HETEROAROTINOIDS: MIMICS OF *TRANS* - RETINOIC ACID

BY

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1984

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May, 1990

HETEROAROTINOIDS: MIMICS OF *TRANS* -

RETINOIC ACID

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ACKNOWLEDGEMENTS

I wish to express my earnest gratitude and special thanks to Dr. Berlin for his guidance, contineous encouragement, and sincere interest which has enabled me to work with boosted motivation. I also wish to express my deep respect and sincere appreciation for Dr. Berlin, for imparting the knowledge and dicipline in the field of Chemistry as well as in certain important aspects of life, outside the lab.

I thank all my colleagues in Dr. Berlin's group: John, Satish, Stan and Tim (for their valuble guidance and inspiration) Prasanna (for all the help in Chemistry) Francis, Marwan and Greg (the 'King') for creating a family like atmosphere and for the willingness to share useful ideas. Special thanks to Stan Zisman for helping me prepare for my seminar.

I am also grateful to Dr. H. A. Motolla and Dr. R. Essenberg for serving as members in my graduate advisory committe. I also thank Dr. Ford for allowing us to use his UV-spectrometer, to collect valuble data and Hui for helping me run the UV experiments. I would also like to express my gratitude to the Department of Chemistry, OSU and the faculty members [and my colleagues: Mike (for putting up with me), Rusty, John, Sanjay, Chris, Jeff (for Mass Spectral data) and to Stan (NMR), Tom (glass shop), Kelly, Vicky, Carolyn and June] for providing an atmosphere of encouragement condusive to research.

A note of thanks to all my friends (thank you Venkey), relatives, all my school and college teachers, my room mate Ganesh (OK) and to a special friend Erin.

No words of appreciation would suffice to express my gratitude to my family [my grandparents, parents, my sister (Uma) and my brother-in-law (Narayanan)] whose love, encouragement and guidance have made it possible for me to come this far in life. This thesis is dedicated to my grand parents (Mrs and Mr. S. V. Appadurai Iyer) whose love

and support in the past years have been exceptional. I finally like to thank God for giving me this opportunity, strength, wisdom and continued encouragement.

TABLE OF CONTENTS

LIST OF TABLES

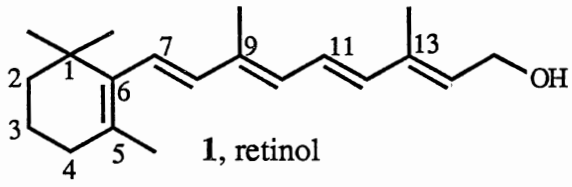
LIST OF PLATES

CHAPTER I

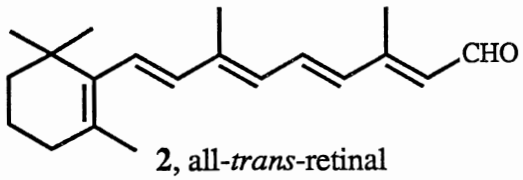
HISTORICAL

Introduction

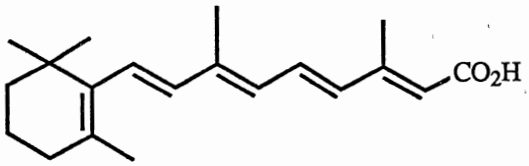
The word "retinoid" is a general term that is commonly applied to both the naturally occurring compounds **1-10** with vitamin A activity and synthetic anologues **10-15**, with or without the biological activity of vitaman A (**1**).[59] Thus a material has been classified



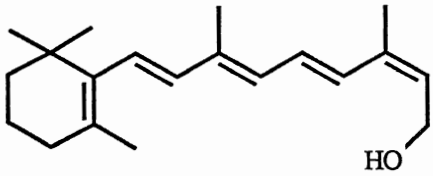
1, retinol



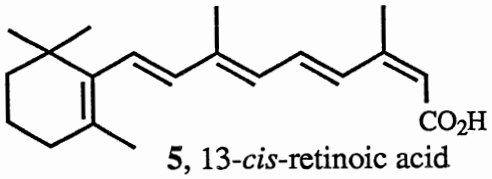
2, all-*trans*-retinal



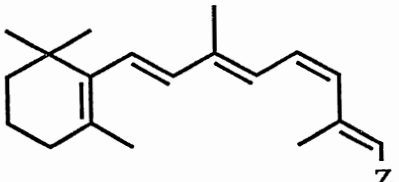
3, all-*trans*-retinoic acid



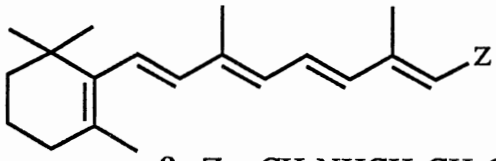
4, 13-*cis*-retinol



5, 13-*cis*-retinoic acid



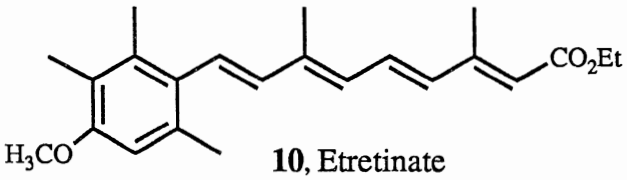
6, Z = CO_2H (11-*cis*-retinoic acid)
7, Z = CHO (11-*cis*-retinal)



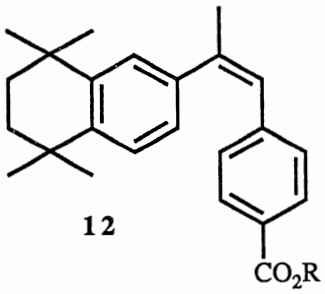
8, Z = $CH_2NHCH_2CH_2OH$
9, Z = $C(O)NHC_2H_5$

under the nomenclature of retinoids if the compound possessed a structural relationship reminiscent of the parent retinol,[64] even if the compound was devoid of activity associated with vitamin A. Retinoids have also been designated structurally as compounds consisting of four isoprenoid units joined in a head-to-tail manner.[34] Arotinoids **10-12** are retinoids with a least one aryl ring in the system.[46,47] Heteroarotinoids **13** and **14** possess an aryl ring and at least one heteroatom in the ring system (or possibly in the side chain in some cases).[13,66,72]

AROTINOIDS



10, Etretinate



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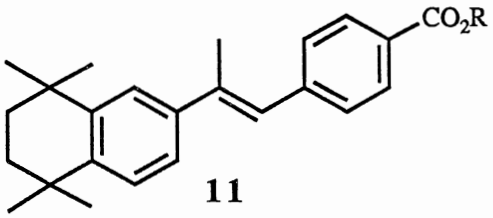
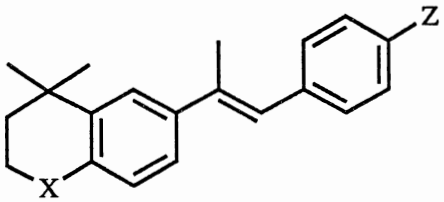
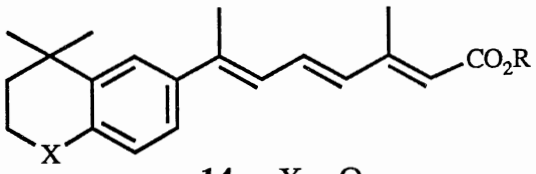


11

HETEROAROTINOIDS



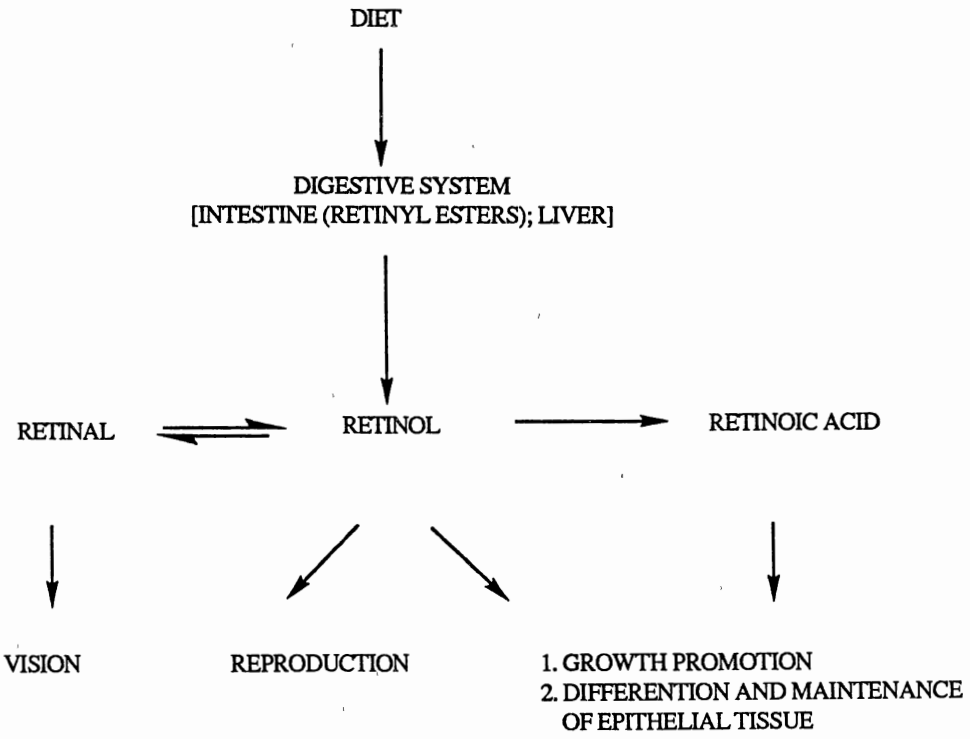
13, X = O, SO_2, NR, S
Z = CO_2H, CO_2Et, CO_2Me



14a, X = O
R = Et
14b, X = S
R = Et

Background

Since ancient times, it was believed that a substance existed in the diet which was necessary for night vision. However, only at the beginning of this century was it shown that a fat soluble compound (later named vitamin A) not only prevented night blindness[32] but also promoted growth in rats.[32] In 1937, Holmes and Corbet were able to crystallize pure retinol (**1**).[32] An important discovery in this field was the identification by Wald (1934)[69] and by Morton (1944)[49] of the chromophore of the visual pigment, namely retinal (**2**). However, it is unclear as to what form or derivatives of retinol is involved in the other biological functions such as growth promotion, reproduction, differentiation, and maintenance of epithelial tissues as illustrated.

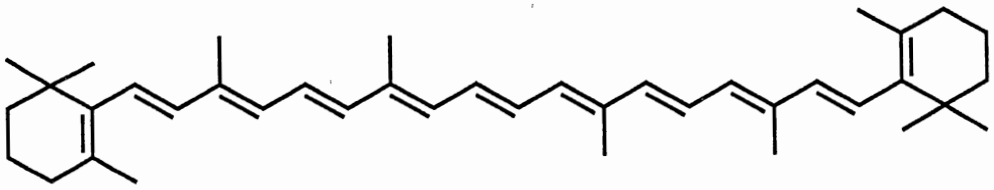


It appears that no single retinoid is able to function alone in all the varied aspects of vitamin A activity which suggests that different metabolites of retinoids may be responsible for vitamin A and related types of activity. Thus it is important to understand the

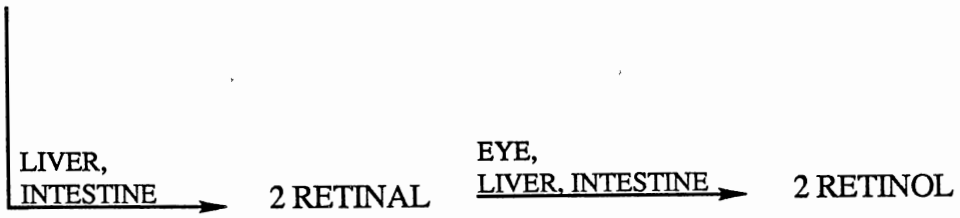
metabolism of natural retinoids and the nature of various metabolites. Recent treatises focus on the potential carcinostatic property of retinoids, metabolites therefrom, and certain structure-activity relationships uncovered.[4,13,54,63]

Metabolism of Natural Retinoids

Until the introduction of synthetic retinoids into food, ß-carotene (**15**), a natural plant product, was the major dietary source of retinol (**1**). This compound is cleaved into two molecules of retinal (**2**)[28,63] by a soluble enzyme (ß-carotene 15,15'-oxygenase) found in the intestine and in the liver.[28,63] The reaction requires molecular oxygen and is inhibited by sulfhydryl group inhibitors and by chelators of ferrous ion.[15,16] The enzyme involved in this conversion (retinaldehyde reductase) is found not only in the intestine but also in the liver[73] and in the eye.[6] Alcohol dehydrogenase can also convert retinol (**1**) to retinal (**2**).[6] It has been postulated that in rat liver both the retinaldehyde reductase and the alcohol dehydrogenase activity reside in the same enzyme.[73]

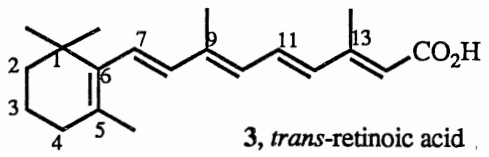


15, ß-Carotene

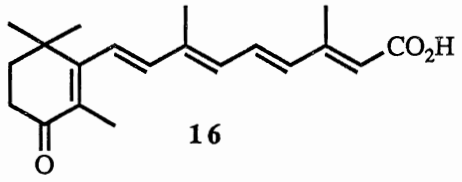


It is believed that after binding to a specific protein to form a complex, retinol (**1**) is transported to cells.[41] The major metabolic pathway for **1** can involve either oxidation or a non oxidative pathway.[65] Many oxidative paths have been suggested by different research groups.[17,20-22,31,48,57,74] It is clear from structures **16-22** that oxidation can occur at

several sites. After analysis of these structures, one might conclude that the metabolic degradation sites are: (1) oxidation at C(4), (2) epoxidation of the double bond in the cyclohexyl ring, (3) oxidation of one of the methyl carbons of the geminal dimethyl pairs,



3, *trans*-retinoic acid



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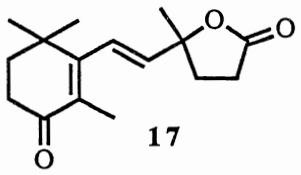

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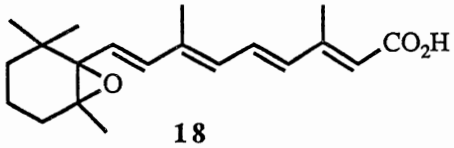

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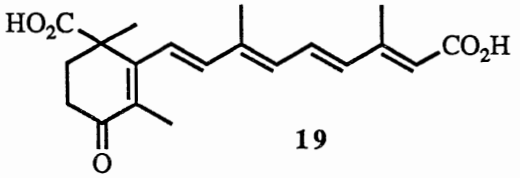

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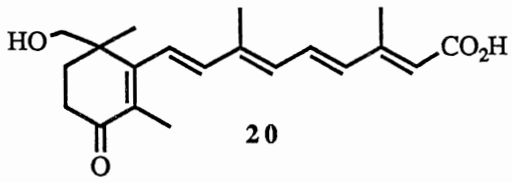

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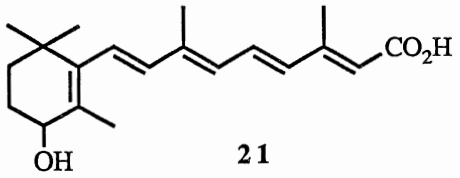

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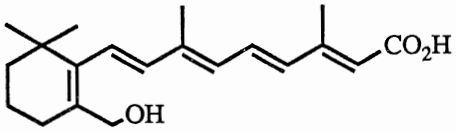
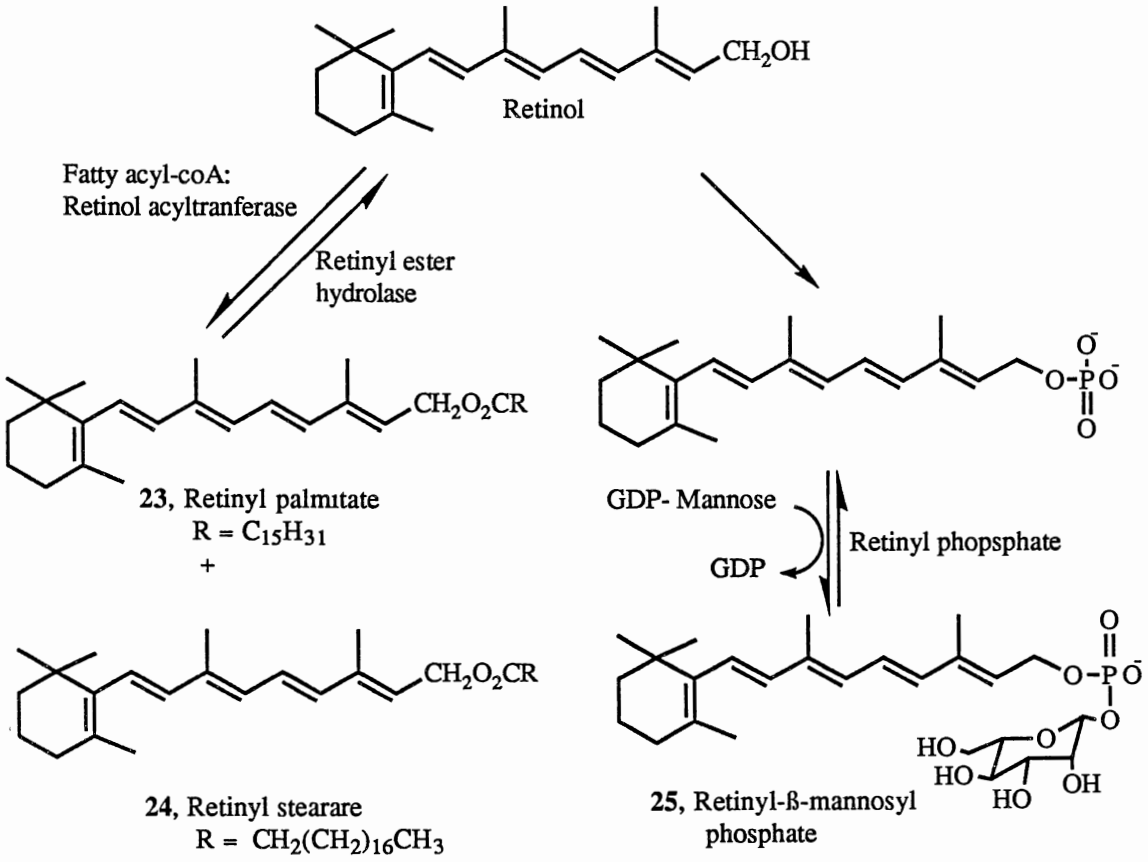


22

(4) oxidation of the methyl group at C(5), and (5) shortening of the polyene side chain with partial oxidation-reduction of the conjugated system. In general, oxidation may occur at a double bond in **3**, such as to give **17** and **18**, at a carbon atom one bond removed from a double bond,such as to give **16** and **19-22**, or at carbon atom two bonds removed from a

double bond such as to give **19** and **20**. Nonoxidative pathways have also been suggested.[1,15,29] These involve either isomerization[17] of the double bond in the ring or formation of an ester involving retinol and a carboxylic acid [like stearic or palmitic[29] as in **23** and **24**] or phosphoric acid (like **25**).



Current research seeks to determine if any metabolite of vitamin A, or that of its synthetic analogues, may have activity equivalent or superior to that of all-*trans*-(**3**) or 13-*cis*-retinoic acid (**5**). Since specific biological activities may in reality reside with metabolities of retinoids, it is very important to understand the metabolic pathways. Such information can aid in strategy to design synthetic analogues to have specific properties. A growing awareness in this area of research includes the structural correlations, especially

the geometric and spatial arrangements of atoms which play a pivotal role in distinguishing metabolites and their specific biological activities. The concept of "a better fit" of the synthetic analogues on a structural basis could result in better binding characteristics of retinoids,[13,25,36] such as involved with the protein complex cellular retinol binding protein (cRBP). This theory is gaining acceptance.[14,25]

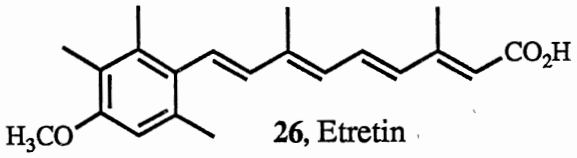
Mechanism of Action

Numerous theories for the mechanism of action of retinoids have been reported in several detailed reviews.[3,54,63] Although the exact mechanism of action of retinoids is not completely understood (especially at the gene level), it is now thought from the many *in vitro* and the *in vivo* studies that retinoids affect the gene expression.[43,58] This process may have a direct relation to the observed biological effects. It is believed that albumin[54] picks up the retinoic acid and the retinol in blood and delivers such to cells. At the cell surface, these retinoids are bound to a protein receptor,[11,58,61] namely, the cellular retinol binding protein (cRBP) and the cellular retinoic acid binding protein (cRABP). The receptors involved have been isolated and characterized.[50-53] The complex (between the retinoid and protein receptor) apparently penetrates the nuclear membrane and enters into the nucleoplasm to deliver the retinol to the chromatin (a type of complex present in the chromosome). Green and co-workers [24] showed that a type of katrtin (67-kDa, fibrous protein) was suppressed by the retinoids at the mRNA level. Later it was discovered[71] that retinoids act at the DNA level in F9 cells (a type of cancer cell). Evans and co-workers [26] and Chambon and co-workers [55] were able to characterize and illustrate the importance of certain human protein receptors which contain a DNA binding site as well as a retinoid binding site. All the above findings suggest that many of the biological activities of the retinoids can be attributed, at least in part, to an ability to act at the gene level (regulation of the gene expression) by forming complexes with specific binding protein receptors,

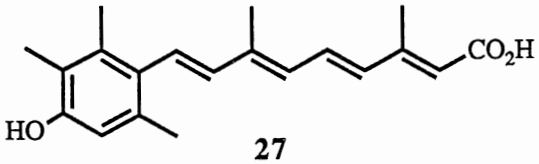
although the exact nature of the binding in the nucleus [that is, whether the binding includes DNA, RNA, or chromatin] is not certain.

Synthetic Retinoids: (Arotinoids)

Retinoids with an aryl ring incorporated in the basic retinoic acid skeleton have been classified under the name of "arotinoids". Early work in this area began via the discovery of the Hoffmann-La Roche Company that the incorporation of an aromatic ring into the basic system caused a dramatic increase in the therapeutic ratio [the therapeutic ratio was determined from the dose (mg/kg) which caused 50% regression of papillomas in Swiss mice relative to that dose (mg/kg) which produced hypervitaminoses A syndrome] of such retinoids.[46,47] This resulted in the development of "Etretinate" (**10**), now a commercial agent.[7,31,46] The success in the commercialization of therapeutically useful retinoids led to investigations and the synthesis of similar compounds.[7,31,47] Compounds **26-31** are metabolites of **10**.



26, Etretin



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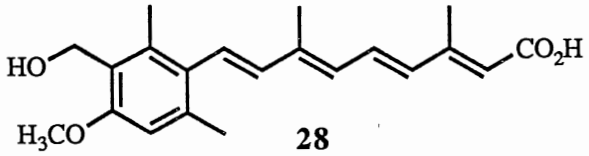

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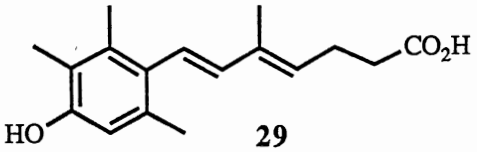

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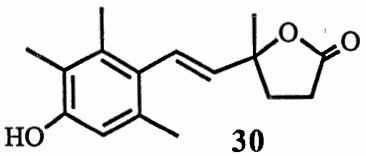

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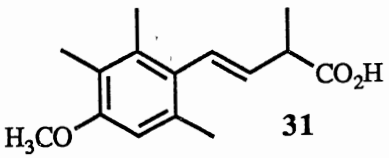

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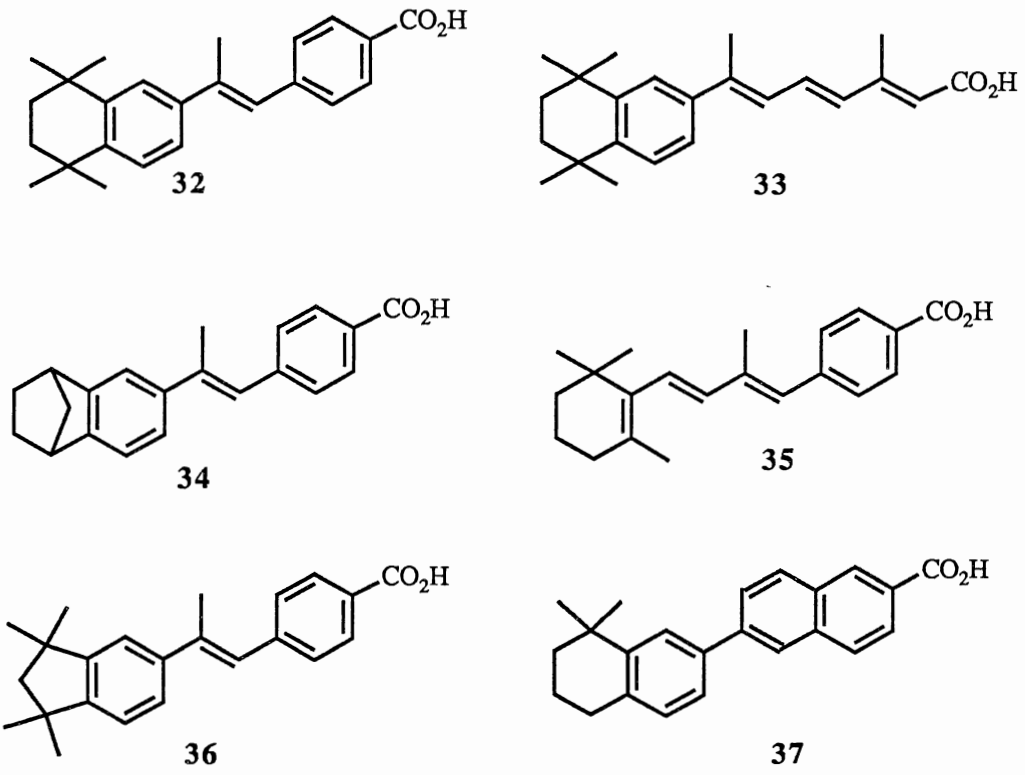
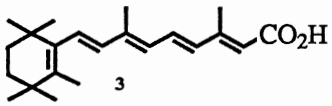
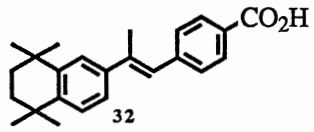
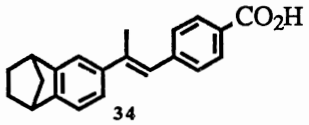
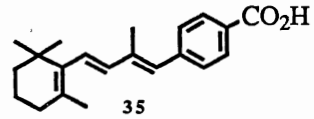
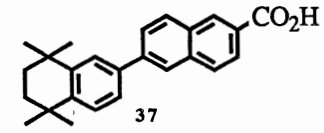
Five- and six-membered rings have also been incorporated into the basic system and biological activities have been determined [7,47] for several compounds. Arotinoids **32-37** are a few examples of novel, synthetic arotinoids reported.



Many arotinoids exibited high antipapilloma activity (ability to cause regression of a certain type of skin tumor).[46] This led to the synthesis of (*E*)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalyl)-1-propenyl]benzoic acid (TTNPB, **32**), an arotinoid with a fused six-membered ring and with another aromatic ring incorporated into the side chain.[13,46] Biological activities, toxicity assays, and various metabolites of Etretinate (**10**), TTNPB (**32**), and other arotinoids have been examined and some of the results are tabulated in Tables I and II. More tests have been reported for retinoids with carboxyl groups since it is believed that the carboxyl group is important for the retinoid to bind *in vivo* to cRABP.[35]

TABLE I

ACTIVITY OF SELECTED RETINOIDS IN TOC AND ODC ASSAYS[a]

Arotinoid	TOC Assay[b] ED_{50}, nmol mg/kg/day	ODC[c] Dose, nmol	ODC[c] %Inhibition[d]
 3	1×10^{-11}	1.7	88
 32	1×10^{-12}	17 1.7	91 81
 34	6×10^{-10}	17 1.7	69 33
 35	3×10^{-10}	17 1.7	77 34
 37	3×10^{-10}	17 1.7	80 58

[a]From Reference 13.

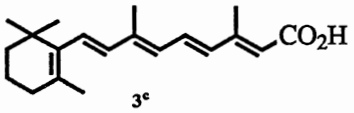
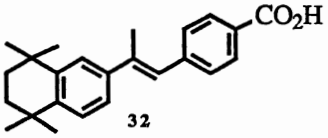
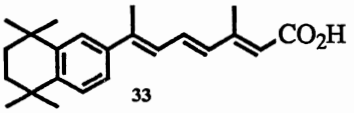
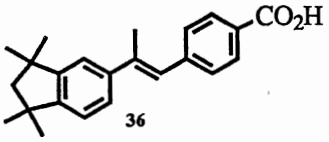
[b]Tracheal organ culture assay, Reference 67 and 68.

[c]Ornithine decarboxylase assay, Reference 67 and 68.

[d]% inhibition = [100 x ODC activity (retinoid)-ODC activity (control)]/ODC activity (control)

TABLE II

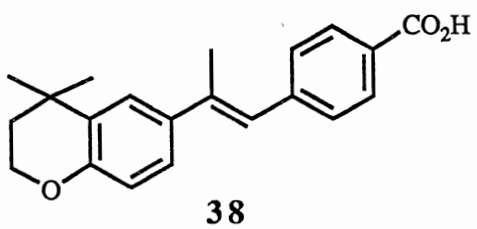
THE ABILITY OF AROTINOIDS TO INDUCE DIFFERENTIATION IN THE HUMAN PROMYELOCYTIC LEUKEMIA CELL LINE (HL-60) AND TO INHIBIT COMPLETELY SCALE FORMATION IN THE SKIN OF THE FOOT OF CHICK EMBRYO[a]

Arotinoid	Induction of Differentiation HL-60 Assay[b], ED_{50}	Complete Inhibition
 3[c]	1×10^{-7} (1×10^{-8})[e]	10^{-5}
 32	7×10^{-8}	10^{-8}
 33	8×10^{-9}	10^{-8}
 36	-[d]	10^{-7}

[a]Reference 7.
[b]Reference 8 and 9.
[c]Not an arotinoid but included for comparision.
[d]HL-60 activity not reported.
[e]Reference 71.

Heteroarotinoids

The undesirable toxicity level of arotinoids has prompted further research in terms of structure modification. Heteroarotinoids are a group of heterocycles which retain some features of the retinoid skeleton but have special characteristics in that at least one aryl ring and one heteroatom are incorporated into the basic system.[13,66,72] Structures of some heteraoarotinoids **38-52** synthesized recently are given below. Several of these heteroarotinoids exhibited marked activity in the ODC (Table III) and TOC assays (see section entitled Activity of Retinoids)[13,45,72] and some (like compounds **38** and **39**) have demonstrated much less toxicity (table IV) than the hydrocarbon standard **32**.[13,45,59]



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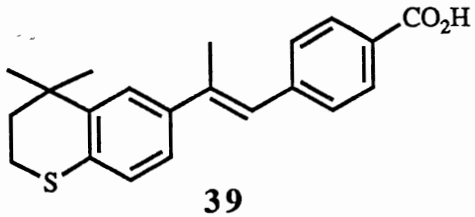

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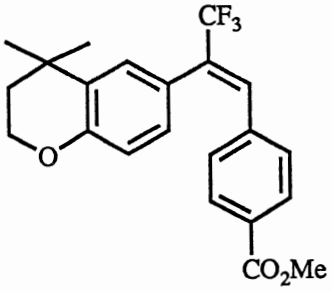

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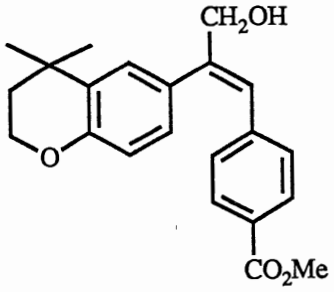

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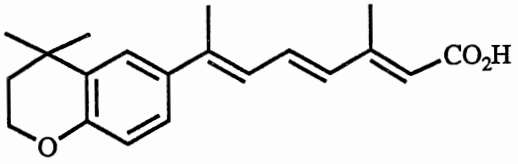

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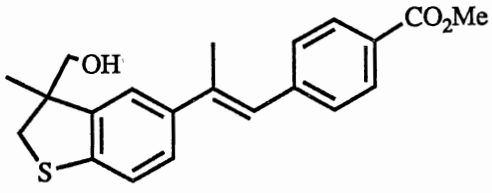

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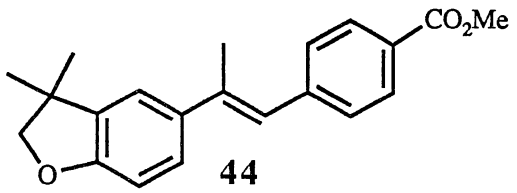

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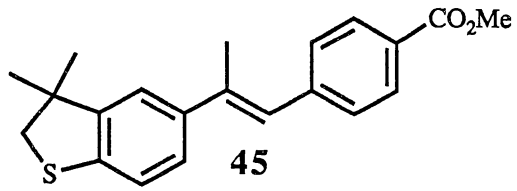

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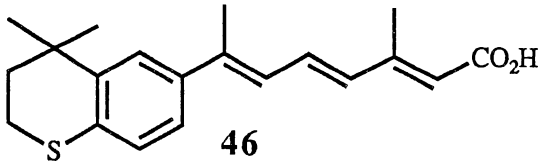

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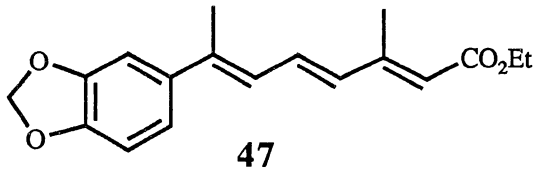

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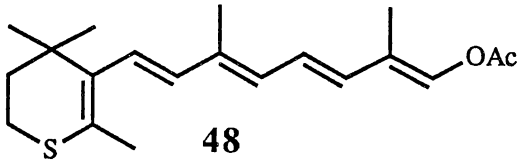

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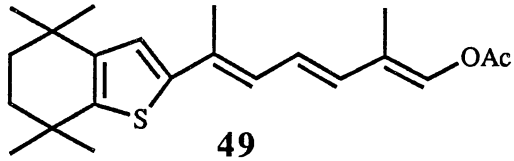

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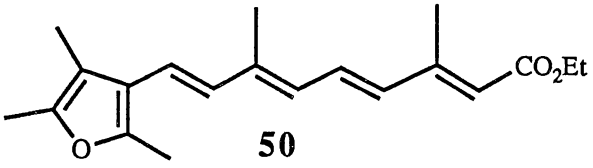

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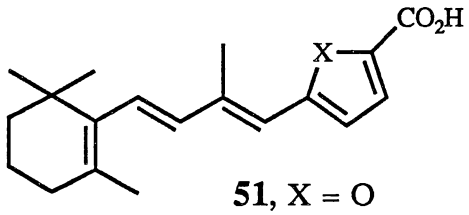
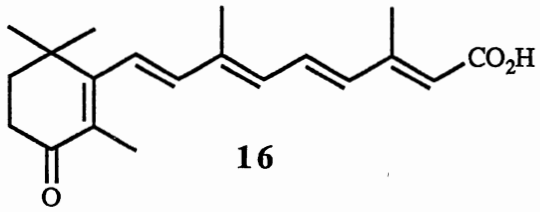
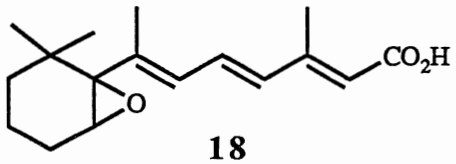


51, X = O
52, X = S

As discussed previously, the oxidation of *trans*-retinoic acid (**3**) occurs at C(4)[57] to give **16** and at the C(5)=C(6) bond[48] to give **18**. Thus, the introduction of a heteroatom at C(4) and protecting theC(5)=C(6) bond by incorporation of an aromatic ring could result in a molecule with increased hydrophilicity, improved transport properties, and hopefully

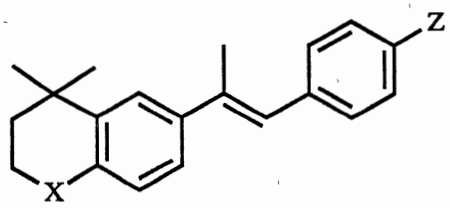


16



18

with reduced overall toxicity. Based on these hypotheses, work of Berlin and co-workers and that of Dawson and co-workers produced heteroarotinoids **13** with X = O, S and S(O) at C(4) and with a fused aryl ring as illustrated.



13, X = O, SO_2, NH, S
Z = CO_2H, CO_2Et, CO_2Me

Activity of Retinoids

One major problem with many retinoids is the acute toxicity.[39] Large doses of retinoids can lead to a symptom called the "Hypervitaminoses A syndrom" which is associated with several toxic side effects such as headaches, nausea, vomiting, dryness, and scaling.[44] Several commercially available retinoids also exhibit undesirable toxicity. For example, Etretinate (**10**) has been reported to cause abnormal liver functions and acute teratogenic properties due, in part, to an enhanced biological half life.[44] Tretinoin [one of the commercial names for all-*trans*-retinoic acid (**3**)] is said to cause hypopigmentation[44] and skin problems (like irritation, redness and scaling) along with other common side effects cited earlier. The toxicity studies are generally conducted on live rats and the LD_{50} values are compared.[13,6]

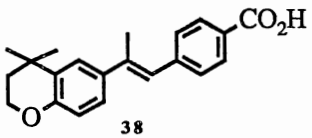
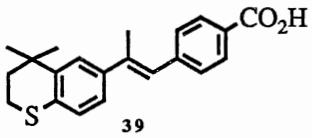
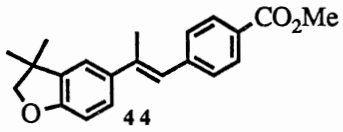
Among the many assays used to assess activity and toxicity of test retinoids, two are prominent and commonly used. They are the ornithine decarboxylase (ODC) assay, which

is an *in vivo* [67,68] method, and the human promyelocytic leukemia cell line (HL-60) assay, which is an *in vitro*.[8,9] method. The compound 12-*O*-tetradecanoylphorbol-13-acetate (TPA) is labelled a cancer promoter and an inducer of the production of the enzyme ornithine decarboxylase, which in turn reacts with ornithine.[67] It is believed that ornithine decarboxylase assists in the transformation of normal to malignant cells.[67,68] Since many retinoids have the ablity to inhibit ornithine decarboxylase induction, the ODC assay has become a reliable method to study antitumor properties of synthetic retinoids.[67,68] The ODC activity is determined by measuring the CO_2 evolved when a suspension of malignant tissue and ^{14}C-labelled ornithine are mixed.[67,68]

The other method (HL-60 assay) involves cell differentiation.[8-10] Normal HL-60 cells do not produce superoxide ions when stimulated by TPA, whereas differentiated HL-60 cells do produce such superoxide ions (O_2^- ions produced by an oxidative metabolic pathway as a part of body's defense mechanism),[10] which can be detected by a change in color of the test dye, nitroblue tetrazolium (from yellow to blue).[9,10] The ODC activity of certain heteroarotinoids (**38, 39** and **44**) in comparison with *trans*-retinoic acid **3** is shown in Table III and the toxicity studies (of heteroarotinoids **38** and **39**) are tabulated in Table IV As illustrated in Table IV the toxicity was determined by measuring the mortality of female Swiss mice.[45] None of the mice survived with arotinoid **32** at a dose of 30 μmol/kg/day for 8 days whereas all the animals survived with a similar dose of *trans*-retinoic acid (**3**, 33 μmol/kg/day). With heteroarotinoid **38**, toxicity levels were determined and compared [with those of retinoic acid (**3**)] at a dose of 600 μmoL/kg/day for 7-10 days with the survival rate being 70% [compared to 95% with retinoic acid (**3**)]. With the sulfur analogue **39**, no deaths were reported until day 8. Toxicity studies have been performed recently[45] to demonstrate the decreased toxicity of certain heteroarotinoids. New varieties of heteroarotinoids have been synthesized[13,14,19,22,37,38,56,71] in an attempt to minimize the toxicity levels.

TABLE III

ACTIVITY OF SELECTED HETEROAROTINOIDS[a]
(ORNITHINE DECARBOXYLASE ASSAY)[b]

Test system	Dose (nmol)	nmol of CO_2/30-min/mg of Protein	% inhibition[c]
Acetone + TPA	10	1.02	Control
Acetone + TPA + t-RA (**3**)	34	0.13	87
 **38**	34	0.090	91
 **39**	34	0.283	72
 **44**	17	-[d]	85

[a]References 13, 25, 61.
[b]Rererences 67, 68.
[c]% inhibition = [100 x ODC activity (retinoid)-ODC activity (control)]/ODC activity (control)
[d]Data not available.

TABLE IV

TOXICITY OF SELECTED RETINOIDS IN FEMALE SWISS MICE[a,b]

Retinoids	Dose μmol/kg day	% Survivers Days 8	% Survivers Days 15	Days of Death	Total Animals
Control	0	100	100	-	30
Retinoic acid (**3**)	600	95	0	7-13	20
	300	100	0	10-14	20
	200	100	63	14-15	30
	100	100	100		30
	67	100	100		20
	33	100	100		10
TTNPB **32**	30	50	0	6-8	20
	10	87	0	7-10	30
	3.3	97	0	7-11	30
	1.0	100	30	10-15	30
Arotinoid **37**	100	100	0	8	10
	30	100	0	9-12	10
	10	100	68	10-15	30
	3.3	100	100		10
Heteroarotinoid **38**	600	70	0	7-10	10
	300	100	50	12-15	10
	200	100	90	14	10
	100	100	100		10
	30	100	100		10
Heteroarotinoid **39**	600	100	0	9-10	10
	300	100	80	14-15	10
	100	100	100		10
	30	100	100		10

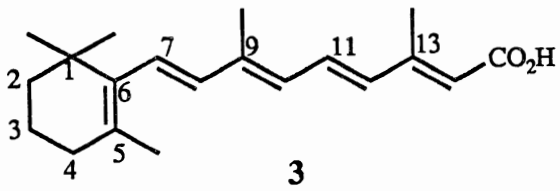
[a]Reference 13.

[b]Retinoids administered by ip injection on week days over period of 2 weeks.

Structure-Activity Correlation of Retinoids.

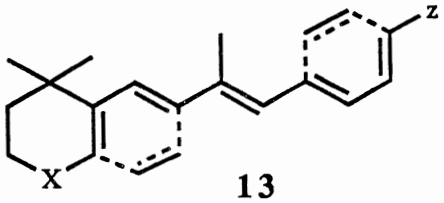
The evolution of heteroarotinoids has involved several logical considerations and are, in part, based on the assumption that the heteroatom might reduce toxicity. For example, a heteroatom was introduced to avoid oxidation at C(4), and an aryl group was incorporated to block oxidation at the C(5)=C(6) position.[13,56,72] These are slight modifications which do not appear to alter appreciably the overall spatial requirement of the system. Understanding the structural and stereochemical properties of a compound and its correlation with biological activity is considered vital in any medicinal and biologically oriented research.[13,14,72]

The structural similarity of a heteroarotinoid **13** to that of the all-*trans*-retinoic acid (**3**), as illustrated below, suggests that the heteroarotinoid could mimic the action of the natural retinoid at the celluar level, or in short, it should have an appropriate "fit". Certain X-ray studies have been performed on a single crystal of **45** [25] and the data have been analyzed



3

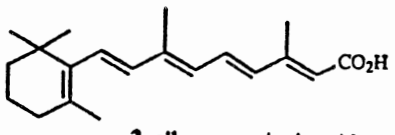
All-*trans*-retinoic acid



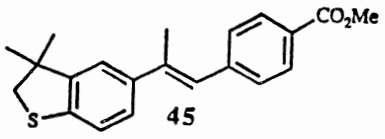
13

Heteroarotinoid

via molecular graphics to determine the "fit" with natural retinoic acid (**3**). Several



3, all-*trans*-retinoic acid



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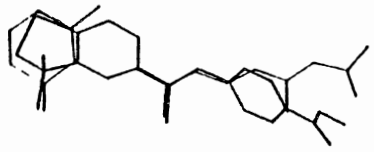
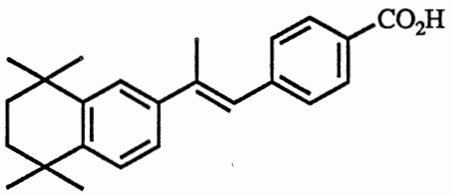


modified retinoids based on certain structure-activity correlations have been reported[13,14,25,36,37,56,60] with most of the work being focused upon reducing the toxicity and increasing the ability of the retinoid to bind with a protein.[14,25] Shudo and co-workers[36,37] replaced the propenyl group of retinoid **32** with hetero functional groups such a C(O)NH and N=N [compounds of the type **53** and **54** (**55**and **56**)]. From the



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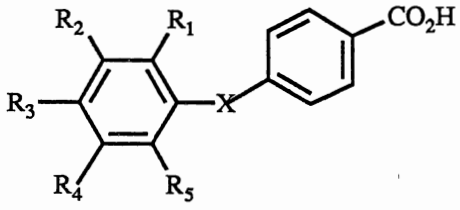

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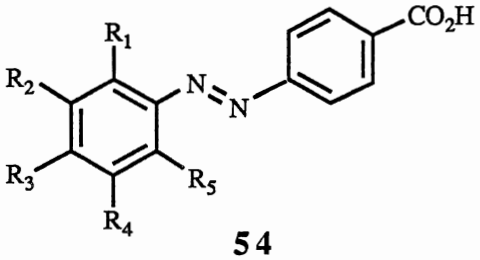

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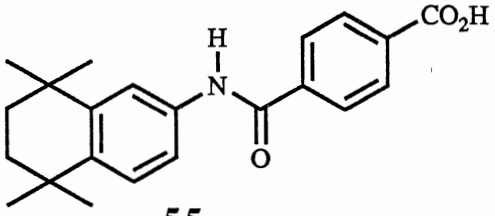

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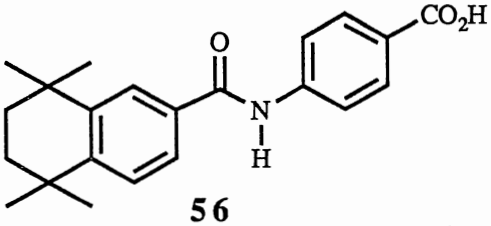
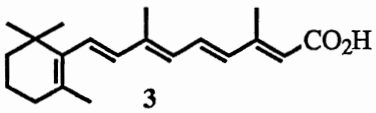
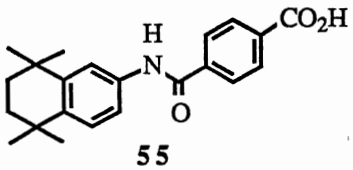
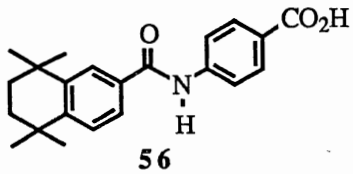


56

activity found for the amides, **55** and **56** (Table V) it was concluded that a hydrophilic group joined by a bridge X to a benzoic acid group,with sufficient steric bulk, was required for biological activity. The testing results did not, however, emphasize any spatial requirements for the the bridge.[36,37] Certain conformationally restricted retinoids (**57** and **58**) with methyl groups on the double bond [C(9)=C(10)] and with rings (cylopropyl and naphthyl, as in **37** and **59**) at the double bond [C(9)=C(10)], retinoic acid

TABLE V

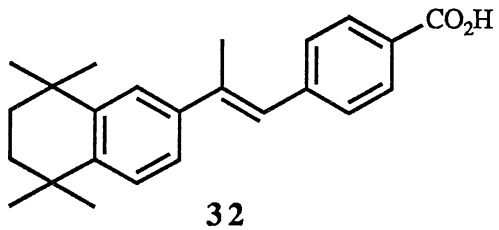
DIFFERENTIATION-INDUCING ACTIVITIES OF RETINOIDAL AMIDE COMPOUNDS ON HL-60 CELLS[a]

Retinoid	ED_{50}, M	Rel. act.[b]
 3	2.4×10^{-9}	100
 55	7.9×10^{-10}	350
 56	3.4×10^{-10}	720

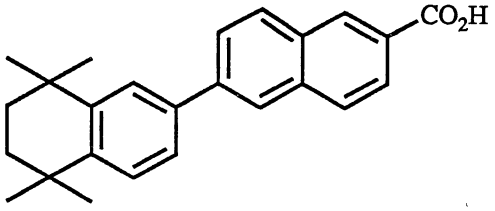
[a]Reference 36.

[b]Relative activity is defined as the mean value of the ratio ED_{50} (retinoic acid) to ED_{50} (test compounds), both values having been obtained in concurrent experiments.

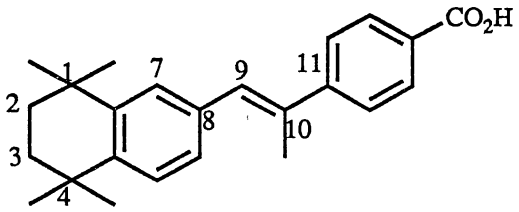
numbering] were synthesised recently by Dawson and co-workers[14] and involved



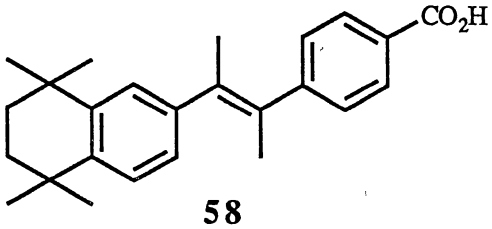
32



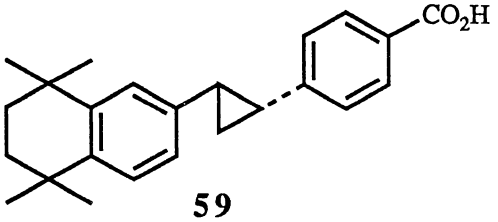
37



57



58

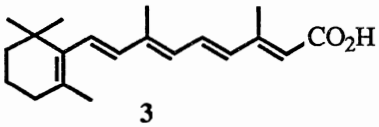
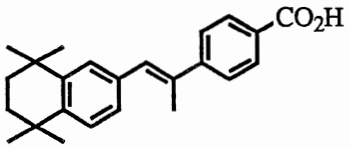
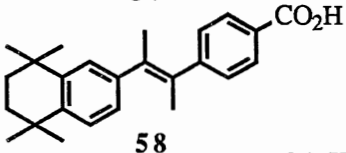
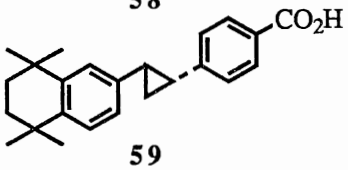
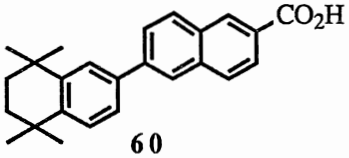


59

modification of **32**. Biological activity was measured in order to establish the conformation preferred by retinoids on binding to various binding protein and receptor sites.(Table VI).[14] From these studies, it was found that **57** and **58** had low activity compared to **32** and **56**. Because the rotational profiles[14] [the restriction in rotation of the aromatic-C bonds around C(8)-C(9) (retinoic acid (**3**) numbering)] of molecules **56-58** were not very different,[14] it was concluded that the presence of a second methyl group on the double bond does not allow the compound to adopt the conformation which favors binding to the protein.[14] Thus, all of these studies reinforce the importance of determining structure-activity relationships in the design of new retinoids. The activities of selected retinoids in ODC, TOC and HL-60 assays are compared in Table VII.

TABLE VI

BIOLOGICAL ACTIVITY OF SELECTED STRAINED RETINOIDS[a]

Retinoid	TOC[b] ID_{50}, nM	ODC ID_{50}, nM	CRABP Binding ID_{50}, μM[c]
 **3**	0.01	0.04	1.0
 **57**	0.007	0.03	1.0
 **58**	> 10[d]	142	1.0
 **59**	> 0.1[d]	3.2	4.8
 **60**	0.003	2.2	1.6

[a]Reference 14.

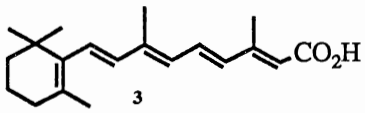
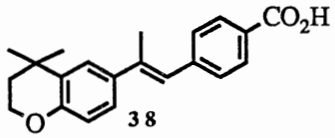
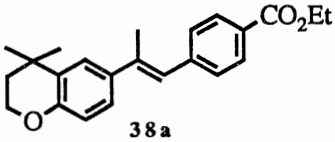
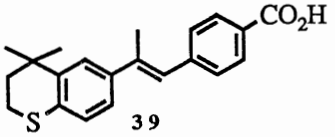
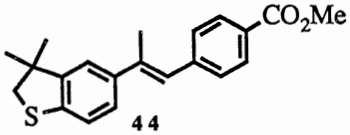
[b]TOC: tracheal organ culture assay (reversal of keratinization).

[c]Concentration of retinoids required to inhibit binding of 2.5 μM all-*trans*-retinoic acid (**3**) by 50%.

[d]Highest concentration of retinoid screened.

TABLE VII

TOC, ODC AND HL-60 ASSAY OF SELECTED HETEROAROTINOIDS

Retinoid	ED_{50}	% Inhibition[a] of control (Dose: 1.7 nmol)	HL-60 (M)
 3	1×10^{-11} [b]	88[b]	1×10^{-7}
 38	6×10^{-6} [c]	42[b]	-[d]
 38a	1×10^{-11} [c]	43[b]	>3 x 10-6 [e]
 39	5×10^{-11} [b]	68[b]	-[d]
 44	-[d]	68[b]	>3 x 10-6 [e]

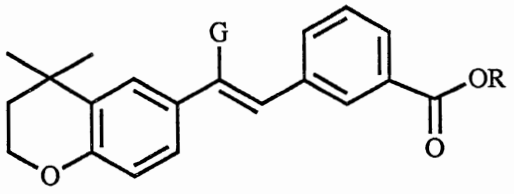
[a]% inhibition = [100 x ODC activity (retinoid)-ODC activity (control)]/ODC activity (control).
[b]See Reference 13.
[c]See Reference 72.
[d]Data not available.
[e]See Reference 66.

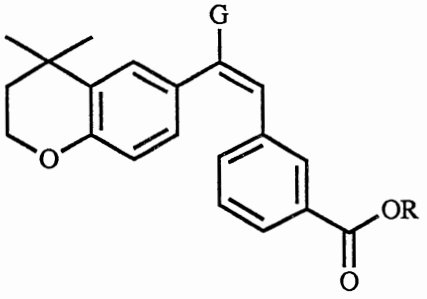
CHAPTER II

RESULTS AND DISCUSSION

Synthesis of New Heteroarotinoids

It has been possible to synthesize several new and novel heteroarotinoids as illustrated

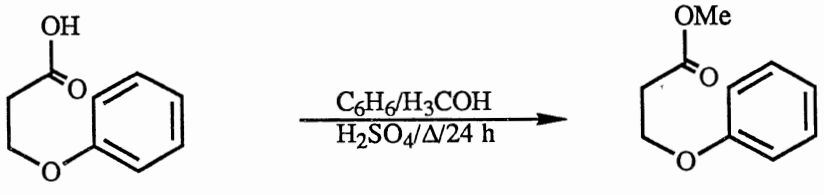




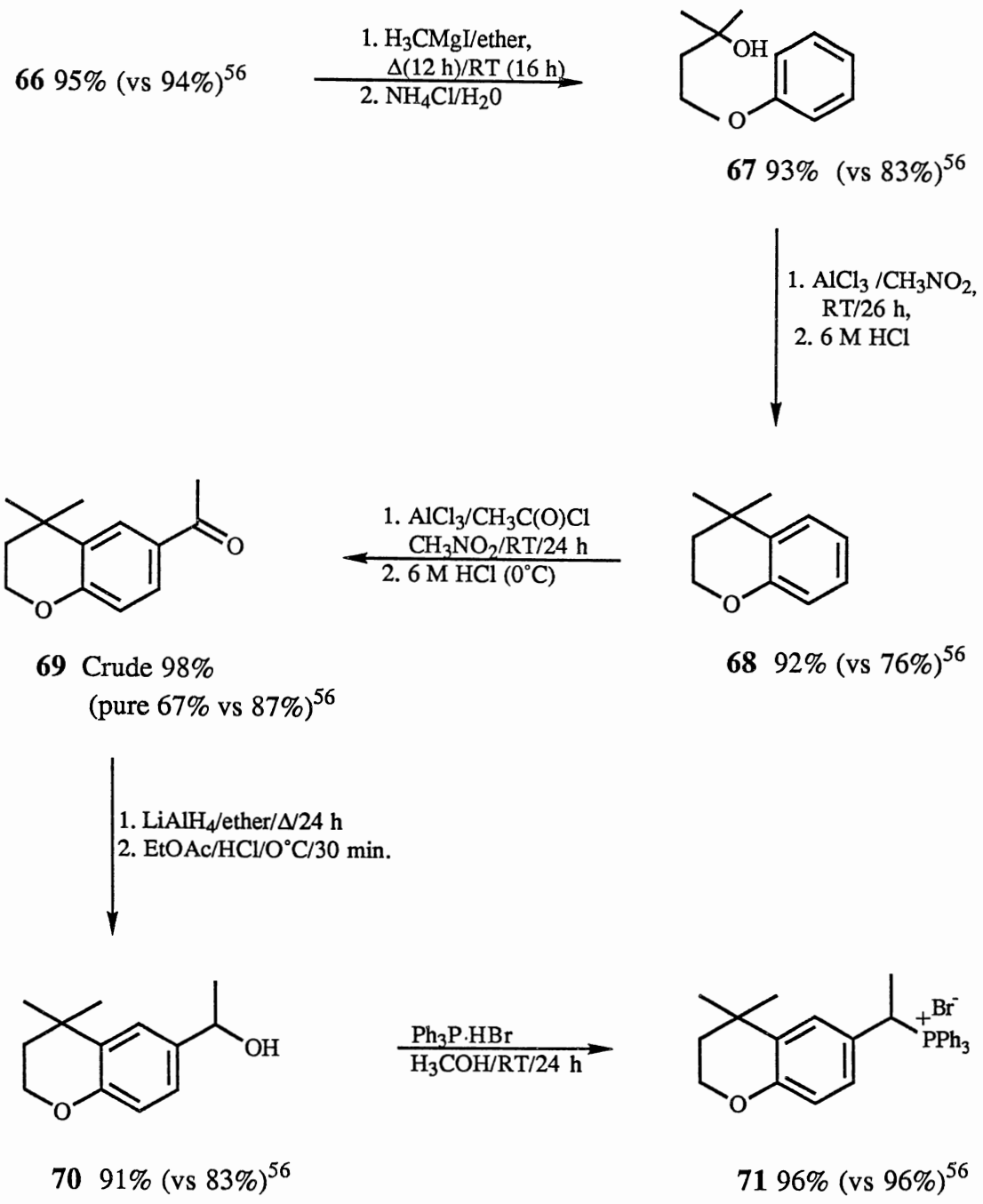
60, G = CH_3, R = Et
61, G = CH_3, R = H

62, G = CH_2OH, R = Et
63, G = CHO, R = Et
64, G = CO_2H, R = Et

in **60-64**. In addition we have been able to optimize certain key steps in our syntheses as shown in the following sequence. A comparison of the yields obtained, via optimized conditions with those previously reported, is also shown in the reaction sequence.

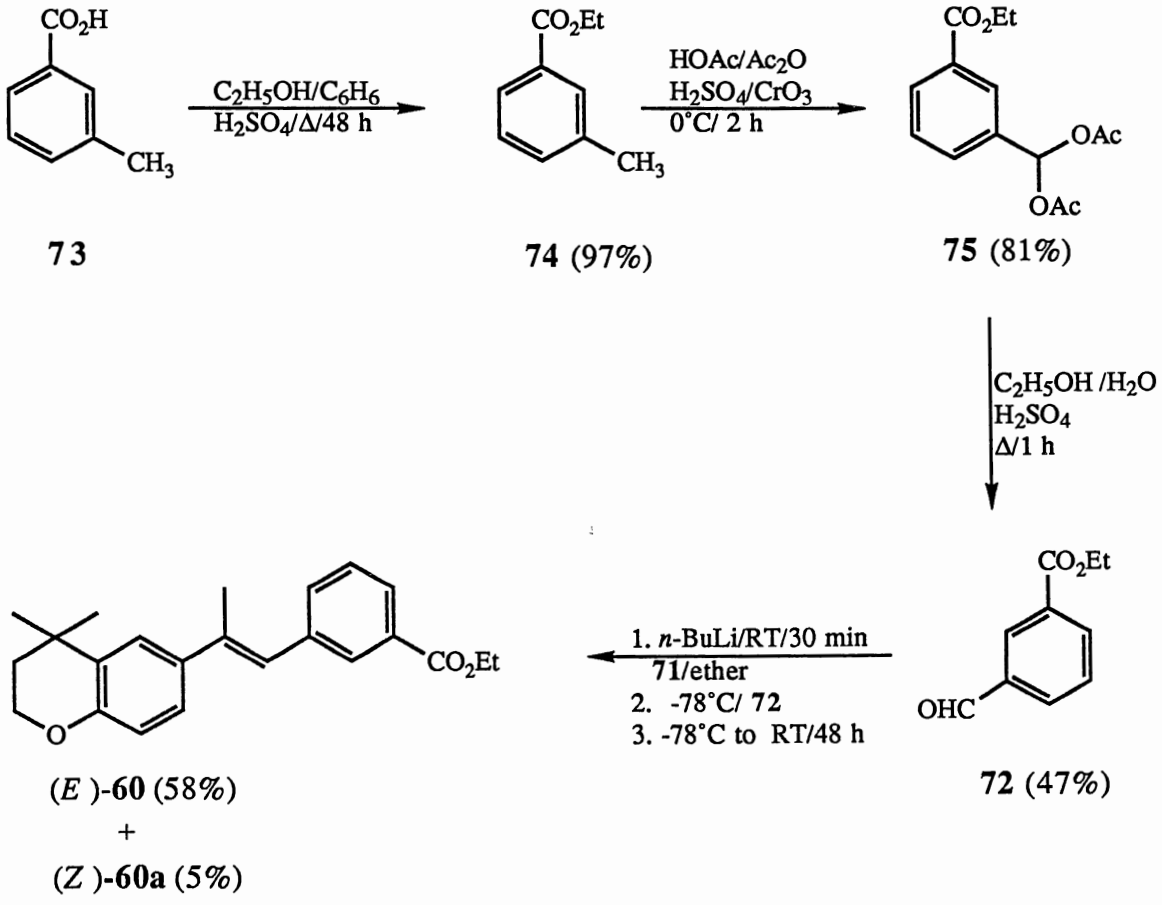


65 **66** 95% (vs 94%)[56]



Since ethyl 3-formylbenzoate (**72**) was an essential reagent to combine with the Wittig reagent from **71**, an independent synthesis for **72** had to be initiated. As a result of X-ray diffraction data and molecular modeling studies, performed in collaboration with Dr. van der Helm at the University of Oklahoma, it was discovered that the superimposition of

methyl (*E*)-[2-(2,3-dihydro-3,3-dimethylbenzo[*b*]thien-5-yl)-1-propenyl]benzoate (**45**)[21] upon the structure of *trans*-retinoic acid (**3**) gave an excellent "fit" except for the carbonyl group in the para position (see structure on page 18). Initial examination suggested that the "fit" would be improved if the carboxyl group were in a meta position. Moreover, with a six-membered oxygen containing ring, rather than a five-membered ring as in **45**, the "fit" might be closer to optimal. Thus, one of the major objectives was to obtain ester (*E*)-**60** and acid (*E*)-**61**. This necessitated the development of synthesis of ethyl 3-formylbenzoate[72] (**72**) which was accomplished by the route shown. Separation of (*E*)-**60** and (*Z*)-**60a** was achieved via the use of the Chromatotron (9.7:0.3, hexane:ether) with silica gel.



73

74 (97%)

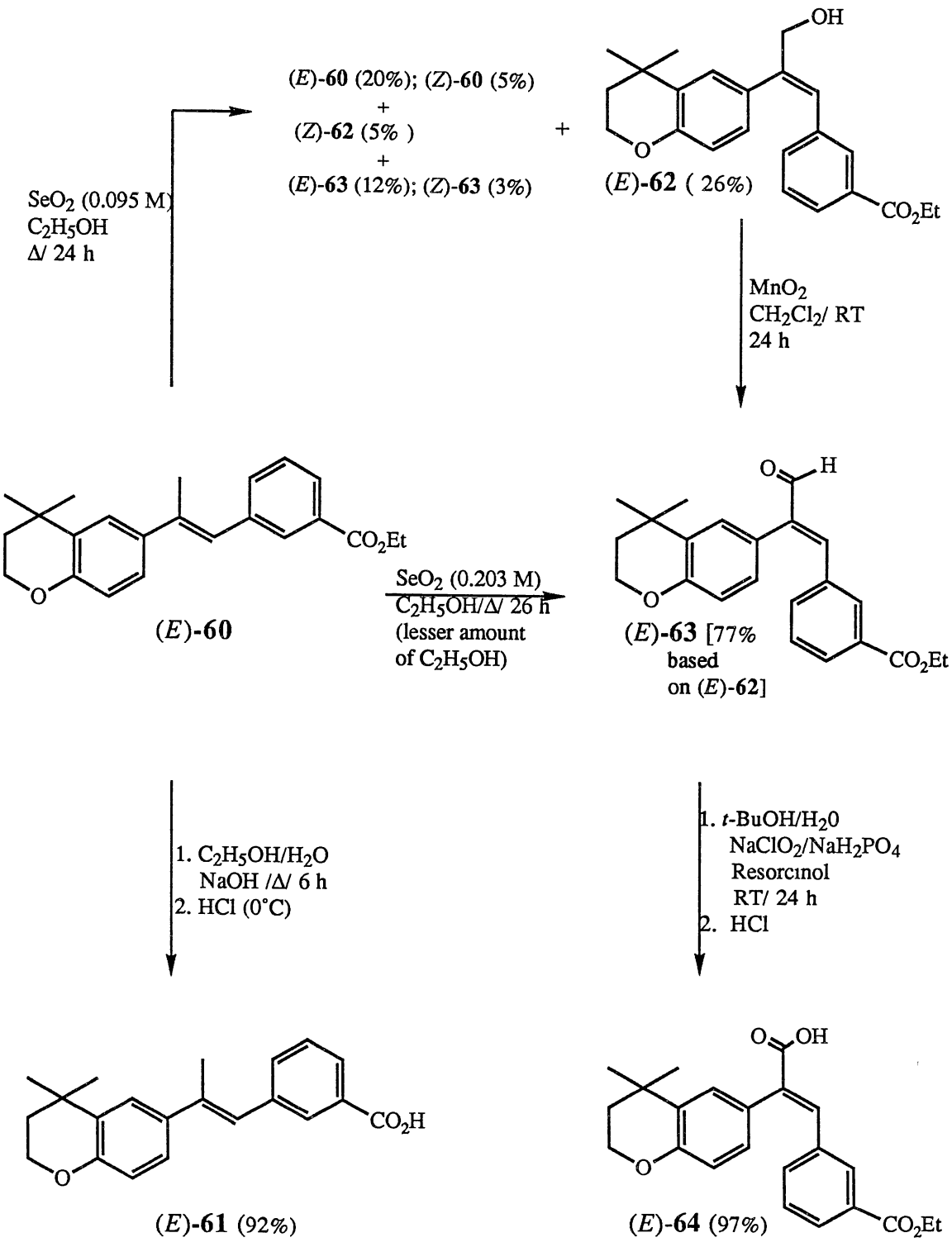
75 (81%)

72 (47%)

(*E*)-**60** (58%)
+
(*Z*)-**60a** (5%)

Since metabolites of heteroarotinoids (*E*)-**60** and (*E*)-**61** may be oxidized *in vivo*, attention was directed towards the synthesis of **62-64**. Synthetic schemes (illustrated on the next page) were developed to utilize certain oxidizing conditions to give control the extent of oxidation. Since it is believed that the carboxyl group is important for a retinoid to bind *in vivo* to cRABP,[35] acid (*E*)-**61** (91%) was obtained by the saponification-neutralization of ester (*E*)-**60** via boiling the mixture (*E*)-**60** in ethanol and sodium hydroxide for 6 h. In order to obtain acid (*E*)-**61** as a solid, devoid of the other (*Z*)-**61**, it was imperative to use pure starting ester (*E*)-**60**. Traces of (*Z*)-**60** were separated via use of chromatography on the chromatotron.

The mixture used to oxidize (*E*)-**60** with high regioselectivity involved six equivalents of SeO_2[12] in 95% ethanol at reflux for 24 h. After filtering off the dark elemental selenium, workup gave a pale yellow oil of alcohol. The oil contained a mixture of (*E*)-**62** (26%) and (*Z*)-**62a** (5%), both the isomers of the aldehyde **63** [12%; (*E*)-**63**:(*Z*)-**63** = 9:1] and some starting ester [20%, (*E*)-**60** and (*Z*)-**60** = 8:2)]. Separation of alcohol (*E*)-**62** and (*Z*)-**62** was effected by using chromatography on 4 mm thick plate of silica gel on the Chromatotron (8:2, hexane:ether was used as the eluting solvent). Evaporation of the solvent gave pure alcohol (*E*)-**62** (26%). An attempt to increase the yield of the (E)-**62** by increasing either the amount of SeO_2 or the reaction time (or both) resulted only in an increase in the ratio of the (*E*)-**62**:(*Z*)-**62** with a slight increase in the yield of aldehyde (*E*)-**63**. It was possible, however, to obtain aldehyde (*E*)-**63** in moderate yields (46%), directly from the ester (*E*)-**60** by decreasing the amount (by 50%) of solvent (95% ethanol) used originally and by increasing the amount of SeO_2 [by 33%, (from 0.095 M to 0.203 M)]. Oxidation of the allylic alcohol (*E*)-**62** to the corresponding aldehyde (*E*)-**63** was achieved in good yields (77%) by using activated MnO_2[2] in methylene chloride at RT (24 h). Clear crystals of the aldehyde (*E*)-**63** were obtained upon recrystallization of the crude product from absolute ethanol. Acid (*E*)-**64** was prepared by oxidation of aldehyde (E)-**63**



with sodium chlorite in a solution of *t*-BuOH/water buffered by sodium monobasic phosphate. Resorcinol was added as a chlorine scavenger, and the reaction mixture was

stirred at room temperature for 24 h.[42] This unique reaction shows promise in these types of systems for effecting regiospecific oxidation of aldehyde groups. Acid (*E*)-**64** was obtained in very high yield (97%) as a yellow solid and could be recrystallized from absolute ethanol.

Purification of New Heteroarotinoids

The heteroarotinoids synthesized were purified using chromatography mostly with the aid of the Chromatotron. Silica gel plates (4 mm thick) were prepared and the separation of the compounds were effected by eluting with suitable solvents. Ester (*E*)-**60** was separated from ester (*Z*)-**60** using hexane:ether (0.97:0.03) as the eluting solvent system. Alcohol (*E*)-**62** was purified by passing the crude product through a 4 mm thick plate of silica gel on a Chromatotron using hexane and ether (8:2). The solvent system used to separate aldehyde (*E*)-**63** was also hexane and ethyl acetate (8:2). Owing to the considerable difference in the R_f values of the ester (*E*)-**60** (highest R_f value), alcohol (*E*)-**62** (lowest R_f value), and aldehyde (*E*)-**63** [R_f value is between the R_f values of (*E*)-**60** and (*E*)-**62**, but closer to the latter], it was possible to separate the compounds using chromatography. Care was exercised to store the purified products quickly in the dark in a freezer. Under these conditions, the melting points did not change over long periods, but the compounds may not be stable at higher temperatures or in light as has been true for other heteroarotinoids.

Synthesis of Precursors of The Heteroarotinoids

Since the precursors **65-70** of key synthon **71** were known and since the expense of the overall synthesis was high, it became an important phase of the work to develop improved methodology to optimize the individual steps for each precursor. This is also a necessity for any future work in which a C-14 label is introduced into the system for metabolic studies. The yield of ester **66** was high and not very much improved from

previous work. However, conversion of the ester to alcohol **67** was increased (from 83% to 93%) by heating the reaction mixture at reflux for 12 hours followed by stirring at room temperature for 16 hours. Cyclization of alcohol **67** to ether **68** was sharply enhanced by periodic addition of **67** to a suspension of $AlCl_3$/nitromethane. Stirring this mixture at RT for 26 hours, followed by careful acidification with 6 *M* HCl, gave the best result. Acetylation of ether **68** with $H_3C(O)Cl/AlCl_3$ was promoted via a new set of conditions. To a stirred solution of ether **68** in nitromethane was added dropwise a mixture of $AlCl_3$ and acetyl chloride in nitromethane. This procedure, followed by very careful acidification and stirring at room temperature for 24 h, led to crude ketone **69** (98%) as a brown oil. Although IR analysis showed this product to be identical with that in the literature, because of the coloration of the oil, vacuum distillation was needed to give pure, colorless ketone **69**. Unfortunately, this latter process resulted in some loss of product (67%). It is conceivable that the brown oil could be used directly without distillation.

Reduction of ketone **69** to alcohol **70** proceeded well with $LiAlH_4$/ether and gave a good return of product (91% versus 83%[56]). The workup involved careful destruction of the excess $LiAlH_4$ with ethyl acetate below 5°C in order to prevent dehydration of the benzyllic alcohol to the corresponding alkene. Previous experience with secondary and tertiary alcohols in related chroman systems demonstrated a clear tendency to undergo elimination under less than neutral conditions as exist in the decomposition of the $LiAlH_4$.

Stirring a suspension of $Ph_3P{\cdot}HBr$ and alcohol **70** in methanol at room temperature for 24 h gave a clear oil which was triturated with dry ether to produce white flakes of salt **71** in essentially the same yield as previously recorded. However, the overall yield of **71** starting from acid **65** was 47.6% which is a slight improvement over that (42.1%)[56] stated earlier. Since aldehyde **72** was somewhat unstable and vulnerable to absorbing water, salt **71** was dried at high vacuum for 12 h prior to immediate conversion to the corrresponding Wittig reagent in ether. Treatment of this Wittig reagent from **71** in ether with aldehyde **72** at -78°C in the dark was initiated at once. The reaction mixture was stirred for 48 hours

with a concomitant increase in temperature from -78° to room temperature. The resulting oil was subjected to chromatography with the Chromatotron from which (*E*)-**60** was isolated.

Aldehyde **72** has been reported but the techniques required were not well delineated in the patent, and thus a scheme had to be devised. The crucial step proved to be the oxidation of ester **74** (from acid **73**) which required the addition of small increments of CrO_3 at 0°C in the presence of $HOAc/Ac_2O$. A white pasty ester **75** formed and was hydrolyzed immediately to the formyl ester **76** in moderate yield (47%). Undoubtedly the lower yield resulted from the susceptibility of aldehyde **76** to air oxidation and to its partial solubility in water from which it had to be extracted.

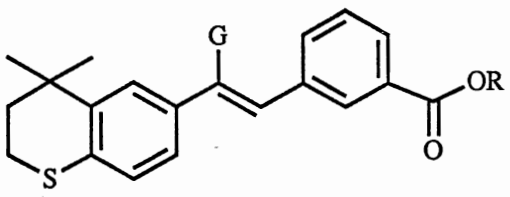
It is worthwhile to mention that all of the above modifications of procedures and the new methods developed are the end results of considerable effort after examination of many variables in terms of reaction conditions. Consequently, the preparations included herein afforded the somewhat light sensitive final products in the most consistent manner to date. All of the members of **60-64** appear to be stable when stored in the dark and very cold but may change with standing. No investigation has been made of the long term stability characteristics of the new heteroarotinoids recorded herein.

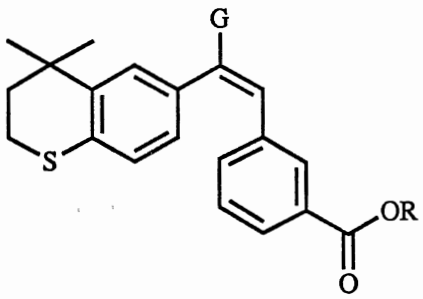
All ultraviolet spectra indicate some conjugation (Plates XXVII, XXXI, XXXVIII, XLII and XXXX1V). A preliminary communication from Dr. van der Helm at the University of Oklahoma suggests that solid (*E*)-**60** has the phenyl ring slightly turned out of the plane of the chroman ring and the central double bond. Molecular graphic strudies have not been completed as yet but again preliminary results suggest the compound may "fit" better with *trans*-retinoic acid (**3**) than did **45** described previously (page 18). However, in (*E*)-**60**, there is some distortion in the solid state. We are currently evaluating these early data and expect the final version shortly to be made ready for publication. This information will also guide our future strategy for designing modified systems of the type represented by (*E*)-**60.** In addition, we have submitted (*E*)-**60** and related systems to Dr.

A. K. Verma of the Cancer Center (Oncology Department) at the University of Wisconsin in Madison, Wisconsin. Preliminary results suggests that several related systems do markedly retard growth of several lines of cancerous cells. Work is also in progress to determine if (*E*)-**60** and related heteroarotinoids will bind to a certain proteins as does *trans*-retinoic acid (**3**) for transport in the cell.

Suggestions for Future Work

The idea of introducing the ester funtionality in the meta position of the aryl ring is based on the concept that a better "fit" would enhance the ability of the heteroarotinoid to mimic *trans*-retinoic acid (**3**), thereby increasing the activity of the heteroarotinoid. If the biological assays confirm the validity of this suggestion, compounds with slightly modified structure should be synthesized for the examination of the activities. Since the sulfur analogues of heteroarotinoids[10,21,41] have proven to be somewhat more active than their oxygen counterparts in certain assays,[41] the sulfur analogues of compounds **60-64** are defensible target molecules, such as structures **76-80** below.





76, G = CH_3, R = Et
77, G = CH_3, R = H

78, G = CH_2OH, R = Et
79, G = CHO, R = Et
80, G = CO_2H, R = Et

CHAPTER III

EXPERIMENTAL

General Information

All reactions were performed under N_2 with magnetic stirring unless otherwise specified. Evaporation of all solvents was effected with a rotary evaporator (Yamato; model RE-46) unless otherwise stated. NMR spectral data on solutions ($DCCl_3$) were obtained using a Varian XL-300 spectrometer with ^1H and ^{13}C data being taken at 299.99 Hz and 75.4 Hz with reference to TMS in ∂ values or ppm, respectively. IR spectra were recorded on a Perkin-Elmer 681 spectrophotometer as films or in KBr pellets while UV data were obtained in 95% ethanol on a Varian DMS 200 UV-Visible spectrophotometer [equipped with an Epson LX-800 professional computer printer]. Melting points were determined with a Fischer-Johns melting point apparatus and were uncorrected.

Compounds **66-71** were prepared by considerable modifications of literature[56] procedures. Elemental analyses were provided by Galbraith Laboratories, Incorporated, of Knoxville, Tennessee. The exact masses of certain compounds were obtained from the mass spectral laboratory with a VG-analytical ZAB 2-SE-high resolution, reversed geometry mass spectrometer.

Methyl 3-Phenoxypropionate (**66**)

A solution of 3-phenoxypropionic acid (**65**, 11 g, 66.19 mmol), concentrated H_2SO_4 (0.3 mL) in methanol (170 mL) and benzene (200 mL) was boiled (28 h) in a single-necked, 500-mL, round-bottomed flask fitted with a Dean-Stark apparatus and a spiral condensor (N_2). A clear yellow solution formed and it was allowed to cool (1 h) to room

temperature (RT). After concentration (to 150 mL), the residual solution was diluted (H_2O, 100 mL) and extracted (ether, 4 x 40 mL). The combined organic phases were washed with saturated $NaHCO_3$ (3 x 40 mL), water (2 x 50 mL), and saturated brine (1 x 50 mL). After the solution was dried (Na_2SO_4, 8 h), the solvent was evaporated [rotovap followed by high vacuum (0.3 mm) at 50-60°C(water bath) for 15 min] to give 11.41 g (95%) of methyl 3-phenoxypropionate (**66**) as a colorless liquid with a strong odor. The compound was used without further purification since spectral data (IR, ^1H, ^{13}C) matched those previously reported (lit[56] bp 69-71°C/0.03 mm). This method proved superior to all others reported[56,72] since it gave a relatively pure product without fractional distillation. Moreover, this ester **66** gave a higher yield of alcohol **67** in the next step than previously recorded.[56,72]

2-Methyl-4-phenoxy-2-butanol (**67**)

To a freshly prepared solution of methylmagnesium iodide [from 0.54 g, (22 mmol) of magnesium and 3.49 g (27.75 mmol) of methyl iodide in dry ether (10 mL)] was added a solution of methyl 3-phenoxypropionate (**66**, 1 g, 5.55 mmol) in dry ether (5 ml) and in a 100-mL, 3-necked, round-botttomed flask equipped with a spiral condenser in tandem with a dry ice condensor, an addition funnel, and a magnetic stirrer.(N_2). [During formation of H_3CMgI, the reaction was started by addition of methyl iodide to magnesium followed by stirring the mixture for 10 min to give a black suspension of the Grignard reagent]. The final mixture (grey in color) was heated to reflux (12 h) with stirring, was then allowed to cool to RT (16 h), and was finally quenched with water (30 mL, after cooling the reaction mixture with an ice-water bath) and a saturated solution of NH_4Cl (30 mL). Two phases separated, and the aqueous phase was extracted (ether, 3 x 25 mL). The combined organic phases were washed with saturated $NaHCO_3$ (3 x 35 mL) and water (3 x 35 mL). This organic solution was dried (Na_2SO_4, with stirring for 10 h). Evaporation (rotovap and

then followed by high vacuum (0.3 mm) at RT for 15 min] gave 0.935 g (93 %) of alcohol **67** as a colorless liquid. (lit[56] bp 85-87°C/0.1 mm). IR (neat) 1740-1750 (C=O) cm^{-1}; ^1H NMR and ^{13}C NMR chemical shift values matched those previously reported.[57,72] The above procedure was superior to reported preparations.[57,72]

4,4-Dimethylchroman or 3,4-Dihydro-4,4-dimethyl-2*H*-1-benzopyran (**68**)

A solution of 2-methyl-4-phenoxy-2-butanol (**67**, 0.93 g 5.16 mmol) in freshly distilled nitromethane (15 mL) was added dropwise (N_2) to a stirred suspension of anhydrous $AlCl_3$ (1.37 g 10.31 mmol, in 15 ml CH_3NO_2) in a 100-mL, 3-necked, round-bottomed flask (over a period of 25 min) equipped with a condenser and an addition funnel. After stirring the deep red reaction mixture (RT, 26 h), ether (40 mL) was added followed by the slow addition of 6 M HCl (60 mL) over a period of 20 min to a chilled (0°C) reaction mixture. The organic layer was separated, and the aqueous layer was extracted (ether, 3 x 30 mL). The organic phases were combined and washed with water (3 x 30 mL) and brine (3 x 25 mL). After drying the solution (Na_2SO_4, 6 h), the solvent was evaporated using a rotovap [then followed by high vacuum (0.3 mm Hg), 55-60°C, water bath, 20 min] to give the ether **68** (0.77 g, 91.59%) as a dark brown oil. Ether **68** was used without further purification since the ^1H and ^{13}C NMR spectra matched with literature data (bp lit[56] 74-80°C/0.7 mm Hg). The above method was more efficient than any reported.[56,72]

4,4-Dimethylchroman-6-yl Methyl Ketone or 1-(3,4-Dihydro-4,4-dimethyl-2*H*-1-benzopyran-6-yl) ethanone (**69**)

A mixture of anhydrous $AlCl_3$ (9.08 g, 68.1 mmol) and freshly distilled acetyl chloride (6.17 g, 78.6 mmol) and nitromethane (15 mL) was added dropwise to 4,4-dimethylchroman (**68**, 8.5 g, 52.43 mmol, N_2) in a 300-mL, 3-necked, round-bottomed

flask equipped with spiral condenser and addition funnel. After stirring the deep red-colored reaction mixture at RT (24 h), 6 M HCl (50 mL) was added dropwise over a period of 20 min to a chilled (0°C) reaction mixture. The resulting mixture was stirred (10 min) and diluted with ether (50 mL). Two phases separated, and the aqueous phase was extracted (ether, 3 x 50 mL). The combined organic phases were washed with $NaHCO_3$, water (3 x 50 mL), and brine (50 mL). Sometimes emulsions formed which could be destroyed by washing with excess brine (100 mL). After drying the solution (Na_2SO_4, 12 h, stirring), the solvent was evaporated [rotovap and high vacuum (0.3 mm Hg), 50-60°C (water bath), 10 min] to a thick reddish-brown oil which was distilled (high vacuum, bp 142-144°C/0.15 mm Hg) to give 7.14 g (67 %) of ketone **69** as a light yellow oil (lit[56] 78-80°C/0.7 mm Hg). The IR, ^1H and ^{13}C NMR spectra matched those of the the reported compound,[56,72] but our method was superior.

α, 4,4-Trimethylchroman-6-methanol or 3,4-Dihydro-4,4-trimethyl-2*H*-1-benzopyran-6-methanol (**70**)

A solution of the previous ketone (**69**, 6.0 g, 24.4 mmol) in anhydrous ether (25 ml) was added (15 min, N_2) to a stirred suspension of $LiAlH_4$ (1.67 g, 44.0 mmol) in dry ether (20 mL) in a 100-mL, 3-necked, round-bottomed flask with the usual setup. The mixture, a grey suspension, was heated to reflux for 24 h. After cooling to RT (1 h), ethyl acetate (15 mL) was added **slowly** and **carefully** to destroy excess $LiAlH_4$ (an ice bath was used to maintain the temperature of the mixture below 5°C during the addition of ethyl acetate). A solution of HCl (5%, 80 mL) was then added **slowly**, and the resulting grey suspension was stirred (15 min). Ether (50 mL) was added and the resulting aqueous layer was separated. The aqueous layer was extracted with ether (4 x 40 mL), and the combined organics were washed with saturated $NaHCO_3$ (3 x 40 mL), water (2 x 50 mL), and saturated brine (2 x 50 mL). After the solution was dried (Na_2SO_4, 8 h), the solvent was

evaporated [rotovap, followed by high vacuum (0.3 mm Hg) 50-55°C (water bath), 15min]. The thick yellow oil solidified in a few minutes (5.66 g, 93.9%), mp 71-72°C (lit[1,2] 70-72°C); IR (KBr) 3140-3640 (OH) cm^{-1}. All ^{1}H and ^{13}C signals matched the literature values for **70**. This procedure proved superior to that reported.[56,72]

1-(4,4-Dimethylchroman-6-yl)ethyl]triphenylphosphonium Bromide
or [1-(3,4 Dihydro-4,4-dimethyl-2*H*-1-benzopyran-6-yl)ethyl]-
triphenylphosphonium Bromide (**71**)

A solution of alcohol **70** (5.60 g 13.8 mmol) and triphenylphosphonium hydrobromide (5.63 g 16.56 mmol) was stirred at RT (N_2, 24 h), in a 250-mL, three-necked, round-bottomed flask. The pale yellow solvent was then evaporated (rotovap), and the resulting clear oil was triturated repeatedly with dry ether (100 mL) until solidification occured. The resulting white solid was suspended with stirring in dry ether at RT (N_2, 4 h). After filtration, a white solid **71** was obtained which was dried (110°C/2 mm Hg) and weighed (13.3 g, 94%); mp 150-152°C.(dec) [lit[56] 149-155°C]. All NMR data (^{1}H and ^{13}C) matched those of the literature.[56] The compound was used without any further purification. The procedure herein was superior to one reported.[56,72]

Ethyl 3-Toluate (**74**)

In a 200-mL, single-necked, round-bottomed flask, equipped with a Dean-Stark apparatus, a spiral condenser, and a magnetic stirrer was placed *m*-toluic acid (**73**, 10 g, 73.4 mmol) in absolute ethanol (15 ml) and benzene (75 mL) with H_2SO_4 (1.5 mL). The solution was heated at reflux (48 h), and then it was allowed to cool to RT (1 h). Water (75 mL) was added, and the aqueous phase was separated and extracted (ether, 3 x 40 mL) and then washed with saturated $NaHCO_3$ (3 x 40 ml), water (2 x 50 mL), and brine (2 x 50 mL). The solvent was evaporated [rotovap and then high vacuum (0.25 mm Hg) at 65°C (water-bath) for 25 min]. A yellow oil obtained was distilled (vacuum, 0.25 mm Hg) to

give 17.7 g (91%) of ester **74** as a colorless liquid (strong and sweet odor), bp 72-74°C/0.250 mm [lit[72] 234°C/760 mm Hg)].

Ethyl 3-Formylbenzoate (**72**)

In a 200-mL, single-necked, round-bottomed flask (N_2) fitted with a condenser was placed ethyl 3-methylbenzoate (**21**, 5.0 g, 7.3 mmol), glacial acetic acid (50 mL), and 50 mL of freshly distilled acetic anhydride with H_2SO_4 (2.0 mL). After stirring for 15 min at RT, the reaction mixture was cooled to 0°C (ice-salt bath). The temperature was maintained below 5°C (1 h) as CrO_3 (8.4 g, 84.2 mmol) was added in small portions (30 min). After stirring (2 h), the dark green reaction mixture was treated **carefully** with ice water (150 mL) and ether (40 mL). The organic phase separated, and the aqueous phase was extracted [$HCCl_3$ (3 x 50 mL) and then ether (2 x 50 mL)]. The combined organic phases were washed with 5% $NaHCO_3$ (3 x 40 mL), water (3 x 30 mL), and brine (2 x 25 mL). After drying (Na_2SO_4, 3 h), the solvent was evaporated (rotovap, followed by high vacuum 0.25 mm Hg, 45°C) to give the diacetate (**75**, 7.5 g, 81%) as a white solid. To ester **75** in a 100-mL, single-necked, round-bottomed flask was added dropwise (RT, stir, 10 min), water (8 mL), and concentrated H_2SO_4 (1 mL). After cooling to RT, water (20 mL) was added, the organic phase separated, and the aqueous phase was extracted with ether (3 x 20 ml) and $HCCl_3$ (25 mL). The combined organic phases were washed with 5% $NaHCO_3$ (2 x 25 mL), water (35 mL), and brine (35 ml). After drying the solution (Na_2SO_4, 4 h), the solvent was evaporated (rotovap, followed by high vacuum 0.2 mm Hg) and gave 2.59 g (47%) of **72** [lit[72] 278-280°C/760 mm Hg)] as a golden yellow liquid. The ester was used without further purification. IR ($DCCl_3$) 2720-2740 (C(O)H), 1700-1740 (C=O) cm^{-1}; ^1H NMR ($DCCL_3$) ∂ 1.43 [t, 3 H, CH_3], 4.41 [q, 2 H,CH_2], 7.64-8.5 [4 H, Ar-H], 10.1 [C(O)H]; ^{13}C NMR ($DCCl_3$) ppm 14.3 [CH_3], 61.4 [CH_2], 129-169 [Ar C], 191.3 [C=O].

Ethyl (*E*)-3-[2,3-Dihydro-4,4-dimethyl-2*H*-1-benzopyran-6-yl)-1-propynyl]benzoate [(*E*)-**60**]

A solution of *n*-butyllithium in hexane (0.90 M, 3.4 mL, 3.14 mmol) was added dropwise (5 min, N_2) to a stirred suspension of the white phosphonium salt **71** (1.29 g, 2.42 mmol) in ether (30 mL dried over sodium ribbon) in a 100-mL, three-necked, round-bottomed flask fitted with an addition funnel and spiral condenser. The resulting reddish-brown mixture was cooled to -78°C (dry ice, acetone, 10 min), and a solution of ethyl 3-formylbenzoate (**72**, 1.0 g, 5.61 mmol) in ether (25 mL) was added dropwise (10 min). This solution was stirred (30 min) at -78°C and then it was allowed to warm slowly to RT (1 h). The color of the reaction mixture was pale yellow. After stirring (48 h), the yellow reaction mixture was filtered, and the residual solid was washed with ether (100 mL). The filtrate was dried (Na_2SO_4) and the solvent was evaporated [rotovap, followed by high vacuum (0.25 mm Hg), 10 min]. The solid was purified by chromatography on a 4 mm thick plate of silica gel (silica gel pF 254 containing gypsum) with the aid of the Chromatotron (Model 7924, Harrison Research). The solvent system used to separate the starting materials and the (*E*)-**60** and (*Z*)-**60** isomers was composed of hexane:ether [0.97:0.3]. The last fraction obtained was concentrated to give 1.4 g (60 %) of a mixture of esters [10:1, (*E*)-**60**:(*Z*)-**60**] which was then treated with boiling ethanol (95%, 3 mL, 5 min). The resulting solution was chilled (dry ice bath) for 24 h. A white solid precipitated and was treated with cold ethanol (95%, 0.5 mL) to give 0.30 g (16.2%) of needle-like crystals of ester (*E*)-**60**; mp 42.5-44°C. IR (KBr) 1715-1725 (C=O), cm^{-1}. ^1H NMR ($DCCl_3$) ∂ 1.39 [s, 6 H, H(9), H(10)], 1.40 [t, 3 H, H(22)], 1.86 [t, 3 H, H(3)], 4.21 [t, 2 H, H(2)], 4.40 [q, 2 H, H(21)], 6.76[s, 1 H, H(13)], 6.80 [s, 1 H, J = 9 Hz, H(8)], 7.25 [dd, J = 9 Hz, J = 3 Hz, H(7)], 7.42 [m, J = 9 Hz, J = 3 Hz, H(5), H(16)], 7.53 [dd, J = 9 Hz, J = 3 Hz, H(17)], 8.03 [dd, J = 9 Hz, J = 3 Hz, 1 H, H(19)] ; ^{13}C NMR ($DCCl_3$) ppm 14.35 [C(22)], 17.55 [C(12)], 30.7 [C(4)], 31.11 [C(9), C(10)], 37.7 [C(3)], 60.99 [C(21)], 63.13[C(2)], 116-153 [C(Ar and vinylic)] 166.72 [C(20)]. Mass

spectral data for $C_{23}H_{26}O_3$: m/e (M^+) 350.1882; Found: 350.1882. Anal.for $C_{23}H_{26}O_3$: C, 78.72; H, 7.47. Found: C, 79.72; H, 7.54.

(*E*)-3[2-(3,4-Dihydro-4,4-dimethyl-2*H*-1benzopyran-6-yl-1-propenyl]benzoic acid [(*E*)-**61**]

In a 2-necked, 25-mL, round-bottomed flask (N_2) fitted with a spiral condenser was placed ester (*E*)-**60** (mp 42-44°C, 0.060 g, 0.17 mmol), ethanol (95%, 1.5 mL), water (3 mL), and NaOH (0.24 g, 0.06 mmol). The resulting solution was boiled (6 h), cooled slowly to RT (30 min),and then chilled (0°C) with an ice bath. Dropwise addition of concentrated HCl (8 drops, pH 2), resulted in the formation of a white solid. This precipitate was then filtered (water aspirator) using a Hersh-funnel with a suitable filter paper (Whatman #1). The solid was then washed with copious amounts of water (150 mL), was air dried (12 h), and was subjected to a high vacuum (Abderhalden with P_2O_5, 60-62°C) to give acid (*E*)-**61** (0.051 g, 92%) as a dry white powder; mp 182-184°C. IR (KBr) 2400-3450 (O-H, CH), 1690-1710 (C=O) cm^{-1}; ^1H NMR ($DCCl_3$) ∂ 1.39 [s, 6 H, H(9), H(10)], 1.86 [t, 2 H, H(3)], 2.27 [s, 3 H, H(12)], 4.21[t, 2 H, H(2)], 6.77 [s, 1 H, H(13)], 7.25-7.81 [Ar-H]; ^{13}C NMR ($DCCl_3$) ppm 17.54 [C(12)], 30.70 [C(4)], 31.9 [C(9), C(10)], 37.6 [C(3)], 63.1 [C(2)], 116.71-153.26 [C(Ar and vinyl)], 172.42 [C(20), C=O].Mass spectral data for $C_{21}H_{22}O_3$: m/e (M^+) 322.1569; Found: 322.1569. Anal for $C_{21}H_{22}O_3$: C, 78.01; H, 6.91. Found: C, 78.23, H, 6.88.

Ethyl (*E*)-3-[2-(3,4-Dihydro-4,4-dimethyl-2*H*-1-benzopyran-6-yl)-3-hydroxy-1-propenyl]benzoate [(*E*)-**62**]

In a 50-mL, 2-necked, round bottomed flask, equipped with a magnetic stirrer, a spiral condenser and addition funnel, were mixed ester (*E*)-**60** (0.500 g, 1.42 mmol) and selenium dioxide (0.950 g, 8.57 mmol) in ethanol (95%, 15 mL, N_2). The reaction was

stirred at reflux (24 h), and then the solution was cooled to RT (30, mint green in color). This green mixture was first filtered through a cotton plug and then through a filter paper (Whatman #1) to remove elemental selenium formed during the reaction. The solution was concentrated using rotovap to a volume of 10 mL, and ether (25 mL) was then added. This mixture was washed with saturated $NaHCO_3$ (3 x 15 mL), water (2 x 25 mL), and brine (2 x 20 mL). After drying (Na_2SO_4, 1 h), the solvent (ether:ethanol, 25:10 mL) was evaporated [rotovap, followed by high vacuum 0.25 mm Hg (50-55°C)] to give a yellow oil which was a mixture of the alcohol [(*E*)-**62** and (*Z*)-**62**] and the starting material (*E*)-**60**. The mixture of alcohols was separated by chromatography on a 4-mm thick plate of silica gel (silica gel PF 254 containing gypsum) with the aid of a Chomatotron. The elution system was a mixture of hexane and ether (8:2). The last fraction from the plate contained alcohol (*E*)-**62** which was devoid of any aldehyde (*E*)-**63** or (*Z*)-**63**. [Isolation of aldehyde (*E*)-**63** was a bonus since it was an original target compound]. Boiling the original reaction mixture with excess of selenium dioxide (greater than 10 fold excess) did not increase the overall yield of the alcohol (*E*)-**62** but instead resulted in an increase of the ratio of both alcohols[roughly 7:3 (*E*)-**62**-:(*Z*)-**62**] and the aldehydes [roughly 7:3 (*E*)-**63**:(*Z*)-**63**]. Alcohol (*E*)-**62** is a pale yellow viscous oil. Solidification attempts with different solvents such as, boiling with ethanol (both 95% and absolute, 1 mL), hexane (1 mL), or heptane (1 mL) failed. IR (neat) 3500-3640 (O-H), 1740 (C=O) cm^{-1}; ^1H NMR ($DCCl_3$) ∂ 1.2 [s, 6 H, H(9), H(10)], 1.33 [t, 3 H, H(21)], 1.77 [m, 2 H, H(3)], 2.24 [s, 1 H, O H], 4.17 [m, 2 H, H(2)], 4.30 [q, 2 H, H(22)], 4.46 [s, 2 H, H(12)], 6.67-7.75 [Ar-H and C=CH]; ^{13}C NMR ($DCCl_3$) ppm 14.2 [C(22)], 30.4 [C(4)], 30.7 [C(9), C(10)], 37.4 [C(3)], 60.8 [C(21)], 63.1, [C(2)], 67.8 [C(12)], 117.3-153.25 [C Ar and C=C], 166.6[C(20), C=O]. Mass spectral data for $C_{23}H_{26}O_4$: m/e (M^+) 366.1834 observed 366.1872. Anal for $C_{23}H_{26}O_4$: C 75.37; H, 7.15. Found: C, 75.00; H, 7.48.

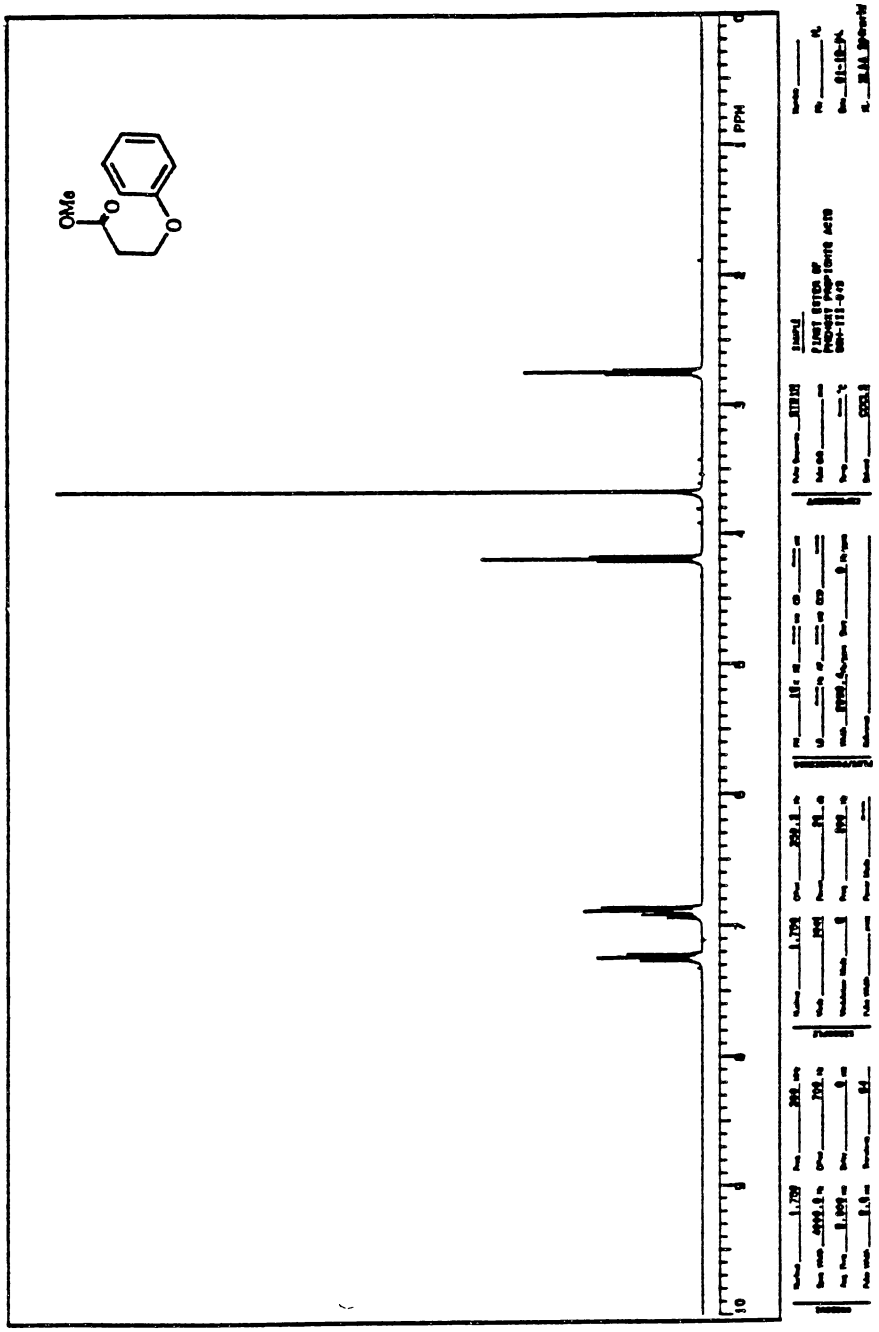
Ethyl (*E*)-3-[2-(3,4-Dihydro-4,4-dimethyl-2*H*-1-benzopyran-6-yl)-1-propenal]benzoate [(*E*)-**63**]

In a 250-mL, 2-necked, round-bottomed flask equipped with a spiral condensor, addition funnel, and magnetic stirrer were mixed alcohol (*E*)-**62** (0.105 g, 0.287 mmol) and activated MnO_2[2] (4 0.52 g, 15.7 mmol) in CH_2Cl_2 (10 mL, N_2). After stirring at RT (24 h), the dark reaction mixture was filtered through a Buchner funnel with filter aid (Celite, Fisher brand, 2 g ,10 mL of CH_2Cl_2). The solid was washed with CH_2Cl_2 (10 mL), and the filtrate (and the washings) was evaporated (rotovap, followed by high vacuum O.25 mm Hg) to give a yellow colored oil which was a mixture of isomeric aldehydes (*E*)-**63** :(*Z*)-**63** [8:2] and the starting material [alcohol (*E*)-**62**]. Separation was effected with chromatography on a 4 mm thick plate of silica gel (silica gel containing gypsum) with the aid of a Chromatotron. The solvent system used to elute the compounds was hexane:ethyl acetate (8:2). The fraction with (*E*)-**63** was collected from the plate, immediately after the (*Z*)-**63** fraction was eluted. The solvent was evaporated [rotovap, followed by high vacuum (0.25 mm Hg), 45-50°C] to give a light yellow oil which was aldehyde (*E*)-**63** (0.8 g, 76.7%). Boiling ethanol (absolute, 0.5 mL) was added to this oil, and the resulting solution was placed in a freezer (48 h). Needle-like crystals (off white in color) of aldehyde (*E*)-**63** formed. These crystals were vacuum dried (Abderhalden, 60-61°C, 0.3 mm Hg, 24 h); mp 87.5-89°C. IR (KBr) 2710 (C(O)H), 1720 (C=O), 1690 C(O)H cm^{-1}. ^1H NMR ($DCCl_3$) ∂ 1.17 [s, 6 H, H(9),H(10)], 1.34 [t, 3 H, H(22)], 1.80 [m, 2 H, H(3)], 4.19 [m, 2 H, H(2)], 4.20 [q, 2 H, H(21)], 6.82-7.95 [Ar-H], 9.7 [s,1 H, H(12)]; ^{13}C NMR ($DCCl_3$) ppm 14.2 [C(22)], 30.4 [C(4)], 30.7 [C(9), C(10)], 37.4 [C(3)], 60.8 [C(21)], 63.0 [C(2)], 117-155 [Ar-C], 165.8 [C(20), C=O], 194.0 [C(12), C=O]. Anal for $C_{23}H_4O_4$: C 75.80; H, 6.63. Found: C 75.85; H, 6.67.

Ethyl (*E*)-3-[2-(3,4-Dihydro-4,4-dimethyl-2*H*-1-benzopyran-6-yl)-1-propanoic acid]benzoate [(*E*)-**64**]

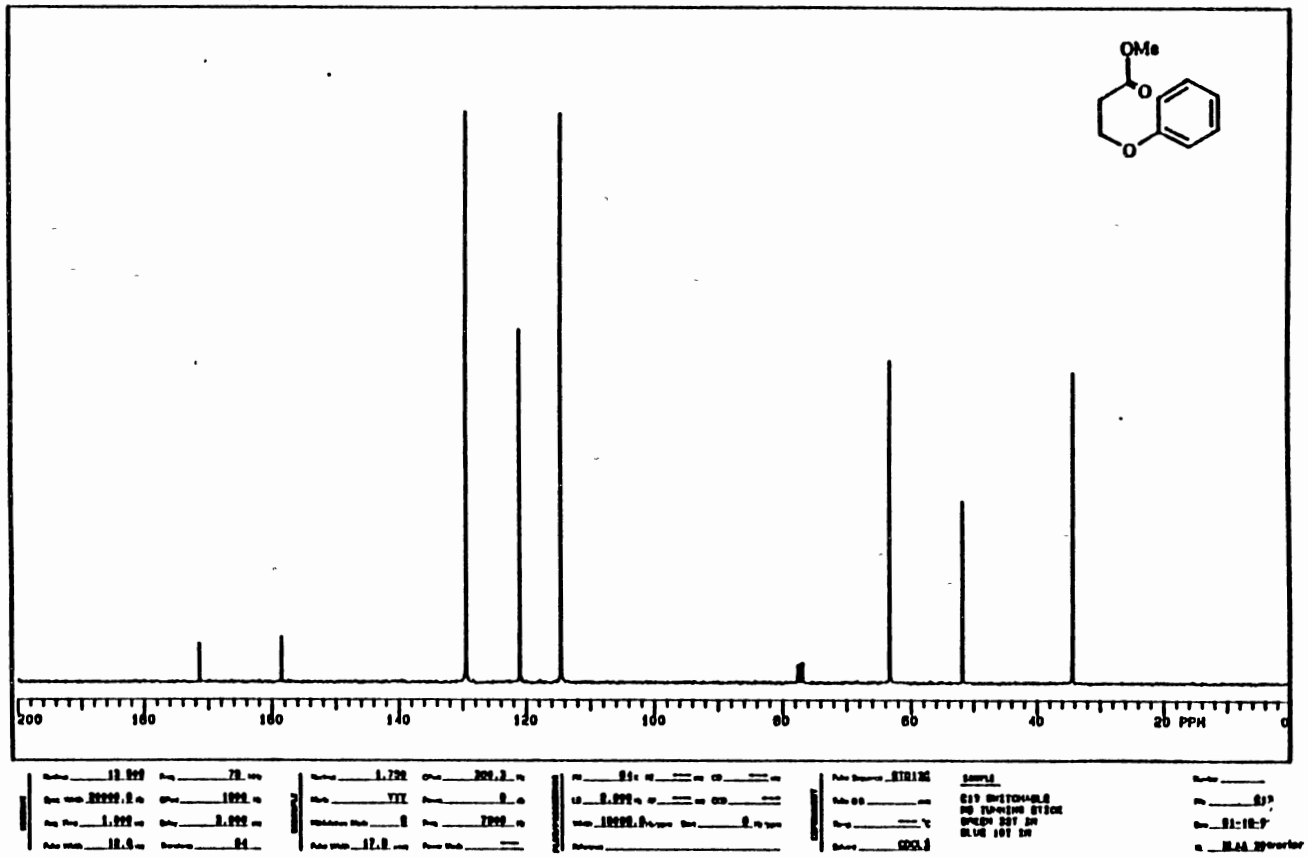
In a 100-mL, 2-necked, round-bottomed flask equipped with a condenser and magnetic stirrer was dissolved aldehyde (*E*)-**63** (0.500 g, 1.3 mmol, N_2) in *t*-butanol (40 mL). After adding resorcinol (0.16 g, 1.51 mmol) to the stirred solution (RT, 10 min), an aqueous solution (45 mL water) containing $NaClO_2$ (1.1 g, 12.35 mmol) and NaH_2PO_4 (1.32 g, 9.61 mmol) was added dropwise (RT, 15 min). After stirring (RT) for 24 h, the reaction mixture was acidified (pH 5, 6M HCl, 1 mL), and then the solvent (*t*-butanol) was evaporated using a rotovap. The solution was extracted with benzene (4 x 35 mL), and the combined organic phases were washed with water (2 x 50 mL) and brine (50 mL). After drying (Na_2SO4, 4 h), the solvent was evaporated using a rotovap [followed by high vacuum (O.3 mm Hg), RT, 24 h] to give acid (*E*)-**64** (0.49 g,1.29 mmol 98%) as a thick yellow solid. Yellow crystals of acid (*E*)-**64** were obtained upon recrystallization with boiling absolute ethanol (2 mL); mp 154-155°C. IR (KBR) 2450-3450 (O-H, CH) 1685-1710 (C=O) cm^{-1}; ^1H NMR ($DCCl_3$) ∂ 1.17 [s, 6 H, H(9),H(10)], 1.35 [t, 3 H, H(22)], 1.80 [m, 2 H, H(3)], 4.20 [m, 2 H, H(2)], 4.31 [q, 2 H, H(21)], 6.83-7.95 [Ar-H], 10.7 [s,1 H, H(12)]. ^{13}C NMR ($DCCl_3$) ppm 14.2 [C(22)], 30.5 [C(4)], 30.8 [C(9), C(10)], 37.5 [C(3)], 61.1[C(21)], 63.3 [C(2)], 117.4-153 [Ar-C], 166.8 [C(20), C=O], 173.3 [C(12), C=O]. Anal for $C_{23}H_{24}O_5$: C 72.61; H, 6.36. Found: C 72.35; H, 6.42.

Plate I



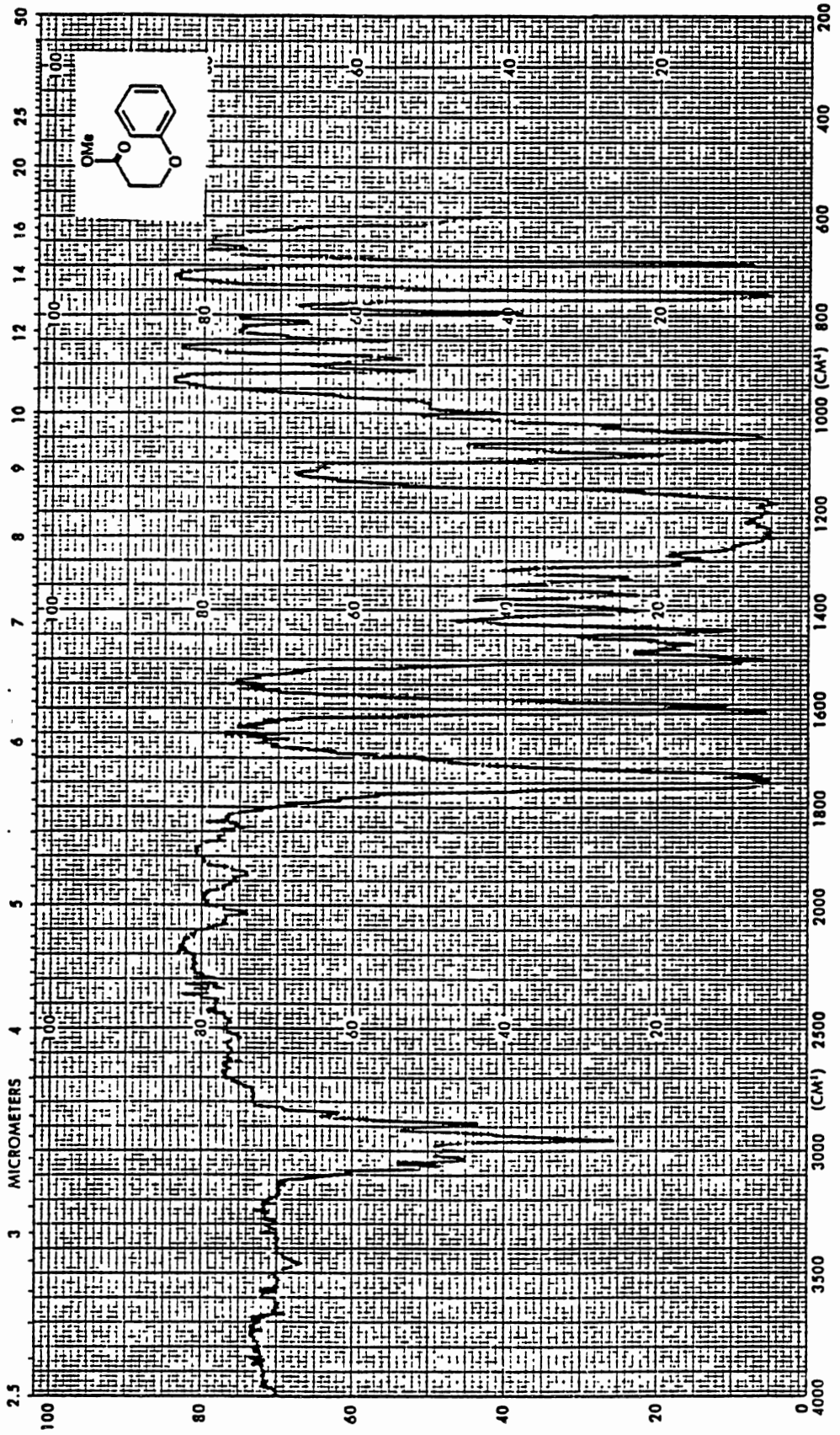
^{1}H NMR Spectrum of 66

Plate II



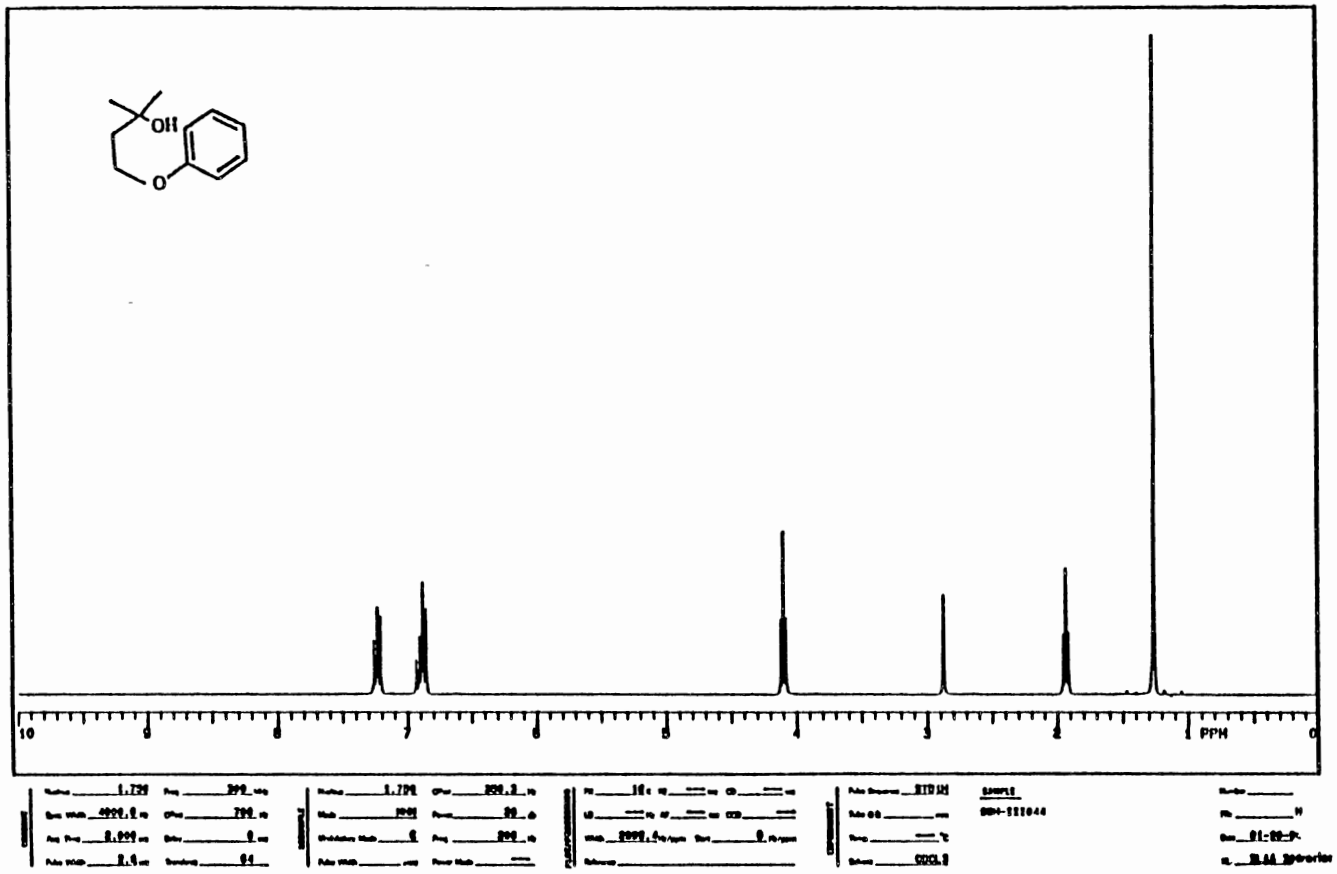
^{13}C NMR Spectrum of **66**.

Plate III



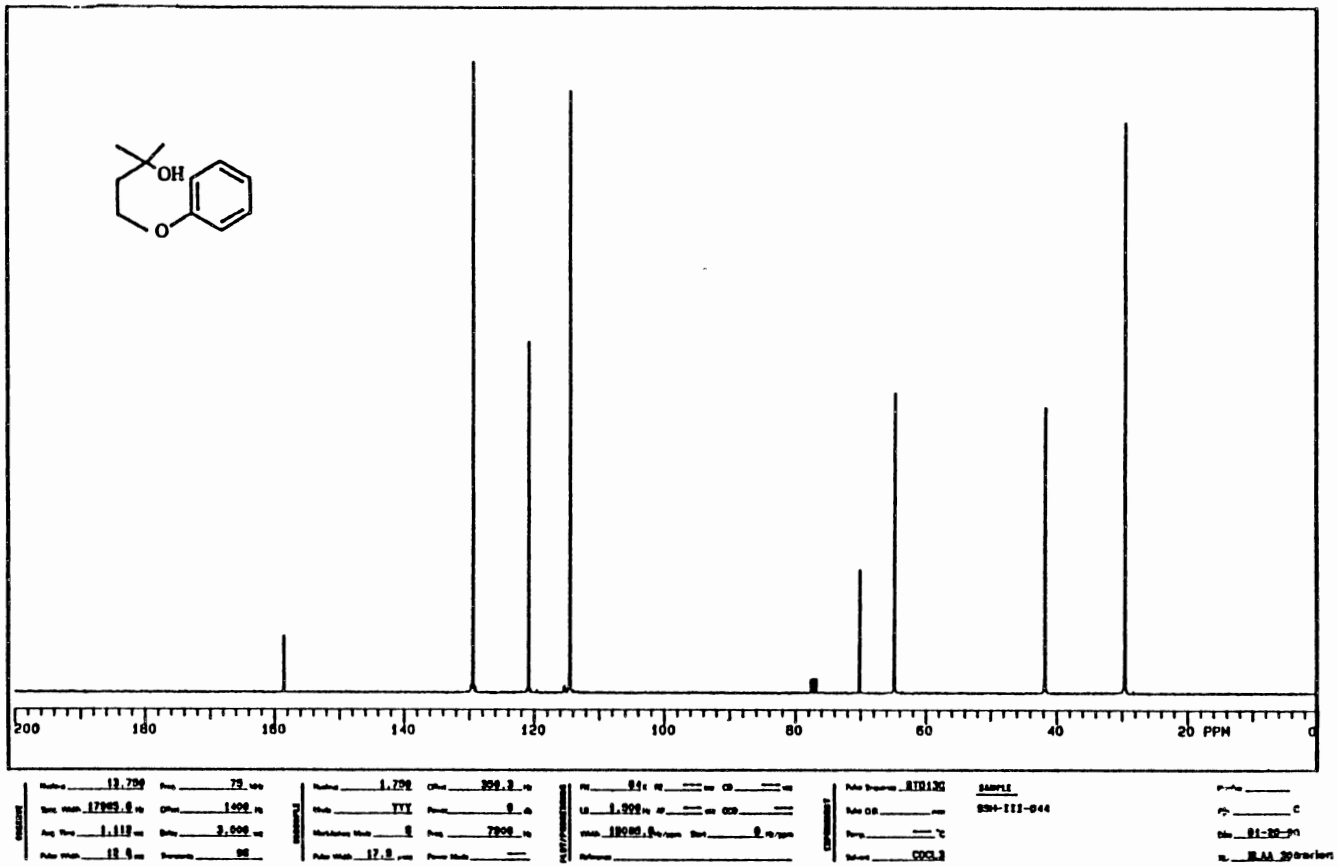
IR Spectrum of **66**

Plate IV



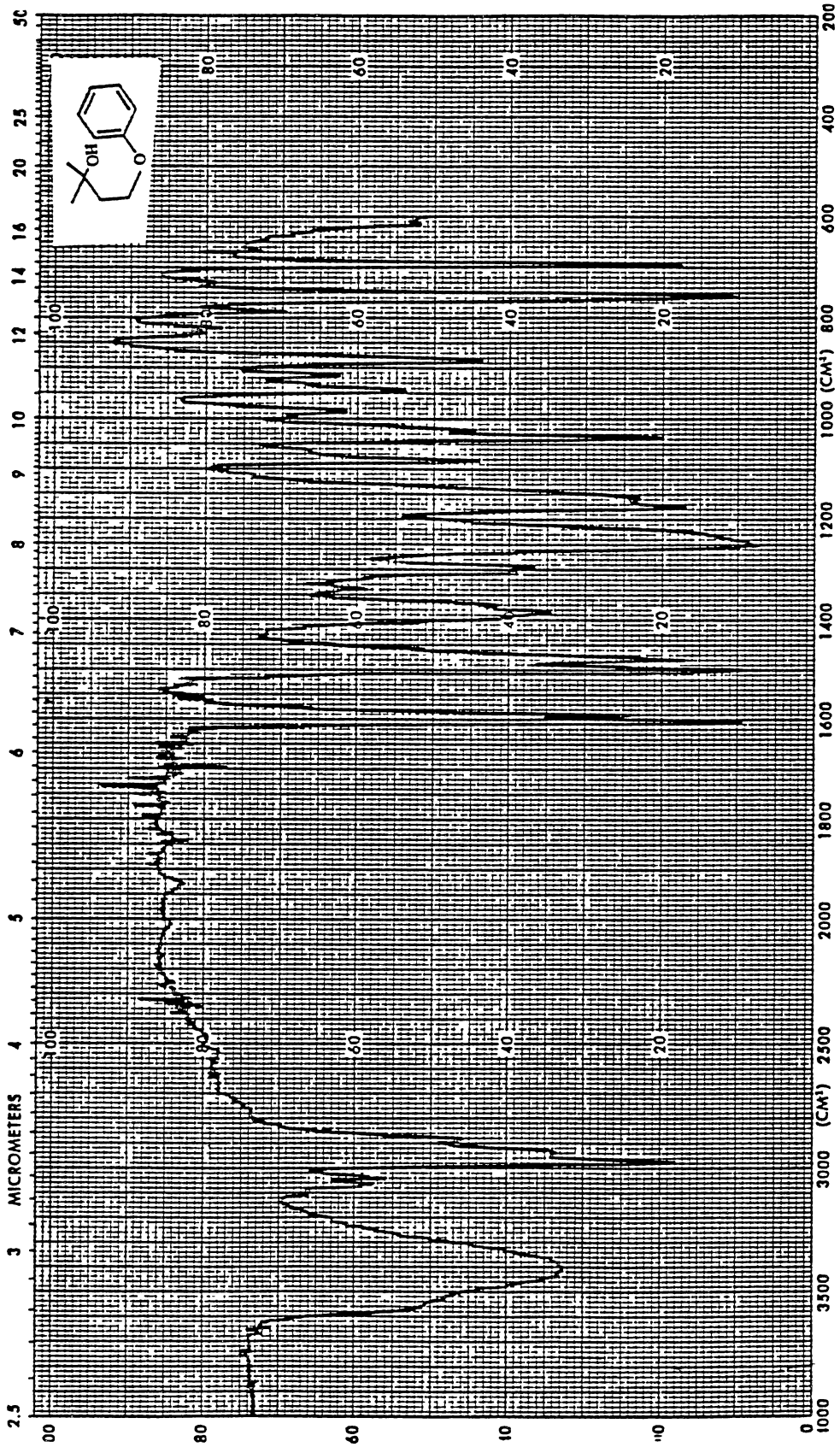
^{1}H NMR Spectrum of **67**

Plate V



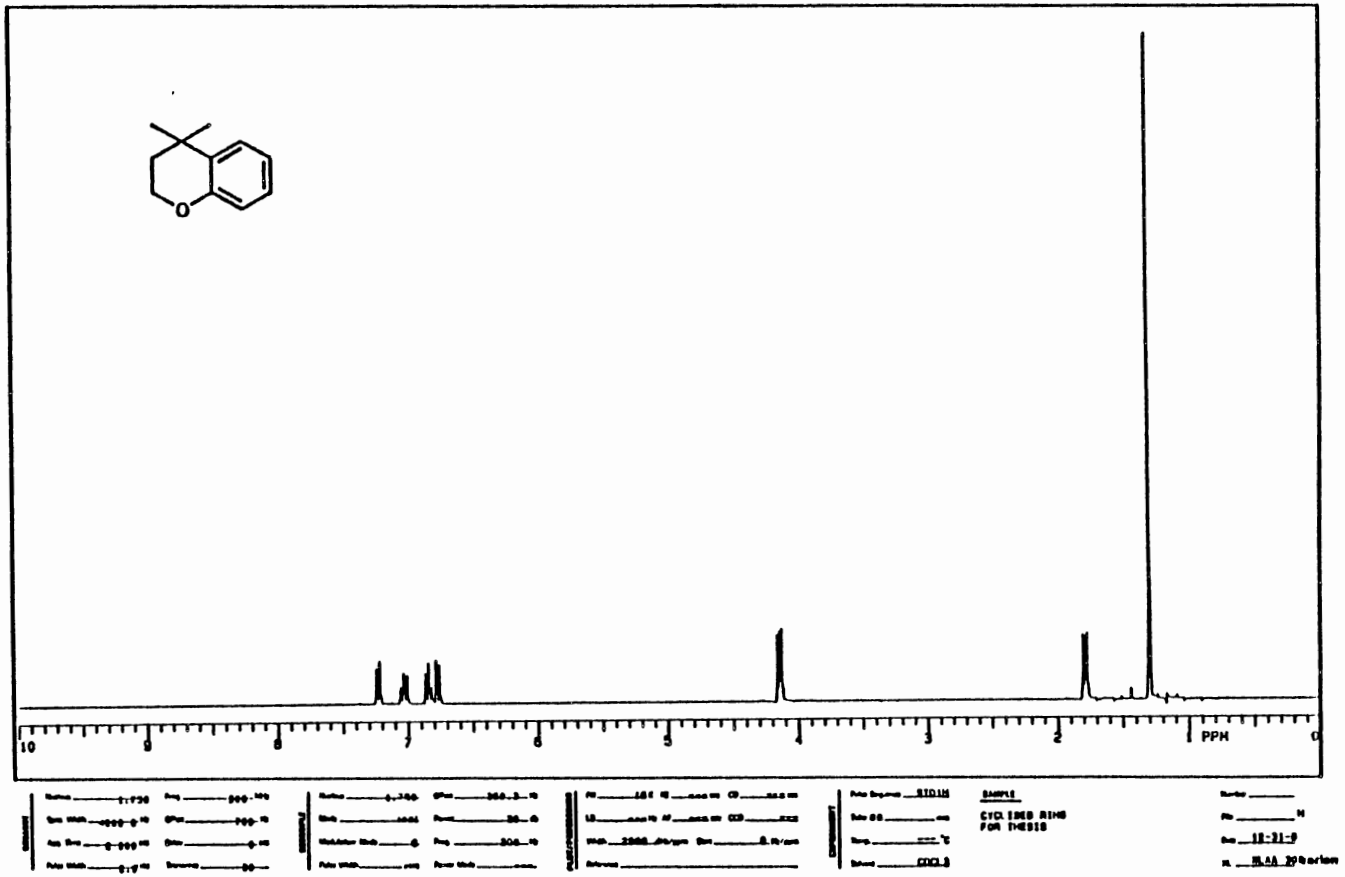
^{13}C NMR Spectrum of **67**

Plate VI



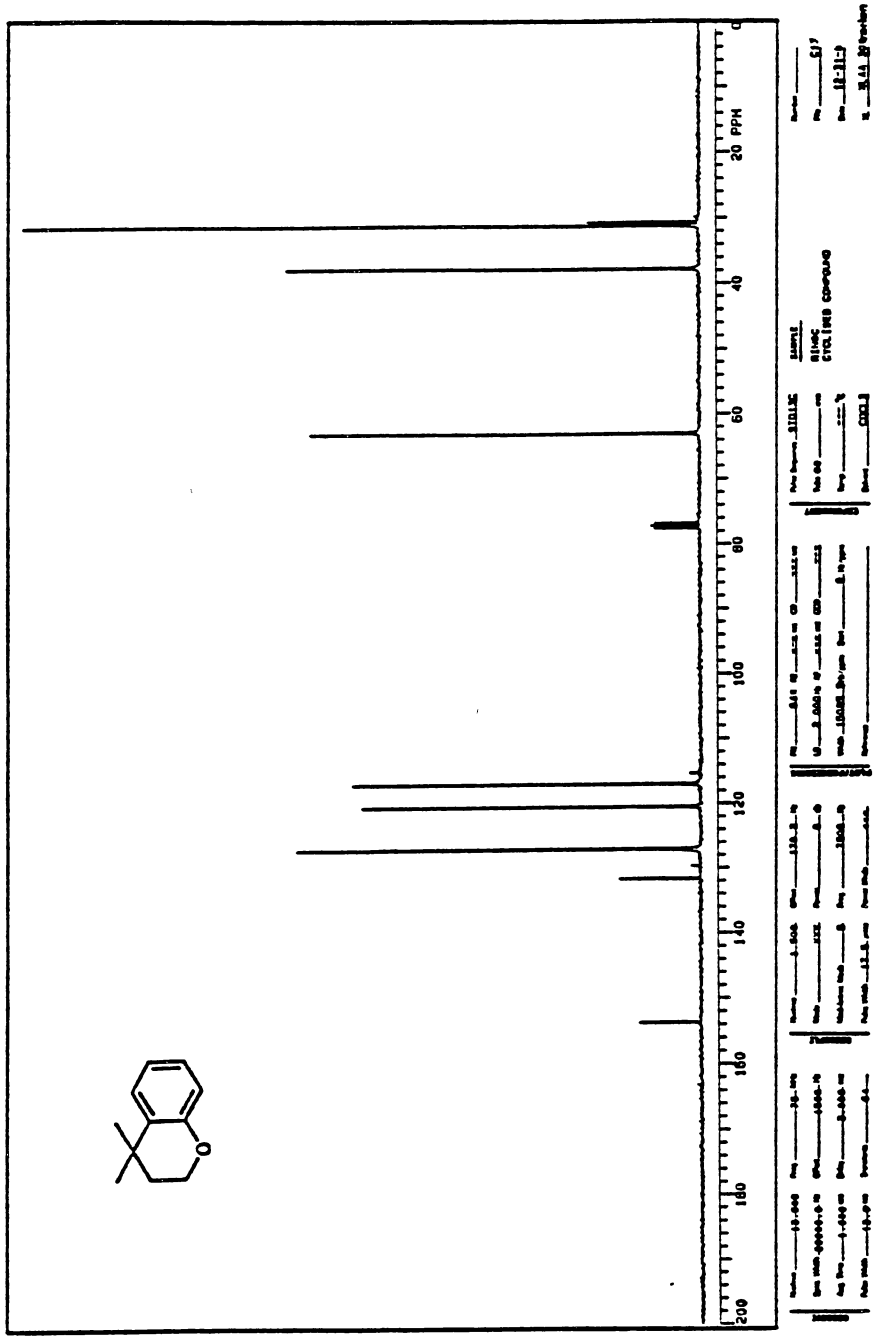
IR Spectrum of **67**

Plate VII



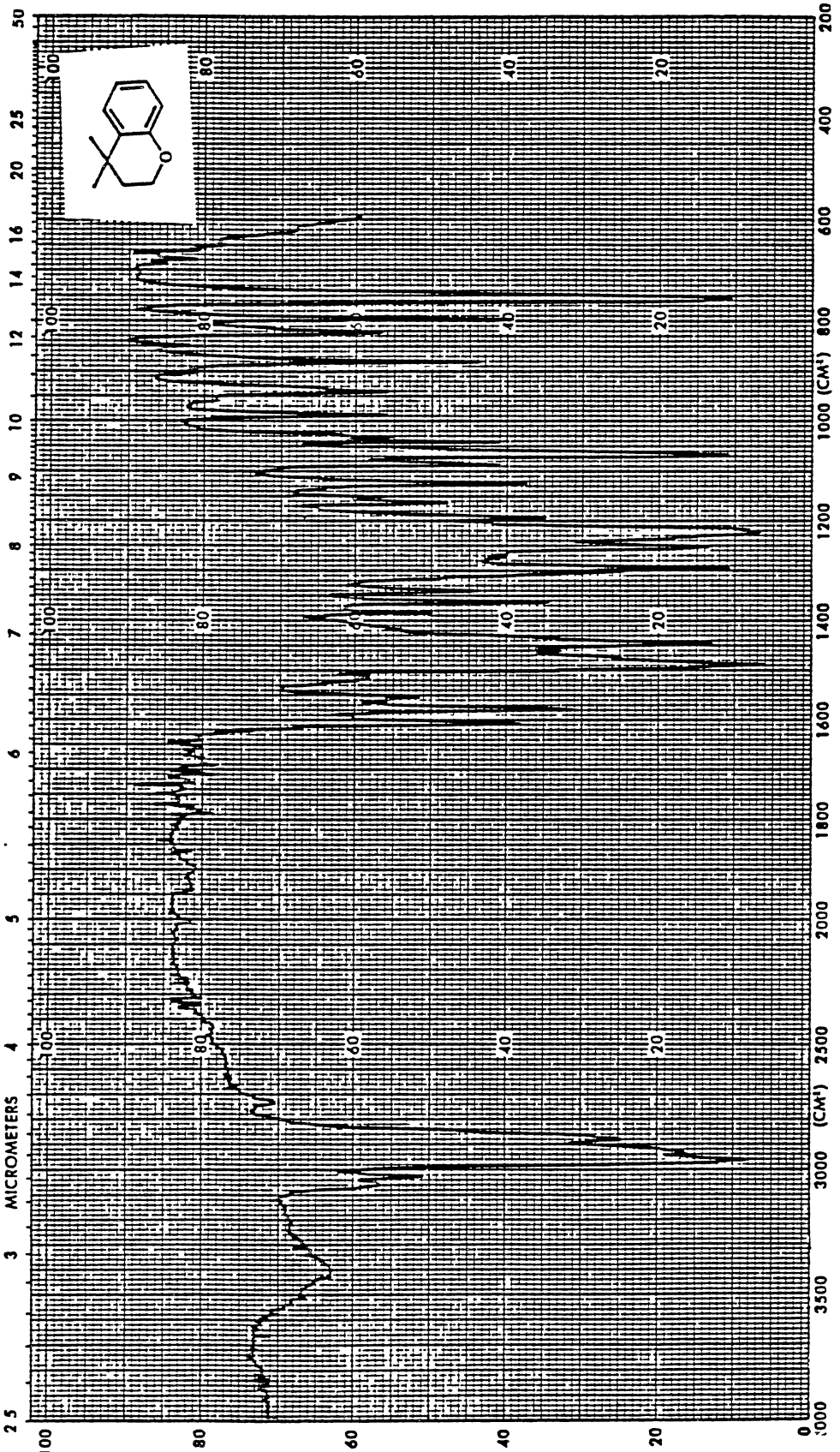
^{1}H NMR Spectrum of **68**

Plate VIII



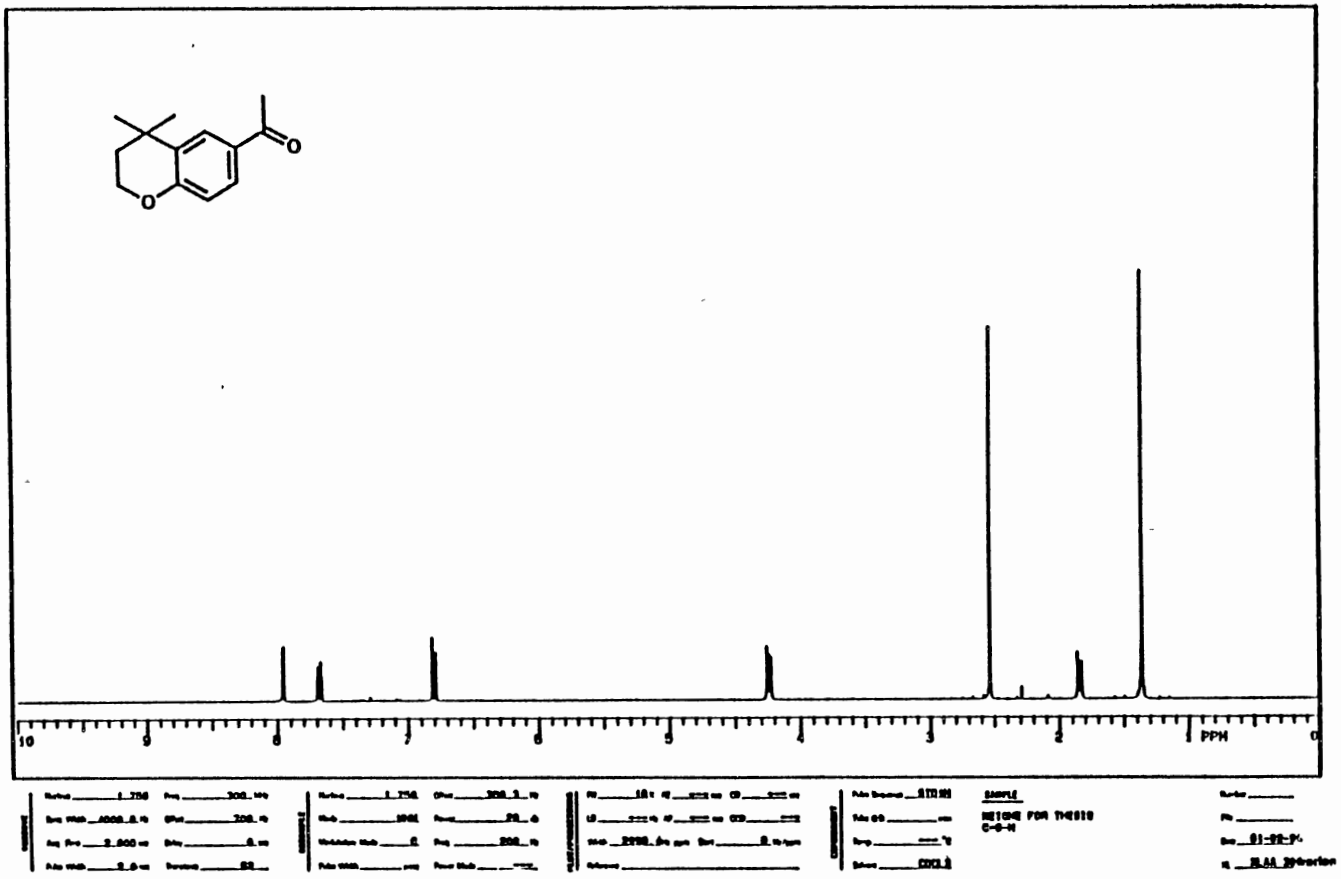
^{13}C NMR Spectrum of 68

Plate IX



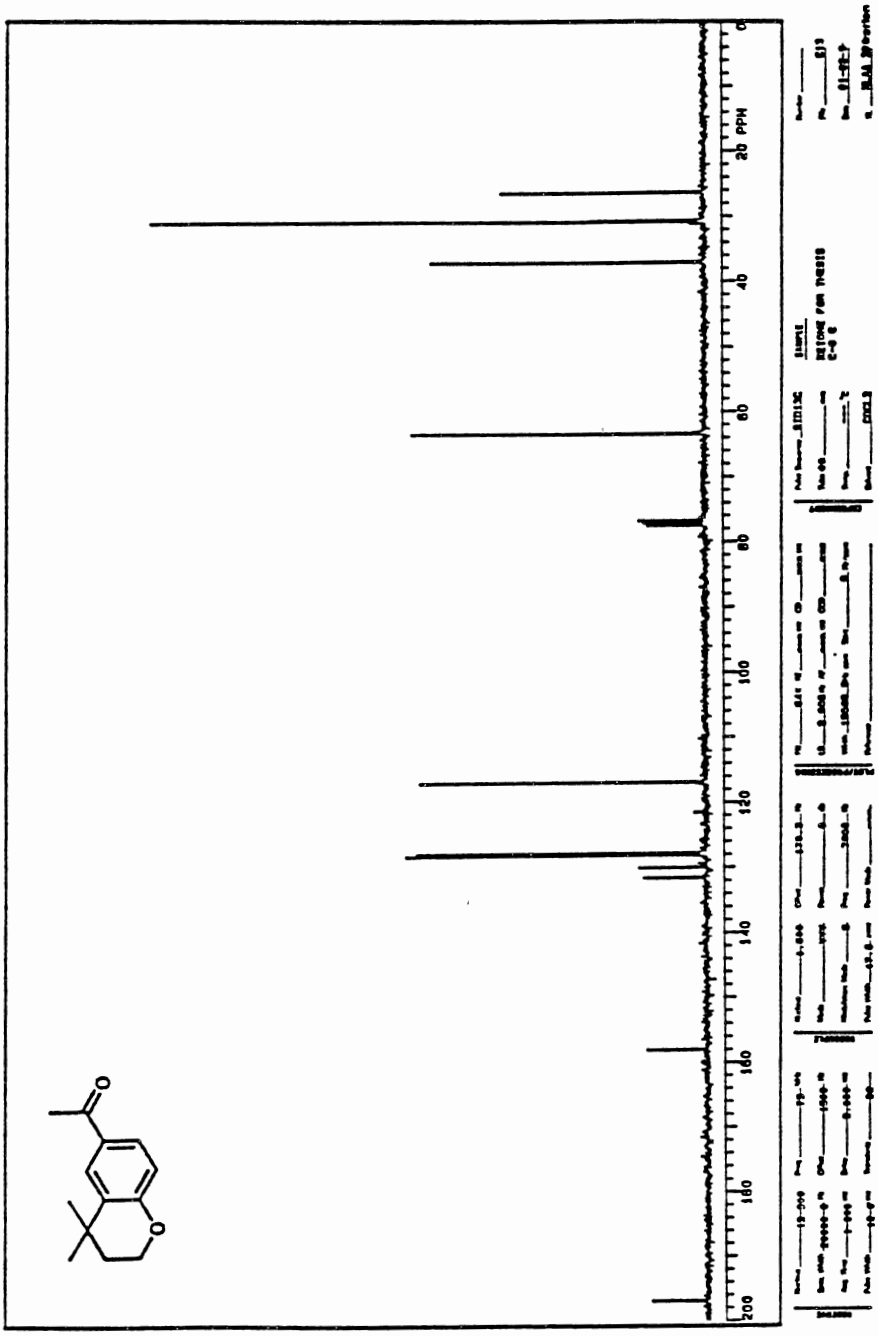
IR Spectrum of 68

Plate X



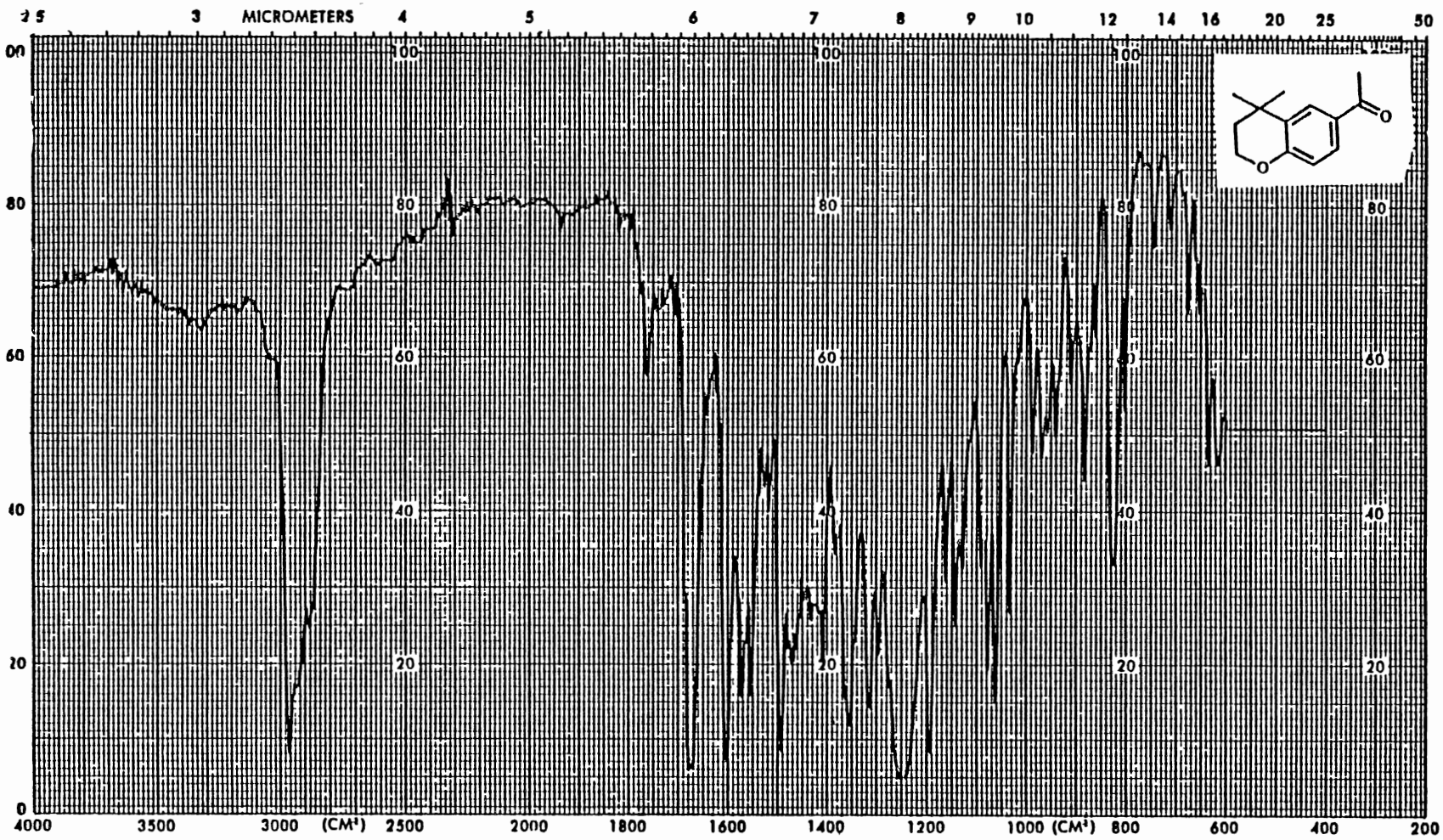
^{1}H NMR Spectrum of **69**

Plate XI



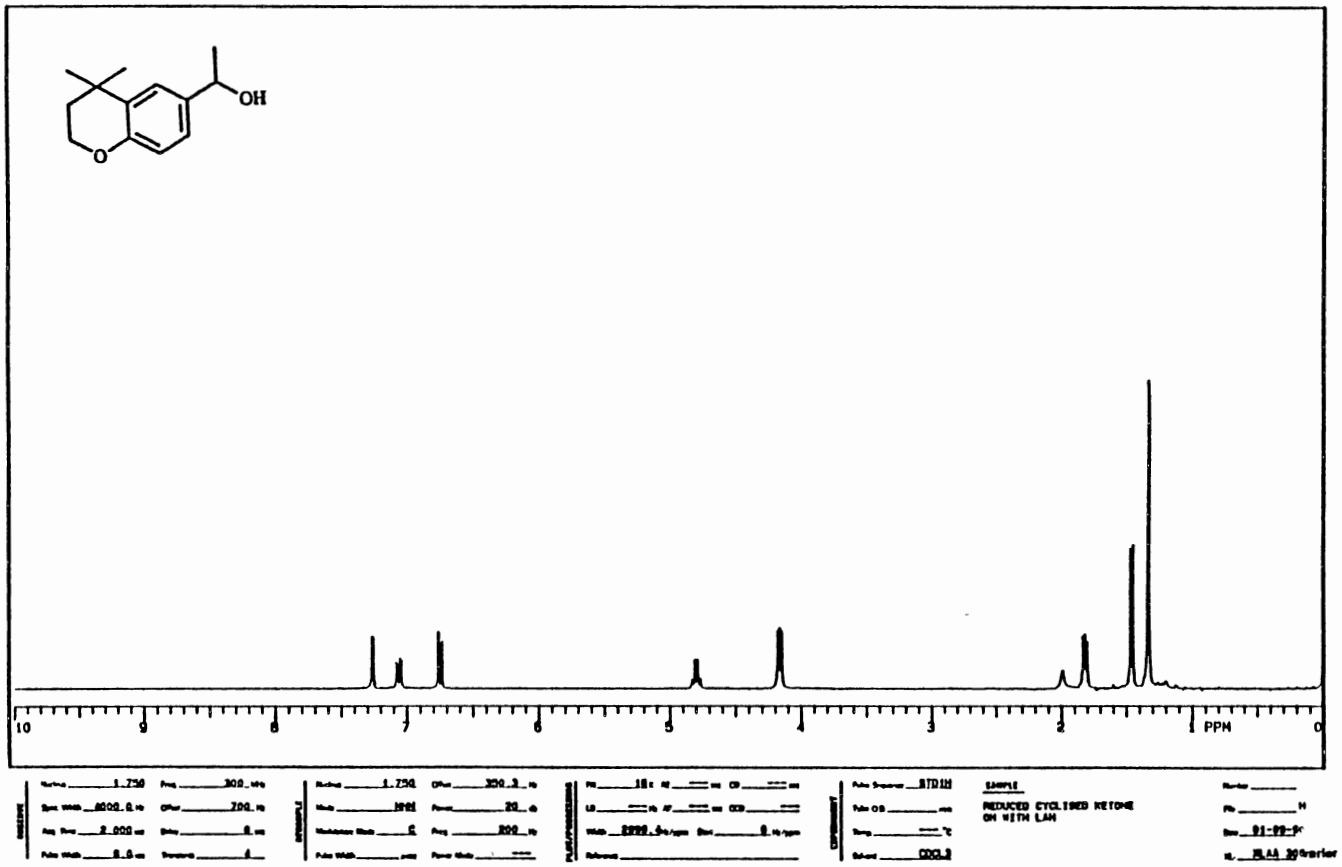
^{13}C NMR Spectrum of **69**

Plate XII



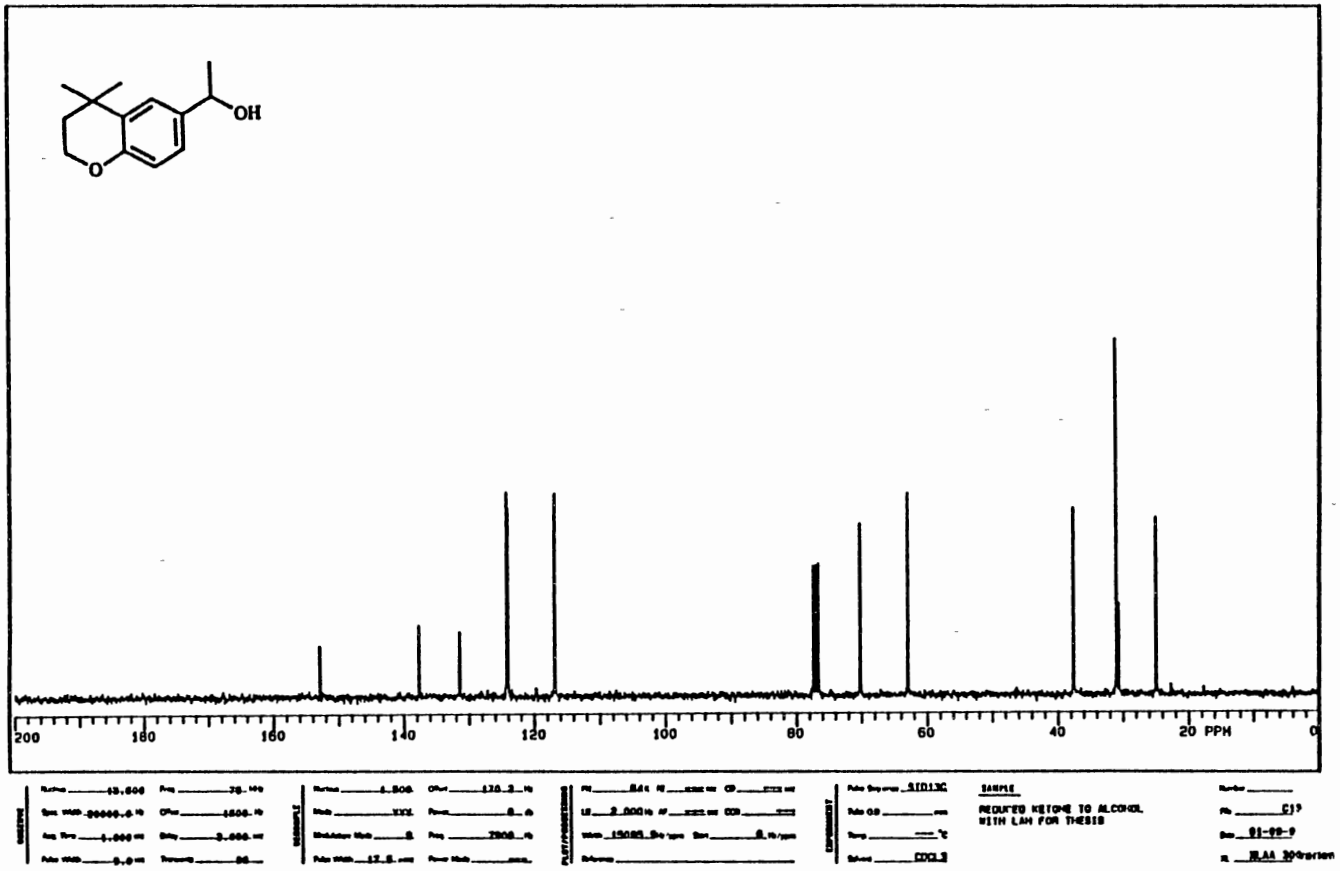
IR Spectrum of **69**

Plate XIII



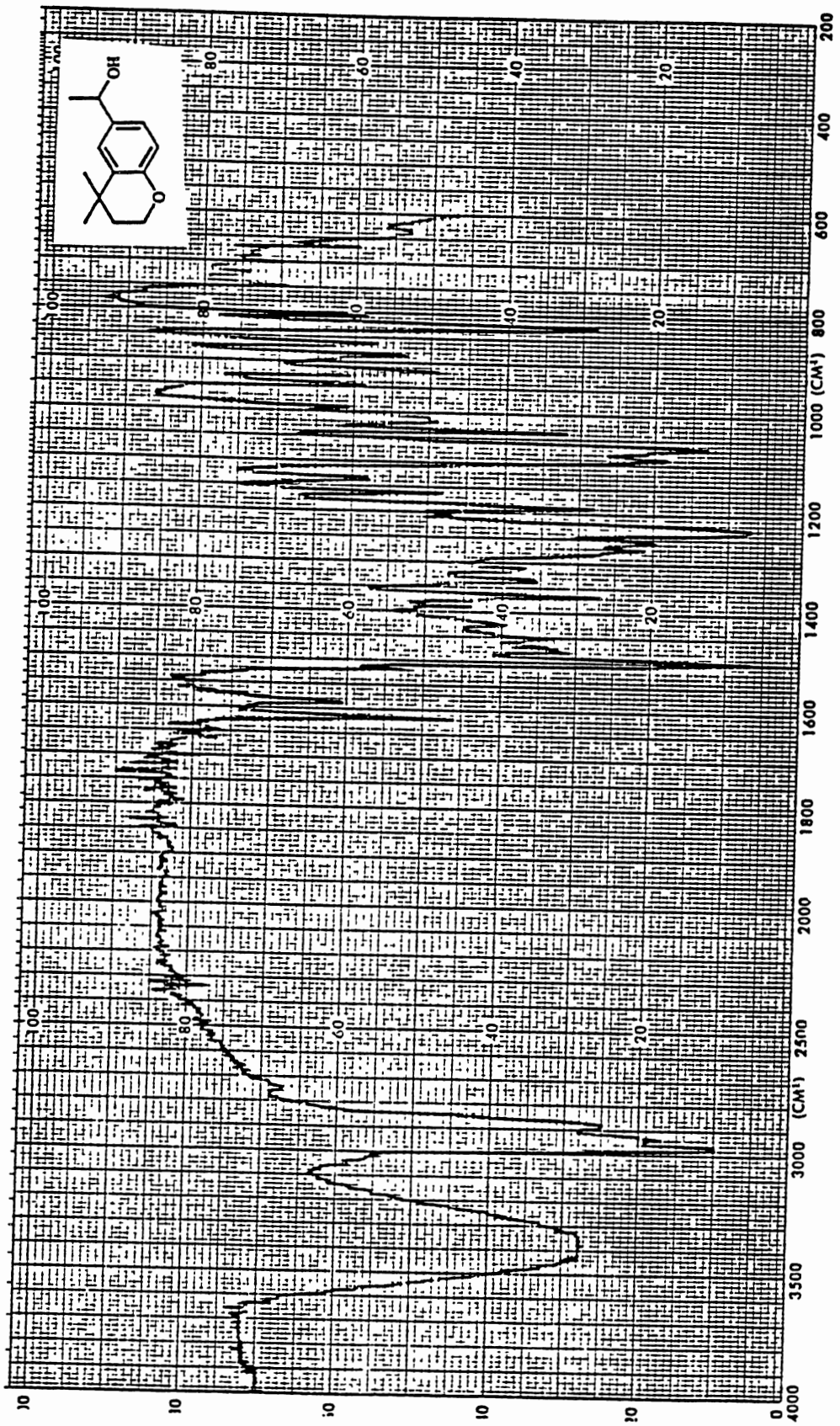
^{1}H NMR Spectrum of **70**

Plate XIV



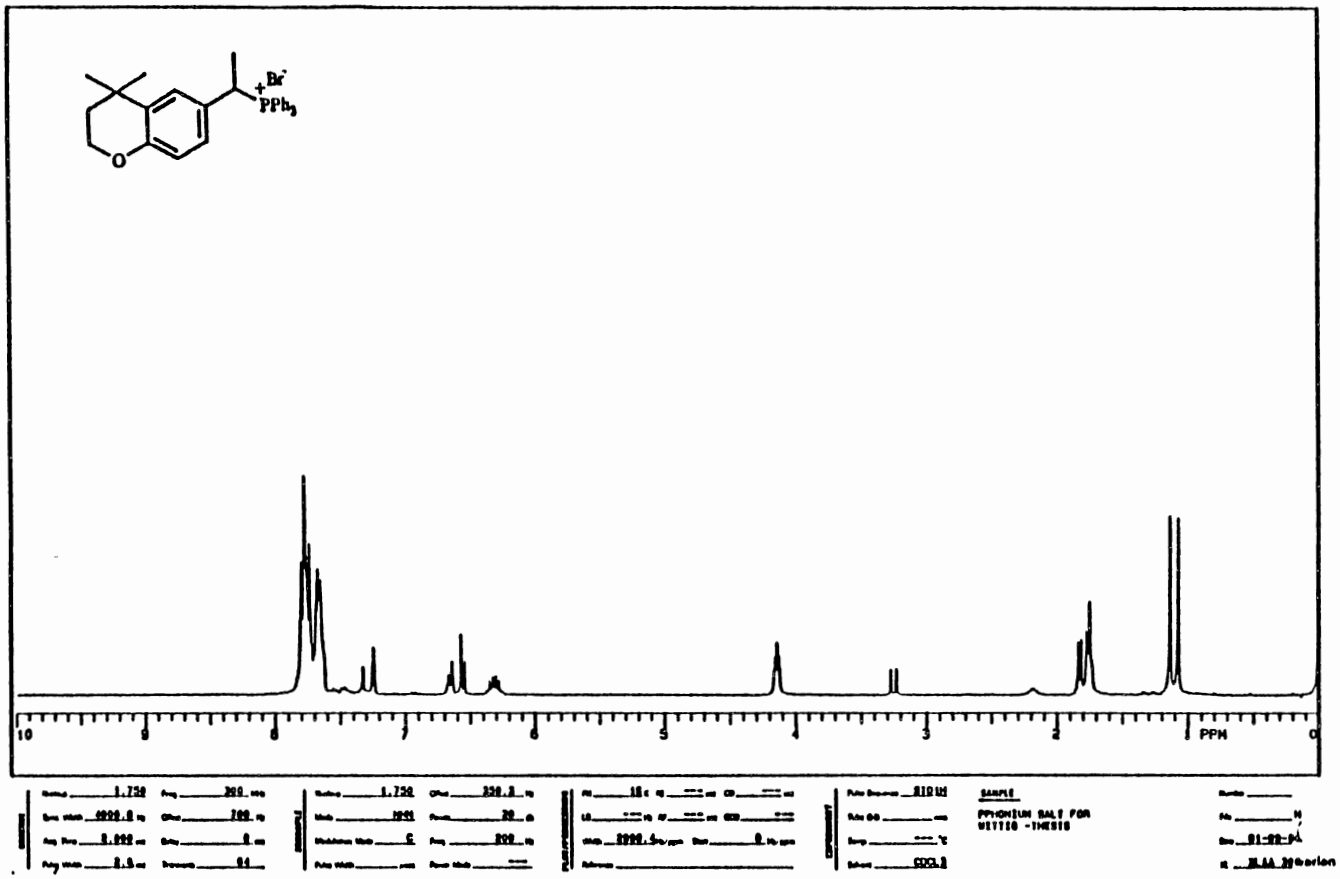
^{13}C NMR Spectrum of **70**

Plate XV



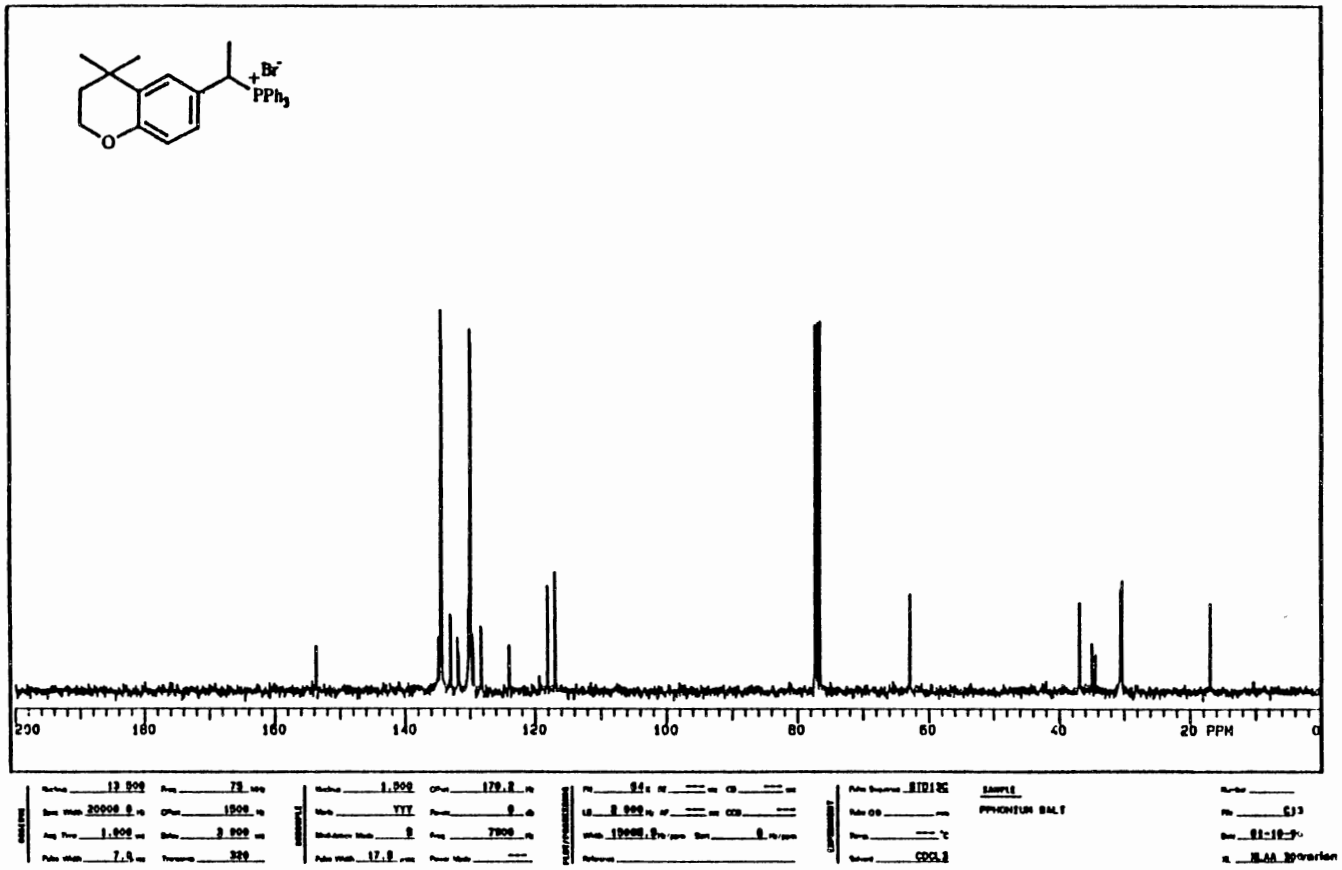
IR Spectrum of 70

Plate XVI



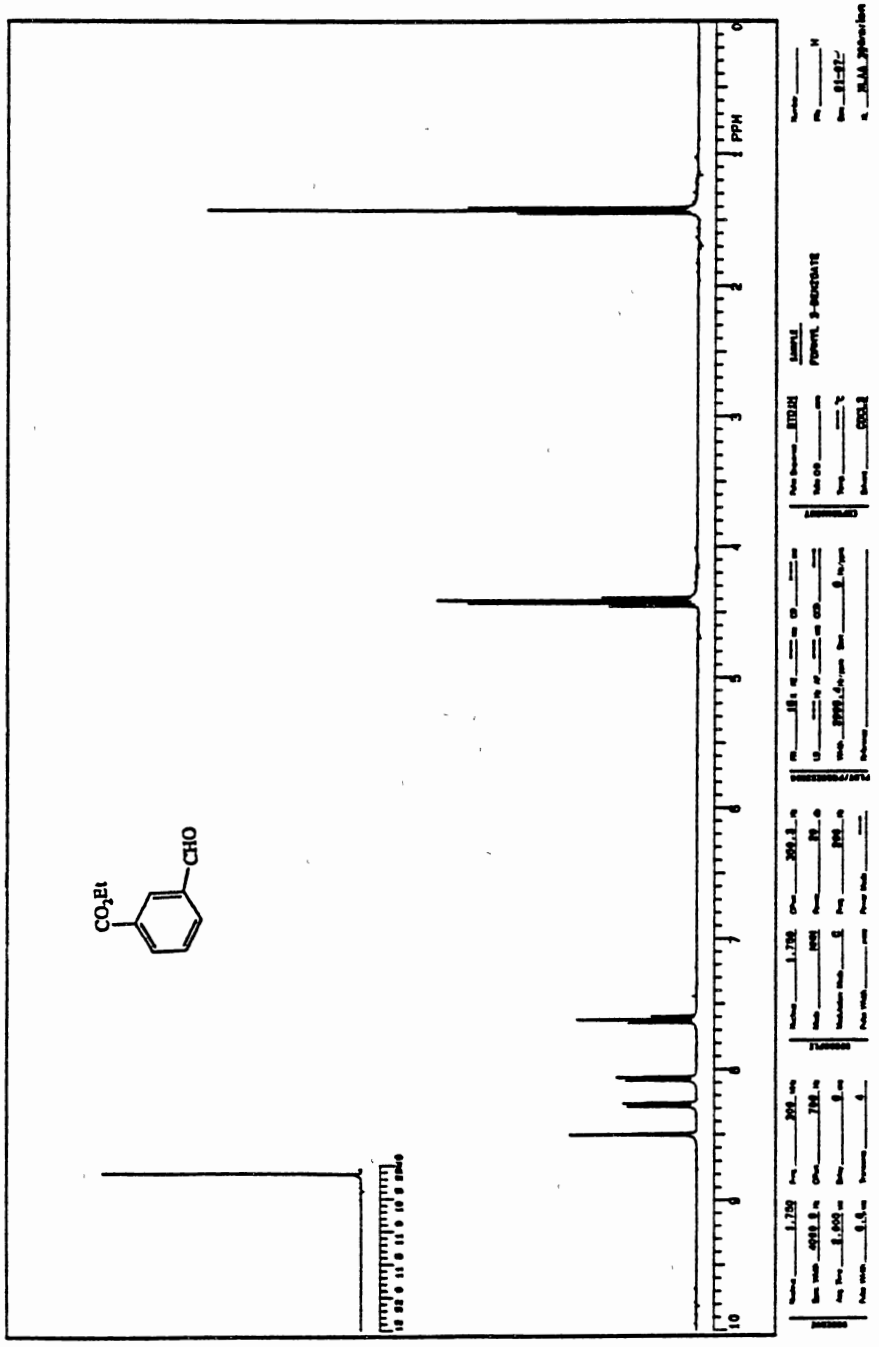
^{1}H NMR Spectrum of **71**

Plate XVII



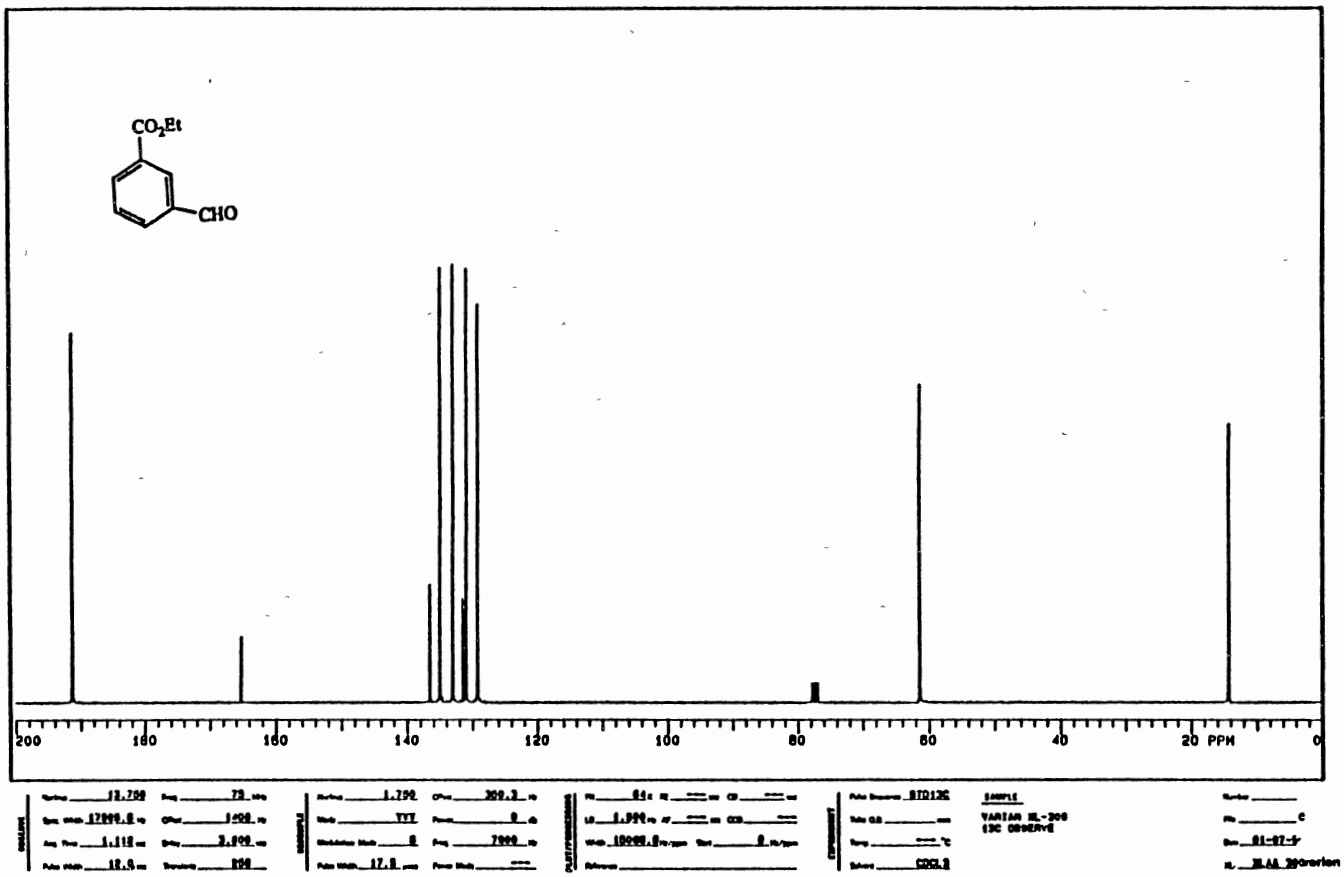
^{13}C NMR Spectrum of **71**

Plate XVIII



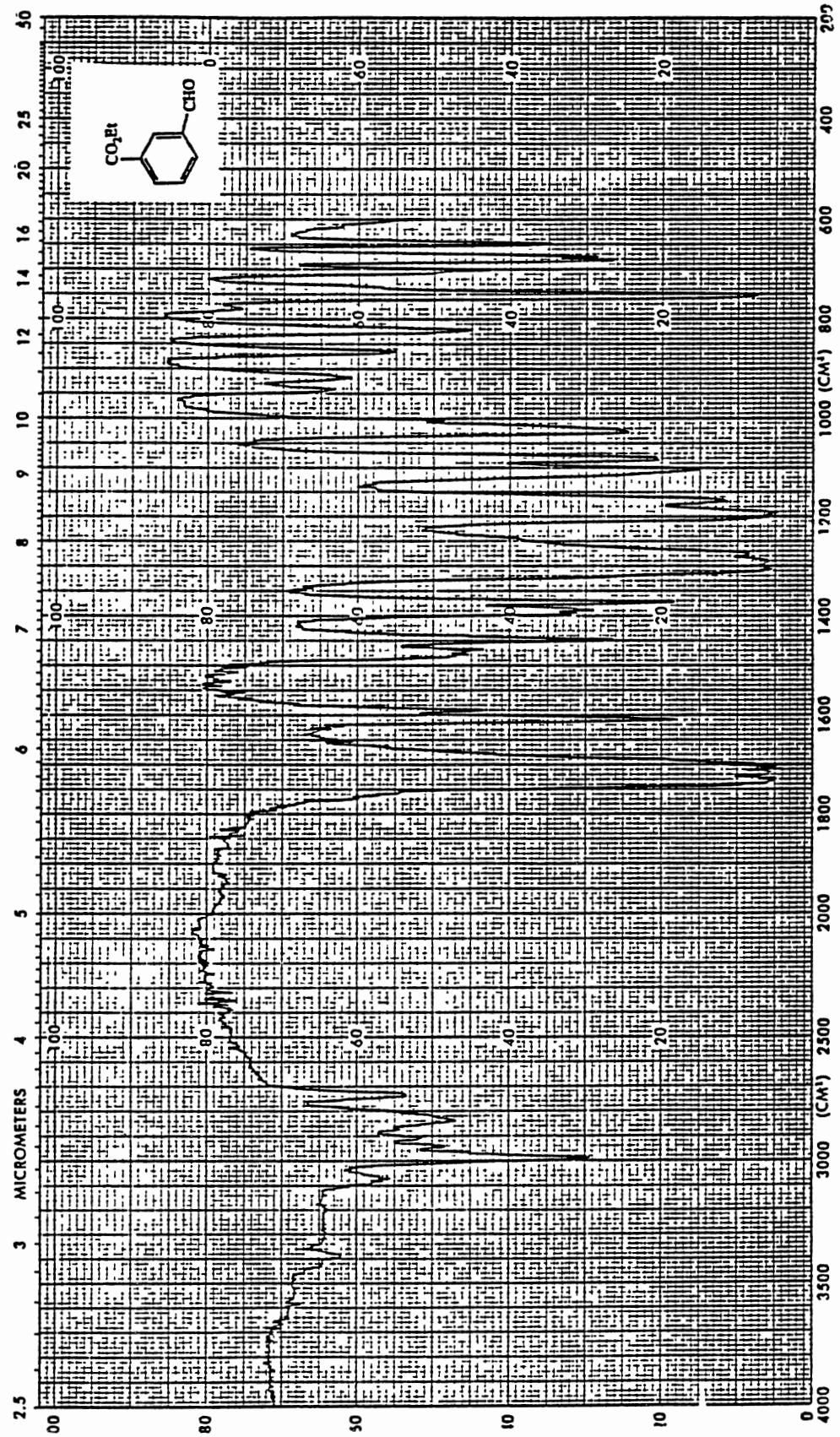
^{1}H NMR Spectrum of 72

Plate XIX



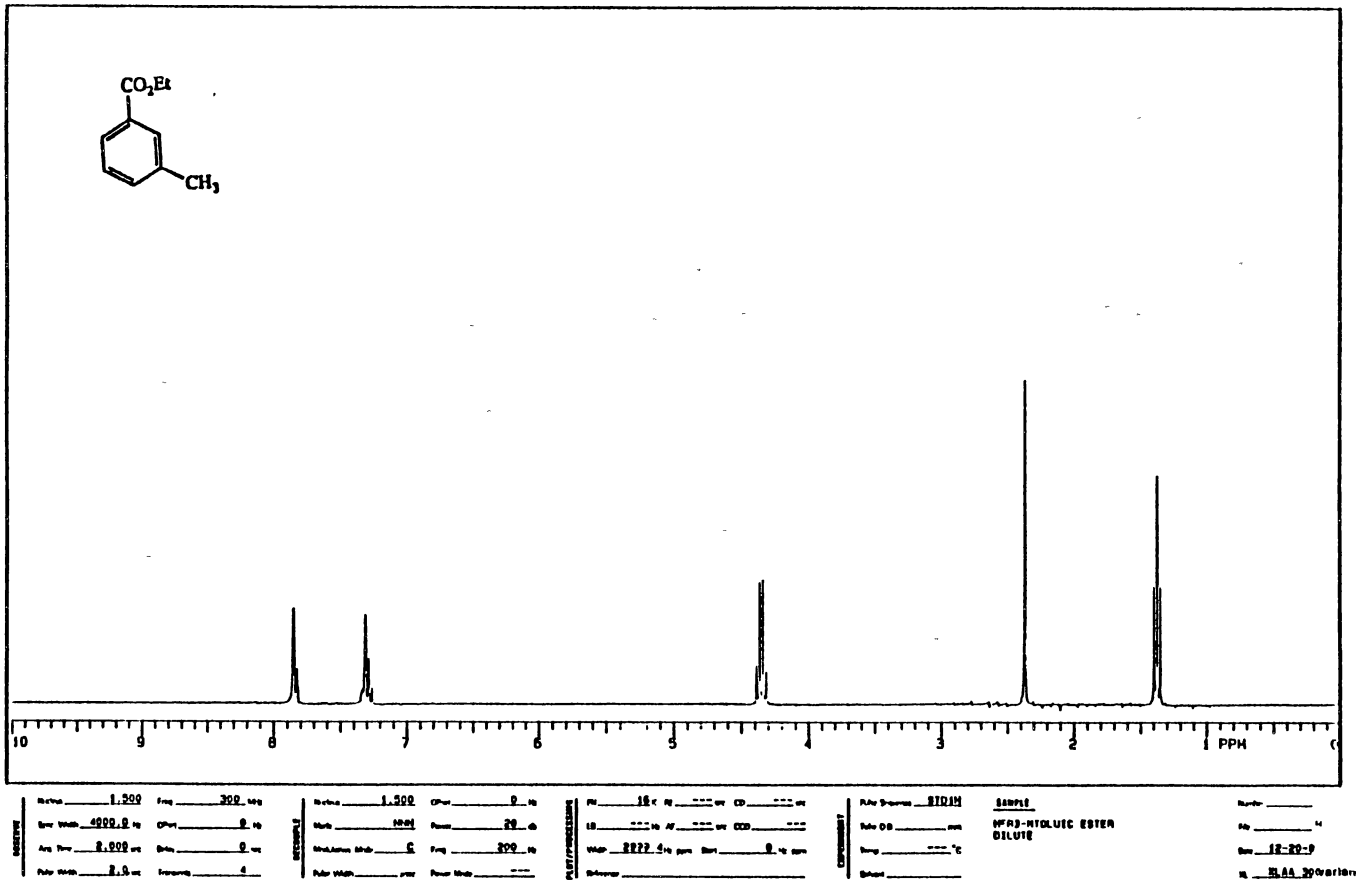
^{13}C NMR Spectrum of 72

Plate XX



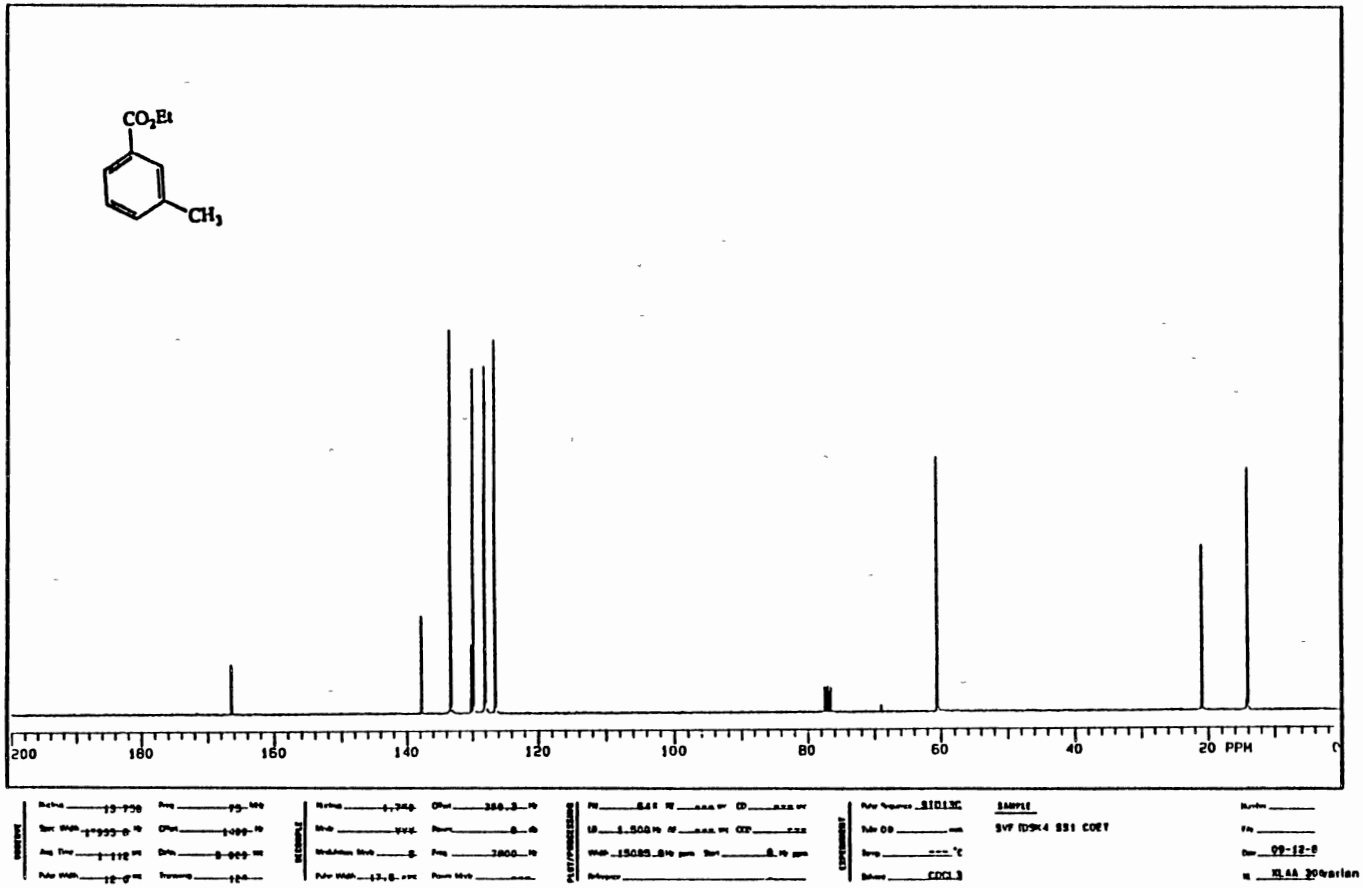
IR Spectrum of 72

Plate XXI



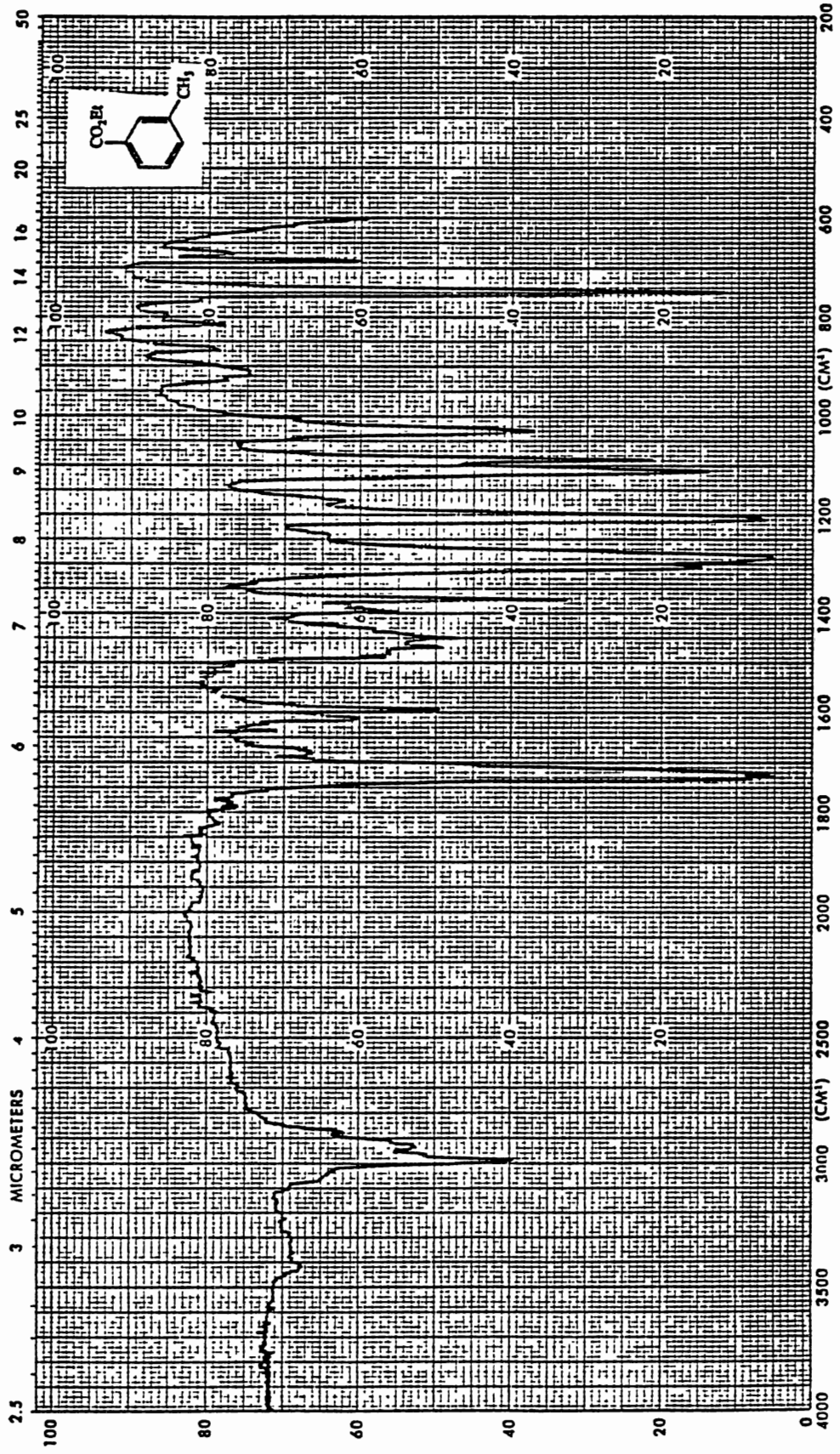
^{1}H NMR Spectrum of **74**

Plate XXII



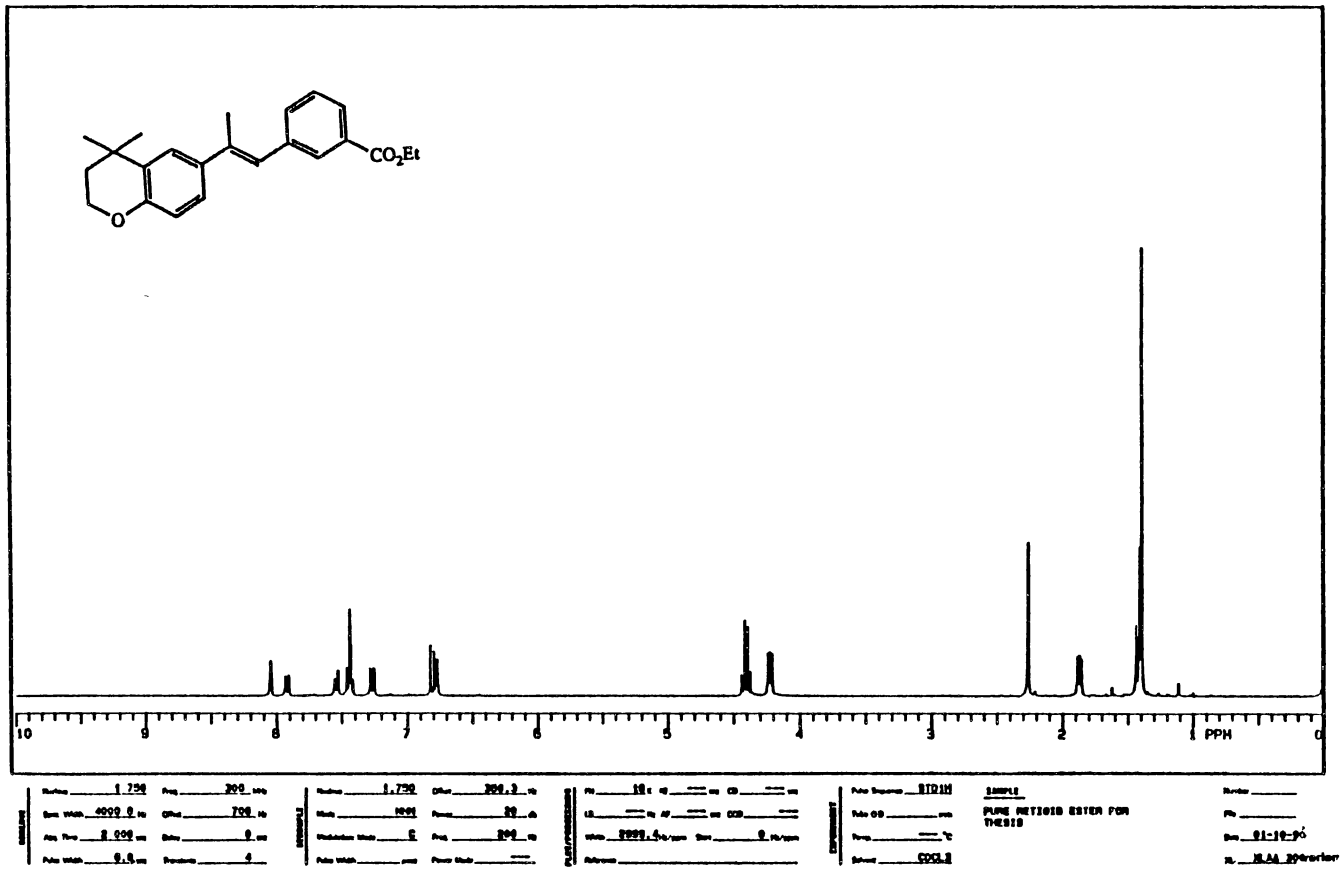
^{13}C NMR Spectrum of **74**

Plate XXIII



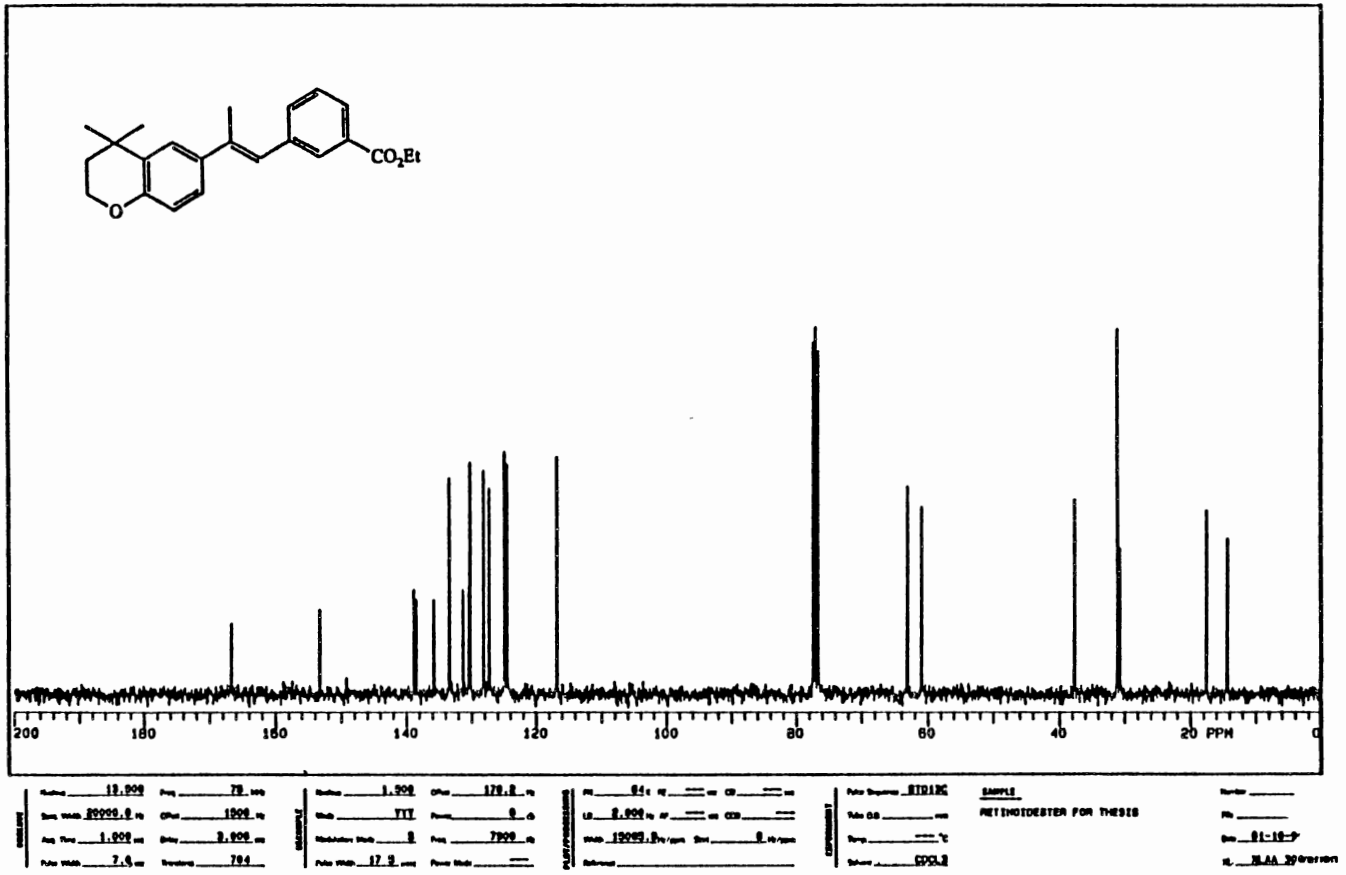
IR Spectrum of 74

Plate XXIV



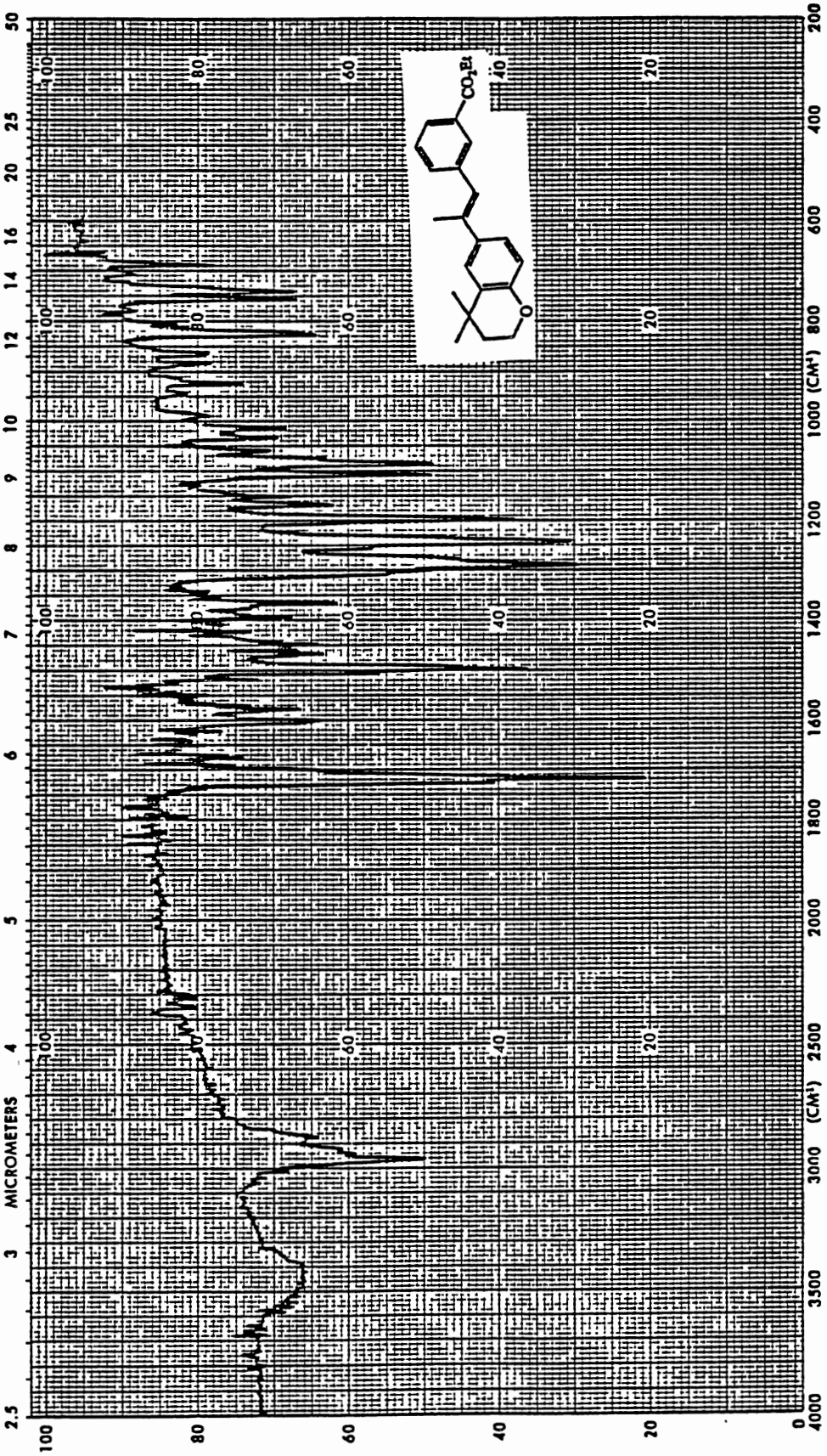
^{1}H NMR Spectrum of **60**

Plate XXV



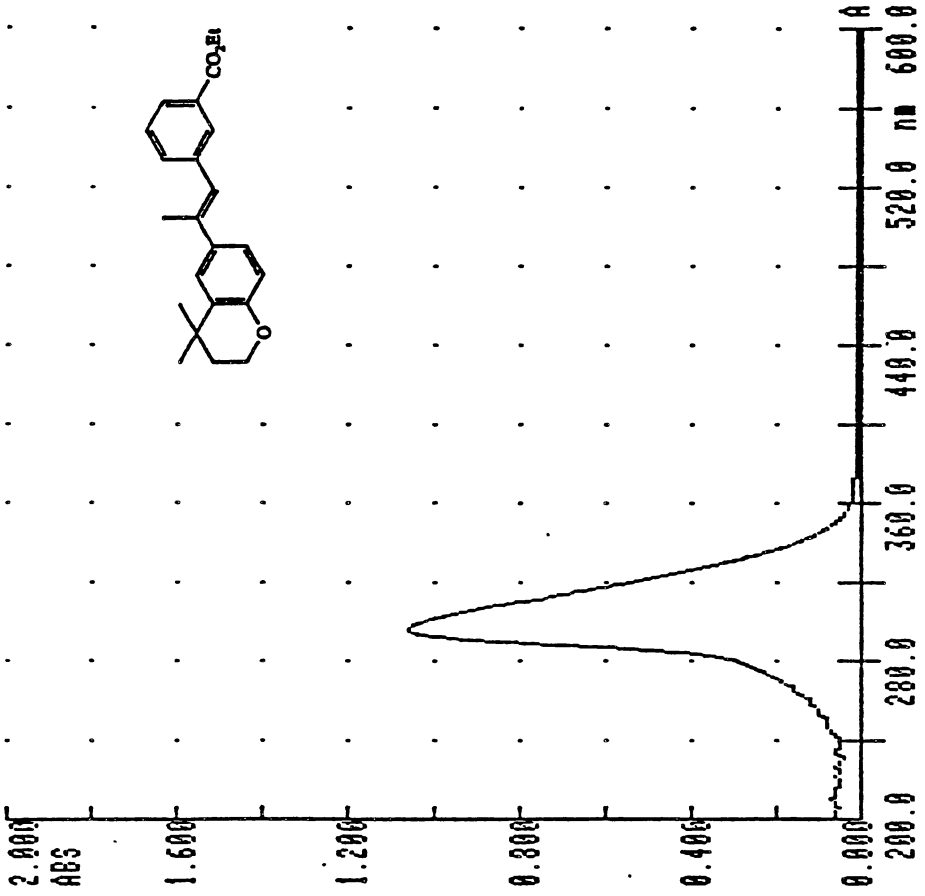
^{13}C NMR Spectrum of **60**

Plate XXVI



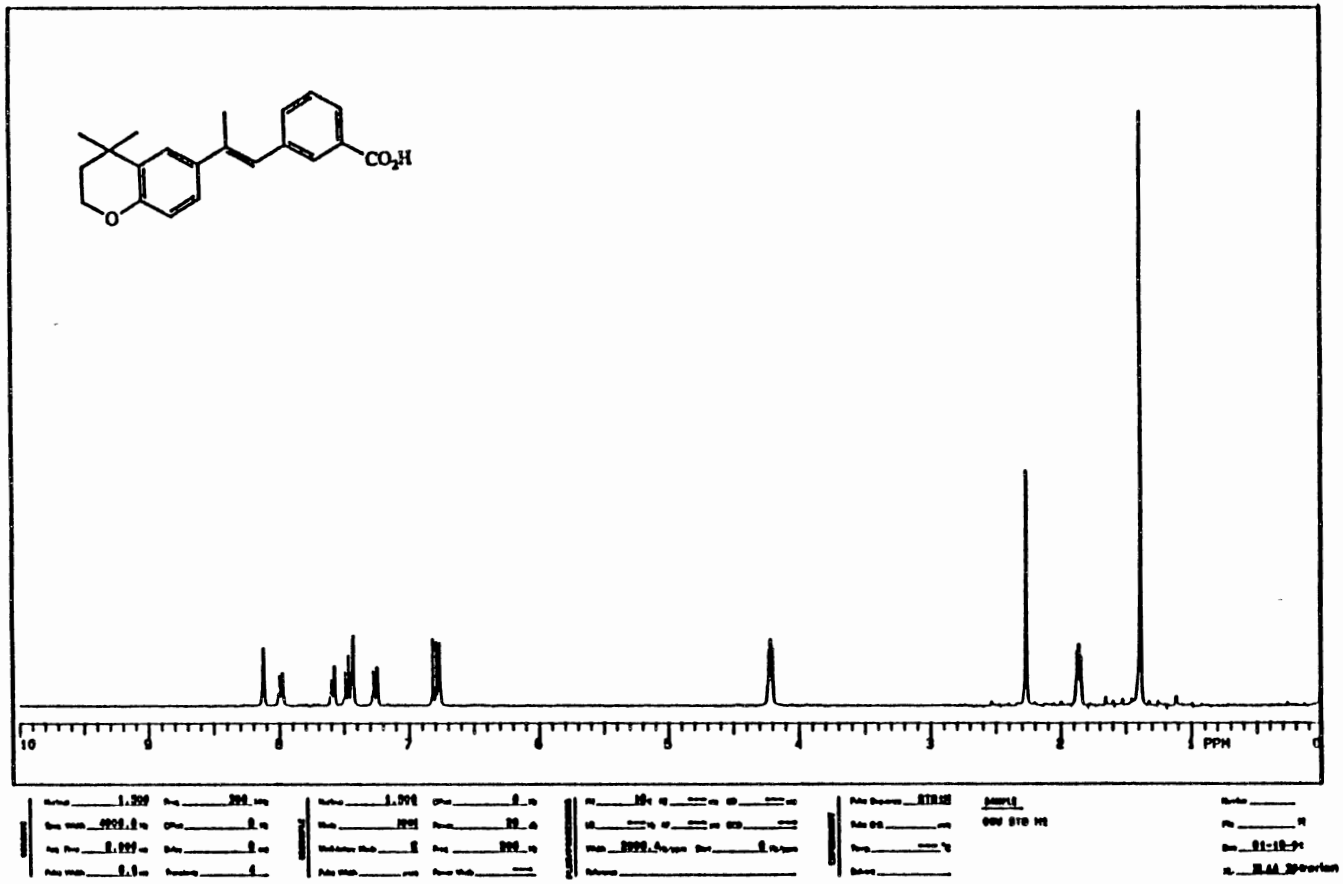
IR Spectrum of 60

Plate XXVII



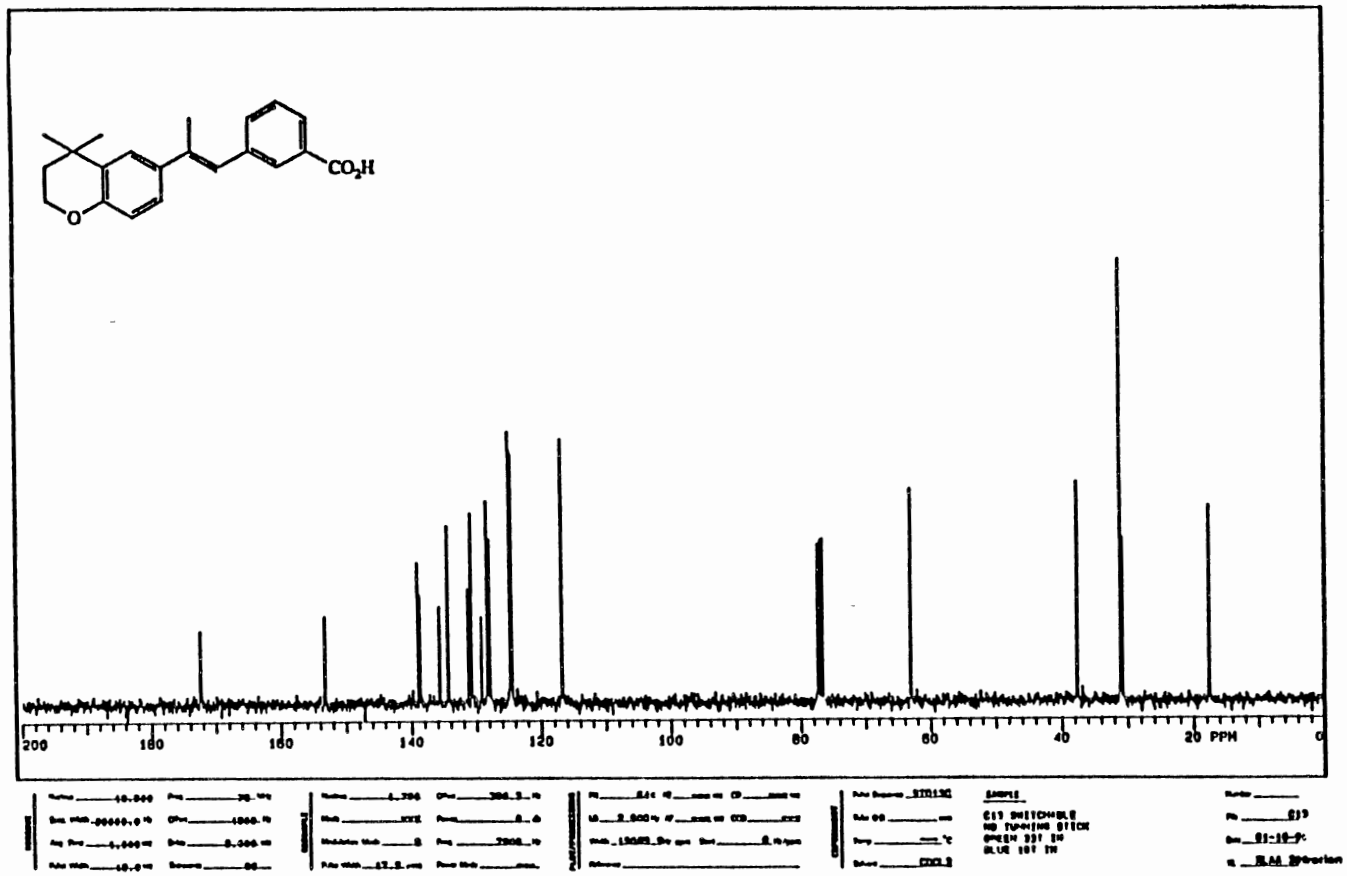
UV Spectrum of 60

Plate XXVIII



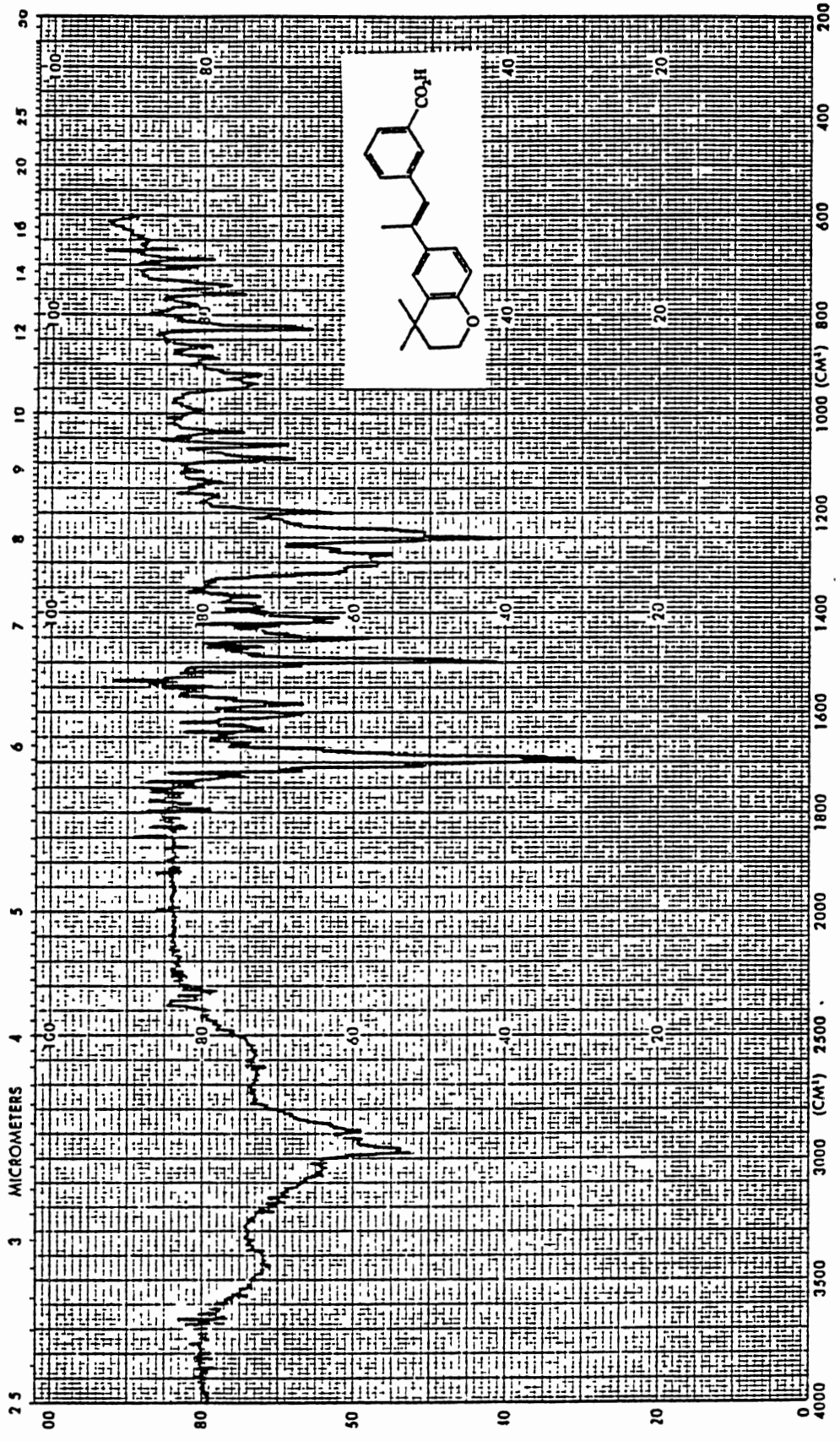
^{1}H NMR Spectrum of **61**

Plate XXIX



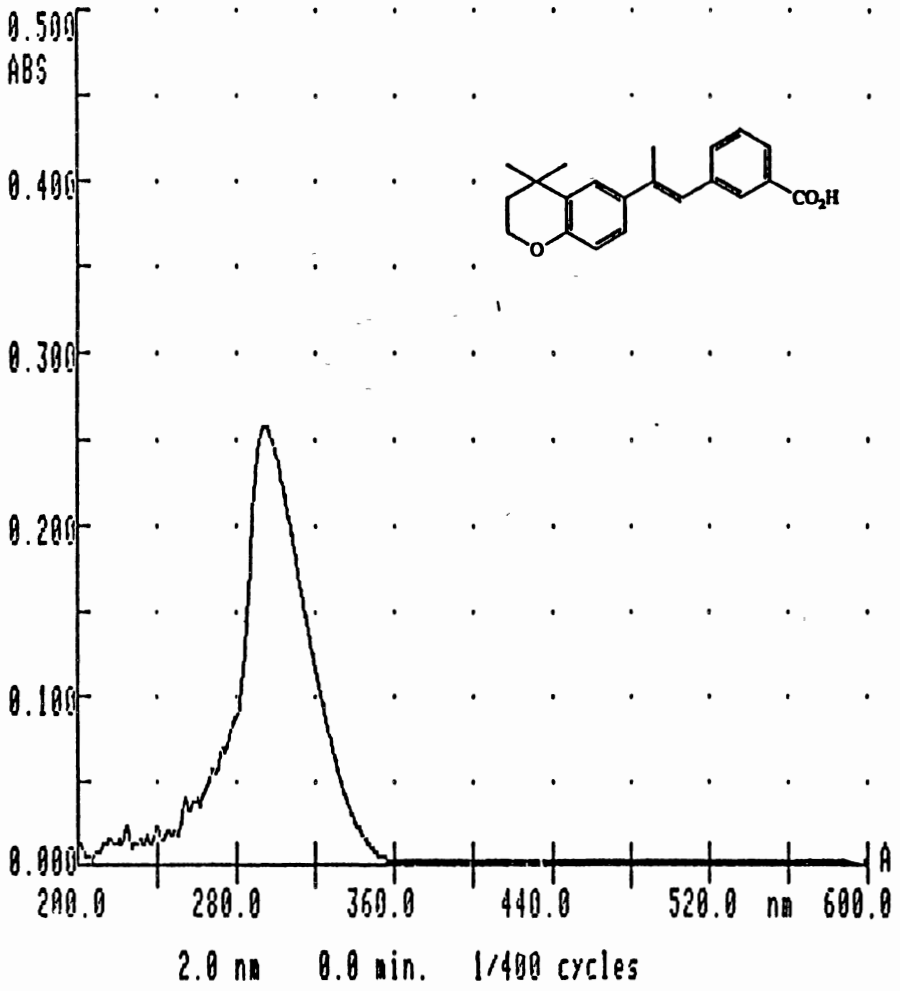
^{13}C NMR Spectrum of **61**

Plate XXX



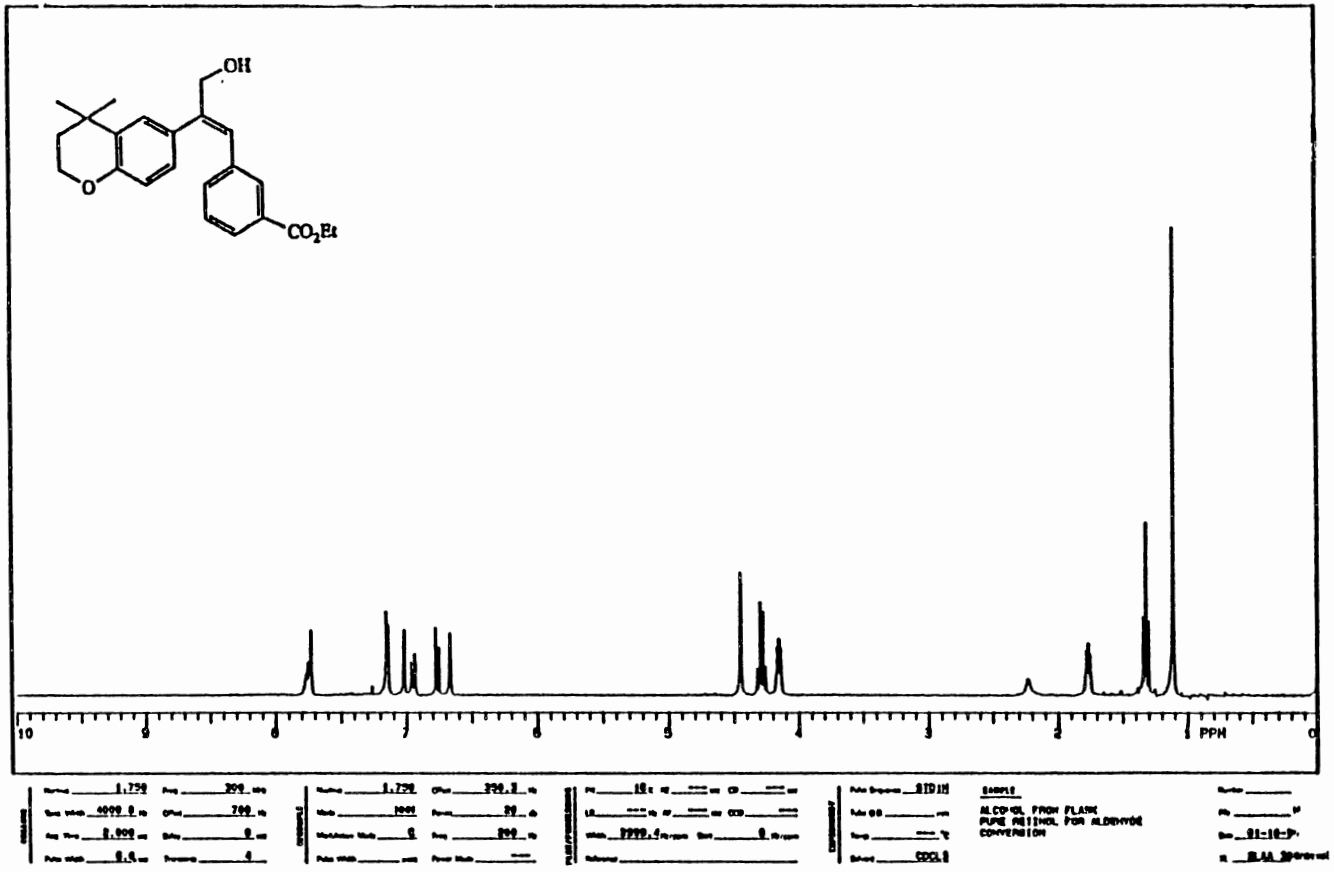
IR Spectrum of **61**

Plate XXXI



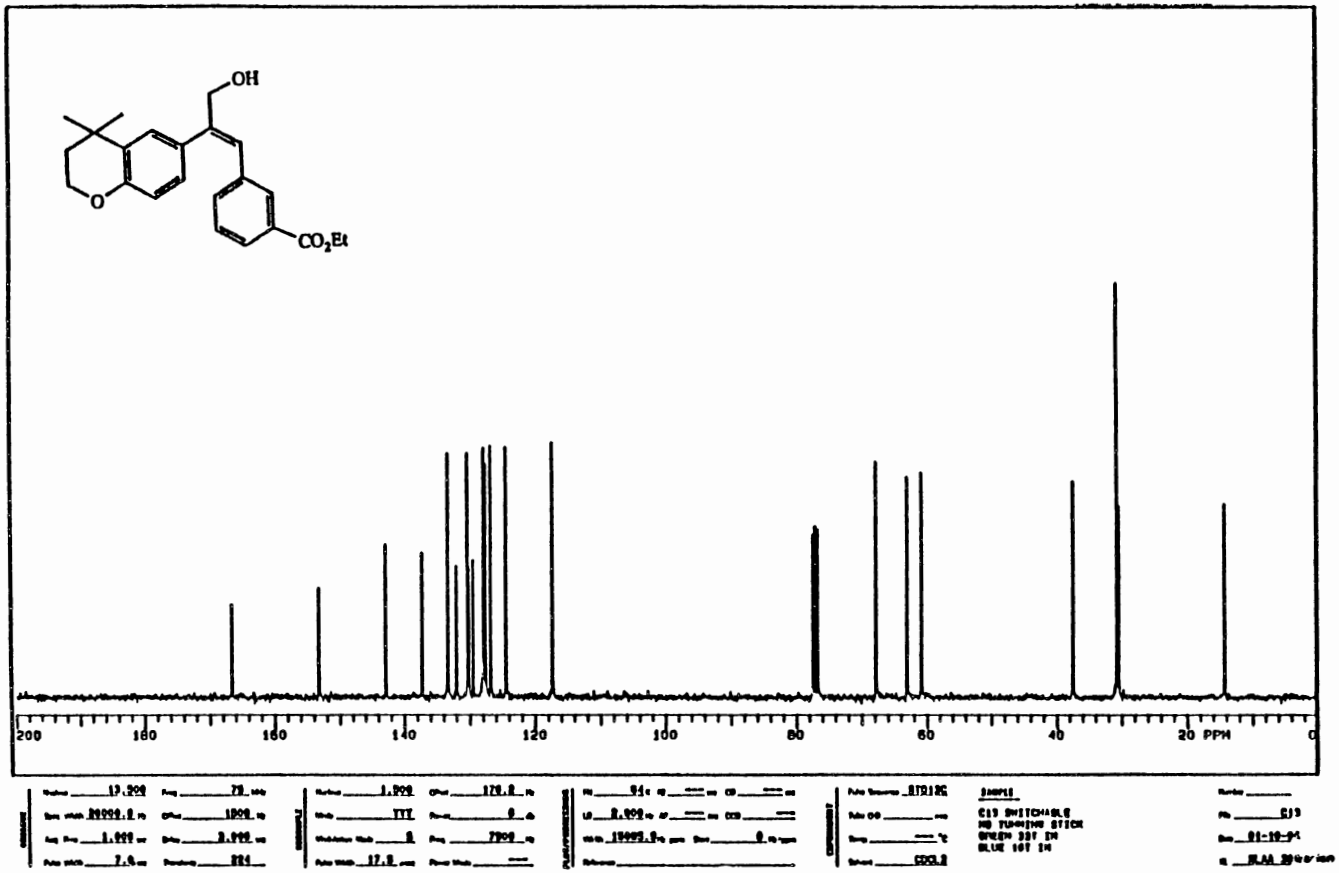
UV Spectrum of **61**

Plate XXXII



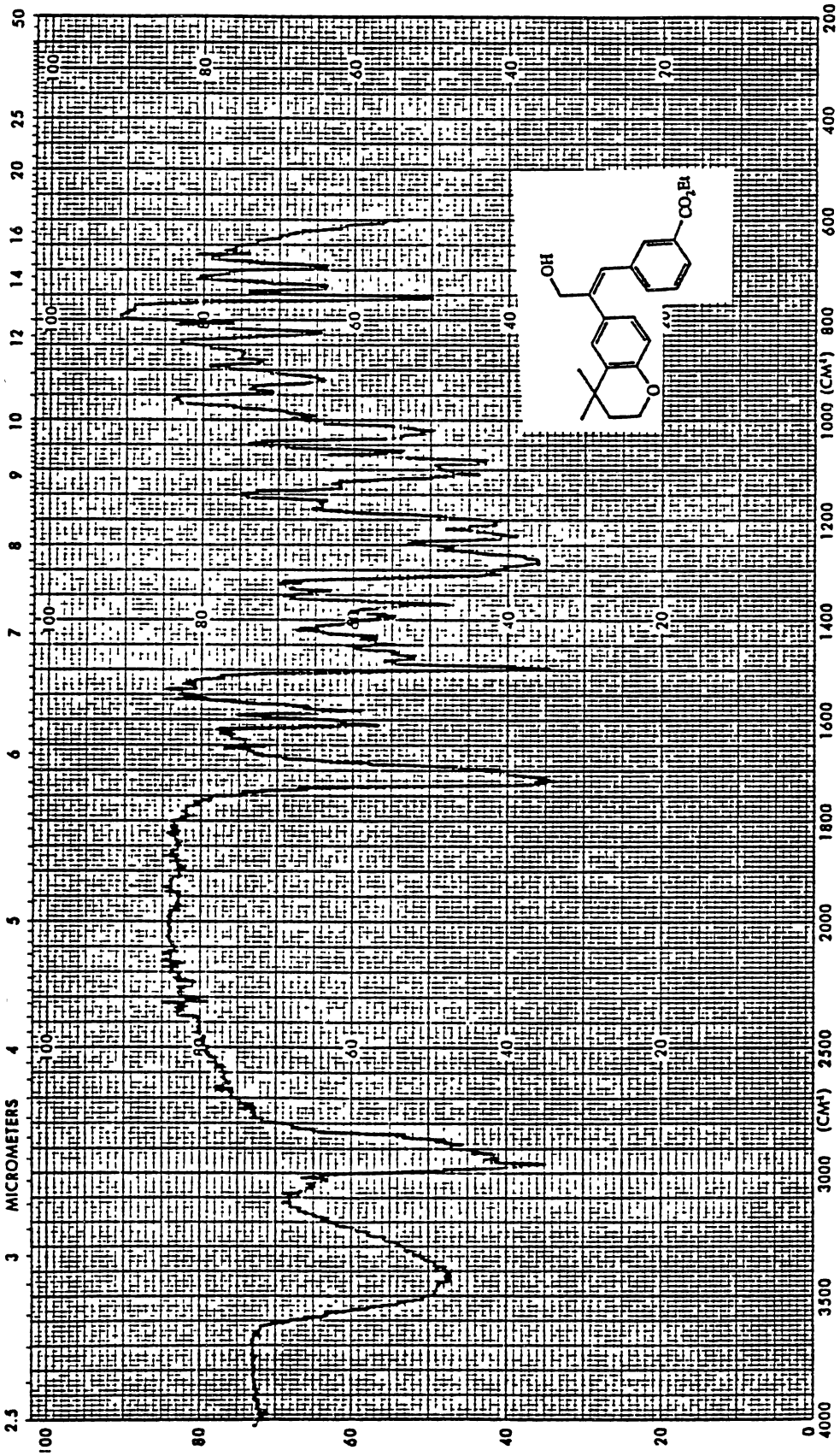
^{1}H NMR Spectrum of **62**

Plate XXXIII



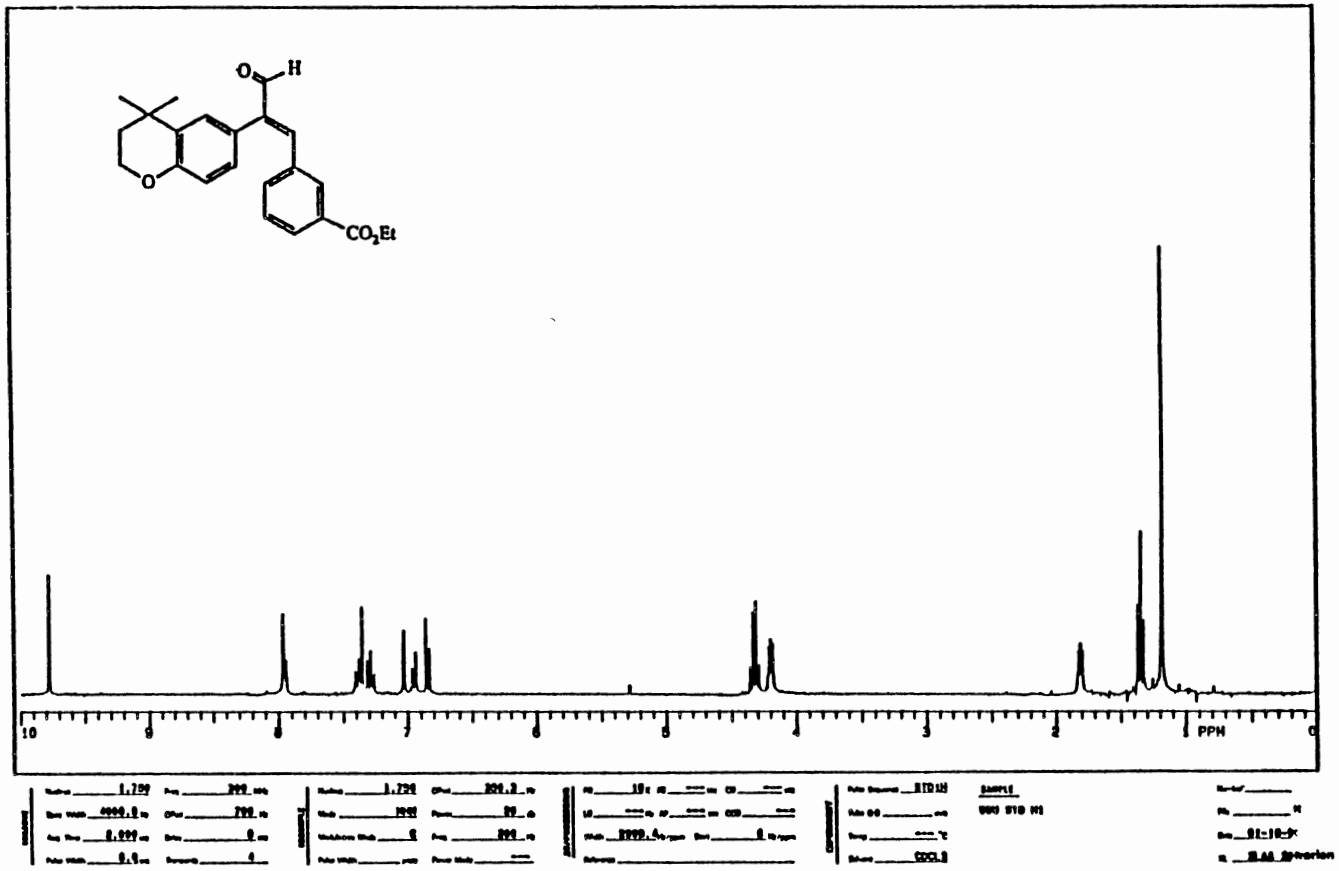
^{13}C NMR Spectrum of **62**

Plate XXXIV



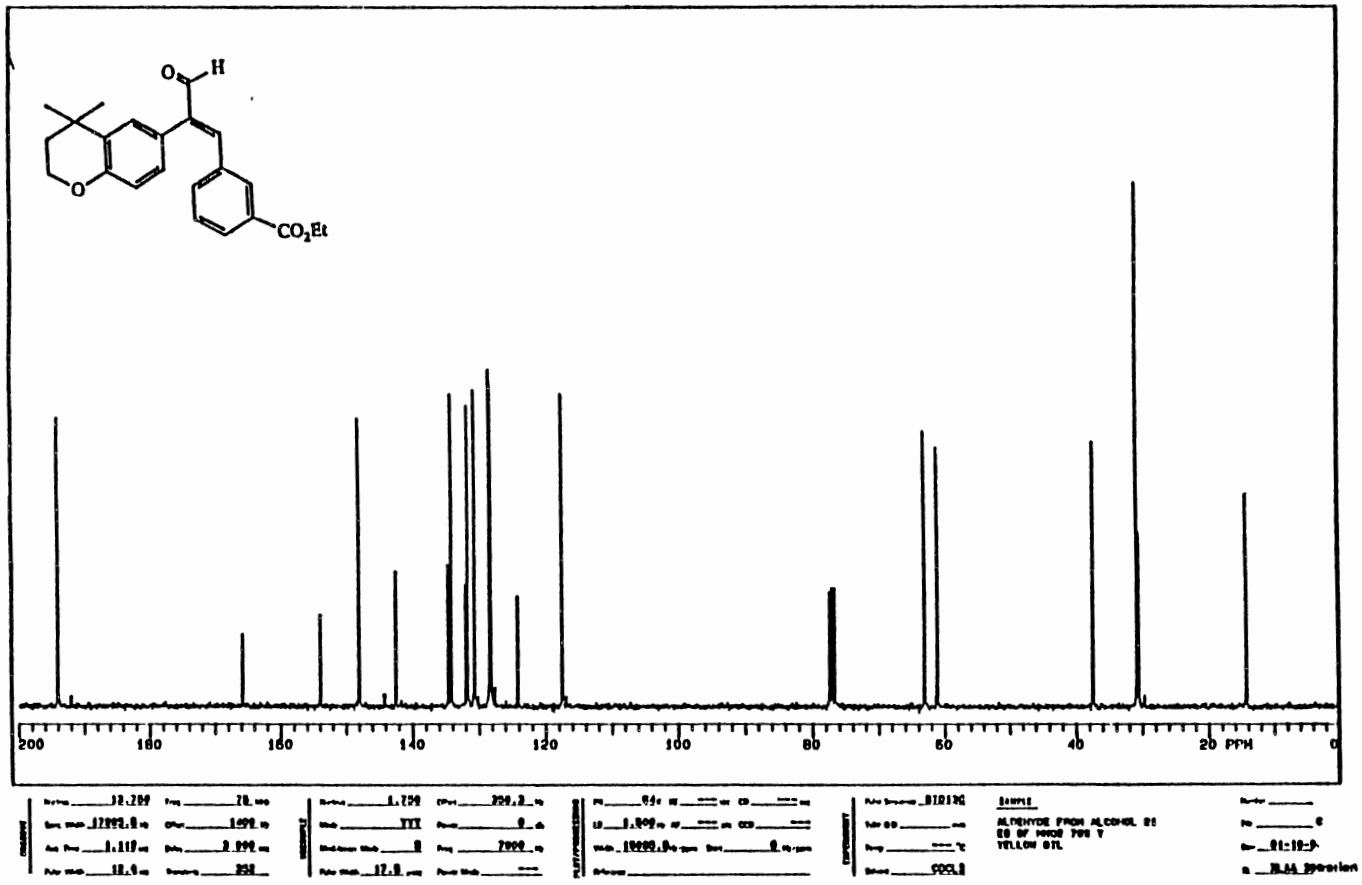
IR Spectrum of 62

Plate XXXV



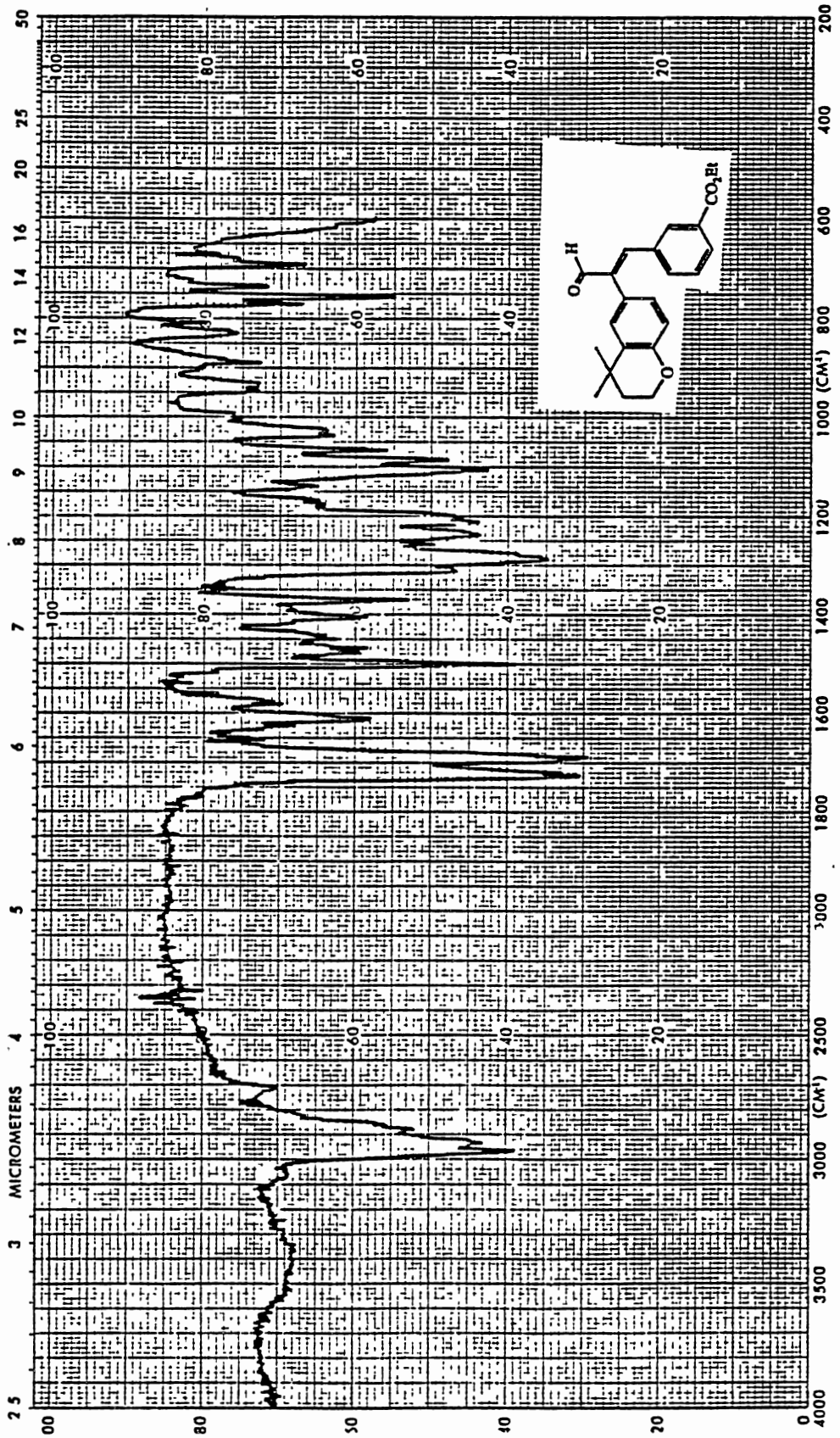
^{1}H NMR Spectrum of **63**

Plate XXXVI



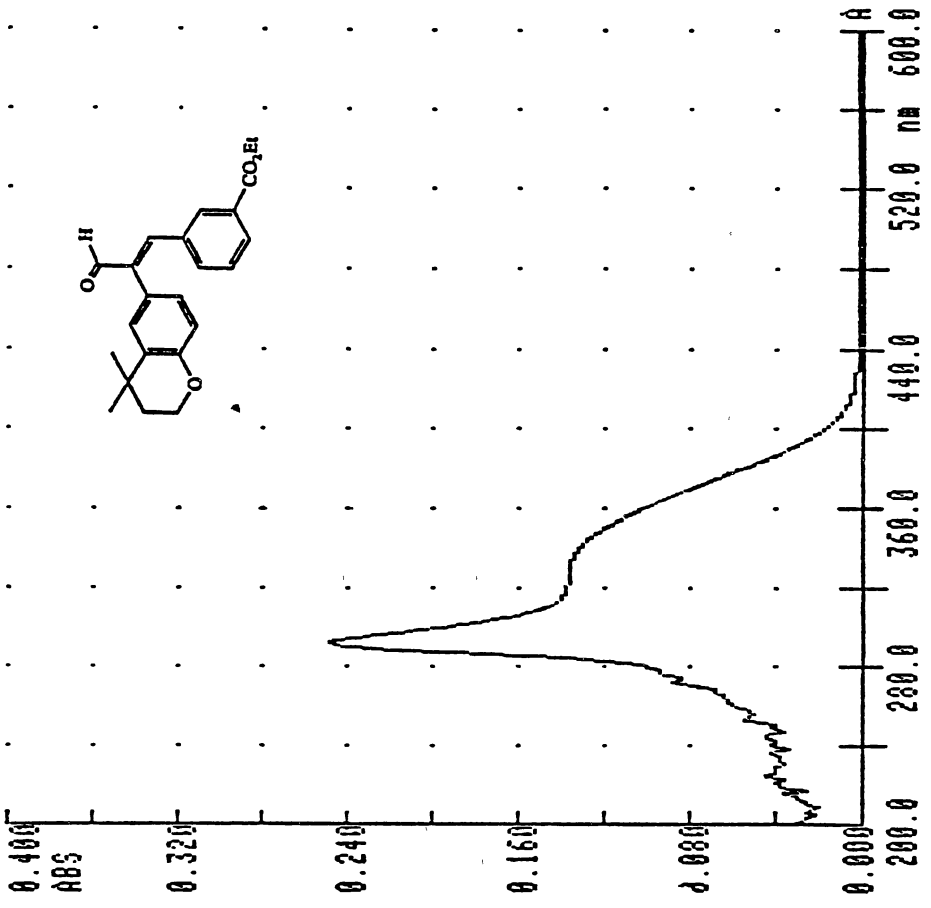
^{13}C NMR Spectrum of **63**

Plate XXXVII



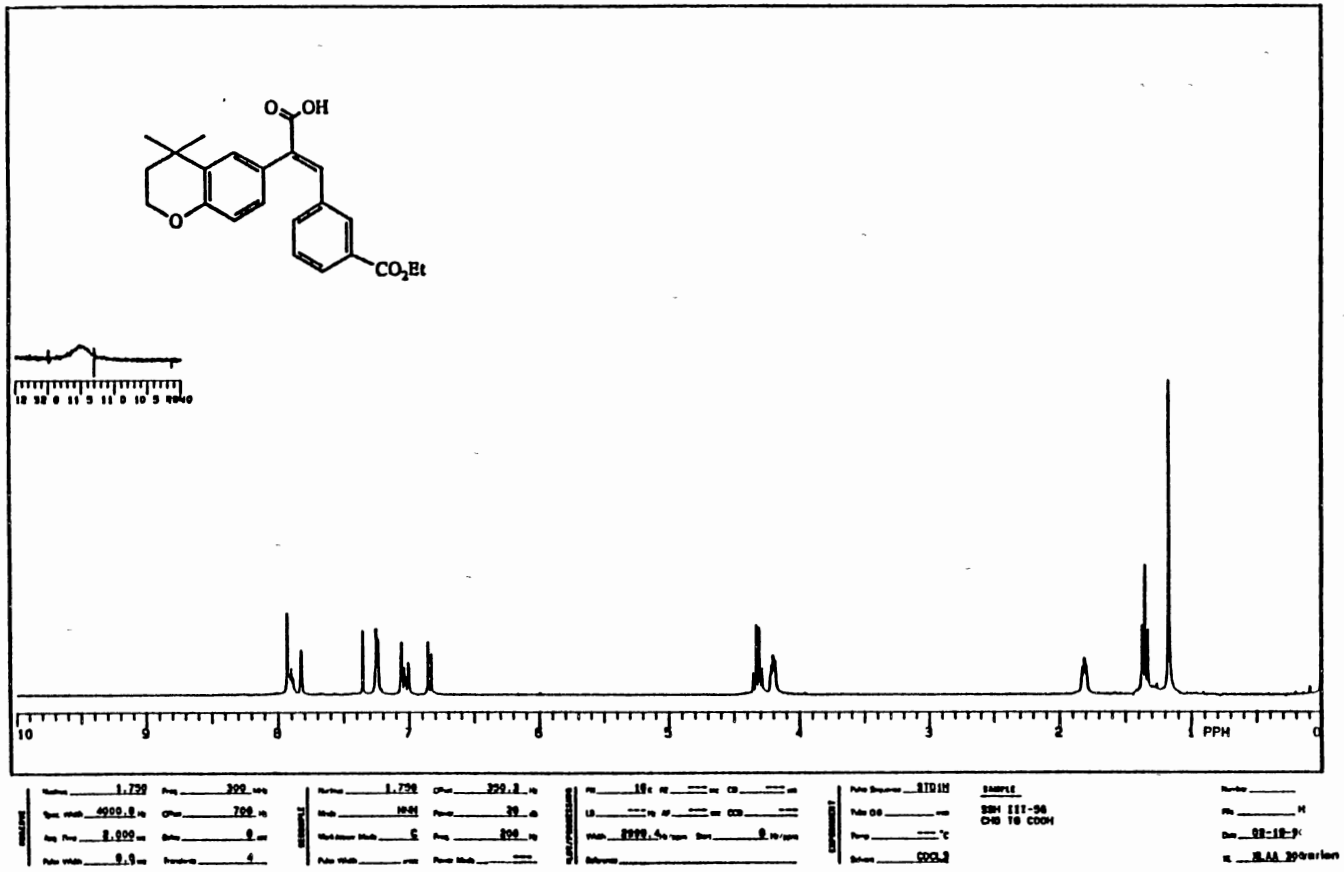
IR Spectrum of **63**

Plate XXXVIII



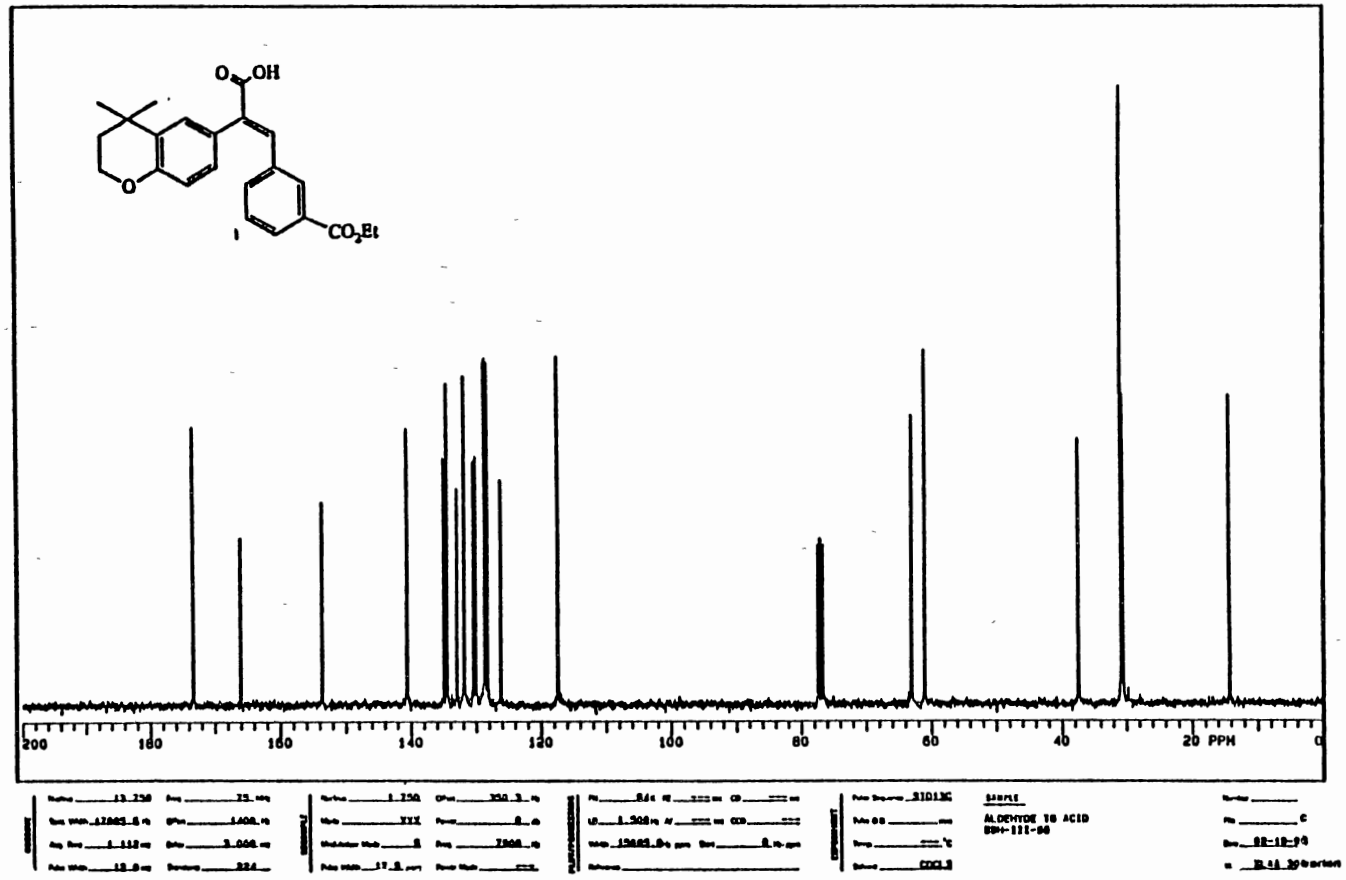
UV Spectrum of 63

Plate XXXIX



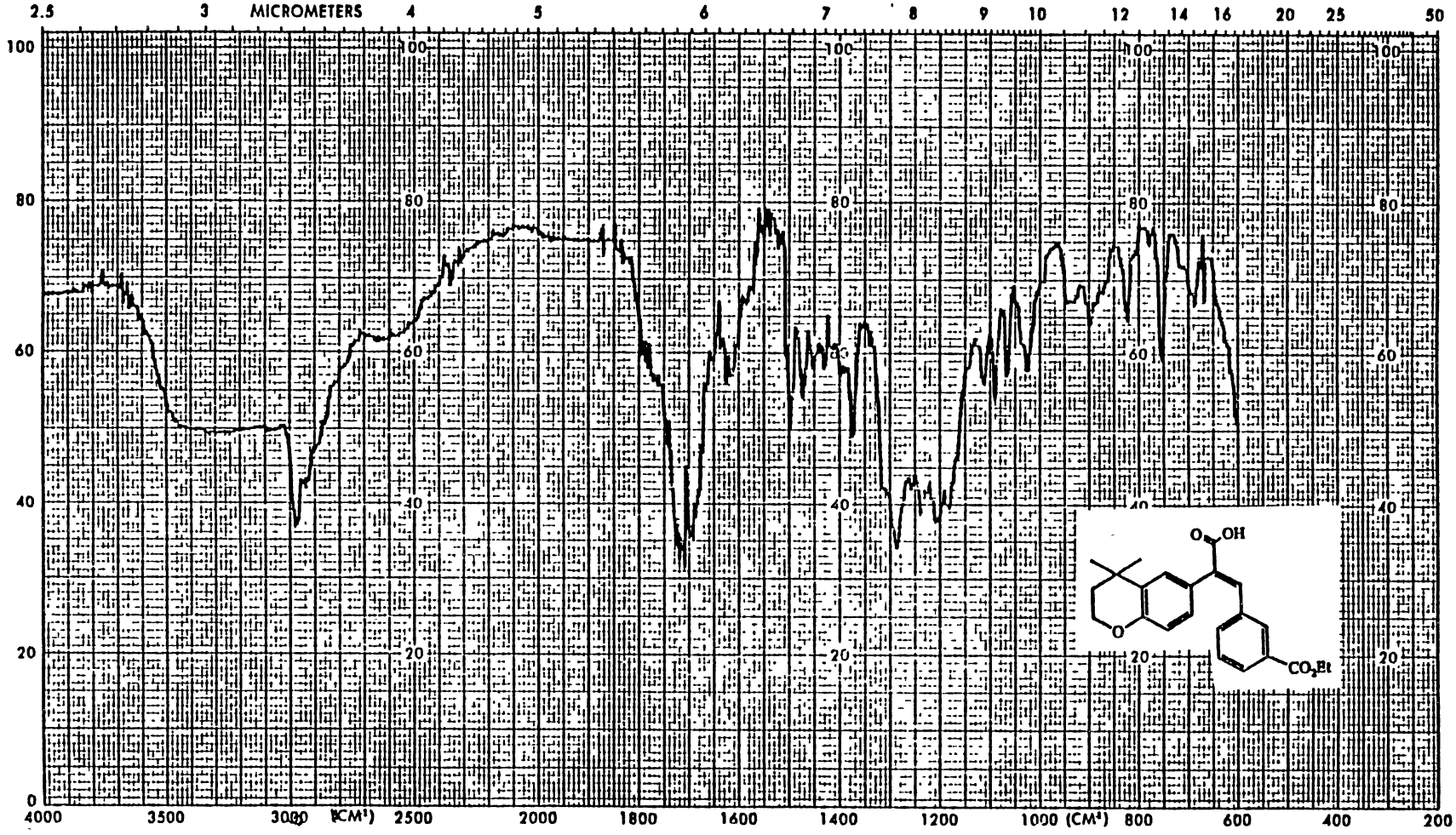
^{1}H NMR Spectrum of **64**

Plate XXXX



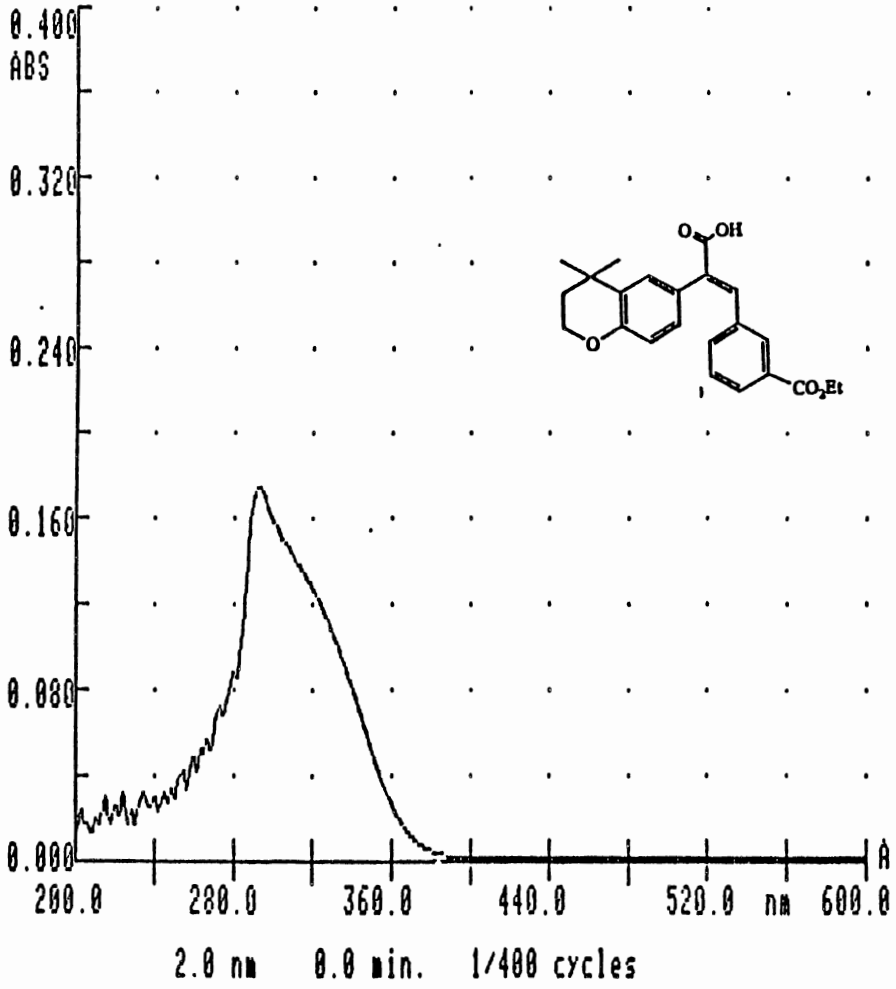
^{13}C Spectrum of **64**

Plate XXXXI



IR Spectrum of **64**

Plate XXXXII



UV Spectrum of **64**

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VITA

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Major field: Chemistry

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