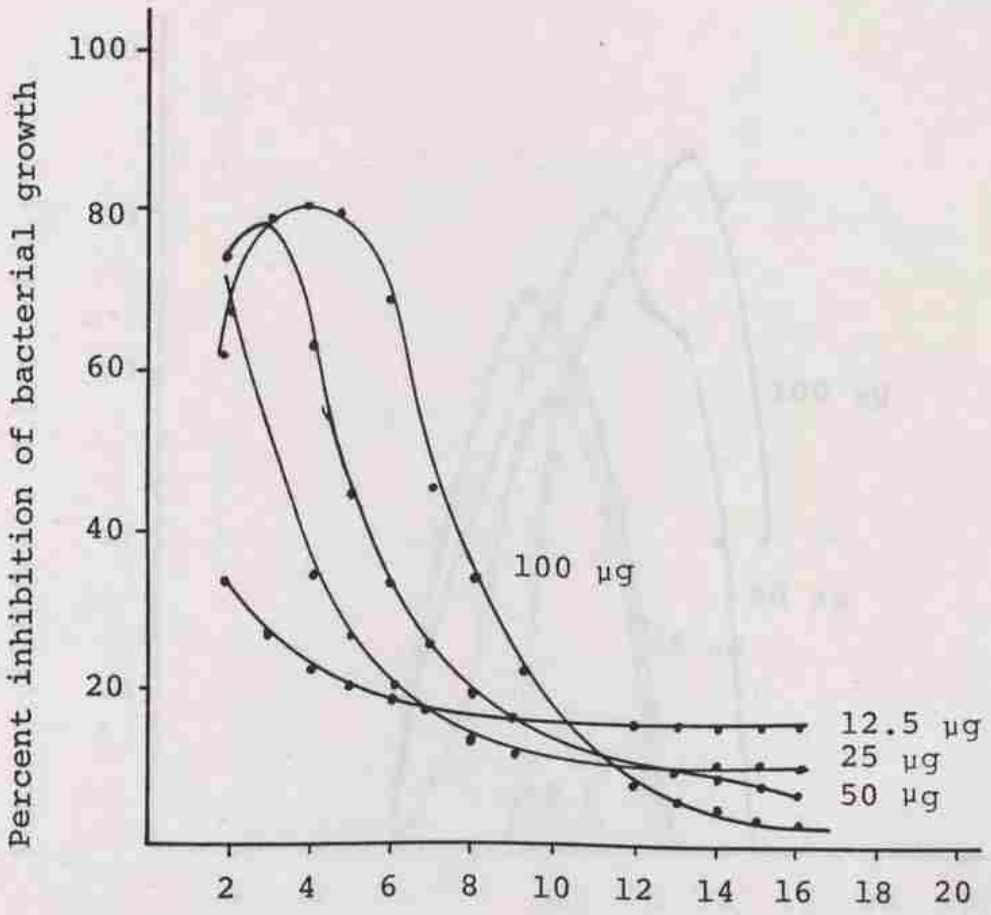
Graph III: Growth inhibition curves
K. pneumonia by p-methoxyphenyltriazene



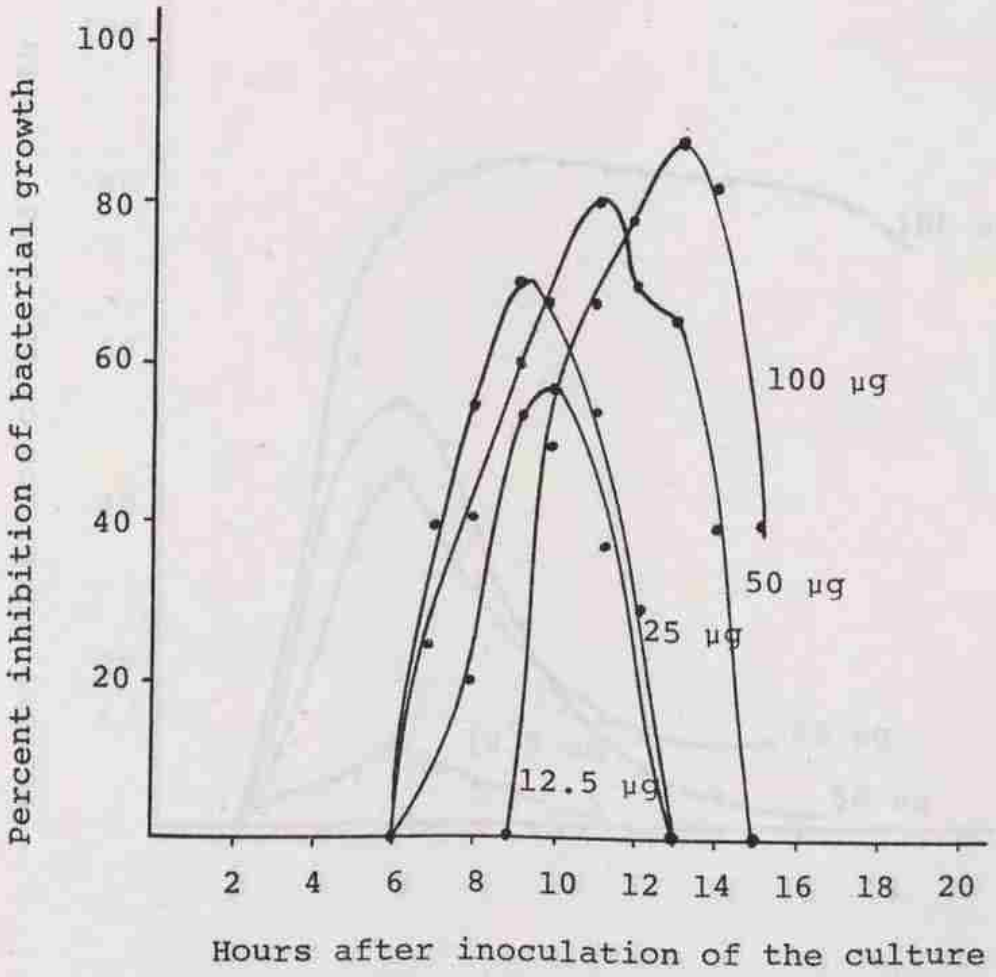
Graph IV: Growth inhibition curves
S. aureus by p-methoxyphenyltriazene



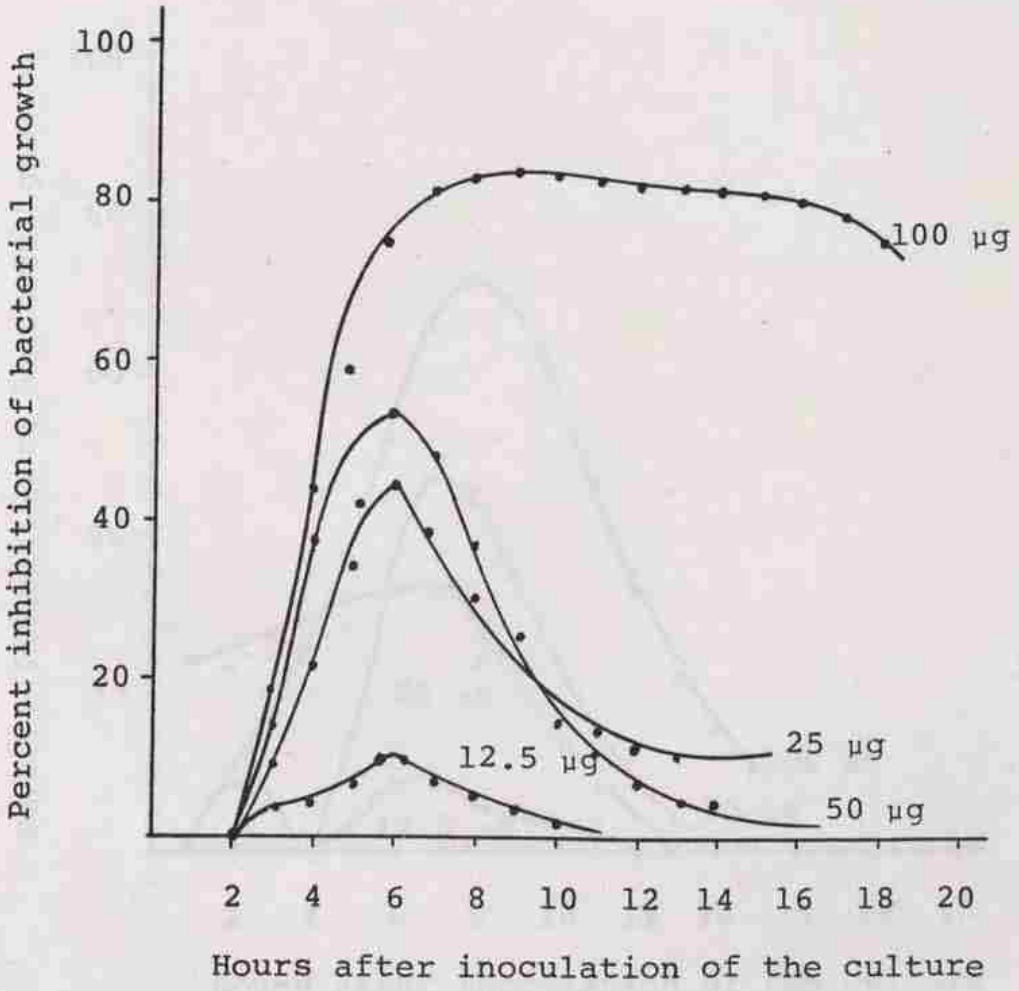
Graph V: Growth inhibition curves
S. group D Enterobacter by p-methoxyphenyl-triazene



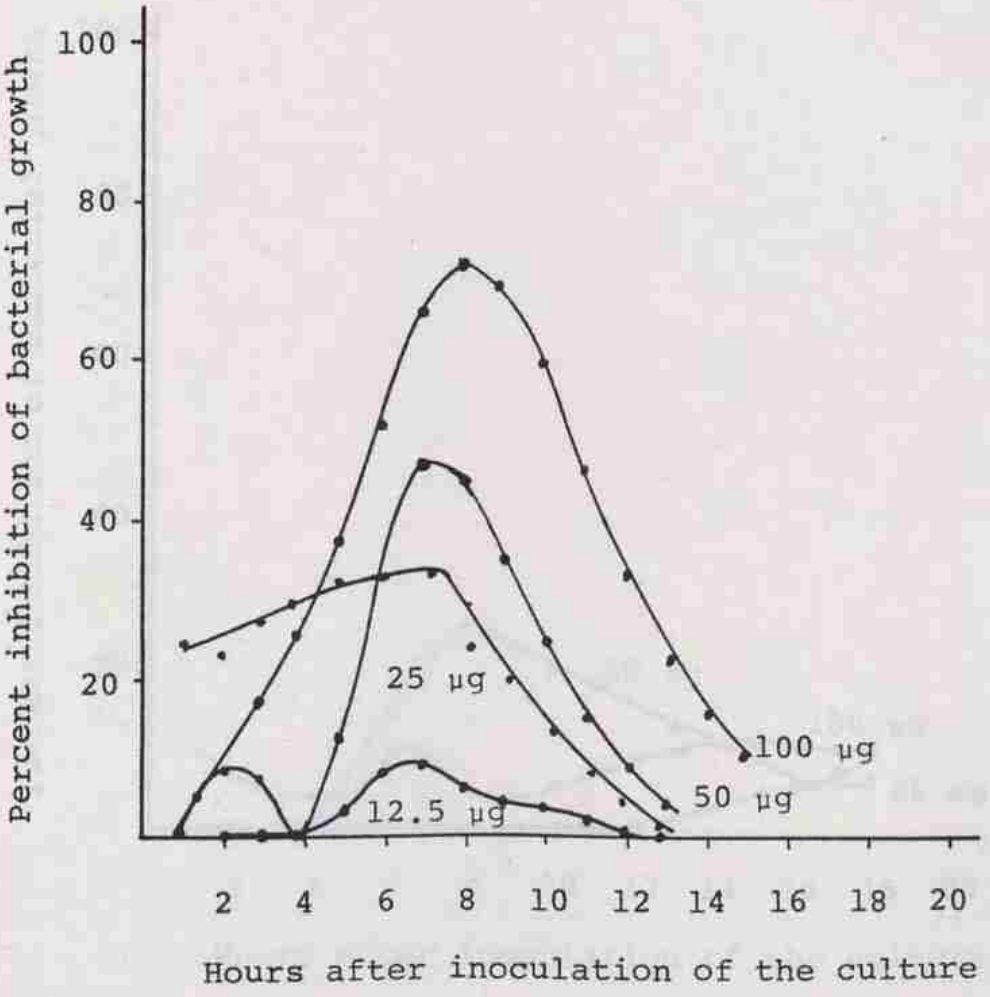
Graph VI: Growth inhibition curves
M. luteus by p-methoxyphenyltriazene



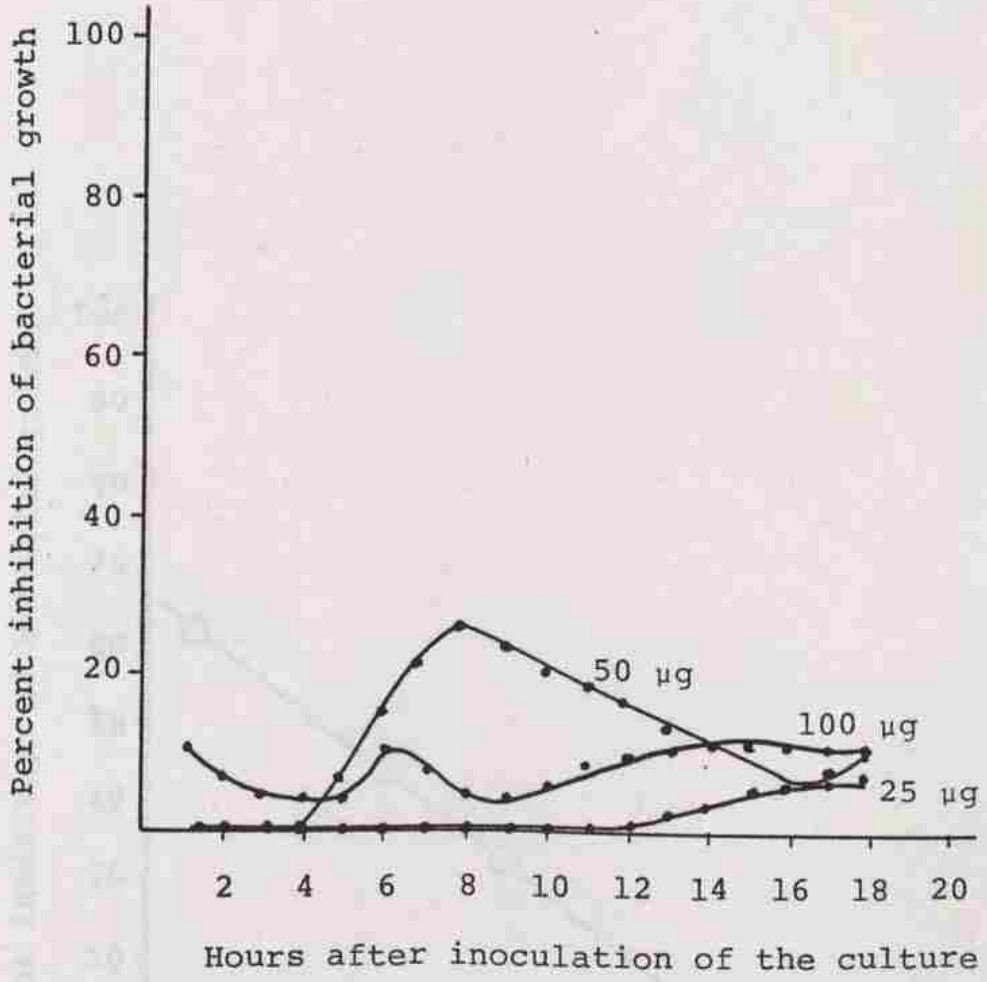
Graph VII: Growth inhibition curves
S. aureus by p-methylphenyltriazene



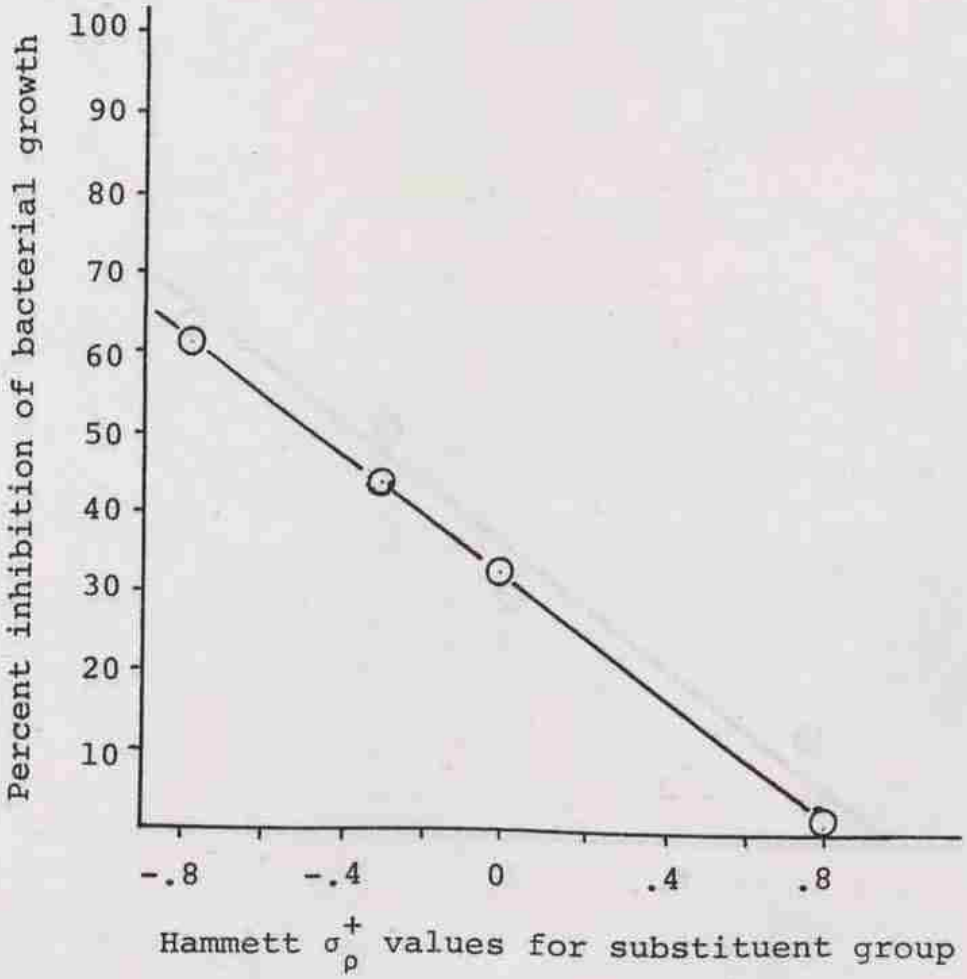
Graph VIII: Growth inhibition curves
S. aureus by phenyltriazene



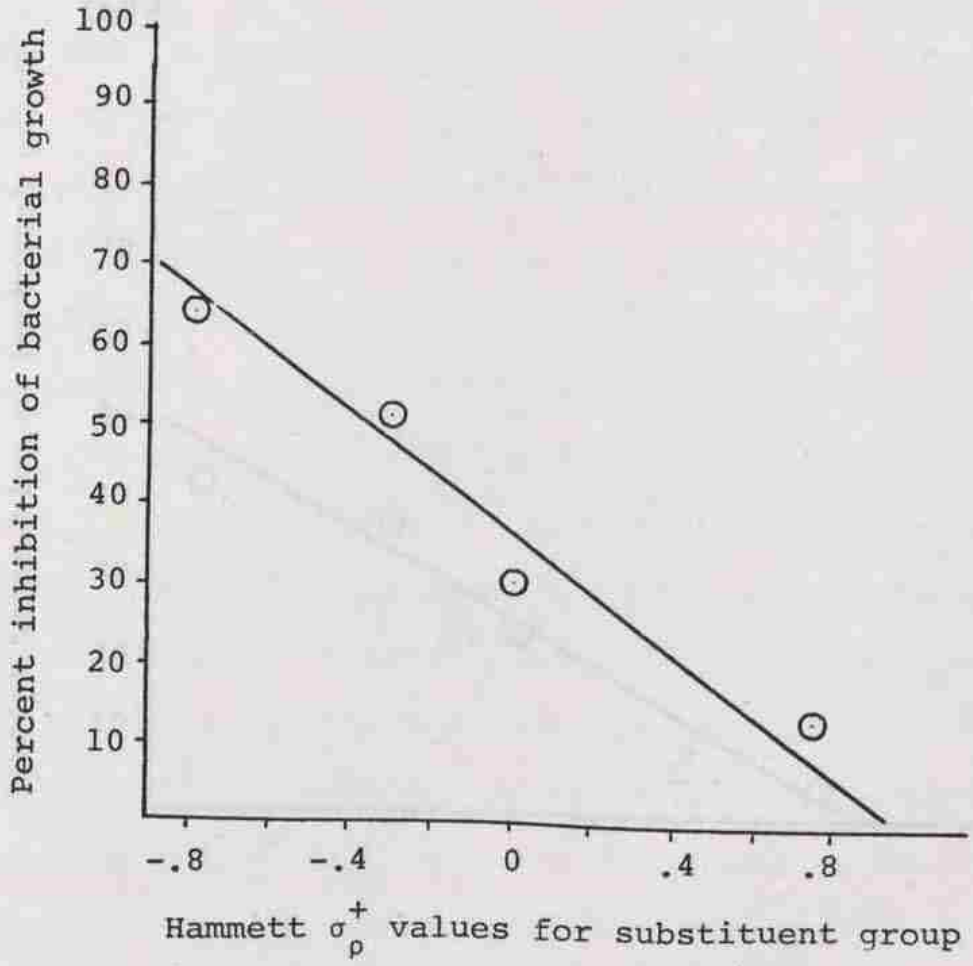
Graph IX: Growth inhibition curves
S. aureus by p-nitrophenyltriazene



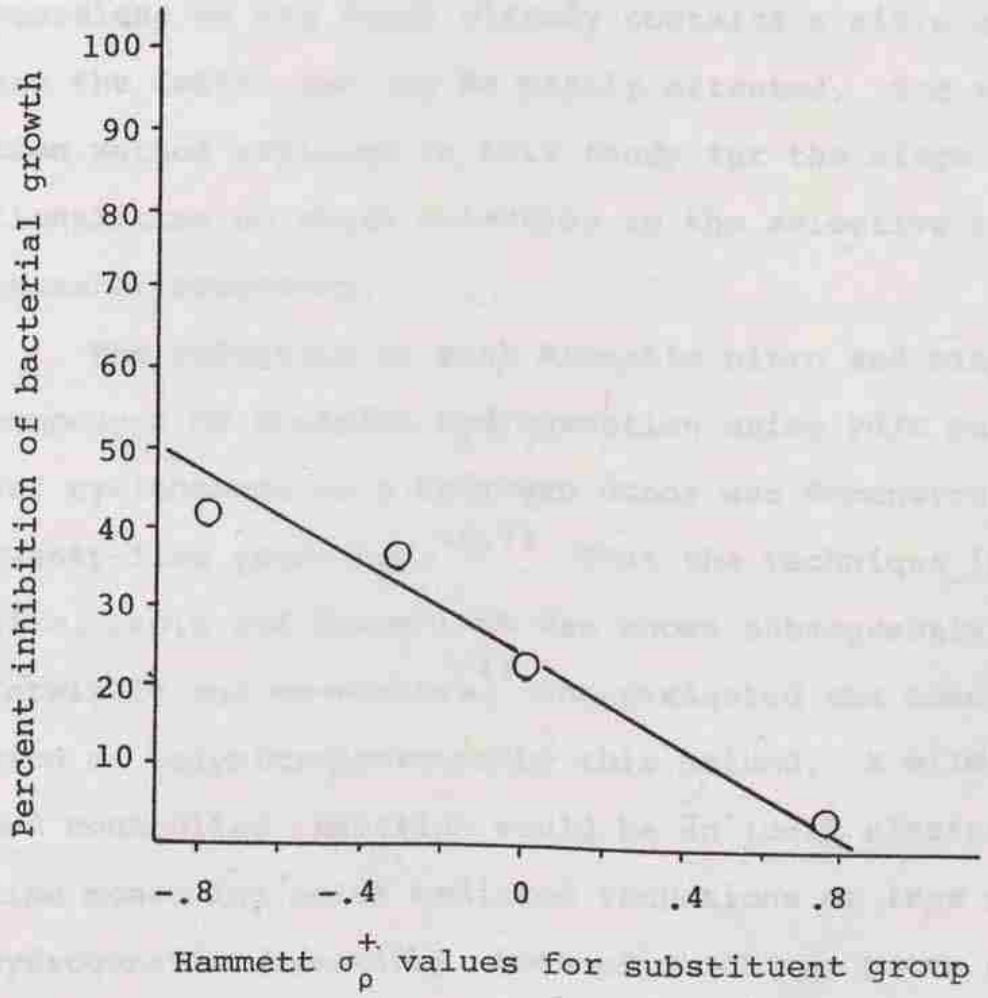
Graph X: Percent inhibition vs. Hammett σ_ρ^+
S. aureus cultures; 25 µg triazene;
6 hr post inoculation



Graph XI: Percent inhibition vs. Hammett σ_p^+
S. aureus cultures; 50 µg triazene;
6 hr post inoculation



Graph XII: Percent inhibition vs. Hammett σ_ρ^+
S. aureus culture; 100 µg triazene;
4 hr post inoculation



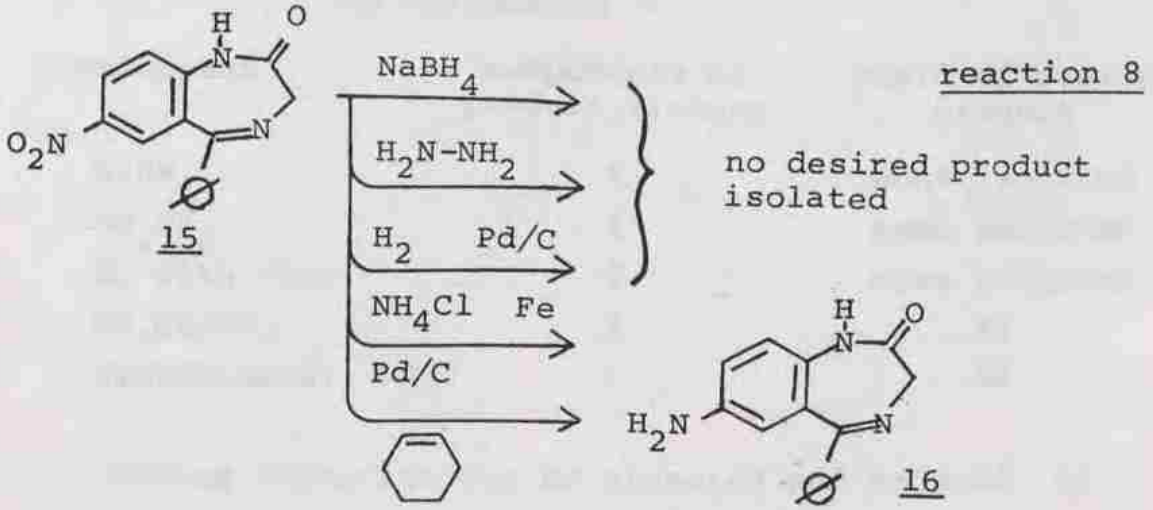
II. Selective Rapid Transfer Reduction and Nitrazepam, Diazoxide and Psoralen Derivatives

Radiolabeling of a compound by the triazene decomposition reaction obviously requires the presence of an amine group on the aryl portion of the molecule. If no amine is present in the compound of interest, the easiest way to introduce that functionality is by nitrating an aromatic moiety within the molecule and then reducing the nitro group to an amine. In the case of the three drug classes listed -- nitrazepam, diazoxide and psoralens -- the first already contains a nitro group and the latter two may be easily nitrated. The reduction method explored in this study for the nitro functionalities on these molecules is the selective rapid transfer reduction.

The reduction of both aromatic nitro and ethylenic compounds by transfer hydrogenation using Pd/C catalyst and cyclohexene as a hydrogen donor was demonstrated over twenty-five years ago.[42,43] That the technique is selective, rapid and convenient was shown subsequently by Entwistle and co-workers[44] who evaluated the mono-reduction of polynitrobenzenes by this method. A mild, swift and controlled reduction would be an ideal alternative to time consuming metal mediated reductions or less selective hydrogenation reactions, both of which may prove problematical in functionally complex drug molecules.

The general procedure in this method involves the dissolution of the substance to be reduced in rapidly refluxing ethanol to which a five- or six-fold stoichiometric excess of cyclohexene has been added. One-half the stoichiometric amount of 10% Pd/C catalyst is then suspended in the medium, and in ten to thirty minutes the reduction of a nitro to an amine is complete. The cyclohexene functions as the hydrogen donor in this reaction and is oxidized to benzene in the process. Although the relatively large amount of Pd/C used may not seem truly catalytic, the same portion of catalyst may be re-used six to eight times without demonstrating any decrease in its efficiency.[44]

The first compound herein on which the selective rapid transfer reduction was applied was nitrazepam (15, reaction 8). Four other reductant systems yielded product mixtures or only small quantities of the desired reduced



reaction 8

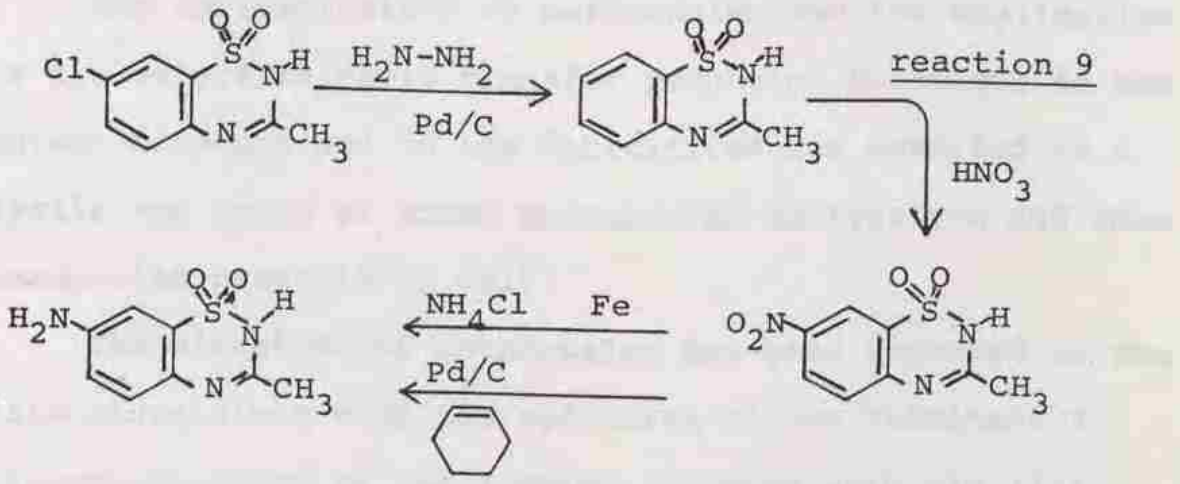
compound (summarized in Table XIII, below). Reduction by sodium borohydride in methanol resulted in a four component mixture that was not successfully fractionated by simple recrystallizations. Hydrazine hydrate resulted in an incomplete reaction (a spot due to starting material is observed in TLC) and the isolation of a glassy solid of unknown composition. Hydrogenation yielded a two component mixture. One component showed the reduction of the -C-N- bond as well as the nitro group; the other component showed a nitro group apparently reduced only as far as an hydroxylamine functionality. Reduction by ammonium chloride and powdered iron yielded some of the desired product, but after a 3.5 hour refluxing period, only 42% of the 7-amino compound was obtained. The selective rapid transfer reduction, however, yielded 95% of the amino compound after only a 30 minute reflux period.

Table XIII: Results of applying various reducing systems to nitrazepam

reductant	components of product mixture	yield of desired product
$NaBH_4$	4	none isolated
NH_2NH_2	2	none isolated
H_2 with Pd/C	2	none isolated
NH_4Cl/Fe	1	42
cyclohexene: Pd/C	1	95

Before diazoxide can be nitrated and reduced, it first must be dechlorinated. This was accomplished by

reaction with hydrazine hydrate and Pd/C catalyst in 95% ethanol. The dechlorinated material was then nitrated, after which two reductant systems were evaluated for suitability (reaction 9). The literature preparation for the



reduction of 7-nitrodiazoxide uses ammonium chloride and iron powder in methanol/water solution. This protocol yielded only 22% of the desired product. Literature precedent regarding the selective rapid transfer reduction stated that nitro-heterocyclic systems containing sulfur (specifically 2-nitrothiophene and 6-nitrobenzothiadiazole) were not reduced by this method.[44] This is not surprising in light of earlier reports[42,43] describing thiophenes as general poisons for the Pd/C catalyst. Interestingly, however, the reduction does proceed to completion in nitro-containing compounds that also have alkyl-bound divalent sulfur ($-CH_2-SH$ or $-CH_2-S-CH_3$, for example), although the reaction is noticeably slower.[44]

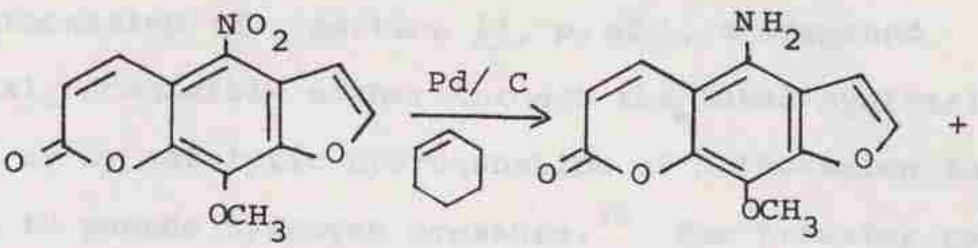
In line with these observations, the selective rapid transfer method did reduce 7-nitrodiazoxide completely, albeit slower than would be expected normally; a two-hour reflux was required.

The derivatization of methoxsalen and the application of the selective rapid transfer reduction technique to the parent compound and to the derivatives has resulted in a facile new route to known methoxsalen derivatives and some unexpected products as well.

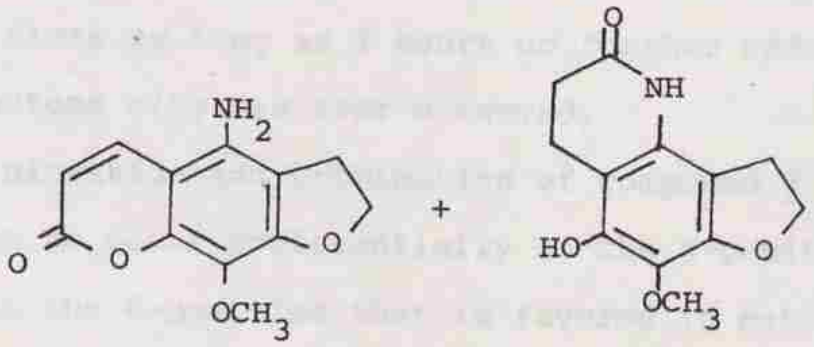
The nitration of methoxsalen has been reported in the literature along with the reduction of the resultant 4-nitromethoxsalen to the 4-amino compound with tin (II) chloride.[36] The application of the selective rapid transfer reduction technique to the 4-nitro compound yielded a three component mixture that was easily separable by fractional recrystallization from ethanol:water solutions (reaction 10, p. 62). Compound 23, 5-hydroxy-4-methoxy-2,3,6,7-tetrahydro-9H-furo[3,2-h]quinolin-8-one, is formed by the reduction and cleavage of the lactone ring in the precursor (compound 22) followed by ring closure to the amine functionality to form a lactam. The formation of this compound could be totally avoided by refluxing the reaction mixture for less than 30 minutes.

In light of the unexpected reduction of the unsaturation in the furan ring the selective rapid transfer

reaction 10

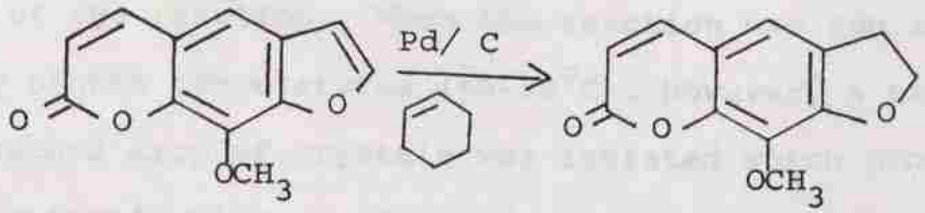


20 21



22 23

reaction 11



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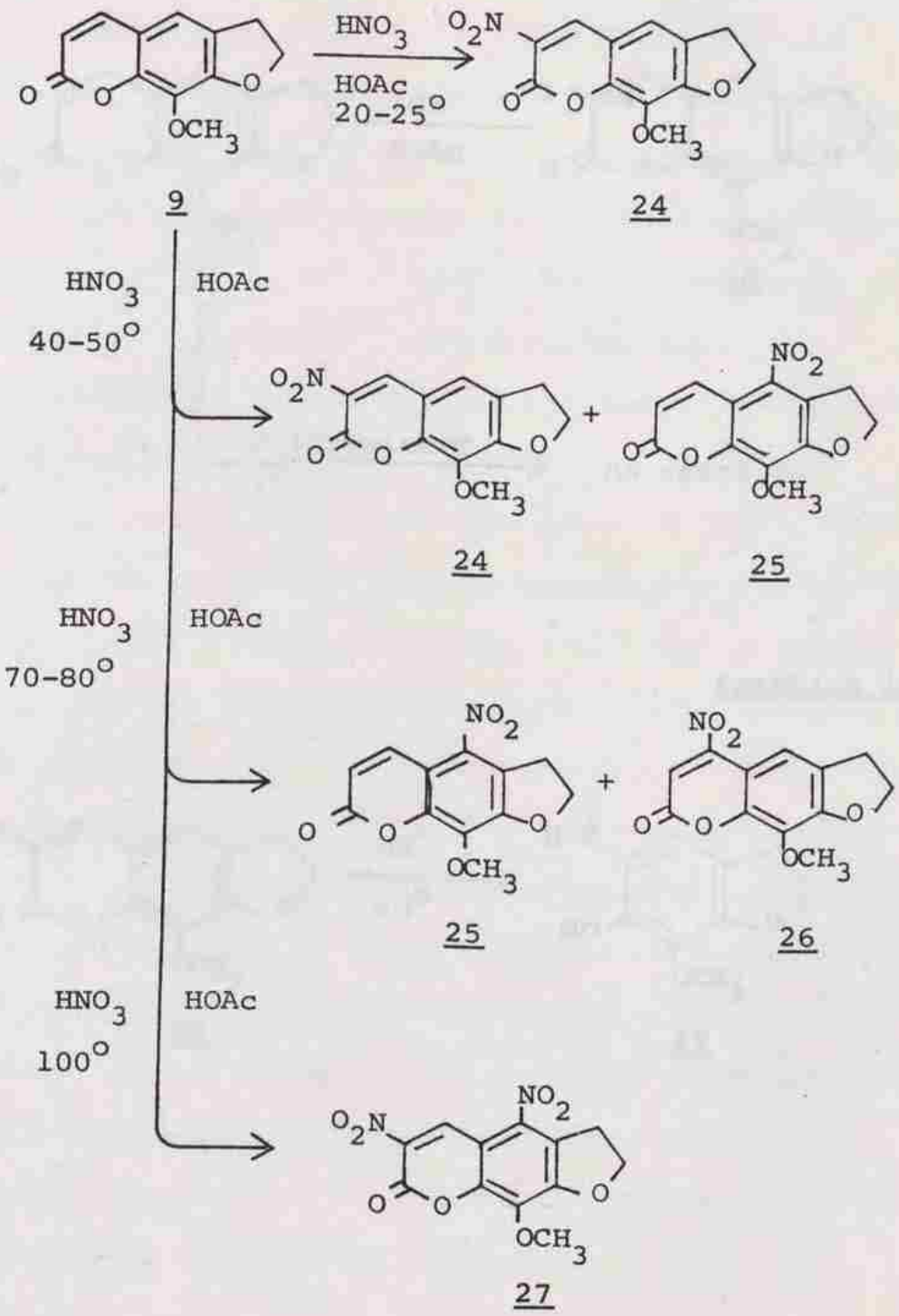
reduction was applied to methoxsalen itself. This proved to be an excellent, facile means of synthesizing 2,3-dihydromethoxsalen (9, reaction 11, p. 62), a compound previously accessible either through the total synthesis route[19] or by catalytic hydrogenation of methoxsalen for 2 hr at 40 pounds hydrogen pressure.[36] The transfer reduction was complete in under 30 minutes. Even with reaction times as long as 2 hours no further reduction of the lactone ring was ever observed.

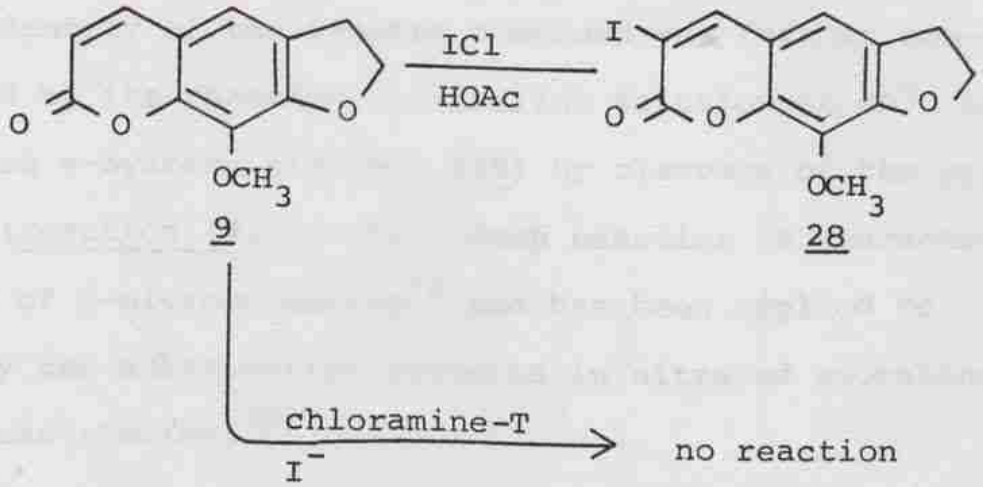
The nitration and bromination of compound 9 have been shown to occur preferentially in the 6-position as opposed to the 4-position that is favored in methoxsalen. This preference for electrophilic substitution at position 6 was substantiated herein in nitration (reaction 12, p. 64) as well as iodination reactions (reaction 13, p. 65).

At room temperature (20-25°C) in a classical nitration reaction, the 6-nitro isomer (24) was the exclusive product of the reaction. When the reaction was run at slightly higher temperatures (40-50°C), however, a significant second crop of crystals was isolated which proved to be a eutectic mixture of the 4- and 6- nitro substitutional isomers. This eutectic melted sharply at 175-176°C and in all likelihood is the material previously identified[38] as 2,3-dihydro-4-nitromethoxsalen. That the substance was a mixture was verified by HPLC analysis

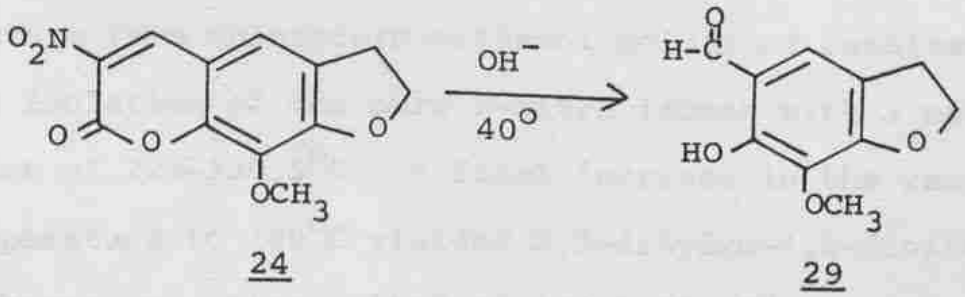
reaction 12


HNO3
HOAc
20-25°
9
24
HNO3
HOAc
40-50°
24
25
HNO3
HOAc
70-80°
25
26
HNO3
HOAc
100°
27

reaction 13



reaction 14



and NMR spectral results. The 4-nitro isomer (25) was successfully separated from the eutectic by preparative HPLC and its melting point determined to be 192-194°C. The identify of the 6-nitro compound was further confirmed by its reaction in alkaline solution at 40°C to form an σ-hydroxy aldehyde (29) by cleavage of the pyrone ring (reaction 13, p. 65). Such behavior is characteristic of 3-nitrocoumarins[72] and has been applied to verify the substitution patterns in nitrated psoralens in previous studies.[38]

Another increase in the temperature of the reaction mixture (70-80°C) yielded another eutectic mixture of the 4- and 5- nitro substitution isomers melting at 174.5-175.5°C. The identity of the components was confirmed by NMR analysis, and the fractional recrystallization of the mixture from chloroform:methanol solutions resulted in the isolation of the pure 5-nitro isomer with a melting point of 229-230.5°C. A final increase in the reaction temperature to 100°C yielded 2,3-dihydro-4,6-dinitro-methoxsalen, which melted at 172.5-174.5°C. Analyses of these mixtures was aided by the fact that all of the substitution products are totally unambiguous in the H^1-NMR. (Reaction results are summarized on Table XIV, p. 67).

Bromination of 9 to yield 6-bromo-2,3-dihydromethox-

Table XIV: Summary of the Reaction Conditions and Resultant Products from the Nitration of 2,3-Dihydromethoxsalen

Temperature	4-NO_2 (25)	5-NO_2 (26)	6-NO_2 (24)	4,6-NO_2 (27)
100°C	----------			----------
	second crop: m. p. 174.5- 175.5°C			first crop: m.p. 172.5-174.5°C
	eutectic mixture of 4- and 5- isomers			
70-80°C	----------			
	mixture of isomers			
40-50°C	----------		----------	
			first crop: m.p. 233-234°C	
	second crop: eutectic mixture of 4- and 6- isomers m. p. 175-176°C			
20-25°C			----------	
			45% yield of 6-NO_2; m.p. 233-234°C	
			starting material recovered	

salen was demonstrated originally by Brokke and Christensen,[36] who could not prove the site of substitution, and later by Kaufman and Worden,[73] who did demonstrate that substitution took place in the 6-position. The electrophilic iodination of 2,3-dihydromethoxsalen (reaction 14, p. 65) was accomplished with ICl dissolved in glacial acetic acid. The silvery white product (28) yielded an H^1-NMR spectrum very similar to that of the known 6-bromo compound. When the same product was sought in a chloramine-T reaction with I_2 and 2,3-dihydromethoxsalen, no product was realized and the starting material was recovered quantitatively. Such reactions with chloramine-T usually require an activated ring; consequently, one must conclude that the 6-position of 2,3-dihydromethoxsalen is too deactivated to react in the desired fashion. It is not too deactivated, however, to react with iodine monochloride.

An electronic justification for the preferential reaction in electrophilic substitution reactions at the 6-position in 2,3-dihydromethoxsalen when compared to the preferential reaction at the 4-position in methoxsalen is at hand in the observation of the C^{13} chemical shifts of the carbons in question. Assignments for the methoxsalen carbons are available in the literature,[74] and the suggested assignments for 2,3-dihydromethoxsalen herein are

verifiable based on comparisons to like compounds described in the literature and by calculations of contributions to chemical shifts by substituent groups with known shift effects. (See Table XV, p. 71-72 for C^{13} chemical shift data for methoxsalen and 2,3-dihydromethoxsalen.) Based on chemical shift differences between the C-4 and C-6 carbons in the two compounds, the relative reactivity of both carbons is understandable. In methoxsalen, C-4 has a chemical shift of 113.1 ppm and C-6 is 114.5 ppm. Thus the carbon further upfield is the most electron-rich and hence the most susceptible to electrophilic attack. In 2,3-dihydromethoxsalen, this order is reversed and C-6 (δ = 112 ppm) is further upfield than C-4 (δ = 117.2 ppm); that nitrations at low temperatures, brominations and iodinations all take place at the 6-position in this compound further substantiate the above statement.

The unique long distance coupling in these compounds is also worthy of note. It is both reported in the literature and observed in the C^{13} NMR spectra in this study that C-6 is split only by its own hydrogen and not by the adjacent hydrogen on C-5. The C-5 resonance, however, is split by the C-6 hydrogen in addition to its own. Long distance coupling also helps to verify the assignment of C-4. In methoxsalen, C-4 is split into a doublet by its

own aromatic proton by a value typical for such systems (163 Hz). The doublet is further split by the proton on C-5 by a value of 4 Hz to yield a doublet of doublets. Upon the reduction of the furan ring, the splitting pattern of C-4 is altered to reflect the presence of two hydrogens at C-3; the J value of 163 Hz is again within the expected range and yields a doublet, but the long range coupling causes a more complex splitting pattern than that of a simple doublet of doublets. The pattern corresponds to a doublet, each peak of which is split into a doublet of triplets. For this to be the case, the C-4 signal must be split by its own hydrogen (J^1 = 162), the C-5 hydrogen (J^3 = 5 Hz), and the two hydrogens on C-3 (J^3 = 2.4 Hz).

The products of the reduction of several of the psoralen derivatives were evaluated as a function of time by HPLC analysis of reaction mixtures. The results are summarized on Table XVI, p. 73-74 . Measured molar extinction coefficients in the UV-visible spectra of the compounds used for calculating concentration ratios from HPLC chromatograms are listed in Table XVII, p. 75.

Reaction I:

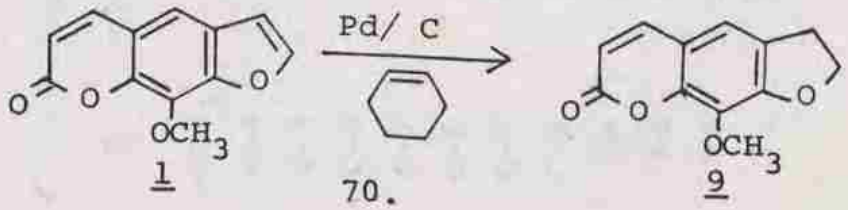


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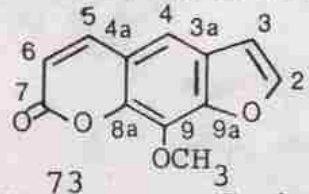
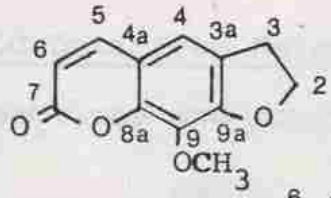
Table XV: C^{13}-NMR Chemical Shift Assignments for Methoxsalen and 2,3-Dihydromethoxsalen (all values in ppm)

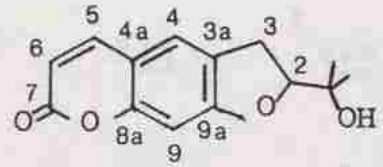
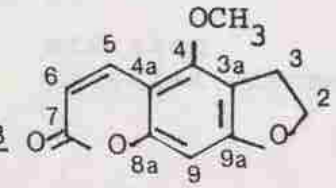
methoxsalen



C	observed	literature [73]	J observed Hz (literature value Hz [73])
$-OCH_3$	61.2	61.2	J^1 146
C-2	146.6	147	J^1=205; J^2=11 (10.5-11.5)
C-3	106.8	106.6	J^1=179; J^2=13 (13-14)
C-4	113.1	114	J^1=162 (163-166); J^3_5=4 (4)
C-5	144.4	145	J^1=164; J^2=6
C-6	114.5	115	J^1=173 (172-174)
C-7	160.4	160.6	J^2= 6 (4-7); J^3=11 (11-12)
C-9	132.7	133	----------
3a	126.2	127	----------
4a	116.5	117	----------
8a	142.9	144	----------
9a	147.6	148	----------

Table XV: (continued)

2,3-dihydromethoxsalen 

literature comparisons :[73] A  B 

C	observed	lit. A	lit. B	calc.	J observed Hz (lit. Hz)
$-OCH_3$	60.8	------	59.4		J^1=145
C-2	73.1	91.0	72.4		J^1=150; J^2=2
C-3	28.9	28.7	28.3		J^1=136; J^2=2.5
C-4	117.2	123.8	158.7	116.1	J^1=163 (163-66); J^3_5=5; J^3_3=2.4
C-5	143.9	144.6	139.2		J^1=162; J^2=5
C-6	112.0	111.2	110.5		J^1=173 (172-174)
C-7	160.7	160.5	161.5		J^2= 4; J^3=11
C-9	131.4	96.7	92.9	128.1	----------
3a	113.4	125.9	105.9		----------
4a	125.6	121.1	110.4		----------
8a	154.4	155.1	156.6		----------
9a	147.0	163.3	165.5		----------

Table XVI: Summary of HPLC Data from Selective Rapid Transfer Reduction

Reactions: reactions and compounds as numbered on p.

	%9	%21	%22	%23	%24	starting material	comments
Reaction I							
10 min	93	--	--	--	--	7	
30	96	--	--	--	--	4	
60	91	--	--	--	--	9	
90	89	--	--	--	--	11	*
Reaction II							
10	13	49	37	--	--	--	
30	9	17	24	--	--	--	
60	7	3	90	--	--	--	
90	9	1	88	2	--	--	
Reaction III							
10	--	--	--	--	73	27	
30	--	--	--	--	74	26	
60	--	--	--	--	70	30	*
90	--	--	--	--	75	25	

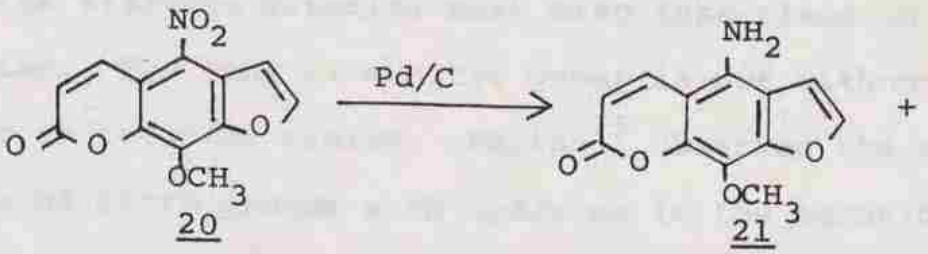
Table XVI: (continued)

	%9	%21	%22	%23	%24	starting material	comments
Reaction IV							
10	12	--	32	--	--	56	
30	6	--	83	--	--	12	
60	5	--	94	trace	--	1	
90	7	--	91	trace	--	1	

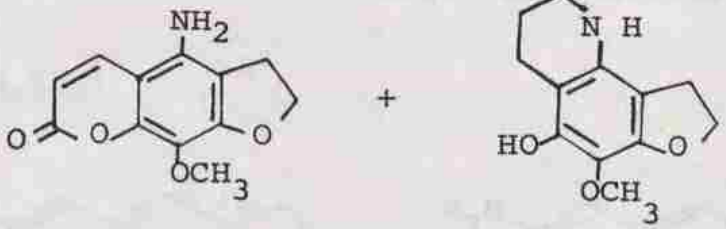
Comments: *A peak of significant proportion that was not one of the known compounds in this study was present in the chromatograph from this run.

Reaction I (p. 70) is essentially complete within 10 minutes. That the amount of product shows a slight decrease with longer reaction times may indicate that the reverse reaction (i.e. dehydrogenation to form a double bond) may also occur with the catalyst in this system. In fact, the catalytic dehydrogenation of 2,3-dihydromethoxsalen with palladium was the final step in the original total synthesis of methoxsalen by Späth and Pailer; they reported only a 13% yield for this step.[19]

Reaction II:



20 21



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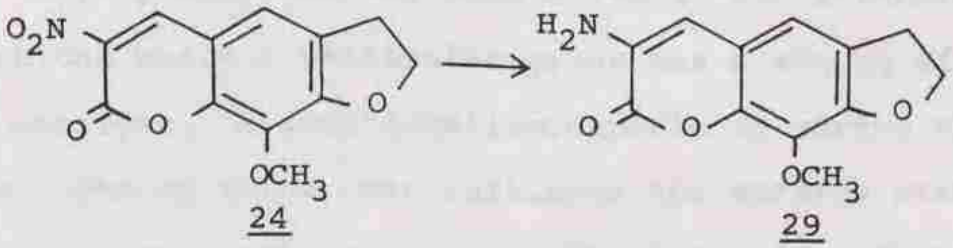
The progression indicated in the HPLC analysis of Reaction II (p. 75) was also demonstrable by TLC and by the actual isolation of products after various reaction times. The reaction after 10 minutes shows a relatively even distribution between products 21 and 22. With longer refluxing times, the balance gradually swings in favor of 2,3-dihydro-4-aminomethoxsalen and only the gradual formation of small amounts of the ring cleavage-rearrangement product (23). The presence of a small but relatively constant amount of 2,3-dihydromethoxsalen indicates that a rapid removal of the nitro group from some of the starting material must also take place in this system. The loss of a nitro group is not without precedent in reducing medium. Kaplan[75] observed the replacement of nitro groups with hydrogen in the borohydride reduction of 1,3,5-trichloro-2,4,6-trinitrobenzene without the reduction of the aromatic ring. In alkaline conditions

Reaction III:

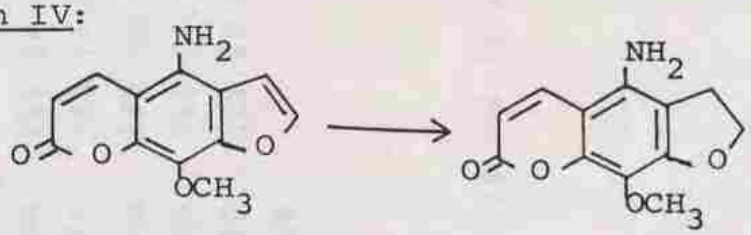


24 29

with sodium borohydride, that compound yielded 1,3,5-trichloro-2,4-dinitrobenzene and 1,3,5-trichloro-2-nitrobenzene.

The reduction of 2,3-dihydro-6-nitromethoxsalen (Reaction III, p. 76) to the amine is complete within the first 10 minutes. It is interesting to note that apparently no removal of the nitro group occurs in this system, as is evidenced by the absence of any 2,3-dihydro-methoxsalen in the product mixture.

Reaction IV:



The gradual decrease in the concentration of starting material in Reaction IV (above) indicates that the reduction of the ethylenic linkage in 4-aminomethoxsalen is proceeding more slowly than the comparable reduction in methoxsalen itself. This phenomenon has been noted in the literature[43] in situations where a particular group has a strong affinity for the catalyst. A functionality capable of strong chemisorbtion, like an amine, may influence the surface stereochemistry at the reactive locus in the molecule and thereby hinder the desired reaction. This may be the reason for the slower reaction in this case.

Table XVII: Summary of UV-visible Spectral Data on Psoralens

wavelength nm (molar extinction coefficient lit/m cm) in methanol

	ε at 254 nm					
methoxsalen	4.37	296 (4.08)*	262 (4.24)	247 (4.58)*		
4-nitromethoxsalen	4.43	338 (3.92)	248 (4.57)			
4-aminomethoxsalen	4.16	321 (4.00)	287 (4.40)	2.28.5 (488)		
2,3-dihydromethoxsalen	3.71	332 (4.03)	260 (3.70)	249 (376)		
2,3-dihydro-4-aminomethoxsalen	3.64	334 (3.90)	271 (3.80)			
2,3-dihydro-6-aminomethoxsalen	3.90	341 (4.16)	253 (3.72)**	240 (3.77)**		
2,3-dihydro-6-nitromethoxsalen	3.70	324 (3.98)	285 (4.40)			
5-hydroxy-4-methoxy-2,3,6,7-tetra-hydro-9H-furo 3,2-h quinolin-8-one	4.07					

* literature values 200 (4.08) and 249 (4.44) in ethanol.[76]

** broad shoulders

EXPERIMENTAL SECTION

General

Melting points, determined on a Fisher-Johns apparatus, are uncorrected. Infrared spectra were obtained on a Perkin-Elmer Model 283 infrared specrtophotometer as nujol mulls or KBr discs. The H^1-NMR spectra were obtained in the indicated solvents with TMS as an internal standard on either a Perkin-Elmer Hitachi R20A spectrophotometer or a Joel FX90Q multinuclear NMR; the latter instrument was used to obtain C^{13}-NMR spectra. HPLC analyses were performed on a Perkin-Elmer Series 2 LC with an LC-75 variable wavelength spectrophotometric detector. UV-visible spectra were obtained on a Beckmann DK-2A with pure solvent as the reference. GLPC studies were performed on a Perkin-Elmer Sigma 2 gas chromatograph with FID detector. Gas chromatography-mass spectral analyses were performed on a Finnegan GCMS with Nova Data Station. Analtech silica gel GF (250 micron) precoated TLC plates were used in all TLC determinations. Combustion analyses were supplied by the G. I. Robertson Microanalytical Laboratory, Florham Park, NJ. All selective rapid transfer reductions not described in detail herein were performed by the general method outlined for methoxsalen.

General Procedure for the Synthesis of Aryl Triazenes

The parent amine (0.10 mol) was suspended by vigorous stirring in cold water. To this was added an ice-cold solution of 7.6 g (0.11 mol) sodium nitrite and the mixture was maintained at 0° to 5°C while 22 ml (0.30 mol) of trifluoracetic acid was added dropwise. After the addition had been completed, the mixture was stirred for an additional 5 min and then slowly added to a cold colution of pyrrolidine (8.5 g, 0.12 mol) in 200 ml of 1.1 M potassium hydroxide. The triazene precipitated immediately but the resultant suspension was stirred for an additional 10 min. If necessary, the mixture was adjusted to neutrality by further addition of 1.1 M potassium hydroxide and the product collected by filtration, dried and recrystallized from 95% ethanol (see Table XVIII, p. 82).

General Procedure for the Preparation of Aryl Iodides from Triazenes

Equimolar amounts of the triazene and potassium iodide (0.10 mol) were suspended in water chilled to 5°C. With this suspension maintained at 5°C, trifluoroacetic acid (0.04 mol) was added in a dropwise fashion. When gas evolution had ceased, the medium was warmed gradually to room temperature and then

neutralized with 6 N ammonium hydroxide. The aryl iodide product was isolated and purified by crystallization, distillation or vacuum sublimation (see Table XIX, p.83).

Pyrrolidyl triazene from 4-amino-9-methoxy-7H-furo[3,2-g][1]benzopyran-7-one (Methoxsalen triazene) (14)

The general procedure outlined above for the conversion of an amine to a pyrrolidyl triazene resulted in a 39% yield of rust brown crystals, mp 180.5-183°C. When the reaction was run in a ten-fold excess of pyrrolidine rather than in aqueous potassium hydroxide the yield of the triazene could be increased to 64%.
^{1}H-NMR ($CDCl_3$): δ2.07 (m, 4, $-C\underline{H}_2C\underline{H}_2-$); 3.85 (m,4,$-C\underline{H}_2C\underline{H}_2$) 4.17 (s,3,$-OC\underline{H}_3$);6.18 (d,1,$C_6\underline{H}$, J=9.1 Hz);7.20 (d, 1, $C_3\underline{H}$, J=2.0 Hz);7.55 (d,1,$C_2\underline{H}$, J=2.0 Hz);8.49 (d,1, $C_5\underline{H}$, J=9.1 Hz).

Anal. Calcd. for $C_{16}H_{15}O_4N_3$:C,61.33;H,4.83;N,13.41
Found:C,61.18;H,5.06;N,13.12

Pyrrolidyl triazene from reduced 7-nitro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one (nitrazepam) (15)

The initial reduction of 7-nitro- to 7-amino-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one was attempted using five different reductant systems. Only one method generated sufficiently pure 7-amino to use in

Table XVIII: Synthesis of Pyrrolidyl Triazenes from Aromatic Amines

$R=NH_2$ in starting material, $-N=N-N(CH_2)_4$ in product

Compound	Yield	m.p. °C	Formula	Analysis
C_6H_4-R pale yellow needles	78%	51.53	$C_{10}H_{13}N_3$	Calc.: C, 68.54; H, 7.48; N, 23.98 Found : C, 68.44; H, 7.69; N, 23.75
4-CH_3O-C_6H_4-R brown needles	62%	56-57	$C_{11}H_{15}N_3O$	C, 64.37; H, 7.37; N, 20.47 C, 64.43; H, 7.38; N, 19.80
4-CH_3-C_6H_4-R peach flakes	67%	78.5-80	$C_{11}H_{15}N_3$	C, 69.81; H, 7.99; N, 22.20 C, 69.68; H, 8.12; N, 22.02
3-NO_2-C_6H_4-R red needles	78%	82.5-83	$C_{10}H_{12}N_4O_2$	C, 54.54; H, 5.49; N, 25.44 C, 54.49; H, 5.61; N, 25.24
3-Cl-4-CH_3O-C_6H_3-R silver leaves	61%	84.5-86	$C_{11}H_{14}N_3OCl$	C, 55.11; H, 5.89; N, 17.53 C, 54.88; H, 6.01; N, 17.47
2-CH_3O-5-NO_2-C_6H_3-R yellow needles	67%	137.5-139	$C_{11}H_{14}N_4O_3$	C, 52.79; H, 5.64; N, 22.39 C, 52.69; H, 5.50; N, 22.21
4-NO_2-C_6H_4-R rust brown flakes	65%	161.5-163	$C_{10}H_{12}N_4O_2$	C, 54.53; H, 5.49; N, 25.44 C, 54.72; H, 5.35; N, 25.20

Table XIX: Synthesis of Aromatic Iodides from Triazenes vs. Yields in Sandmeyer Reaction on Same Parent Amine

Compound	Triazene	Sandmeyer	Formula	Identification
C_6H_5-I	78%	49%	C_6H_5I	b
4-CH_3O-C_6H_4-I	63%	40%	C_7H_7OI	b
4-CH_3-C_6H_4-I	51%	56%	C_7H_7I	b
3-NO_2-C_6H_4-I	26%	11%	$C_6H_4NO_2I$	b
3-Cl-4-CH_3O-C_6H_3-I	66%	23%	C_7H_6OClI	a*
2-CH_3O-4-NO_2-C_6H_3-I	95%	68%	$C_7H_6NO_3I$	b
2-CH_3O-5-NO_2-C_6H_3-I	85%	75%	$C_7H_6NO_3I$	b
4-NO_2-C_6H_4-I	46%	51%	$C_6H_4NO_2I$	b

Sandmeyer reactions run as published for the preparation of iodobenzene from aniline.[77]

Compounds were identified by (a) elemental analysis, or (b) melting point or boiling point comparison with published values[52] and IR and NMR spectral data.

*Anal. calcd. : C, 31.31; H, 2.25;

Found : C, 31.50, H, 2.27.

the preparation of the pyrrolidyl triazene.

(1) Nitrazepam (1.35 g; 4.80 mmol) was dissolved in 100 ml of methanol in which 0.10 g Pd/C catalyst had been suspended. Sodium borohydride (0.285 g, 7.5 mmol) was dissolved in 15 ml of basic methanol and added dropwise to the rapidly stirring suspension. After 10 min. the mixture was made acidic by the addition of dilute HCl and then stirred 10 additional minutes. The suspension was filtered and washed with diethyl ether (2 x 25 ml). The bright red solution was concentrated and refrigerated. A pale yellow powdery solid (0.914 g) was collected, and a TLC of this material revealed four spots. Several recrystallizations from methanol did not successfully fractionate the mixture.

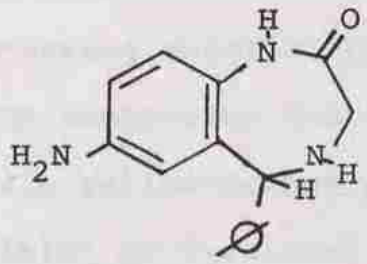
(2) While a solution of nitrazepam (1.40 g; 4.98 mmol) in 100 ml of 95% ethanol was being stirred constantly at 50°C, Pd/C catalyst (0.10 g) was suspended in it. Over a 10 min period, hydrazine hydrate (85%; 10 ml) was added dropwise. Additional catalyst (0.10 g) was added to the suspension and the mixture was gently refluxed for 2 hr. The suspension was filtered through Celite while still hot. The yellow filtrate was concentrated to an oil, taken up in $CHCl_3$, dried with magnesium sulfate, and again concentrated to an oil. The oil was dissolved in a small quantity of ethanol and

and refrigerated for several days. No crystals formed during the refrigeration, but slow evaporation of solvent at room temperature yielded sharp, transparent brown glassy needles which exhibited no sharp melting point but gradually softened and liquefied from 162-183°C. TLC analysis with acetone as elutant showed two spots, the major one at R_f=0.80 and a minor one at R_f=0.70; the latter corresponds to starting material.

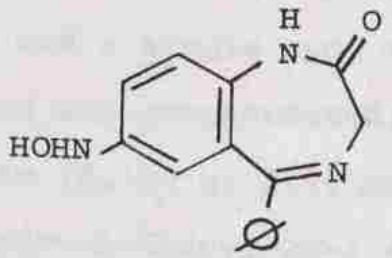
(3) Nitrazepam (1.00 g; 3.56 mmol) was dissolved with mild heating in 250 ml of 95% ethanol. After the addition of 10% Pd/C catalyst (0.50 g), the system was pressurized with hydrogen (52 psi) and then constantly agitated for 5 1/2 hr at room temperature. The mixture was filtered and the yellow filtrate concentrated to a greenish oil that ultimately yielded 0.618 g of grey crystals. TLC of the crystals (acetone eluate) showed two spots with R_f=0.39 and R_f=0.56. A small quantity of the solid was passed through a 30 cm column containing silica gel (grade 63; 60-200 mesh) with acetone as a moving phase. Upon concentration of the collected aliquots, the spot with R_f=0.56 yielded somewhat impure orange-yellow flake-like crystals, m. p. 217-221°C; R_f=0.39 yielded a tan granular solid which softened at 130°C and melted at 185-190°C. H^1-NMR of the material with R_f=0.39 indicated that the -C=N- bond in the

seven-membered ring had been reduced in addition to the nitro group in the 7-position of the aromatic ring. The material with R_f=0.56 may have experienced only partial reduction of the nitro to a nitroso functionality. Neither NMR corresponded to that for the 7-amino derivative obtained by method (5). For the component with R_f 0.39, the following NMR assignments are suggested: H^1-NMR ($CDCl_3$); δ3.40 (v br s, 2, NH_2, vanishes in D_2O); 3.52 (s, 2, CH_2); 5.31 (s, 1, CH); 6.03 (d, 1, C_6H, J_m=2.5 Hz); 6.55 (d of d, 1, C_8H, J_m=2.5 Hz, J_o=8.3 Hz); 6.82 (d, 1, C_9H, J_o=8.3 Hz); 7.84 (br s, 1, NH, vanishes in D_2O). For the component with R_f 0.56: H^1-NMR (DMSO-d_6): δ4.01 (br s, 2, CH_2); 5.12 (v br s, NHOH, 2, vanishes in D_2O); 6.36 (d, 1, C_6H, J_m=2.8 Hz); 6.73 (d of d, 1, C_8H, J_m=2.8 Hz, J_o=8.5 Hz); 7.48 (s, 5, aryl); 9.92 (b s, 1, NH, vanishes in D_2O).

Corresponding structures:



R_f 0.39



R_f 0.56

(4) Nitrazepam (1.250 g; 4.45 mmol) was suspended in a 50% methanol-water solution. Ammonium chloride (1.600g) was added and the suspension was heated to reflux with constant stirring. Powdered iron (1.250 g) was added over a 20 min period, after which the mixture was refluxed for 3 1/2 hr. The hot solution was filtered through Celite, diluted with water, boiled and refiltered. Upon concentration and subsequent recrystallization of the resulting solid material, pale yellow crystals of the desired 7-amino compound (0.472 g; 1.88 mmol; 42.3% yield) melting at 245-247°C were collected. (lit. m. p. 228-231°C.)[78] TLC-acetone eluate-R_f=0.50.

(5) Nitrazepam (0.843 g; 3.00 mmol) was dissolved in 25 ml of 95% ethanol, stirred rapidly and brought to a vigorous reflux. After the addition of cyclohexene (approx. 2 ml), 10% Pd/C catalyst (1.59 g) slurried in 15 ml of 95% ethanol was poured into the solution. After 30 min, TLC with acetone as the moving phase revealed no starting material present and a single spot at R_f=0.50. The suspension was filtered and concentrated, yielding pale yellow-orange crystals (0.720 g; 2.86 mmol; 95.3% yield) of 7-amino-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one, m.p. 241-244°C; mixed m. p. with the product of trial (4), 242-244°C; NMR identical with (4).

H^1-NMR (DMSO-d_6):δ3.45 (br s, 2, NH_2, vanishes in D_2O), 4.06 (s, 2, CH_2), 6.42 (d, 1, C_6H, J=2 Hz), 6.89 (d of d, 2, C_8H, C_9H, J=5 Hz, J=2 Hz), 7.48 (s, 5, aryl), 10.02 (br s, 1, NH, vanishes in D_2O).

The reduced nitrazepam (16) (2.27 g; 9.04 mmol) was suspended by stirring in cold water to which an aqueous solution of 0.698 g (10.0 mmol) of sodium nitrate was added. The mixture was maintained at ice-bath temperature while trifluoroacetic acid (2.1 ml, 27 mmol) was added dropwise. The resultant solution was then added slowly to an ice cold solution of pyrrolidine (0.75 ml, 9.0 mmol) in a 10 ml of 1.1 M potassium hydroxide. After stirring for 5 min at ice-bath temperature, the mixture was slowly brought to room temperature, neutralized and filtered. The solid triazene (17) was recrystallized from 95% ethanol to yield 2.25 g (6.57 mmol, 72.7% yield) of golden yellow crystals, m. p. 248-249.5°C.

H^1-NMR ($CDCl_3$): 1.98 (m, 4, CH_2), 3.73 (m, 4, CH_2), 4.33 (br s, 2, CH_2), 7.45 (m, 8, aryl), 9.29 (s, 1, NH).

Anal. Calcd. for $C_{19}H_{19}N_5O \cdot 1/2H_2O$: C,66.67; H,5.85; N,20.47

Found : C,66.91; H,5.89; N,20.13

Pyrrolidyl triazene from 7-chloro-3-methyl-2H-1,2,4-benzothiadiazine-1,1-dioxide (diazoxide) (17)

To a solution of diazoxide (13) (2.31 g, 0.010 mol) in 275 ml of 95% ethanol at a temperature of 50-55°C was added a slurry of 0.10 g Pd/C catalyst in 15 ml of 95% ethanol. Over a 30 min period, 56 ml of 85% hydrazine hydrate was added dropwise followed by an additional 0.10 g of catalyst. The resultant suspension was refluxed for 5 hr, then filtered hot through Celite. The filtrate was concentrated to one-half its volume, diluted with 150 ml water, and neutralized with 6 N HCl. Upon further concentration, this solution yielded 1.32 g (67% yield) of white crystals of 3-methyl-2H-1,2,4-benzothiadiazine-1,1-diazoxide, which melted at 269-271°C after one recrystallization from methanol (lit. m. p. 263-264°C [79]).

The dechlorinated diazoxide (1.315 g, 0.671 mmol) was stirred vigorously in a solution of 14 ml concentrated nitric acid and 40 ml concentrated sulfuric acid and heated to 50°C for 1 1/2 hr. This lemon yellow solution was poured on 300 g of crushed ice and neutralized with 6 N NH_4OH. The neutral solution was extracted with ethyl acetate, after which the organic layer was concentrated, yielding 1.06 g (66%) of pale yellow flakes of 7-nitrodiazoxide, m.p. 264-266°C(lit. 263-264°C [80]).

The literature preparation for the reduction of 7-nitrodiazoxide (iron powder and ammonium chloride in methanol/water[76] resulted in only a 22% yield of 7-aminodiazoxide.[18] The selective rapid transfer reduction was applied to this system, although literature precedent[44] warned that nitro-heterocyclic systems containing sulfur (specifically 2-nitrothiophene and 6-nitrobenzothiadiazole) were not reduced by this method. To a boiling solution of 7-nitrodiazoxide (0.315 g, 1.31 mmol) in 75 ml of 95% ethanol was added a slurry of 0.69g of 10% Pd/C catalyst and 1.0 ml cyclohexene. The mixture was refluxed vigorously for 2 hr, after which the suspension was filtered and concentrated to yield 0.100 g (0.498 mmol, 38% yield) of light tan flakes of 7-aminodiazoxide with a m. p. $>360^{\circ}C$ (lit. $>360^{\circ}C$)[77] and which was identical in both IR and NMR with the material synthesized via the literature preparation.

The triazene of 7-aminodiazoxide was prepared in a fashion analogous to the general procedure for the synthesis of aryl triazenes, which resulted in a 61% yield of product. The peach-colored solid (17) decomposed at $279^{\circ}C$. H^1-NMR: (DMSO-d_6) δ 1.65 (m, 4, $C\underline{H}_2$), 2.28 (s, 3, $C\underline{H}_3$), 3.54 (m, 4, $C\underline{H}_2$), 7.39 (m, 3, aryl).

Anal. Calcd. for $C_{12}H_{15}N_5O_2S$: C,49.13;H,5.15;N,23.88

Found : C,48.88;H,5.10;N,23.61

Reduction of 9-methoxy-7H-furo [3,2-g] [1]benzopyran-7-one (methoxsalen) (1)

Methoxsalen (1.00 g, 4.63 mmol) was dissolved in 100 ml refluxing 95% ethanol. Cyclohexene (2.5 ml, 24.7 mmol) was added to the boiling solution followed by 2.45 g of Pd/C catalyst, the mixture was stirred vigorously and refluxed for 30 min, and then filtered. The catalyst was washed with several portions of hot ethanol; the solution was evaporated and the product recrystallized from 95% ethanol to yield 0.72 g (3.3 mmol, 71% yield) of transparent pale yellow needles of 2,3-dihydromethoxsalen (9), m. p. 164-165°C (lit. 160-161°;[37] 163°C [19]).

Reduction of 4-nitromethoxsalen[20]

4-nitromethoxsalen was prepared by the literature method.[36] The reduction of 4-nitromethoxsalen (1.00 g, 3.83 mmol) by the selective rapid transfer method yielded a mixture of two products, successive fractional recrystallization of which from 95% ethanol yielded 4-aminomethoxsalen (21) (0.40 g, 1.7 mmol, 44%) and 2,3-dihydro-4-aminomethoxsalen (22) (0.29 g, 1.2 mmol, 31%). The 4-aminomethoxsalen (m. p. 242-243°C; lit. 234-235°C)[36] was demonstrated by mixed melting point determination and spectral comparison to be identical with the product synthesized via the literature method using stannous chloride as the reductant. The 2,3-dihydro-4-amino-

methoxsalen (m. p. 241-243°C, lit. 232-234°C)[38] was shown to be different from the other product by mixed melting point and chromatographic analysis. 2,3-dihydro-4-amino-methoxsalen: H^1-NMR (DMSO-d_6): δ3.04 (t, 2, $C\underline{H}_2$, J=9.0Hz), 3.76 (s, 3, $-OCH_3$), 4.71 (t, 2, $C\underline{H}_2$, J=9.0 Hz), 5.82 (br s, 2, $N\underline{H}_2$, exchangeable in D_2O), 6.02 (d, 1, =C$\underline{H}$, J=9.5Hz), 9.24 (d, 1, =C$\underline{H}$, J=9.5 Hz).

When run again at a reflux time of 50 min, in addition to the above two products a silver white solid, 5-hydroxy-4-methoxy-2,3,6,7-tetrahydro-9H-furo 3,2-h quinolin-8-one (23), was isolated (0.12 g, 0.51 mmol, 13%), m. p. 267-268°C; H^1-NMR (DMSO-d_6):δ2.10-2.55 (m, 4, $-C\underline{H}_2-C\underline{H}_2-$), 2.81 (t, 2, $C\underline{H}_2$, J=8.5 Hz), 3.48 (s, 3, $-OC\underline{H}_3$), 4.30 (t, 2, $C\underline{H}_2$, J=8.5 Hz), 8.53 (s, 1, -O$\underline{H}$, exchangeable rapidly in D_2O), 9.42 (br s, 1, N$\underline{H}$, exchangeable slowly in D_2O).

Anal. Calcd. for $C_{12}H_{13}NO_4$: C,61.27; H,5.57; N,5.96
Found : C,61.01; H,5.54; N,5.72

Synthesis of the pyrrolidyl triazene of 2,3-dihydro-4-aminomethoxsalen

The general procedure for the conversion of an amine to a triazene resulted in a 67% yield of shiny tan crystals m. p. 194-195°C.

H^1-NMR ($CDCl_3$): 2.00 (m, 4, $-C\underline{H}_2-C\underline{H}_2-$), 2.83 (t,2, $C\underline{H}_2$,

J=8.0 Hz), 3.70 (m, 4 , $-C\underline{H}_2-C\underline{H}_2-$), 3.82 (s, 3, $-OC\underline{H}_3$), 4.61 (t, 2, $C\underline{H}_2$, J=8.0 Hz), 6.12 (d, 1, =C<u>H</u>), 8.36 (d, 1, =C<u>H</u>).

<u>Anal</u>. Calcd. for $C_{16}H_{17}N_3O_4$: C, 60.94;H, 5.44; N, 13.33

Found : C, 60.87;H, 5.58; N, 13.10

<u>Synthesis of 2,3-dihydro-6-nitromethoxsalen</u> (<u>24</u>)

Glacial acetic acid (15 ml) was warmed slightly to effect the dissolution of 2,3-dihydromethoxsalen (0.75 g, 3.4 mmol) and then cooled to room temperature. Concentrated nitric acid (3 ml dissolved in 7 ml glacial acetic acid) was added dropwise with constant stirring to the solution, which was cooled slightly during the first few minutes of the reaction. After 1.5 hr, cold water was added and two crops of crystals were collected and dried. Upon recrystallization from methanol, the first crop yielded 0.41 g (1.56 mmol, 45% yield) of 2,3-dihydro-6-nitromethoxsalen (<u>24</u>), m. p. 233-234°C (lit. 220-222°C [38]). The remaining material proved to be recovered starting material.

H^1-NMR (DMSO-d_6): 3.28 (t, 2, $C\underline{H}_2$, J=9.0 Hz); 3.90 (s s, 3, $-OC\underline{H}_3$); 4.78 (t, 2, $C\underline{H}_2$, J=9.0 Hz); 7.50 (b s, 1, aryl); 9.11 (s s, 1, =C<u>H</u>).

<u>Anal</u>. Calcd. for $C_{12}H_9NO_6$: C, 54.76; H, 3.45; N, 5.32

Found : C, 54.69; H, 3.56; N, 5.33

In addition to the analysis and the NMR verification of the site of substitution (the singlet hydrogen of the lactone ring at 9.11), the identity of the 6-substituted isomer was confirmed in the manner described in the literature for 3-nitrocoumarins.[72,81] The 2,3-dihydro-6-nitromethoxsalen (0.178 g. 0.652 mmol) was treated with 10 ml of a 5% KOH solution and heated to 40°C for 15 min on a water bath. The mixture was cooled, acidified with dilute HCl, and the resultant solid was collected and recrystallized from aqueous ethanol. The grey-tan needles melted at 70-73°C, gave a positive ferric chloride test, and were therefore identified as 5-formyl-6-hydroxy-7-methoxy-2,3-dihydrobenzofuran (28) (lit. m. p. 72-72°C).[38]

H^1-NMR ($CDCl_3$): δ 3.20 (t, 2, $C\underline{H}_2$, J=9.1 Hz); 3.98 (s s, 3, $-OC\underline{H}_3$); 4.74 (t, 2, $C\underline{H}_2$, J=9.1 Hz); 7.08 (s, 1, aryl); 9.65 (s s, 1, O=C$\underline{H}$).

When this preparation was repeated at a slightly higher temperature of 40-50°C, the first crop of crystals recovered was recrystallized from methanol to a melting point of 227-229°C and was therefore identified as the 6-nitro substitution product. A second crop of crystals from this preparation was recrystallized to a sharp melting point of 175-176°C and was thought to be the 2,3-dihydro-4-nitromethoxsalen (m. p. 175-176°C) as

reported in the literature.[38] HPLC analyses of this material, however, revealed two components; NMR analysis identified the substances to be a 1:1.2 eutectic mixture of the 4- and 6-nitro isomeric substitution products respectively.

Characterization of 2,3-dihydro-4-nitromethoxsalen (25)

This isomer was separated by preparative HPLC separation of the eutectic mixture which resulted from the synthesis of compound 24 (p. 93). On a C-18 RP Whatman Magnum 20 Preparative Column with 50% methanol: water as a moving phase, at a flow rate of 18 ml/min, the peak elutes at 9.90 min. Upon recrystallization, the yellow solid melts at 192-194°C.

H^1-NMR ($CDCl_3$): 3.66 (t, 2, CH_2, J=6 Hz); 4.17 (s, 3, $-OCH_3$); 4.81 (t, 2, CH_2, J=6 Hz); 6.44 (d, 1, -CH, J=6.7 Hz); 8.35 (d, 1, -CH, J=6.7 Hz).

Anal. Calcd. for $C_{12}H_9NO_6$: C, 54.76; H, 3.45; N, 5.32

Found : C, 54.48; H, 3.40; N, 5.39

Reduction of 2,3-dihydro-6-nitromethoxsalen (24)

Upon reduction by the selective rapid transfer method, the title compound (0.26 g, 1.0 mmol) yielded a light tan solid (29) (0.18 g, 0.77 mmol, 77% yield) m. p. 154-155°C.

H^1-NMR ($CDCl_3$): δ 3.17 (t, 2, CH_2, J=8.5 Hz); 3.20-3.40

(br s, 2, NH_2, exchangeable in D_2O); 3.97 (s, 3, $-OCH_3$); 4.58 (t, 2, CH_2, J=8.5 Hz); 6.56 (s, 1, =CH); 6.70 (br s, 1, aryl).

Anal. Calcd. for $C_{12}H_{11}NO_4$: C, 61.80; H, 4.76; N, 6.01
Found : C, 61.82; H, 4.69; N, 5.78

Synthesis of 2,3-dihydro-4,6-dinitromethoxsalen (27)

To a constantly stirring solution of 2,3-dihydro-methoxsalen (0.750 g, 3.34 mmol) in 10 ml glacial acetic acid at 100°C was added dropwise a solution of concentrated nitric acid (3.0 ml, 47 mmol) in 7 ml glacial acetic acid. After 1.5 hr, water was added to the dark orange solution causing the formation of a bright yellow precipitate. Multiple recrystallizations from methanol yielded shiny yellow felted needles of the desired compound (0.58 g, 1.9 mmol, 56% yield), m. p. 172.5-174.5°C. H^1-NMR ($CDCl_3$): δ3.72 (t, 2, CH_2, J=8.5 Hz); 4.18 (s, 3, $-OCH_3$); 4.88 (t, 2, CH_2, J=8.5 Hz); 9.40 (s, 1, =CH).

Anal. Calcd. for $C_{12}H_8N_2O_8$: C, 46.76; H, 2.62; N, 9.09
Found : C, 47.05; H, 2.80; N, 8.85

A repeat of this preparation was analyzed by HPLC on a C-18 RP Preparative Column with 50% methanol:water as the moving phase and a flow rate of 16 ml/min. This analysis revealed three major components present in the second crop of crystals collected. NMR analysis of the mixture demonstrated the presence of not only the 4,6-

dinitro compound sought but also of two mon-substituted isomers, tentatively identifed and later confirmed as the 4-nitro- and 5-nitro-isomers (25, 26).

When the reaction was repeated at a slightly lower temperature of 70-80°C, a yellow solid was isolated and recrystallized repeatedly from methanol to yield fluffy yellow crystals melting sharply at 174.5-175.5°C. Analysis revealed the material to be a mono-substituted substance; NMR, however, showed the presence of two compounds in a 1:1 ratio. These compounds were tentatively identified and later confirmed as the 4-nitro- and 5-nitro- substitution isomers (25, 26). Hence the sharply melting solid was a eutectic mixture of the two isomers.

Characterization of 2,3-dihydro-5-nitromethoxsalen (26).

This isomer was the second crop of crystals separated from the eutectic mixture by fractional recrystallization of the eutectic using chloroform:methanol solutions. The granular yellow-orange solid melted at 229-230.5°C.

H^1-NMR ($CDCl_3$): δ3.36 (t, 2, CH_2, J=6.0 Hz); 4.07 (s, 3, $-OCH_3$); 4.85 (t, 2, CH_2, J=6.0 Hz); 7.22 (br s, 1, aryl); 8.72 (s s, 1, =CH).

Anal. Calcd. for $C_{12}H_9NO_6$: C, 54.76; H, 3.45; N, 5.32

Found : C, 54.76; H, 3.49; N, 5.14

Synthesis of the triazene of 2,3-dihydro-6-amino-methoxsalen

The preparation of the pyrrolidyl triazene was attempted in the general manner outlined for aryl triazenes. However, the only solid material recovered from the triazene preparation was a very small quantity of unreacted starting material. HPLC analysis of the reaction mixture revealed no fewer than eight peaks, three of which could be considered major components.

The reaction was attempted again using a 10-fold molar excess of pyrrolidine and no aqueous potassium hydroxide, but no product was isolated.

Synthesis of 2,3-dihydro-6-iodomethoxsalen (29)

A solution of ICl (0.372 g, 2.30 mmol) in 15 ml glacial acetic acid was added dropwise to a solution of 2,3-dihydromethoxsalen (0.500 g, 2.29 mmol) in glacial acetic acid warmed to 50°C. The solution was allowed to stir at 50°C for 24 hr. Upon cooling, the solution was treated with 5% aqueous sodium bisulfite until the iodine color was discharged, was diluted further with water and then filtered. The silvery white precipitate (0.472 g, 1.37 mmol, 60% yield) melted at 204.5-205.5°C after two recrystallizations from chloroform:methanol. H^1-NMR ($CDCl_c$): δ 3.29 (t, 2, CH_2, J=8.5 Hz); 4.04 (s s,

3, $-OC\underline{H}_3$); 4.74 (t, 2, $C\underline{H}_2$, J=8.5 Hz); 6.92 (br s, 1, aryl); 8.18 (s s, 1, =C<u>H</u>).

<u>Anal</u>. Calcd. for $C_{12}H_9IO_4$: C, 41.88; H, 2.64; I, 36.88

Found : C, 42.10; H, 2.80; I, 37.05

An alternative synthesis of the compound that would be acceptable as a radiolabeling method was attempted by dissolving equimolar amounts of 2,3-dihydromethoxsalen (0.050 g, 0.229 mmol) and sodium iodide (0.034 g, 0.229 mmol) in 50% aqueous ethanol and treating the solution initially with an equimolar amount of chloramine-T. After refluxing for 24 hr, no product was observed on TLC. A four-fold excess of chloramine-T was added, followed by three hours of reflux; ultimately a fifteen-fold excess of chloramine-T was added to the solution, but no reaction was observed after 4.5 hr of refluxing. The starting material was recovered quantitatively.

General Protocol for Radiometric Susceptibility Testing of Triazenes

For each assay a stock solution of the test compound was prepared by dissolving the triazene (10 mg) in ethanol (0.5 ml), Tween 80 (0.1 ml) and culture medium without glucose (0.4 ml), and diluting this solution to a total volume of 10 ml by the final addition of 9 ml of culture medium without glucose. Aliquots of this solution were used to innoculate vials containing

1 μCi U-C-14 glucose and 1 x 10^6 bacteria. A control culture containing the same quantities of materials but minus the triazene was prepared for each sample. The resultant cultures were placed in a "bactec" autoanalyzer and the quantity of C-14 CO_2 released by test samples and controls was determined at hourly intervals.

Because of the lower solubility of the p-nitrophenyltriazene, 4 ml of ethanol was needed to dissolve 10 mg of solid, 0.4 ml Tween 80 was added and 5.6 ml of culture medium without glucose used to bring the sample to a total volume of 10 ml.

General Procedure for HPLC Evaluation of Product Mixtures in Selective Rapid Transfer Reduction Reactions of Psoralens

The selective rapid transfer reductions were run in the fashion previously described for methoxsalen; 0.100 g of starting material was used in all reactions. At the times indicated with the moment of addition of the Pd/C catalyst taken as t_o, approximately 3 ml of liquid was removed from the suspension, filtered and evaporated to dryness. The dried material was redissolved in a minimum amount of HPLC grade methanol and injected on a C-8 RP analytical column with 37% methanol:water as the moving phase at a flow rate of 2.5 ml/min. Peaks were identified by comparison of t_r values with known standards.

Figure I: C^{13}NMR of Phenyltriazene (completely decoupled)

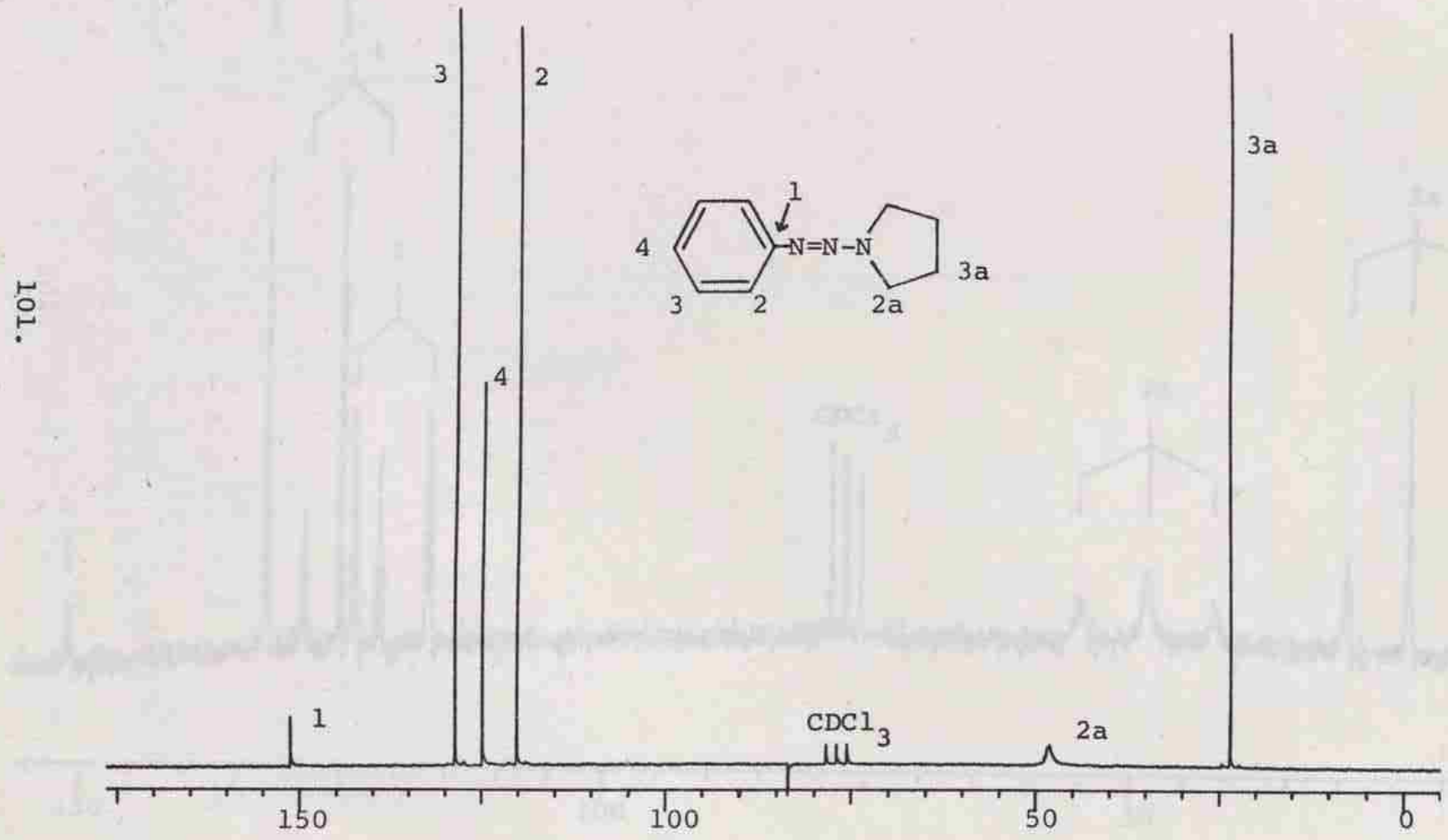


Figure II: C^{13} NMR of Phenyltriazene (NOE--proton coupled)

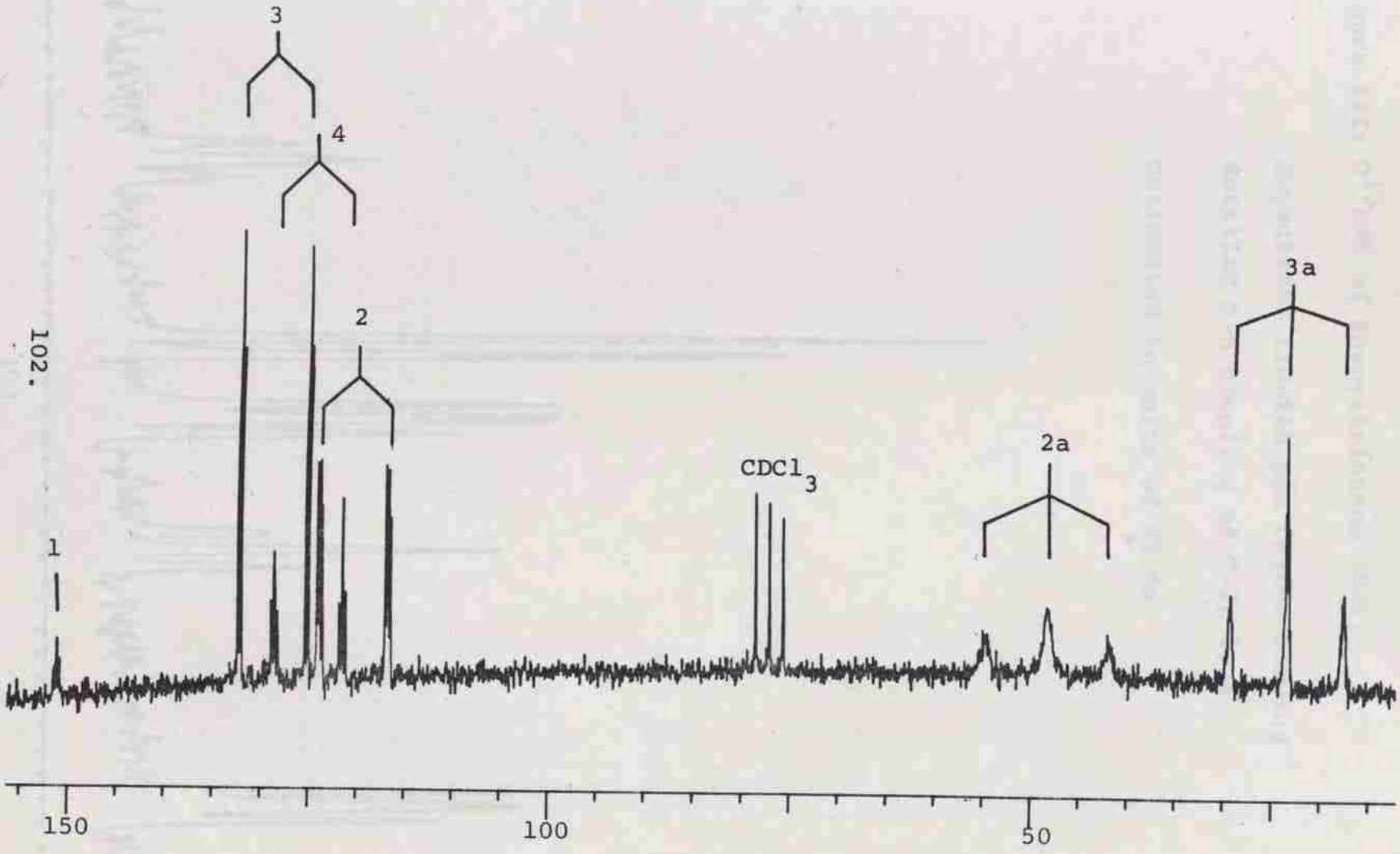


Figure III: C^{13}NMR of Phenyltriazene (NOE)

Expanison: 110-140 ppm region showing detailed C-H coupling of C-2, 3, 4

Calibrated in units of 10 Hz

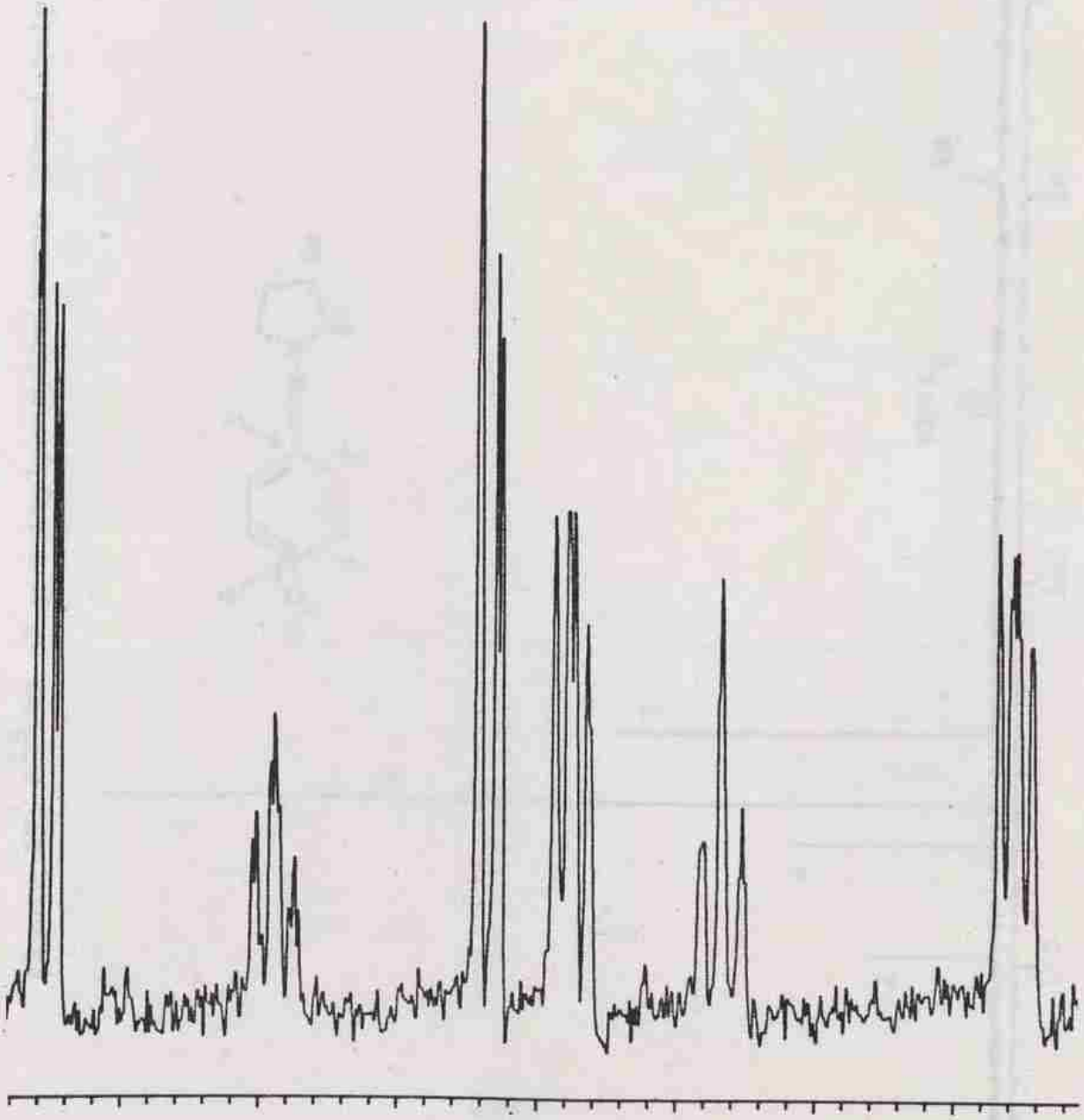


Figure IV: C^{13}NMR of p-Methylphenyltriazene (completely decoupled)

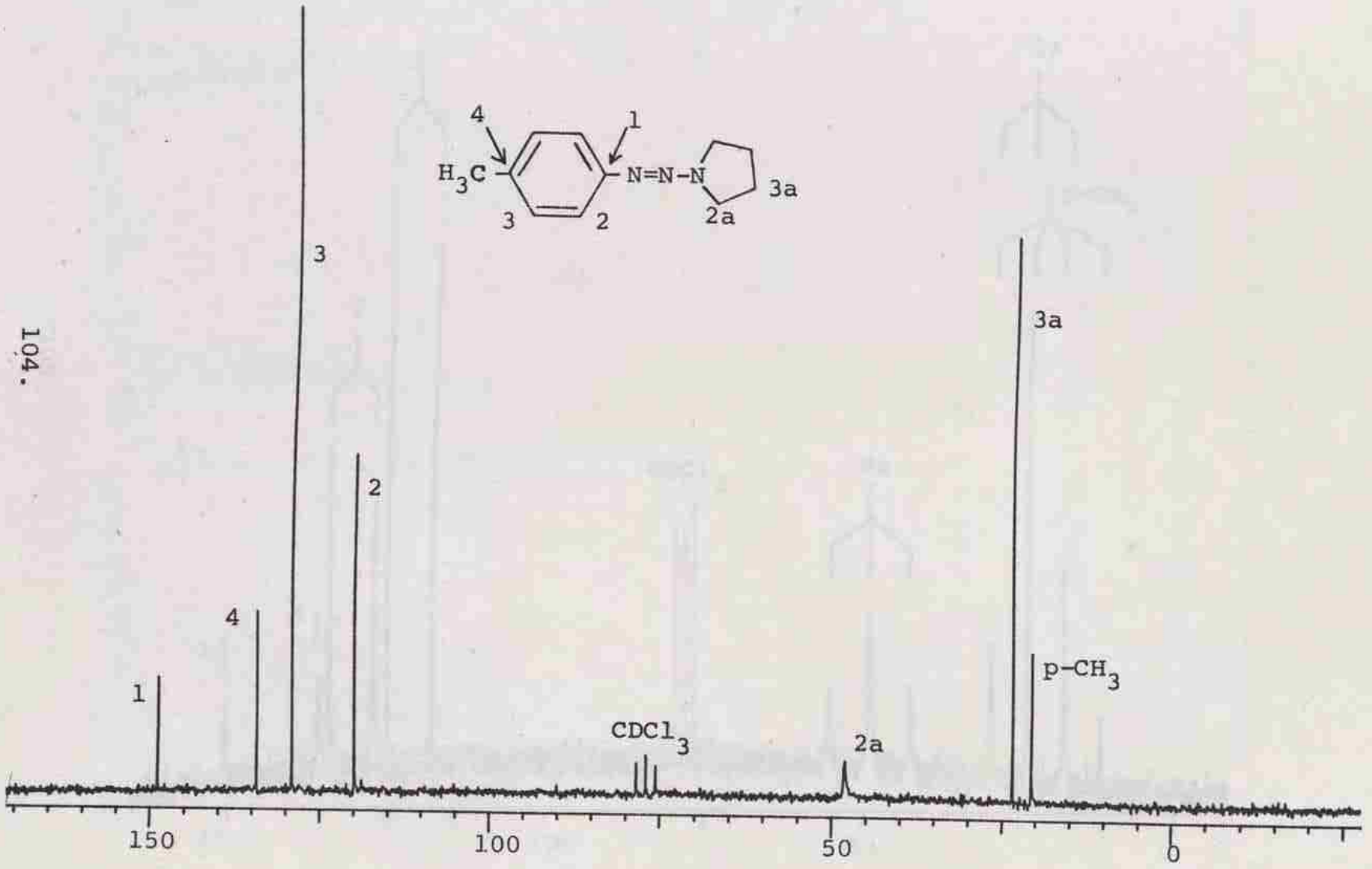


Figure V: C^{13}NMR of p-Methylphenyltriazene (NOE)

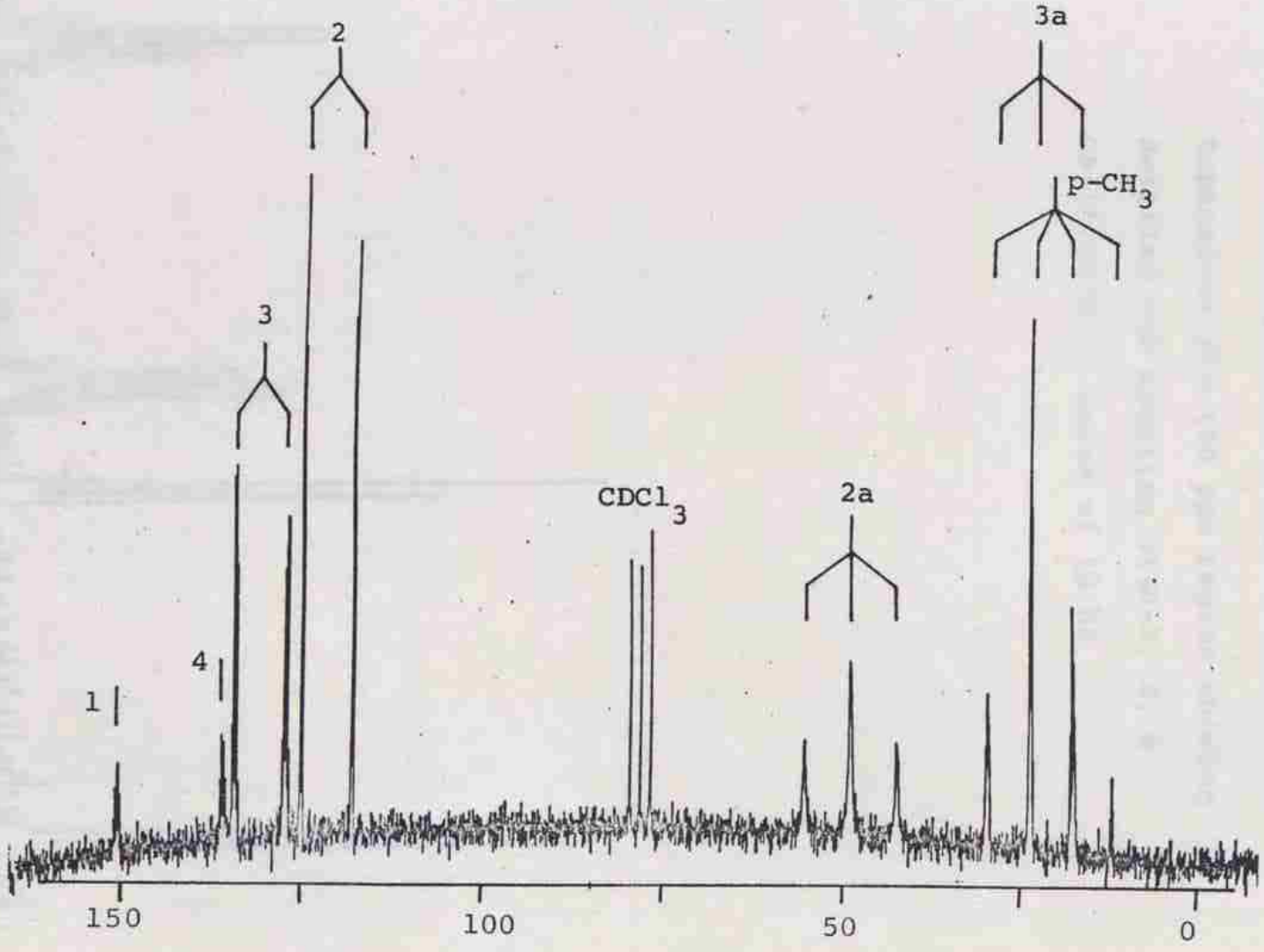


Figure VI: C^{13}NMR of p-Methylphenyltriazene (NOE)

Expansion: 110-140 ppm region showing detailed C-H coupling of C-2, 3, 4

Calibrated in units of 10 Hz

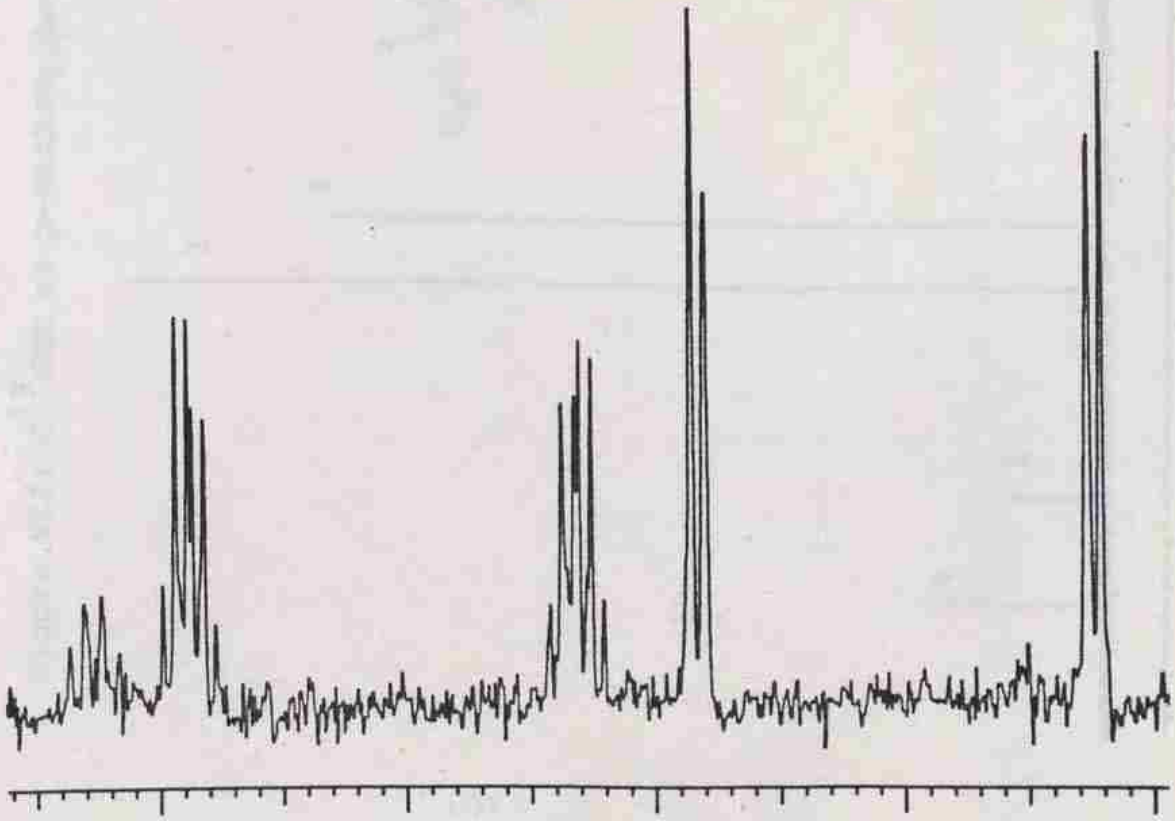


Figure VII: C^{13} NMR of p-Methoxyphenyltriazene (completely decoupled)

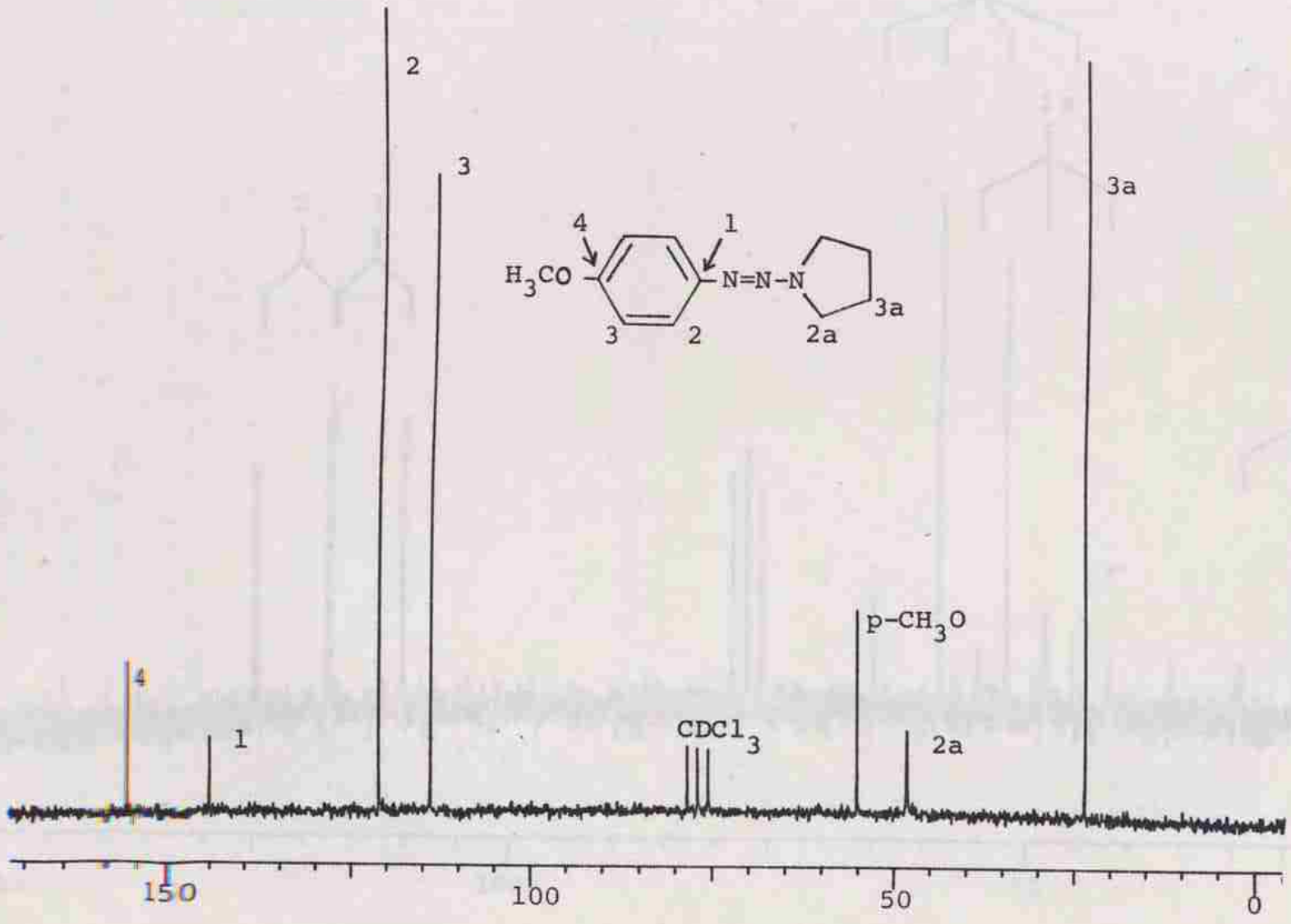


Figure VIII: C^{13}NMR of p-Methoxyphenyltriazene (NOE)

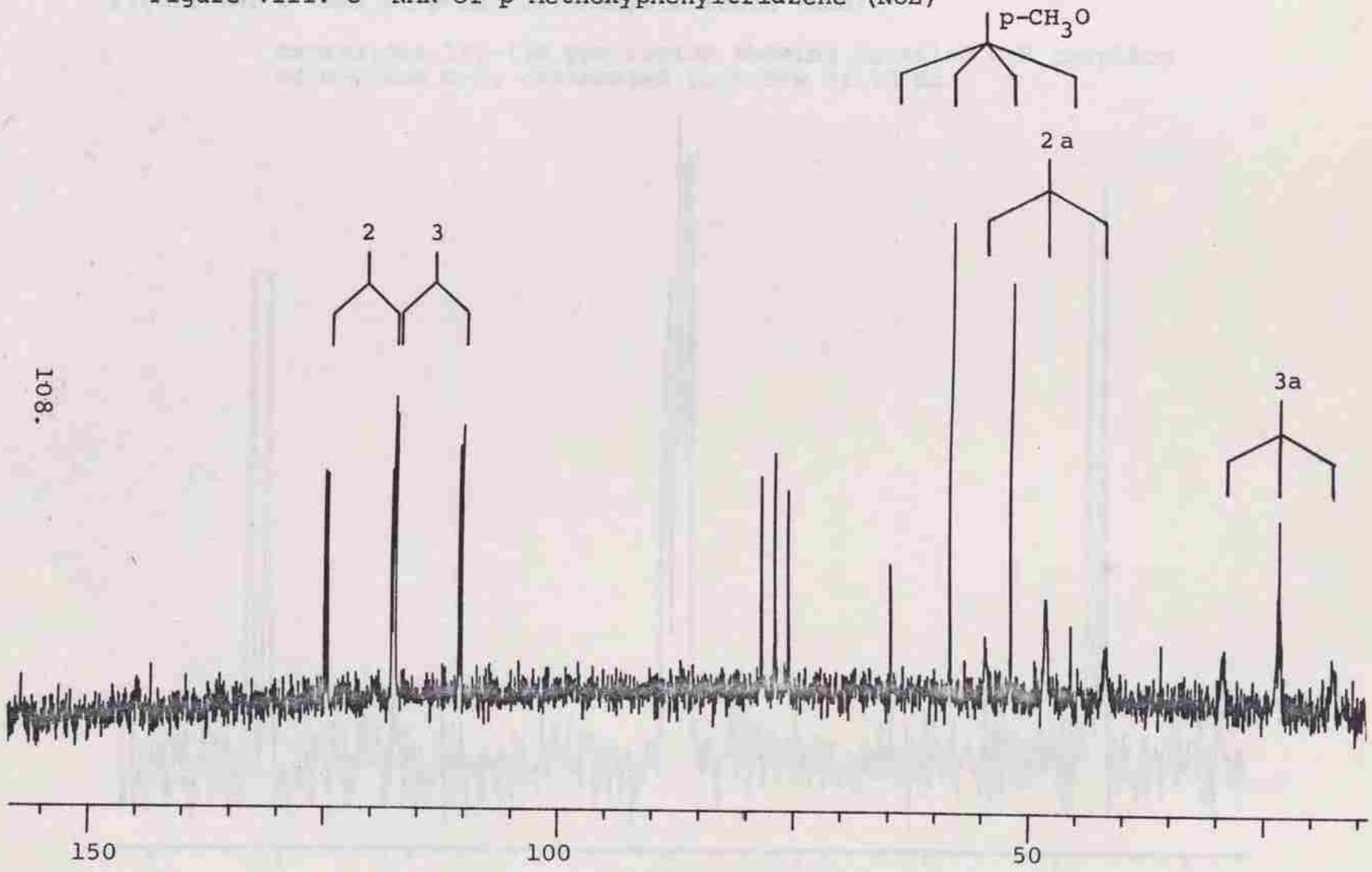


Figure IX: C^{13}NMR of p-Methoxyphenyltriazene (NOE)

Expansion: 105-130 ppm region showing detailed C-H coupling of C-2 and C-3; calibrated in units of 10 Hz

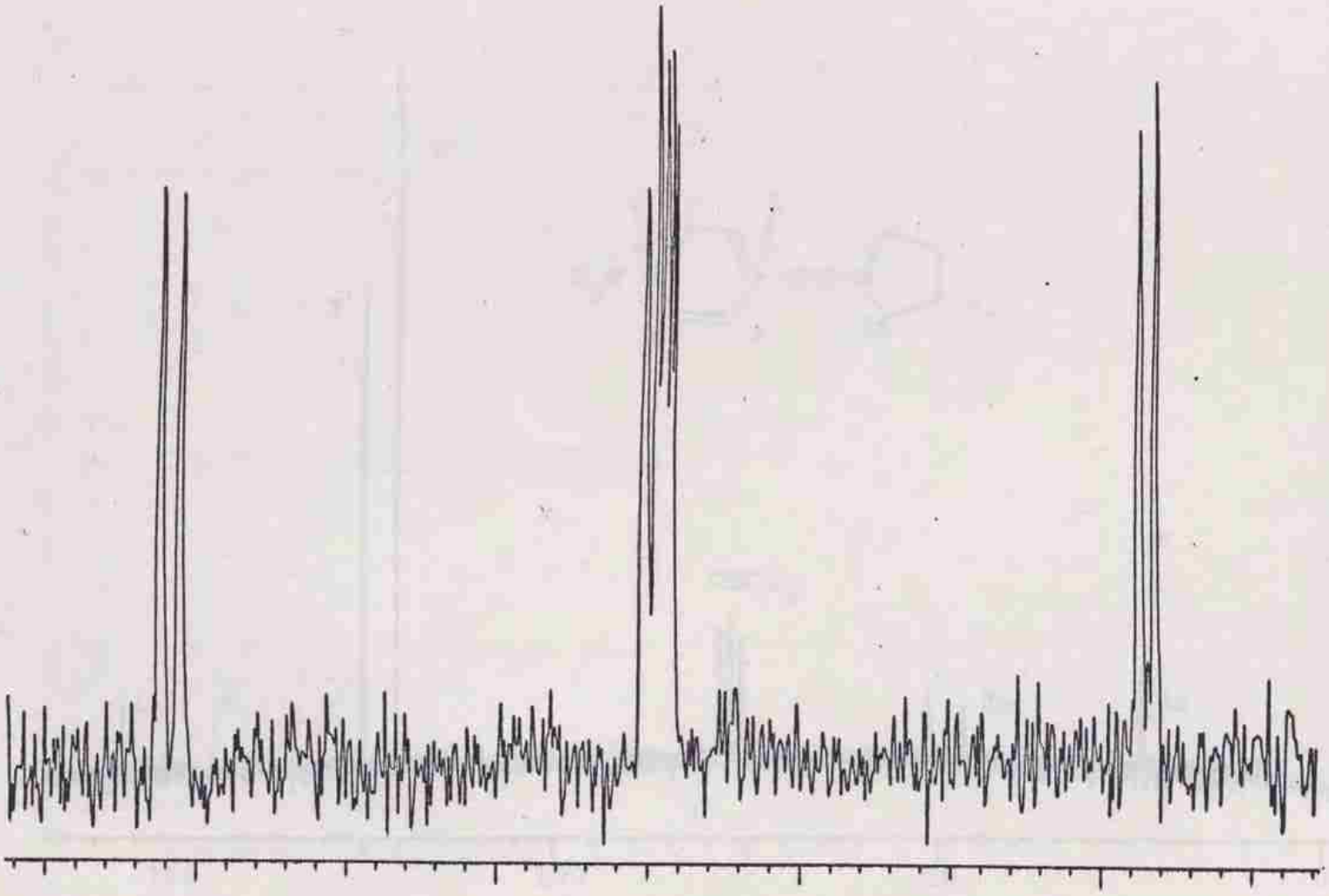


Figure X: C^{13}NMR of p-Nitrophenyltriazene (completely decoupled)

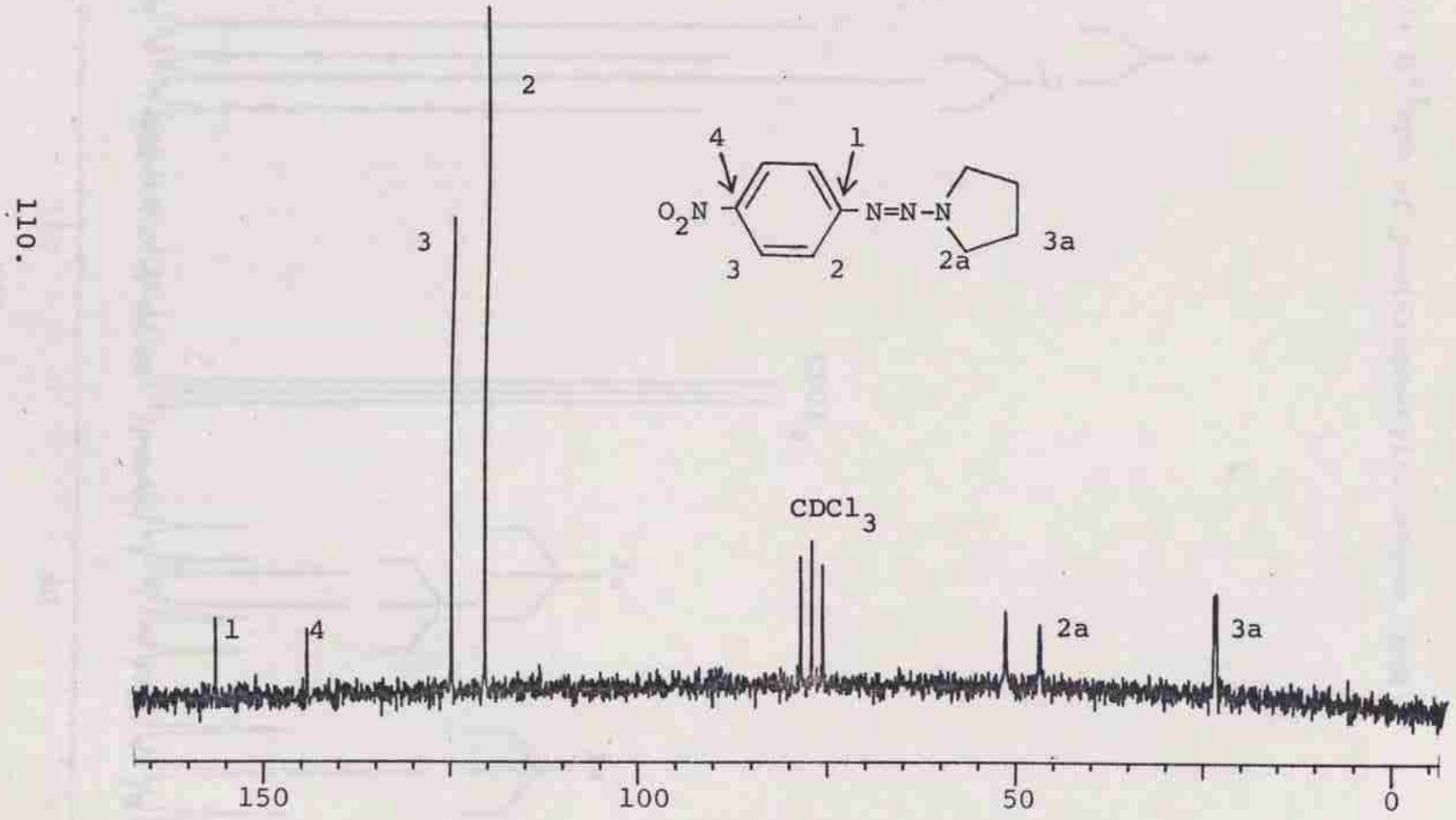


Figure XI: C^{13}NMR of p-Nitrophenyltriazene (NOE)

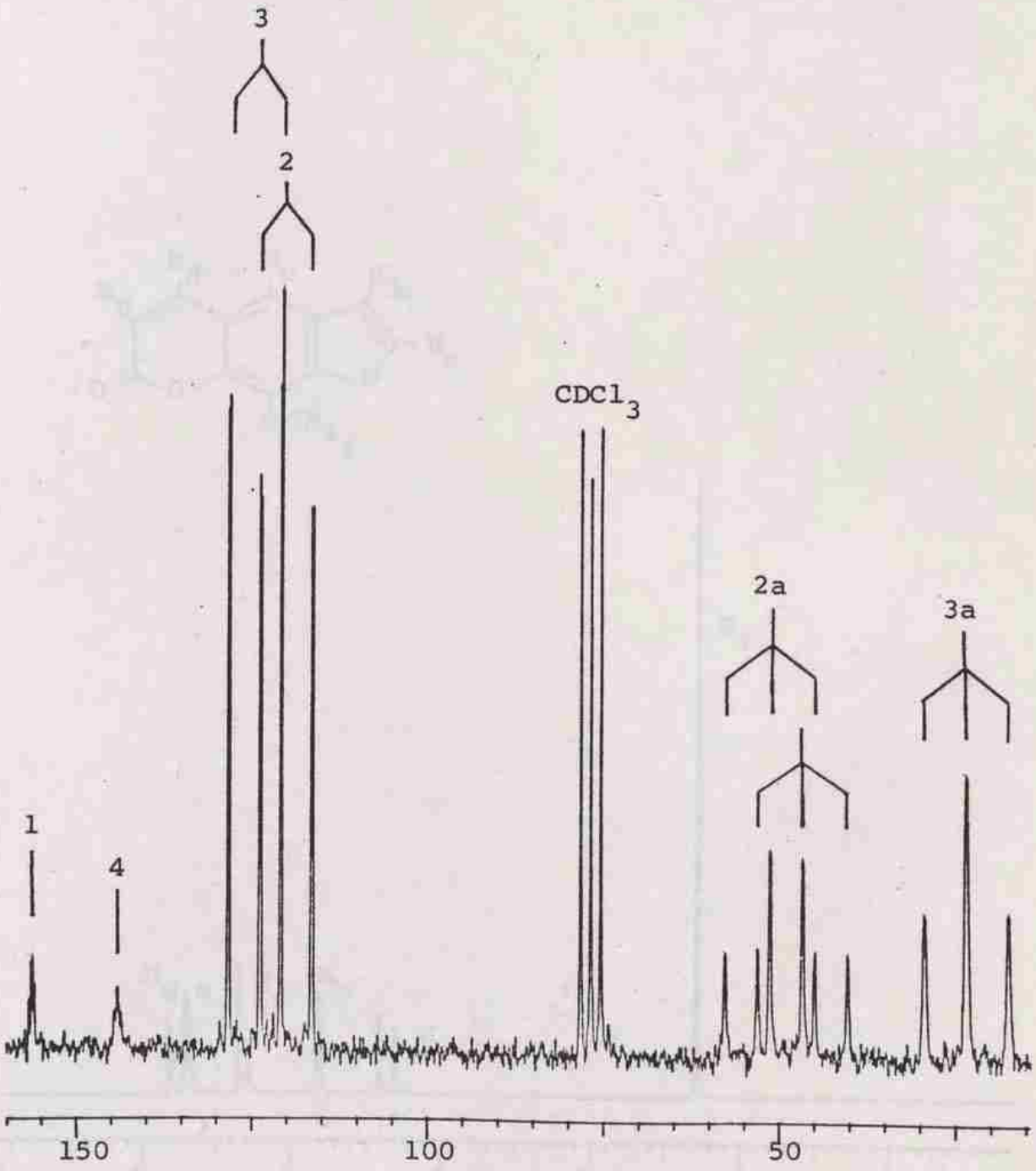


Figure XII: H^1NMR of Methoxsalen

(small peak at ca. 7.27 is $CDCl_3$)

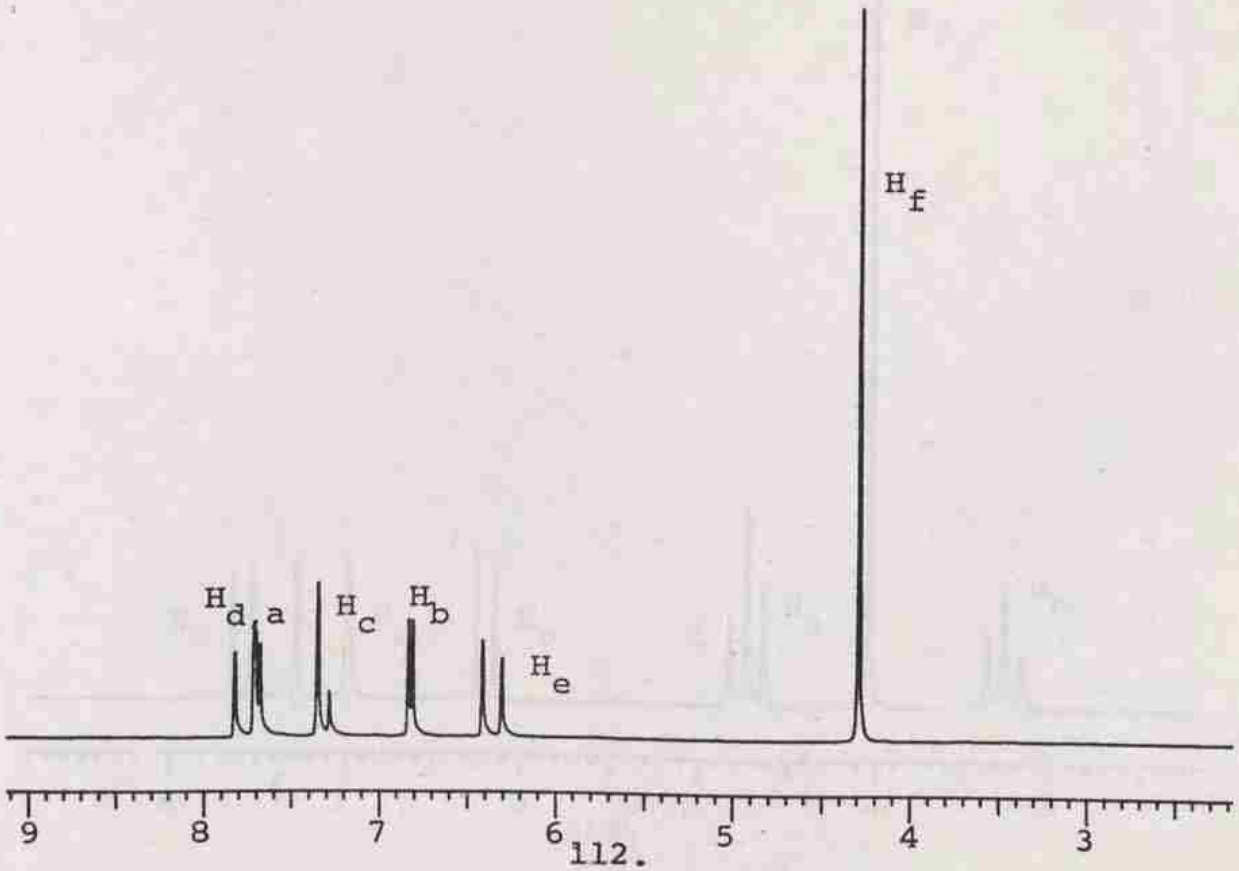


Figure XIII: H^1NMR of 2,3-Dihydromethoxsalen

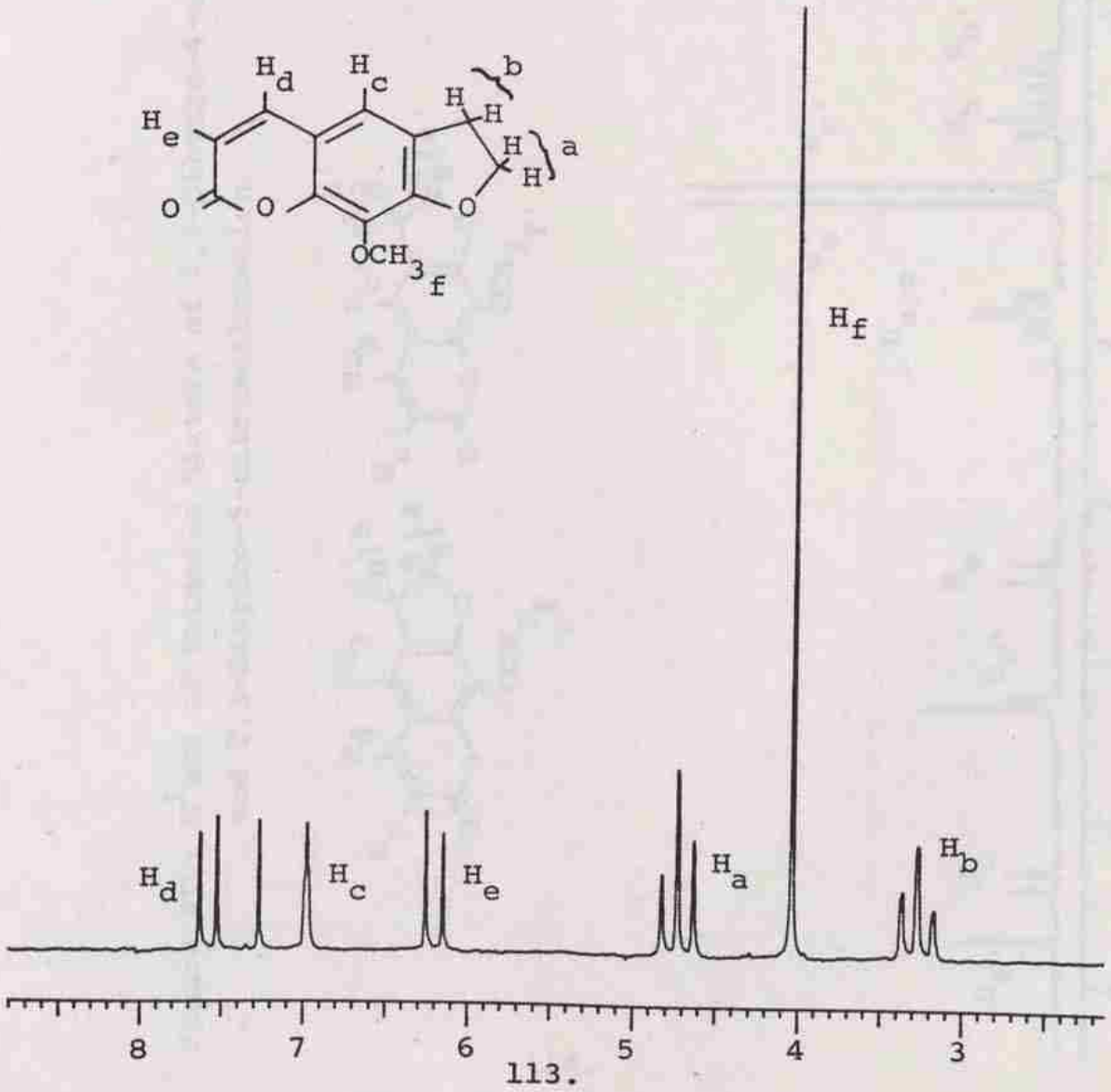
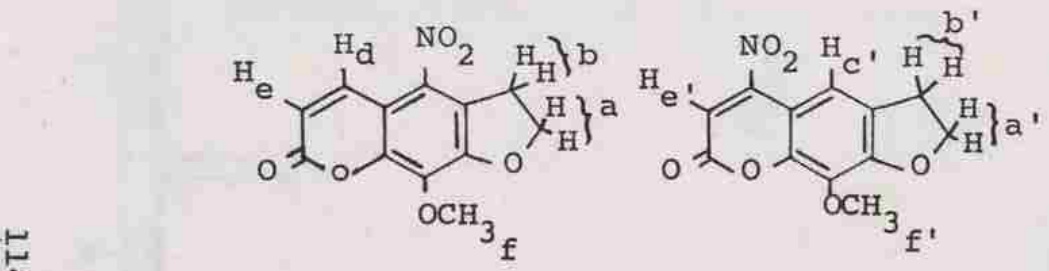


Figure XIV: H^1NMR of Eutectic Mixture of 2,3-Dihydro-4-nitromethoxsalen and 2,3-Dihydro-5-nitromethoxsalen



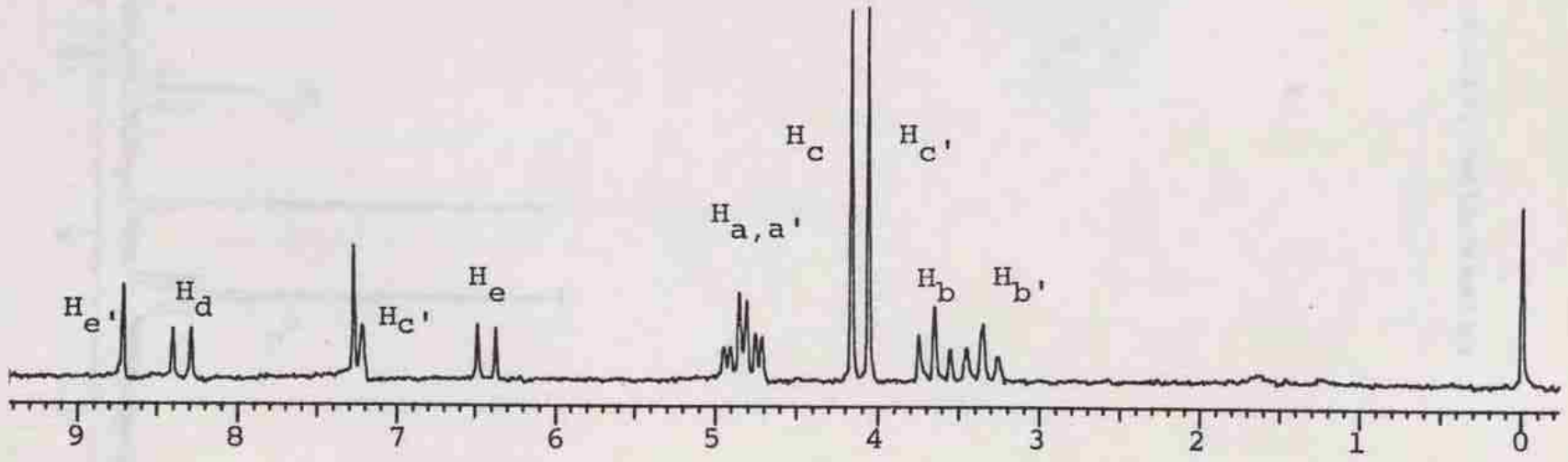


Figure XV: H^1NMR of 2,3-Dihydro-4-nitromethoxsalen

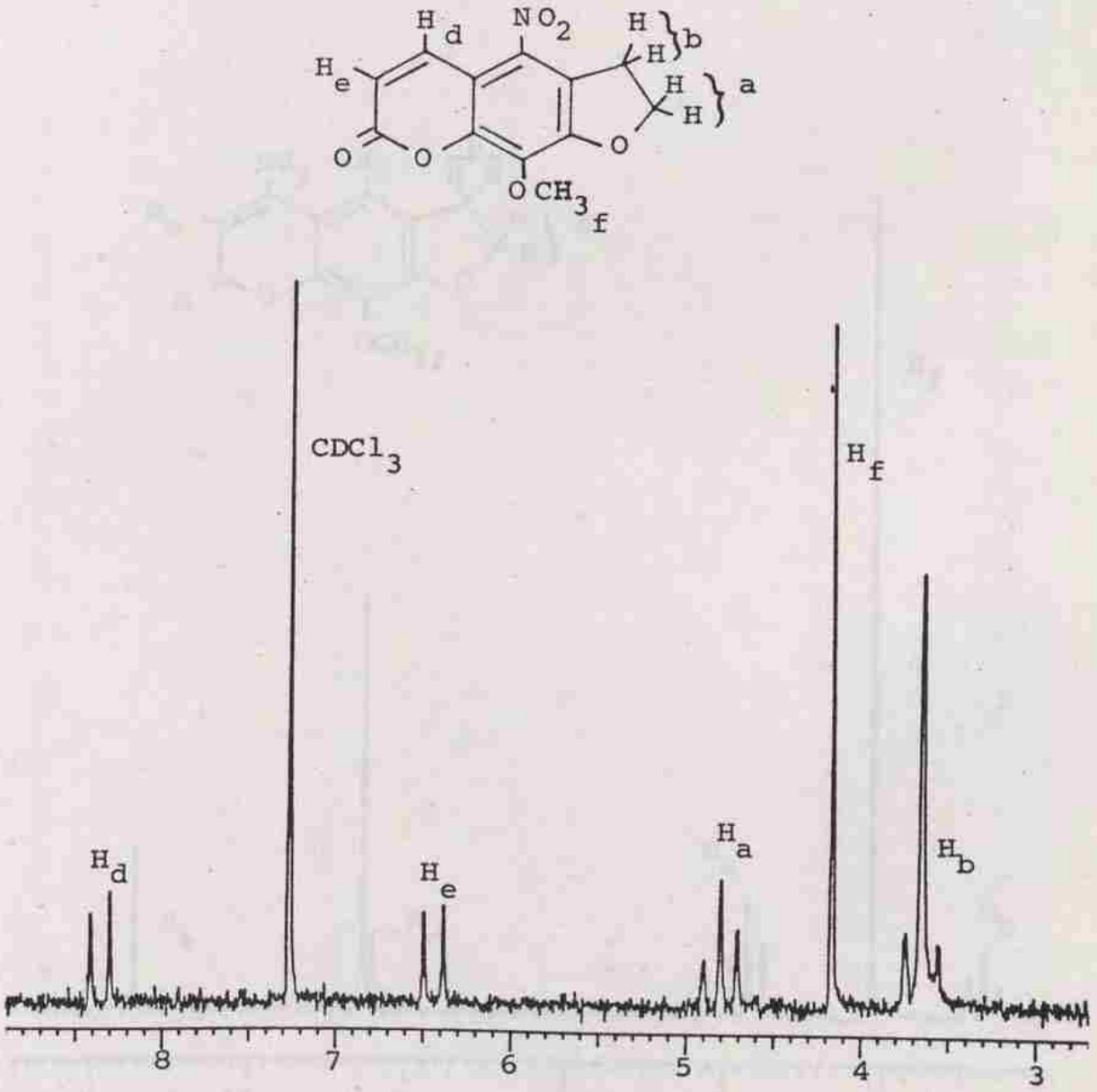


Figure XVI: H^1NMR of 2,3-Dihydro-5-nitromethoxsalen

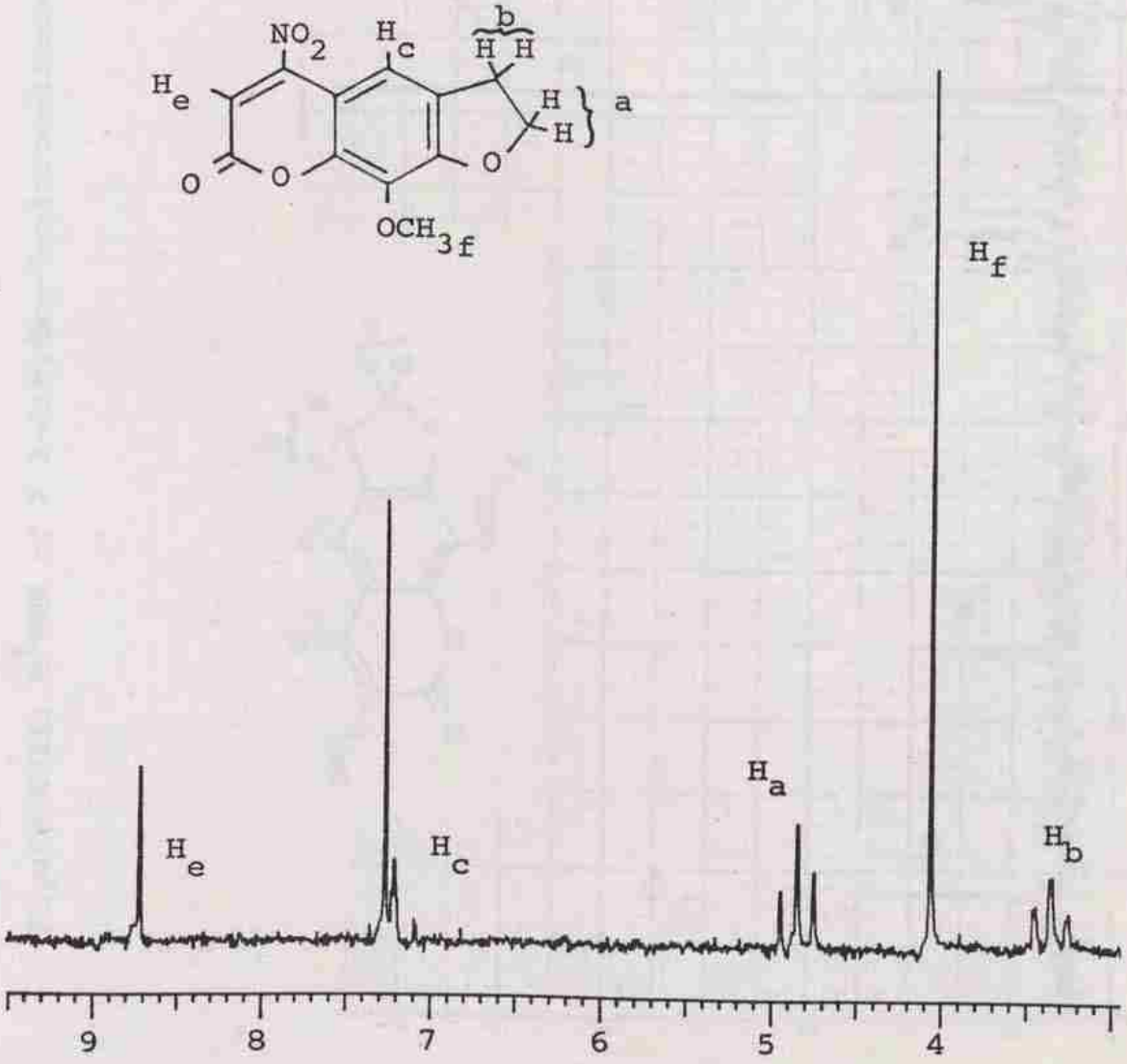


Figure XVII: H^1NMR of 2,3-Dihydro-6-nitromethoxsalen

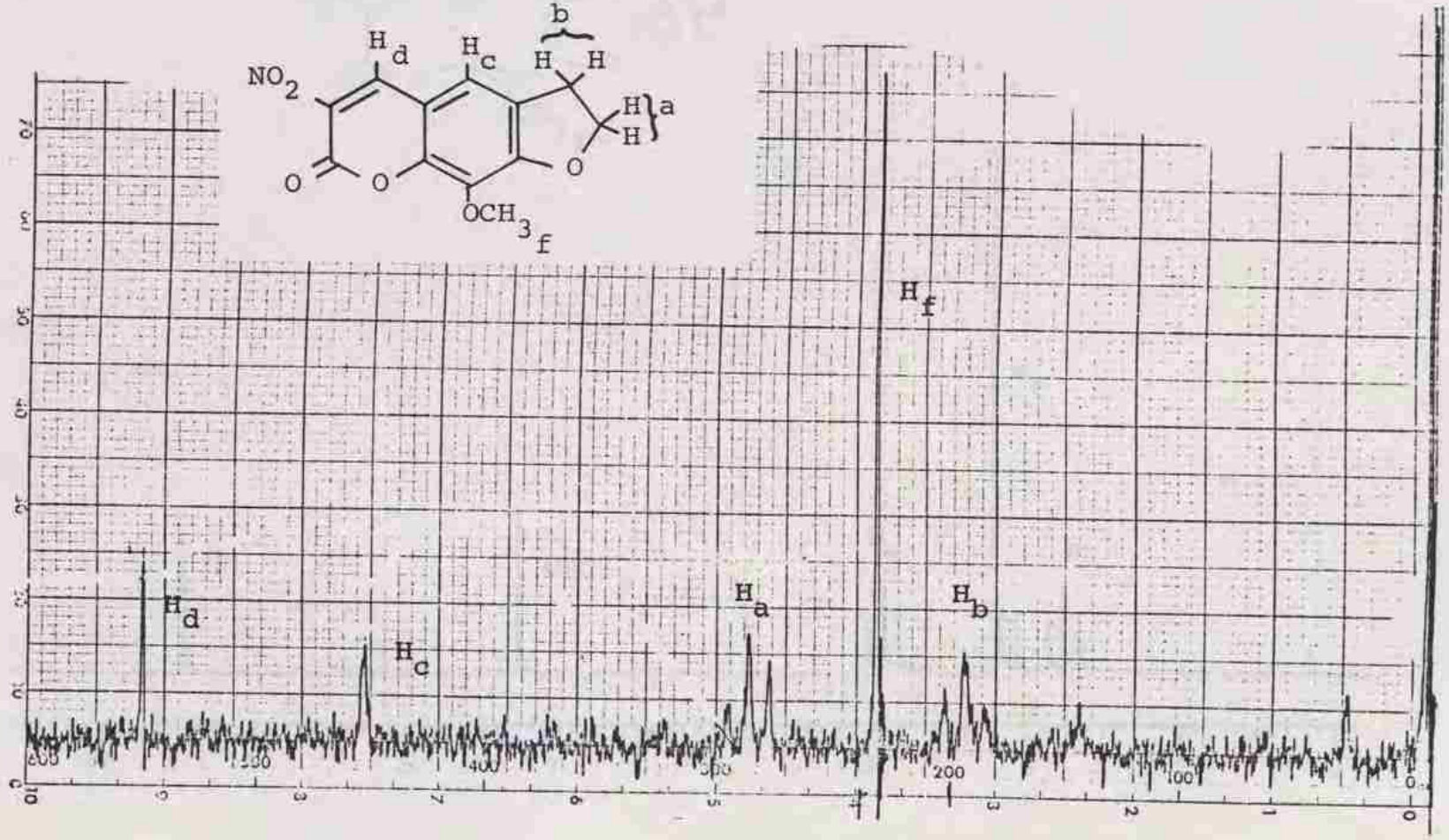


Figure XVIII: H^1NMR of 2,3-Dihydro-4,6-dinitromethoxsalen

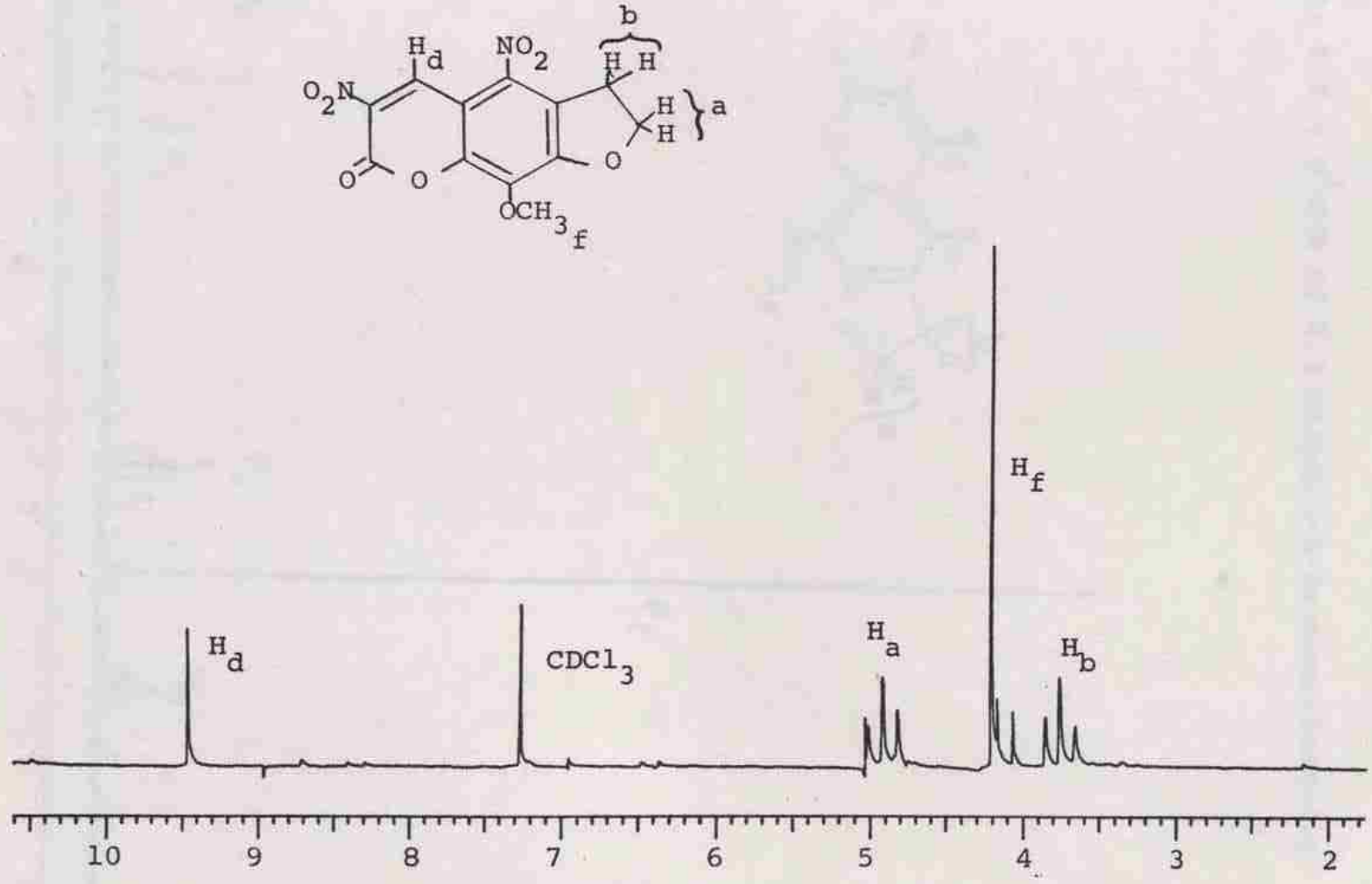


Figure XIX : H^1NMR of 2,3-Dihydro-6-bromomethoxsalen

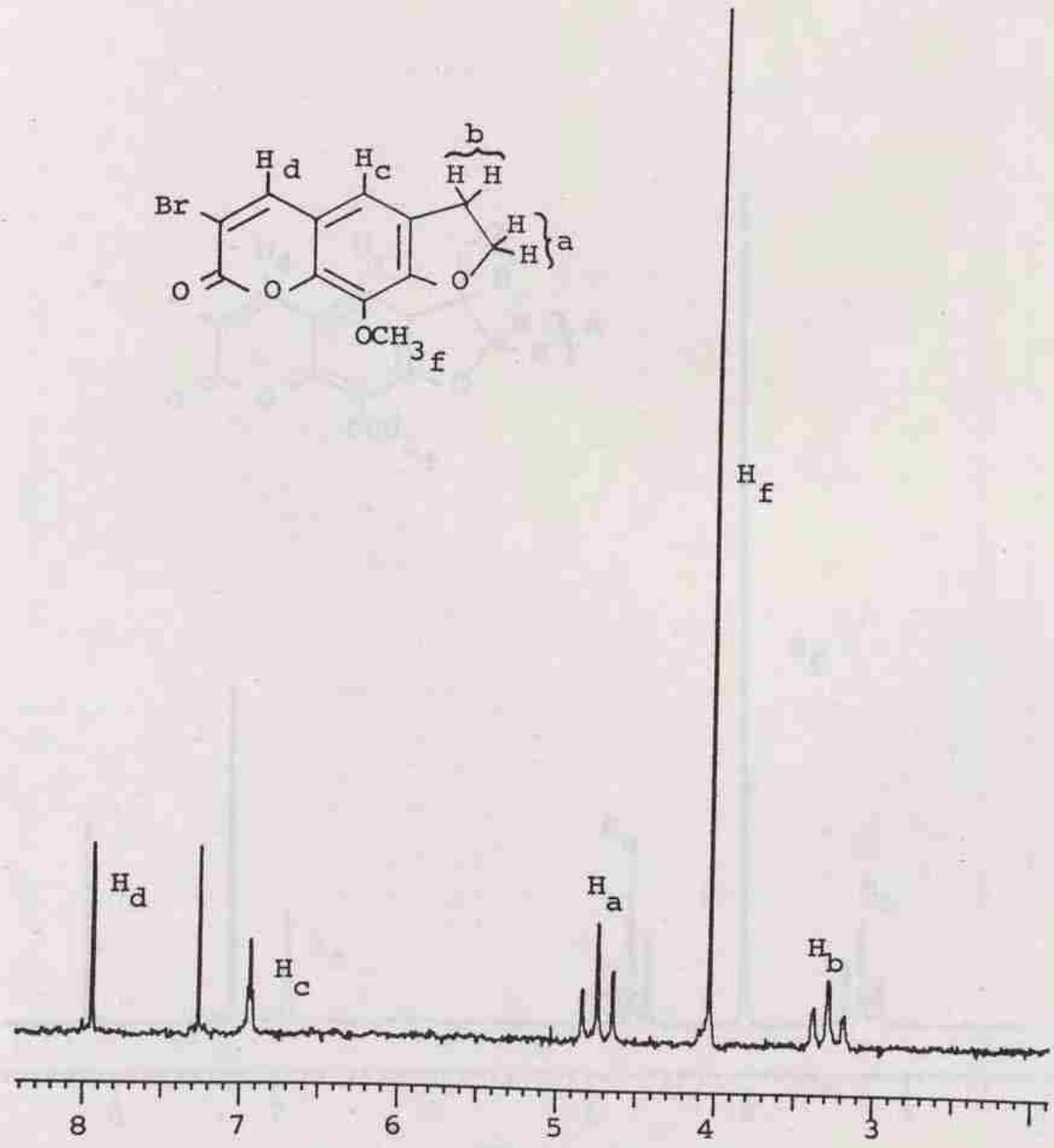


Figure XX : H^1NMR of 2,3-Dihydro-6-iodomethoxsalen

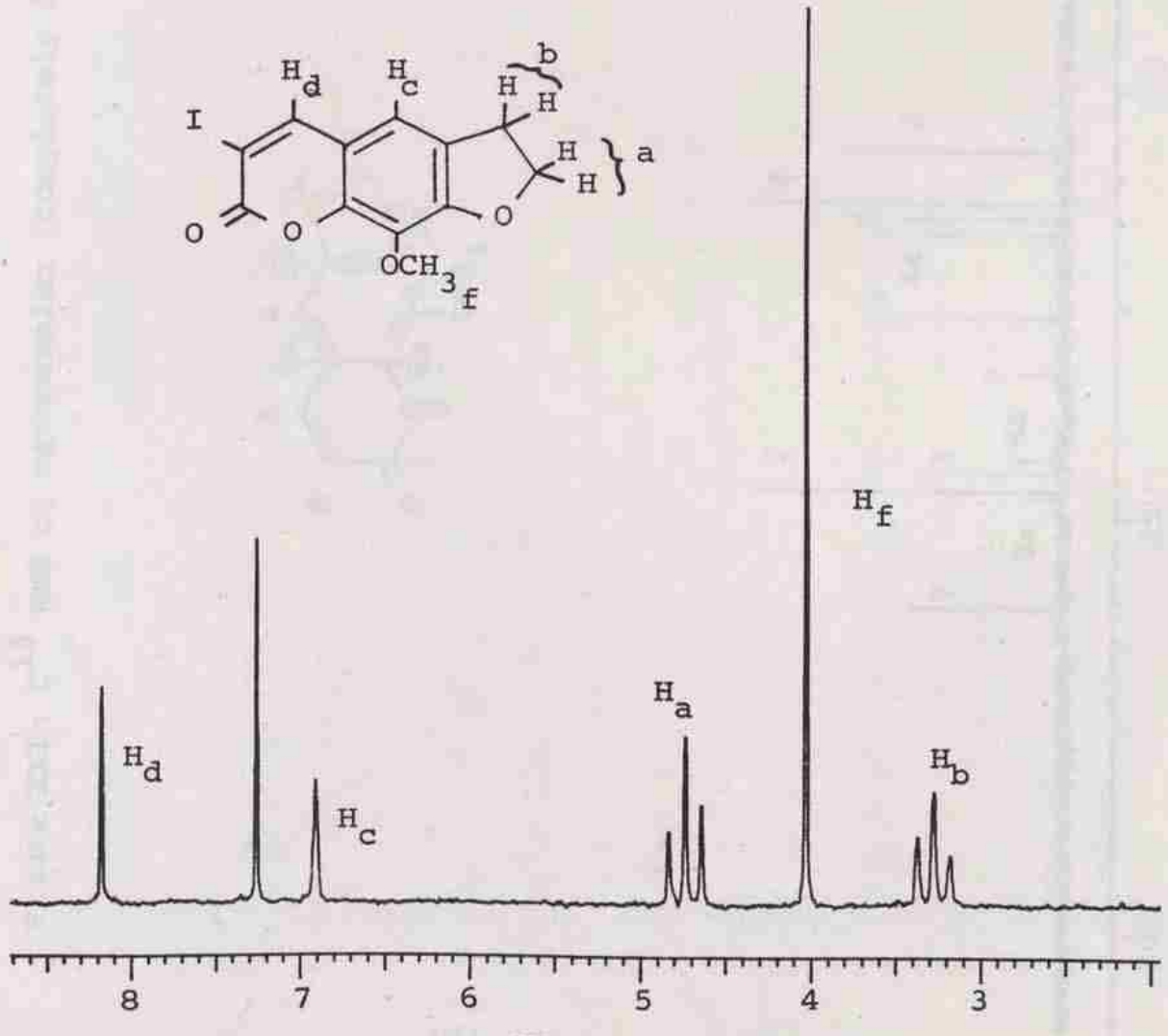


Figure XXI: C^{13} NMR of Methoxsalen (completely decoupled)

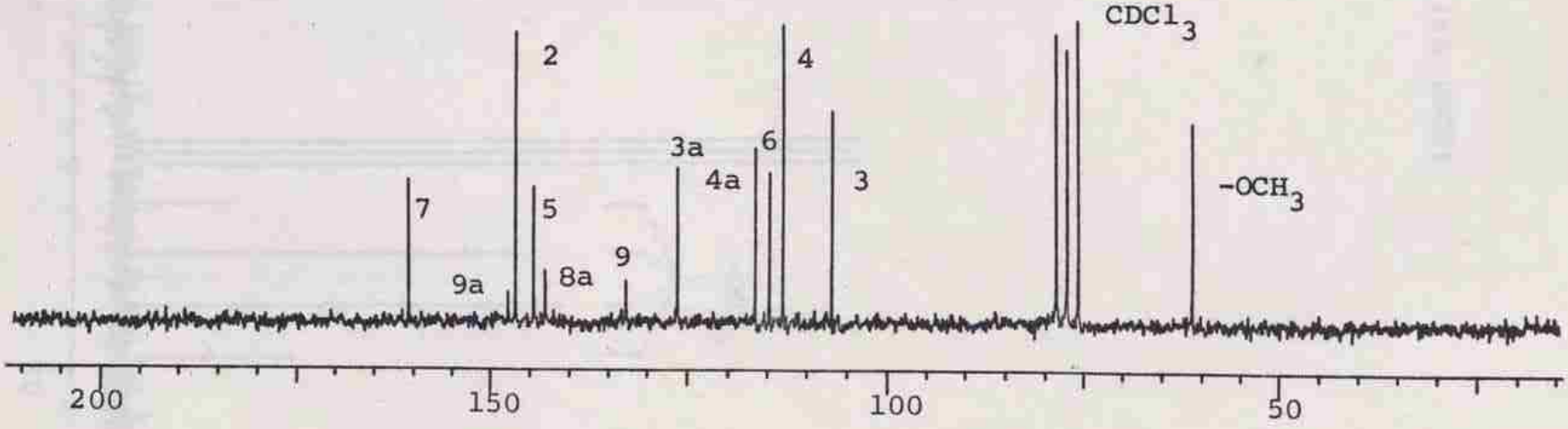


Figure XXII : C^{13} NMR of Methoxsalen (NOE)

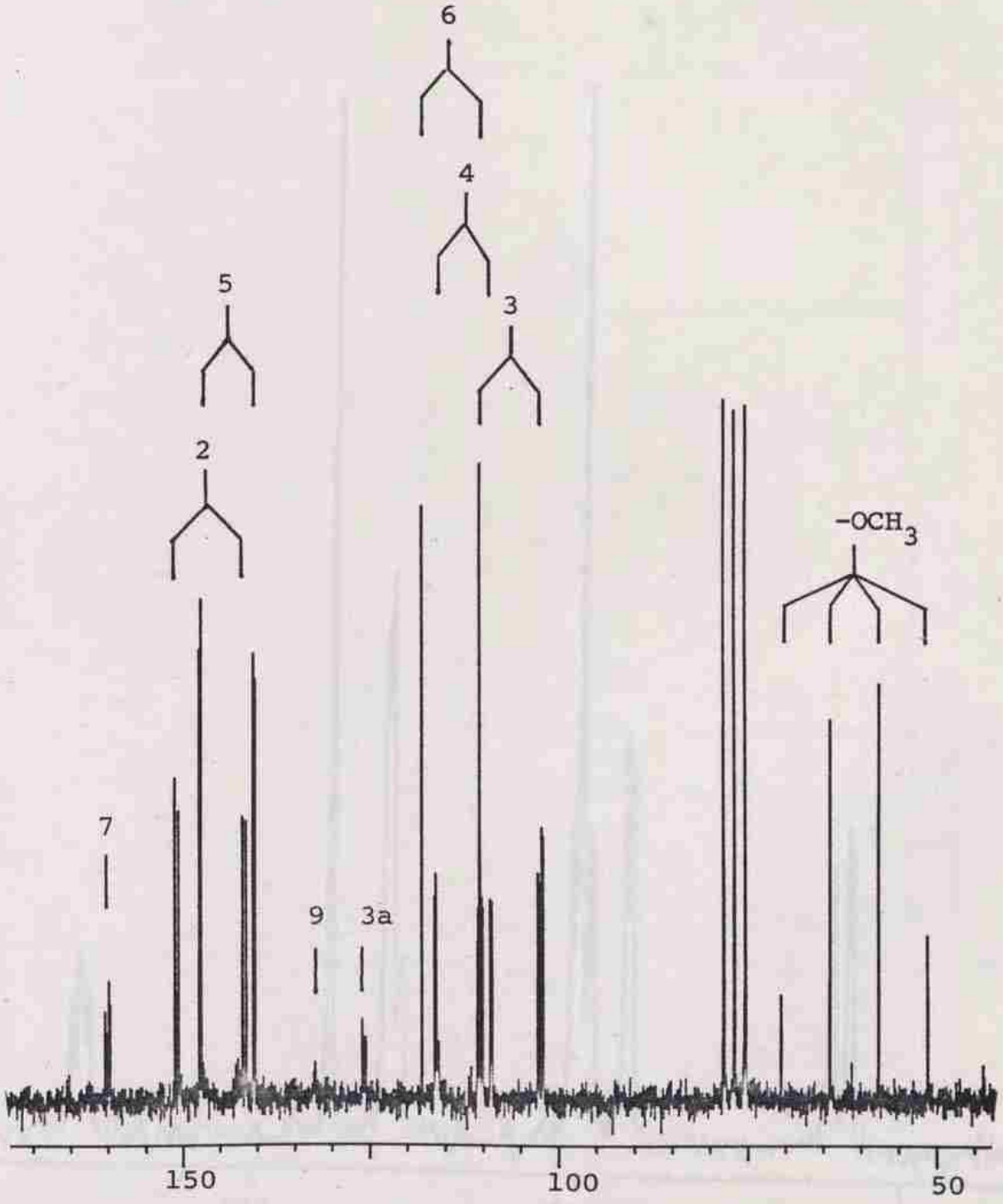


Figure XXIII : C^{13}NMR of Methoxsalen (NOE)

Expansion: 100-130 ppm region showing detailed C-H coupling of C-3, 4, 6

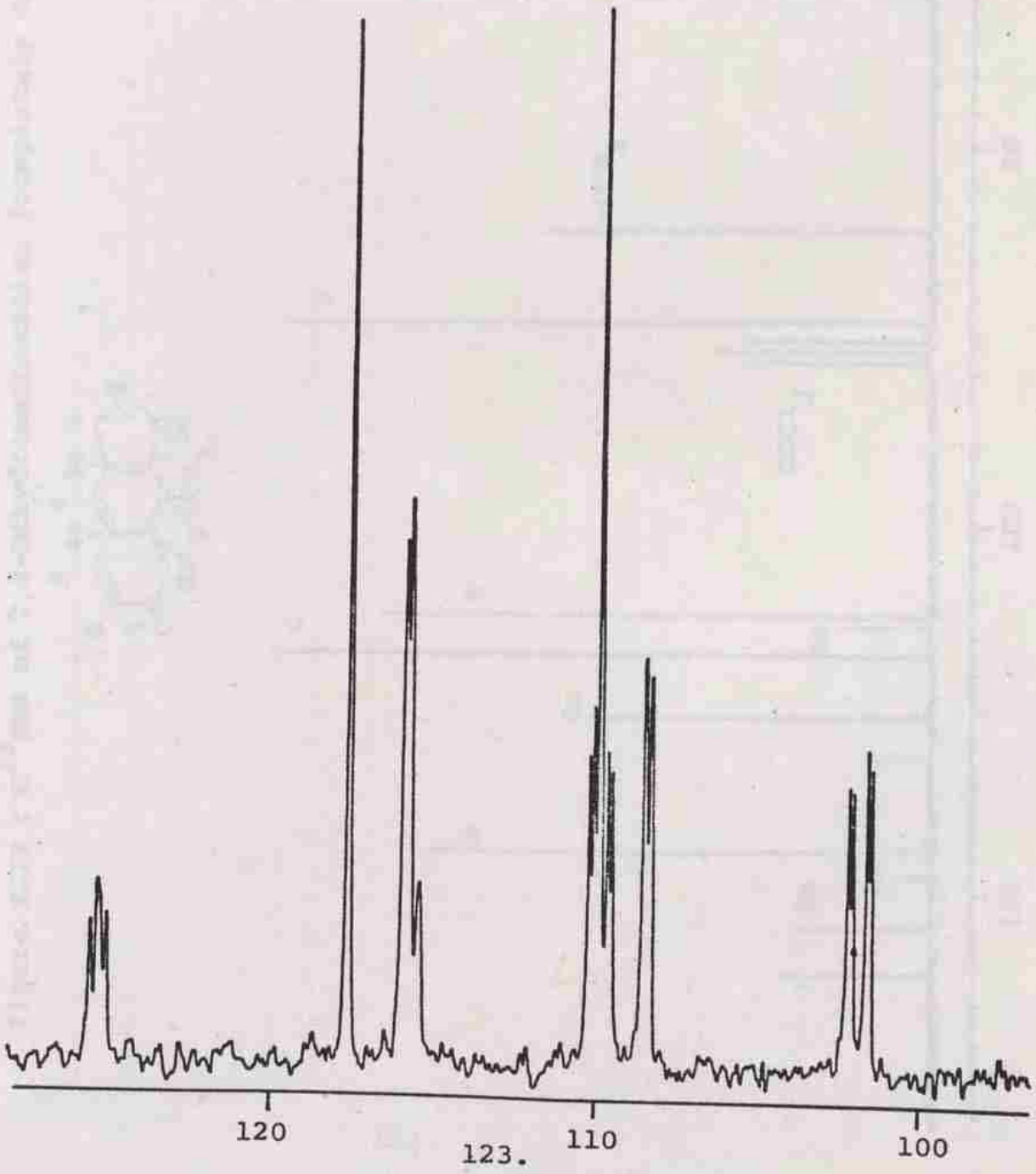


Figure XXIV : C^{13}NMR of 2,3-Dihydromethoxsalen (completely decoupled)

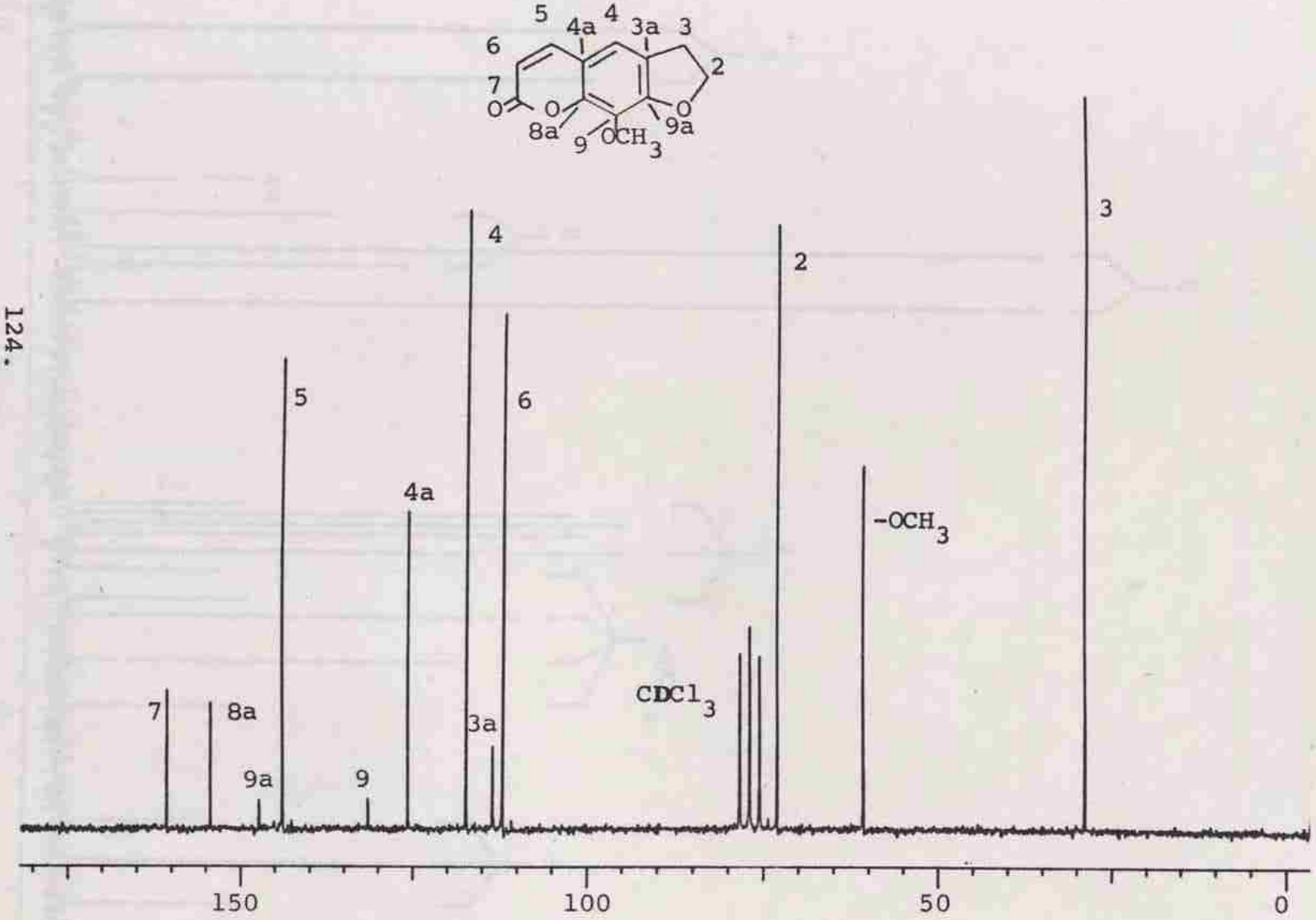


Figure XXV : C^{13}NMR of 2,3-Dihydromethoxsalen (NOE)

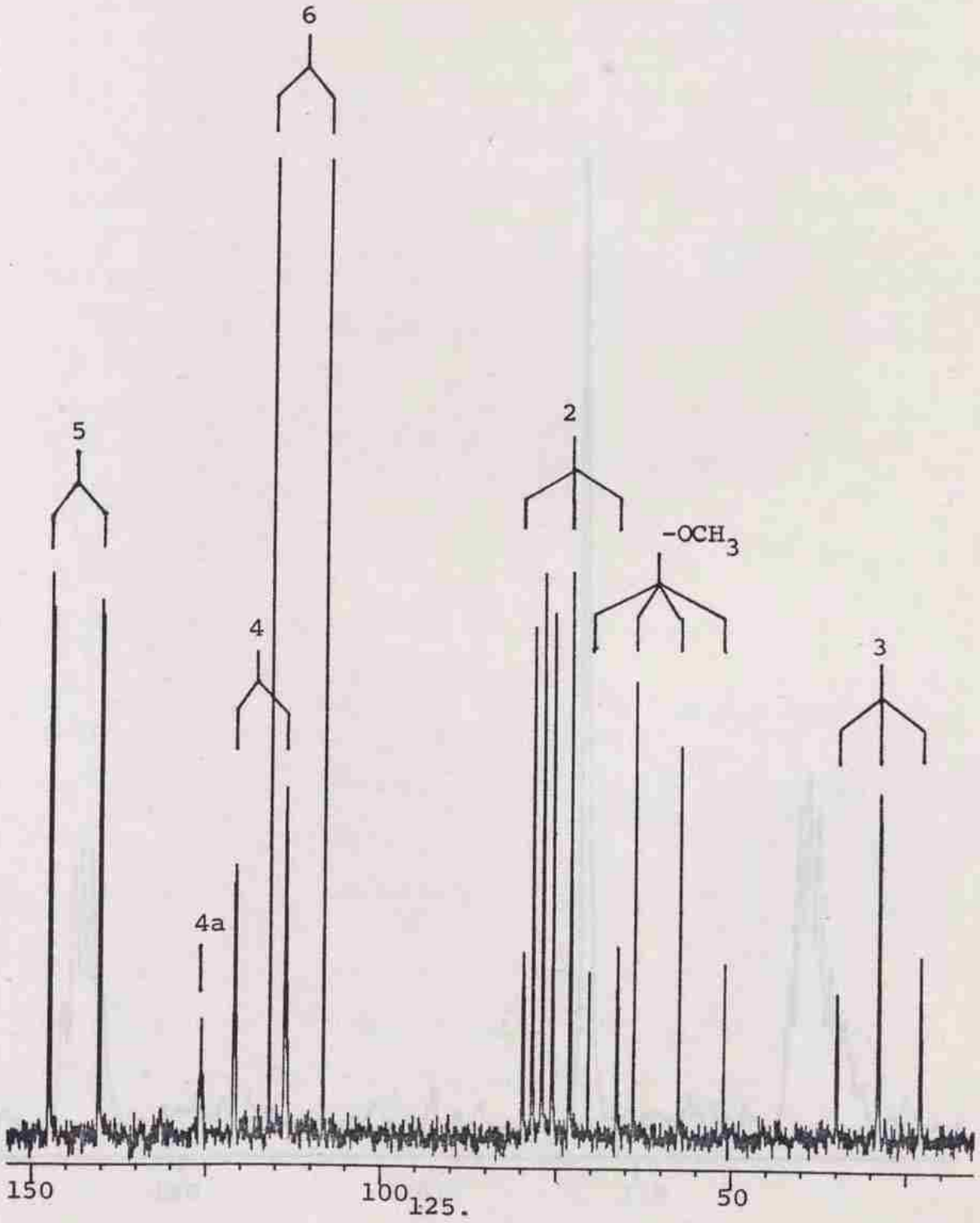
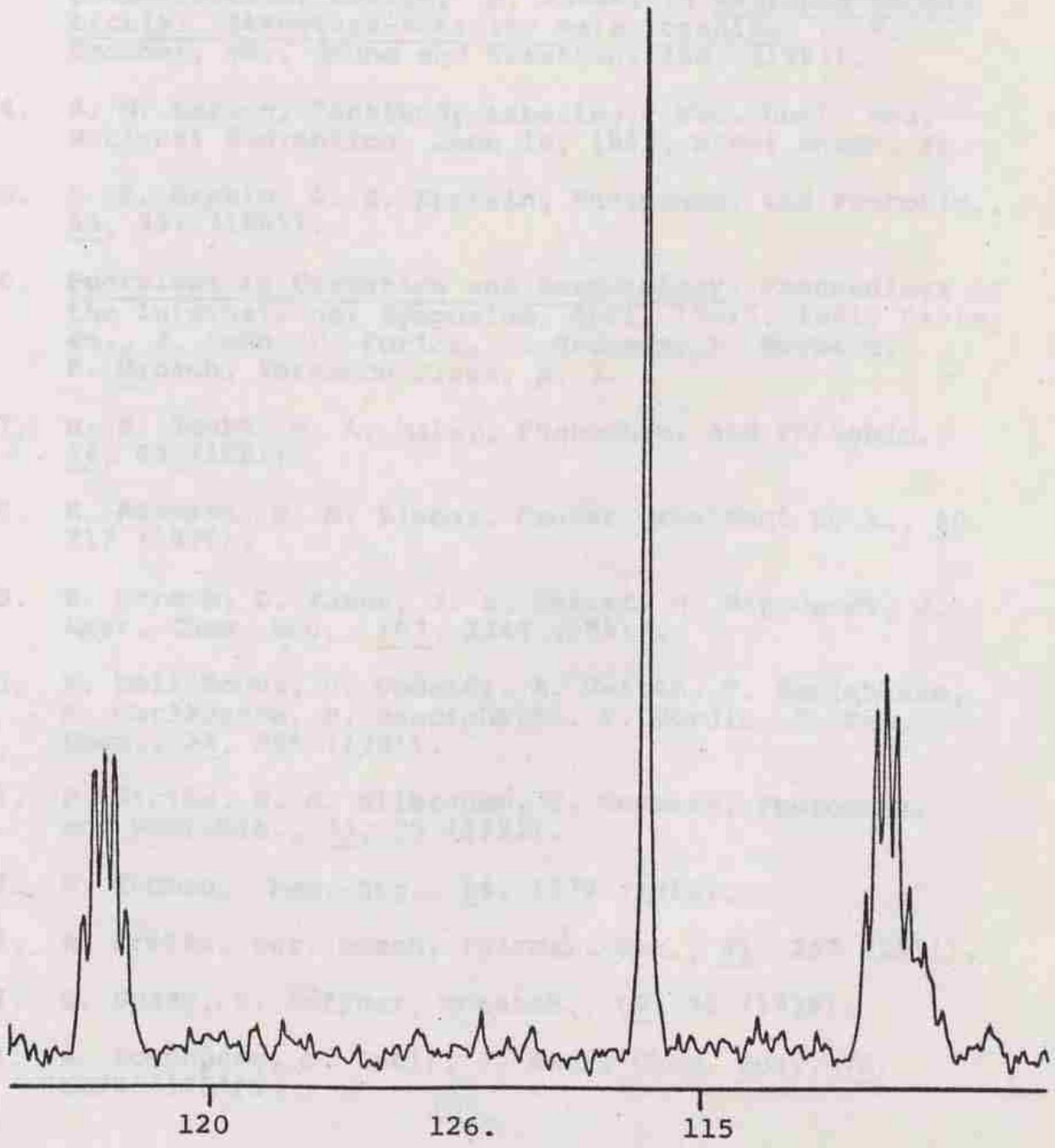


Figure XXVI : C^{13}NMR of 2,3-Dihydromethoxsalen (NOE)

Expansion: 110-125 ppm region showing

detailed C-H coupling of C-4



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APPENDIX

Below are definitions of words and phrases used in the text whose meaning as applied may not be immediately clear.

hot--descriptive of a compound which bears a radioactive atom, or of the radioactive atom itself

cold--descriptive of a compound bearing no radioactive atoms in its structure, or of a stable isotope itself

tracer level--descriptive of a reaction done with a radioisotope in solution in which the concentration of the isotope is several orders of magnitude less than the concentration of the species to be labeled. This phrase denotes both non-stoichiometric with respect to the isotope-coreactant concentration ratio, and nanomolar or lower with respect to the isotope concentration.

mass level--descriptive of a reaction, normally with a stable isotope, in which the molar concentration of both reactive species is comparable. This denotes stoichiometric with respect to the isotope-coreactant concentration ratio, and millimolar or higher with respect to the concentration of both reactants.

VITA

Natalie Foster, the daughter of Josephine N. and the late Claude R. Ingraham, was born July 18, 1948 in Montrose, Pennsylvania.

She received her elementary and secondary education in the Montrose Area School District, and graduated from Montrose Area High School in 1966. After studying one year at the Gymnasium der Humboldtschule in Bremerhaven, Germany, she attended Muhlenberg College, Allentown, Pennsylvania, where she received a Bachelor of Science degree in chemistry in 1971. While teaching at the Swain School in Allentown, Pennsylvania from 1971-1973, she completed the requirements for a Master of Arts degree in Secondary Education at Lehigh University. In 1974, she entered the Chemistry Department at Lehigh, from which she was graduated in 1977 with a Doctor of Arts in Chemistry.

After teaching for three years at Cedar Crest College in Allentown, Pennsylvania, she returned to Lehigh in 1980 as a Visiting Assistant Professor in the Chemistry Department. In 1981, she became an instructor in the department and at that time completed the requirements for the degree of Doctor of Philosophy in Chemistry.

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