MODIFIED HETEROAROTINOIDS: POTENTIAL ANTICANCER AGENTS

BY

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Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
December, 1993

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ACKNOWLEDGEMENTS

I wish to express my deepest and most sincere gratitude to Dr. K. D. Berlin for his complete and total commitment toward my entire career. I wish to express my deep respect and heartfelt appreciation for his untiring encouragement and constant guidance which enabled me to work to my best ability. I would like to thank him for all the knowledge he has imparted to me in the field of chemistry as well as in certain important aspects of life, outside the lab.

I am also grateful to Dr. Ford, Dr. Mottola, and Dr. Nelson for being on my graduate committee; Dr. Gholson, Dr. Holt, Dr. Sangiah, and Dr. Nelson for their never-ending patience and prompt help in sending out reference letters to various companies; a special thanks to Dr. Mottola for putting in a kind word about me to my future employers. I would also like to thank the secretaries of the Department of Biochemistry for all their help, and Mr. Pat Anderson for his useful suggestions about thesis formatting and meeting deadlines.

I thank all my colleagues in the Department: Greg (my Buddy) for being a family to me and for his willingness to share everything with me. The lab would never be so much fun without him; Marwan (my other buddy) for showing me that hard work and humor can go hand in hand; Mike Cagle for being a great friend; John Schemenaur and Tim Smith for all the friendly advice; Matora, Mahika, Sameer and Kevin for being part of our 'crazy' group at the lab; Thayne, Paul, and Lloyd for all their help with Mass Spec. I extend my appreciation to June (store room), Stan (NMR and personal advice); Tom (glass shop) for their timely help and to the Department of Chemistry for the overall support and encouragement.

No words of appreciation would suffice to express my gratitude to my family: my grandparents, parents, sister Uma and brother-in-law Narayanan whose love, patience and

encouragement has been the greatest pillar of strength and support to me. I would like to dedicate this thesis to my grandparents and parents for their endless love and for believing in me. I thank my beautiful wife Deepa, for always being there for me and putting up with my late nights at the lab. Finally, I thank God for giving me the strength, wisdom and courage to go through my thesis and complete it successfully.

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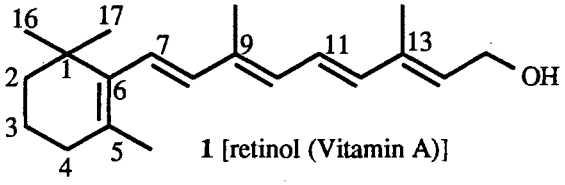
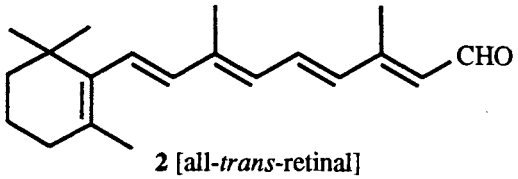
HISTORICAL

Introduction

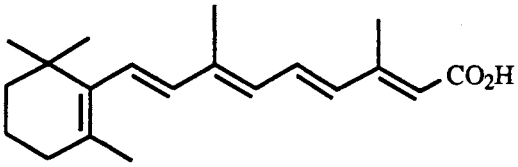
All compounds that exhibit biological activities of retinol (**1**) are termed "vitamin A derivatives". However, the term "retinoids" includes both natural (**1**-**9**) and synthetic analogs (**10**-**15**) of retinol, whether or not they have biological activity.[5] A retinoid can



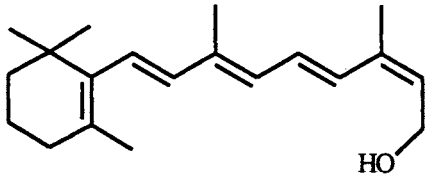
1 [retinol (Vitamin A)]



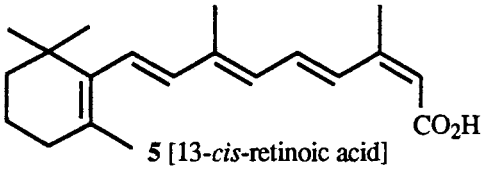
2 [all-*trans*-retinal]



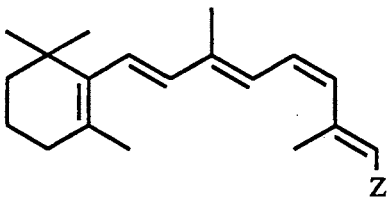
3 [all-*trans*-retinoic acid]



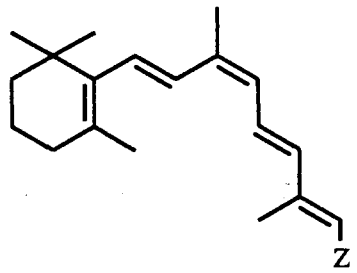
4 [13-*cis*-retinol]



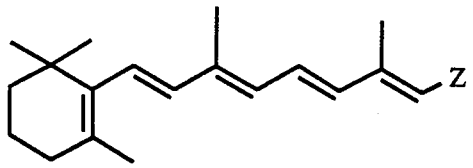
5 [13-*cis*-retinoic acid]



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



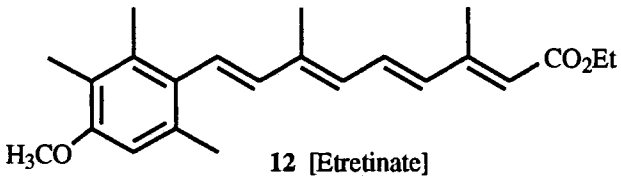
8 [Z = CO_2H (9-*cis*-retinoic acid)]
9 [Z = CHO (9-*cis*-retinal)]



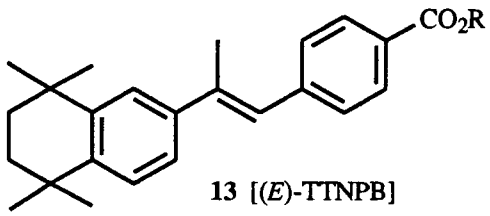
10 [Z = $CH_2NHCH_2CH_2OH$]
11 [Z = $C(O)NHC_2H_5$]

also be defined as a "substance that can elicit specific biological responses by binding to and activating a specific receptor or set of receptors".[62] Currently there are several classes of synthetic retinoids, such as the 'arotinoids' (**12** and **13**; retinoids with at least

AROTINOIDS

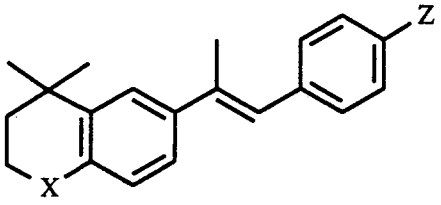


12 [Etretinate]

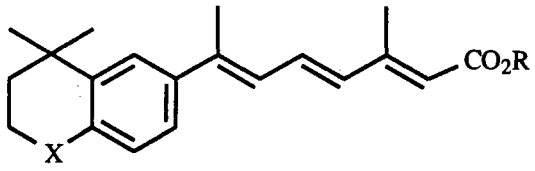


13 [(*E*)-TTNPB]

HETEROAROTINOIDS



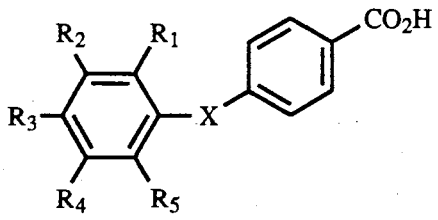
14 [X = O, SO_2, NR, S]
15 [Z = CO_2H, CO_2Et, CO_2Me]



16 [X = O, S]
17 [R = H, Et]

one aryl ring in the basic structure), 'heteroarotinoids' (**14-17**; retinoids with at least one aryl ring and a heteroatom in the fused ring system), and 'retinobenzoic acids' (**18**; retinoids with different functional groups connecting two aryl rings).

RETINOBENZOIC ACIDS



18 [R = Medium sized alkyl group
X = -NHC(O)-, -C(O)NH-,
-C(O)C=C-, -N=N-]

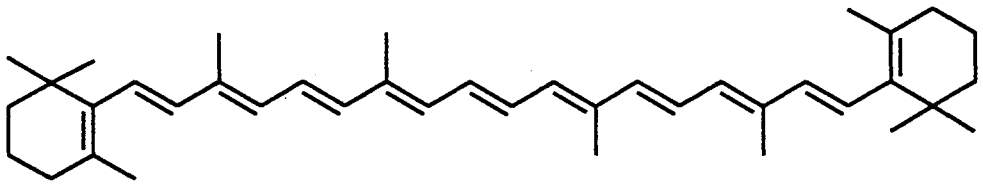
Although several biological functions are associated with vitamin A, such as growth promotion, reproduction, differentiation and maintenance of epithelial cells, it is not clear as to what form of vitamin A is involved in these biological activities. Some of the biological functions associated with the vitamin A and its natural analogs are:

1) the aldehyde form, retinal (**2**), which participates in the vision process (retinal (**2**) binds with protein, opsin, which is required for vision),[51,73]

2) retinol (**1**) which is believed to be involved in the maintenance of reproduction functions by regulating the development of the germinal epithelium, and[51,71]

3) retinoic acid (**3**) which is associated for growth and differentiation of various types of epithelial cells including skin, intestine, lungs, etc.[51,57]

It appears that different metabolites of retinoids may be responsible for vitamin A and related type of activities, and therefore it is important to understand the nature of the metabolism and the properties of metabolites. In the digestive system, it is believed that β-carotene (**19**) is cleaved centrally by certain enzymes.[26] Retinal (**2**), generated through the central cleavage of β-carotene, is presumably reduced by a reductase (enzyme) to retinol. The enzyme involved in the conversion of β-carotene to retinal (**2**) (retinaldehyde reductase) is found in the liver, intestine and the eye.[27] The mechanism of *in vivo* conversion of β-carotene to retinol (**1**) is controversial and is being investigated.[31] The two major pathways for the degradation of retinoids are oxidative and non-oxidative pathways. Many oxidative paths have been suggested by different research groups from both *in vitro* and *in vivo* studies.[19]



19 [ß-CATOTENE (From diet)]

↓

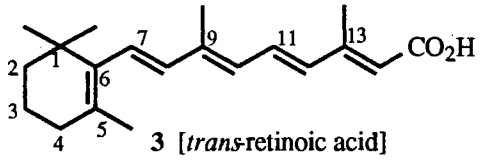
DIGESTIVE SYSTEM
[INTESTINE (RETINYL ESTERS); LIVER]

↓

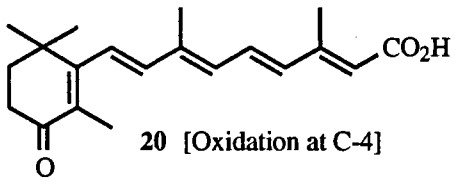
RETINAL ⇌ RETINOL → RETINOIC ACID

(Vision) (Reproduction) (1. Growth promotion
2. Differentiation and maintenance of epithelial cells)

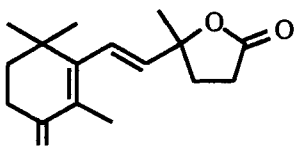
From structures **20-26** it is clear that oxidation can occur at several sites in **3**: (a) oxidation at C(4) [as in **20**, **21**, **23-25**], (b) epoxidation of the double bond in the cyclohexyl ring [as in **22**], (c) shortening of the polyene side chain with partial reduction of the double bonds [as in **21**], (d) oxidation of one of the methyl groups of the geminal



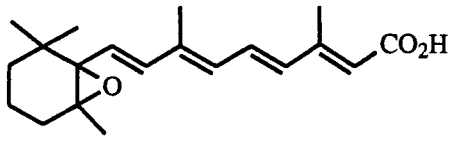
3 [*trans*-retinoic acid]



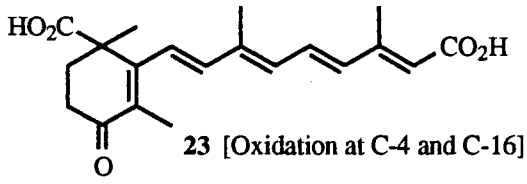
20 [Oxidation at C-4]



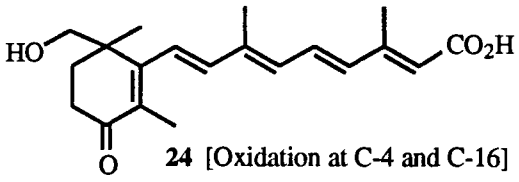
21 [Shortening of Polyene Side Chain]



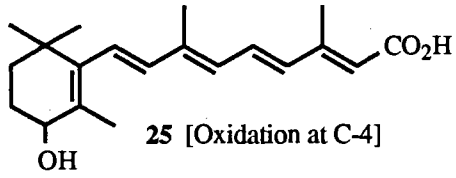
22 [Epoxidation of Double Bond in the Ring]



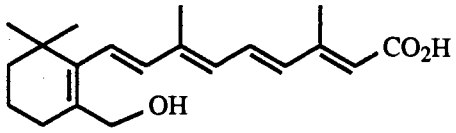
23 [Oxidation at C-4 and C-16]



24 [Oxidation at C-4 and C-16]

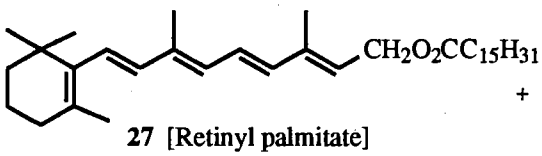


25 [Oxidation at C-4]



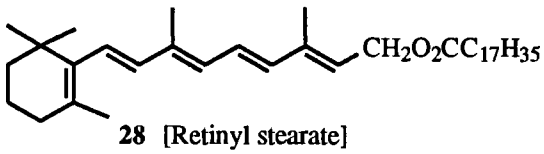
26 [Oxidation at C-5 Methyl]

dimethyl pairs [as in **23** and **24**] and (e) oxidation of the methyl group at C(5) [as in **26**].[30] A non-oxidative pathway involves modification of the polar end of retinol (**1**).[19] The alcohol group is converted into fatty esters (**27** and **28**, palmitate and stearate) or phosphates (**29** and **30** with or without a sugar unit).[19,15] Isomerization of the side-chain double bonds is also believed to occur.[20]

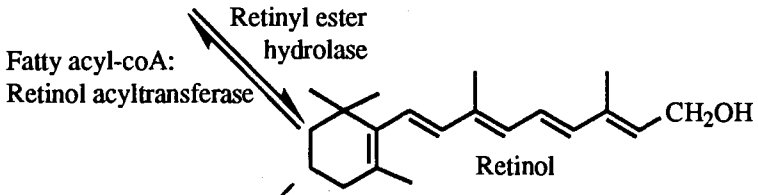


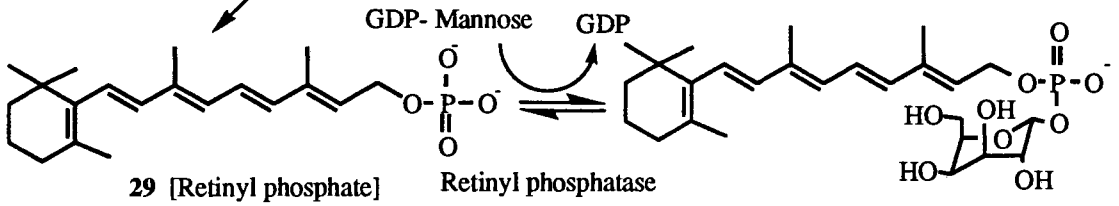
27 [Retinyl palmitate]

+



28 [Retinyl stearate]





29 [Retinyl phosphate]

Retinyl phosphatase

30 [Retinyl-ß-mannosyl phosphate]

Natural retinoids and their metabolites have received considerable attention in the treatment of various cancers of skin,[33,43] head and neck,[33] and lungs[33] and bladder.[33] Thus retinoids possess pharmaceutical importance as chemopreventive agents in the treatment of epithelial, psoriasis, and cystic acne.[51,61] Unfortunately, due to the acute toxicity (hypervitaminosis A syndrome) and various side effects of natural retinoids (and their analogs), they are available only as topical formulations for various skin disorders. The characteristic symptoms of chronic hypervitaminoses A in laboratory animals are weight loss, erythemea, hair loss, internal hemorrhage and fractures.[47] Hypervitaminosis A has also been associated with teratogenicity. In order to minimize the toxicity, while maintaining the carcinostatic properties of natural retinoids, the basic retinoidal structure has been extensively modified. These modifications have led to the discovery of synthetic retinoids like arotinoids (compounds like **12** and **13**), heteroarotinoids (compounds like **14-17**) and retinobenzoic acids (compounds of the type **18**).[13,40,74]

Many important aspects of retinoids are widely discussed in detail in various books and reviews.[50,51,64,71] These include historical, isolation, characterization, and synthesis of retinoids along with their biological activities and related toxicities. With the isolation of various receptors, research is being focused on the ability of retinoids to specifically bind to different receptors.[17] The scope of our research is directed to the modification of retinoids in order to achieve higher activity, reduced toxicity and specific receptor binding abilities.

Modification of Retinoids: Arotinoids and Heteroarotinoids

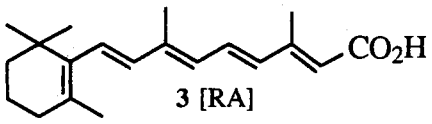
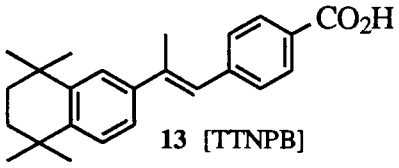
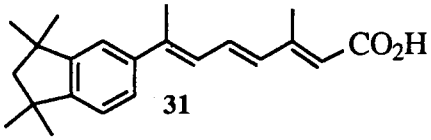
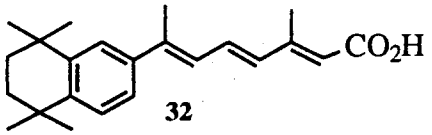
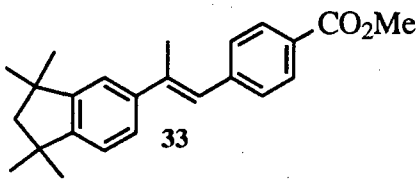
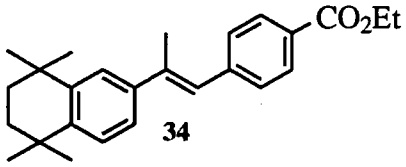
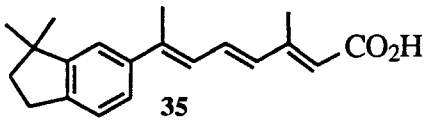
When an aromatic ring is incorporated into the basic retinoid skeleton, the compound is classified as an "arotinoid' as cited previously. Etretinate (**12**) is an arotinoid which contains an aromatic ring in lieu of the usual six-membered ring of the natural retinoid [like in retinol (**1**)]. Etretinate (**12**), now commercially available, was discovered in the late 1970s by Bollag and co-workers[6] (Hoffmann-La Roche), who found that

incorporation of an aromatic ring into the retinoid skeleton could improve the therapeutic ratio [the ratio of dose (mg/kg) that induced 50% regression of papillomas in Swiss mice to that of the dose (mg/kg) which induced hypervitaminoses A syndrome] values of retinoids compared to RA (**3**). TTNPB [(*E*)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalyl)-1-propenyl]benzoic acid (**13**)] had a therapeutic ratio much higher (> 8 see Table I) than that of RA (**3**, therapeutic ratio = 5). Table I shows the therapeutic ratio of selected arotinoids, and Table II shows the activity of a few arotinoids. Five- and six-membered analogs were prepared and various metabolites of Etretinate (**12**) have been identified. Actually the high anti-papilloma activity (ability to cause regression of a tumor) led to the discovery of the arotinoid, TTNPB (**13**), which contains an aromatic ring fused to a six-membered ring with another aromatic ring replacing the polyene side chain. Although the therapeutic ratios (based on the ability of the arotinoids to cause regression of papilloma) of many arotinoids were high, the values did not reflect the acute toxicity of the test compounds (arotinoids). For example, different activities were obtained with different tests for arotinoid TTNPB (**13**; 300 times more active than RA in chick embryo test;[63] 20 times more active than standard RA in the F9 and TOC assays).[63] However, the toxicity level of TTNPB remained significantly high in all of the tests (compared to the standard RA), thus indicating that the therapeutic ratio does not reflect the acute toxicity of the arotinoids.

Further modification of the retinoid structure to achieve enhanced activity and lower toxicity has lead to the discovery of 'heteroarotinoids' by two research groups, namely those of Berlin and co-workers[74] and Dawson and co-workers.[13] Heteroarotinoids are a group of heterocycles which retains some features of the retinoid skeleton but have special characteristics in that at least one aryl ring and one heteroatom are incorporated into the system. Replacement of C(4) by a heteroatom (like oxygen or sulfur) and protecting the C(5)=C(6) bond by fusing an aromatic ring to the six-membered ring might enhance the hydrophilicity, decrease toxicity and improve the

TABLE I

THE THERAPEUTIC PROFILE OF SELECTED AROTINOIDS IN ODC ASSAY[a]

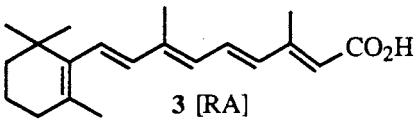
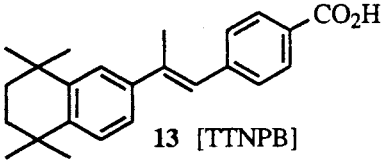
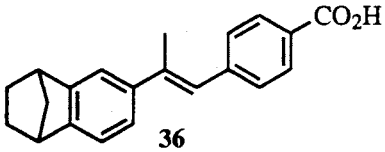
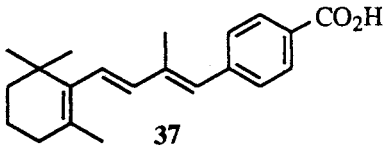
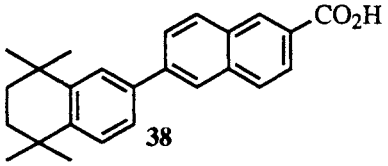
Arotinoid	ED_{50} (mg/kg)	Hyper-vitaminosis A mg/kg/day	Therapeutic[b] Ratio
 3 [RA]	400	80	5
 13 [TTNPB]	> 0.8	0.1	> 8
 31	3	3	1
 32	1.5	0.75	2
 33	< 0.2	0.1	< 0.1
 34	0.05	0.1	0.5
 35	12.5	12.5	1

[a]Reference 48.

[b]Therapeutic ratio = The ratio of dose (mg/kg) that induced 50% regression of papillomas in Swiss mice to that of the dose (mg/kg) which induced hypervitaminoses A syndrome.

TABLE II

ACTIVITY OF SELECTED AROTINOIDS 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 [RA]	1×10^{-11}	1.7	88
 13 [TTNPB]	1×10^{-12}	17.0 1.7	91 81
 36	6×10^{-10}	17.0 1.7	69 33
 37	3×10^{-10}	17.0 1.7	77 34
 38	3×10^{-10}	17.0 1.7	80 58

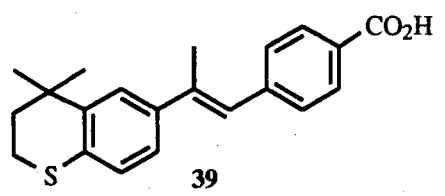
[a]From reference 13.

[b]Tracheal Organ culture assay, reference 63.

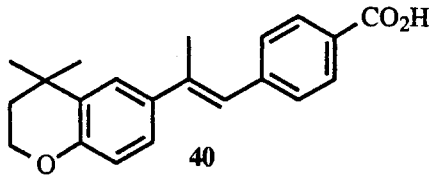
[c]Ornithine decarboxylase assay, reference 72

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

transport properties of retinoids. Heteroarotinoids with five- and six-membered rings containing heteroatoms (sulfur and oxygen) have been synthesized.[74] Both the polyene and the aromatic side-chain derivatives of heteroarotinoids have been prepared. Compounds **39-51** are examples of some of the heteroarotinoids obtained in our labs. Several of the heteroarotinoids exhibited marked activity in the ODC (Table III)[74,66] and



39



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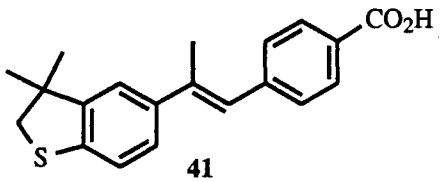

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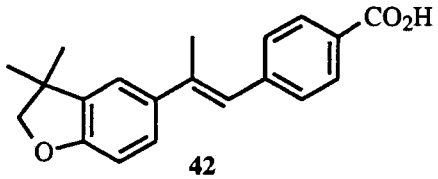

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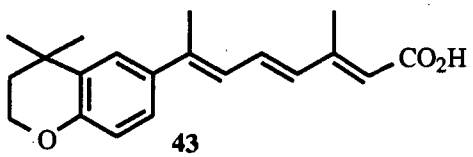

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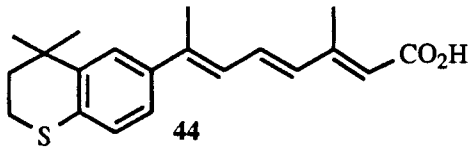

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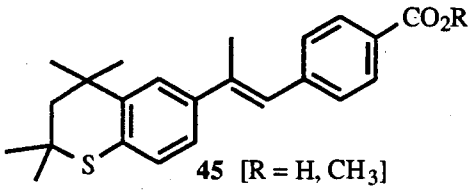
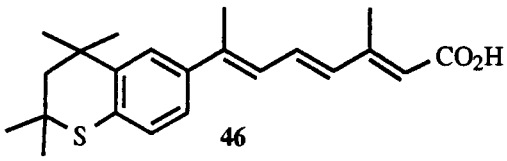
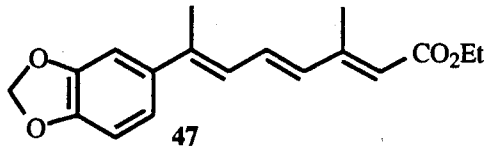


45 [R = H, CH_3]



46



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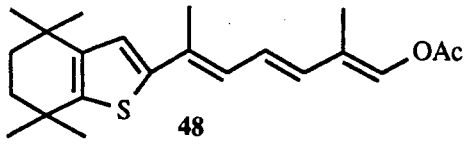
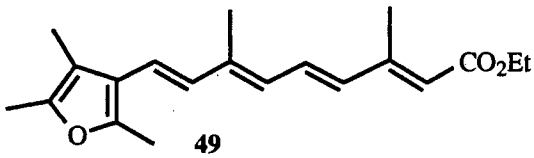
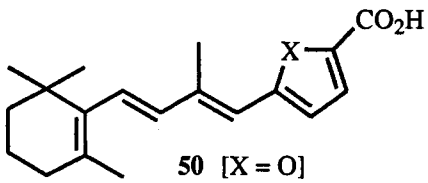


48



49

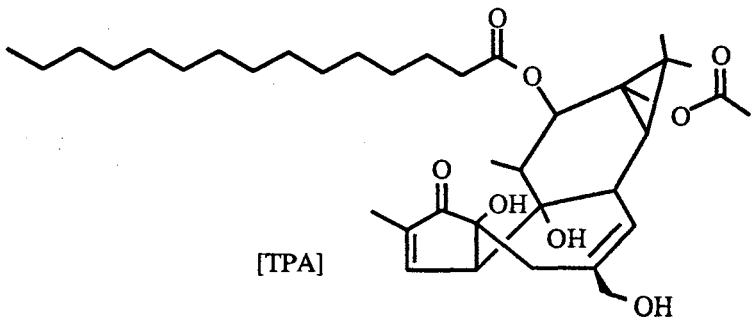


50 [X = O]
51 [X = S]

TOC activity (Table IV),[74] and heteroarotinoids **39** and **40** were much less toxic (Table V)[13] than the arotinoid TTNPB (**13**) and RA.

Assays of Heteroarotinoids. Many assays have been used to assess the activity of retinoids, and among them, the two most common assays are the ornithine decarboxylase (ODC, *in vitro)* assay and the HL-60 (human leukemic cell line, *in vitro*) assay. In the ODC assay, the activity of the retinoid is determined by measuring the amount of $^{14}CO_2$ evolved from ^{14}C labeled ornithine by the enzyme ornithine decarboxylase. A phorbol ester, 12-O-tetradecanoyl phorbol-13-acetate (TPA shown below), induces production of the enzyme ornithine decarboxylase, which in turn assists in the transformation of normal to malignant cells by reacting with ornithine.[72] Thus, TPA is a known cancer promoter.[75] A suspension of malignant tissue and ^{14}C labeled ornithine are mixed, and then the amount of $^{14}CO_2$ released is measured.[72] Thus, the smaller the amount of $^{14}CO_2$ released the greater the potential anticancer activity of the test retinoid.

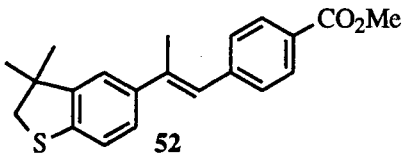
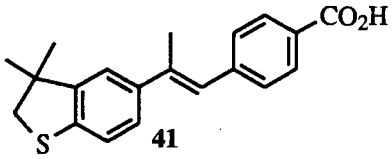
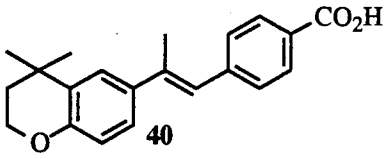
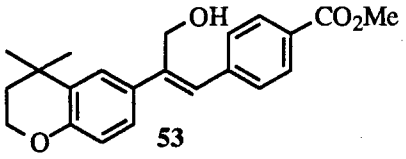
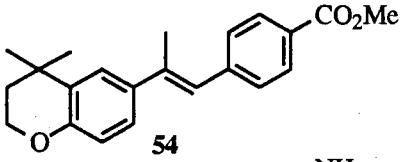
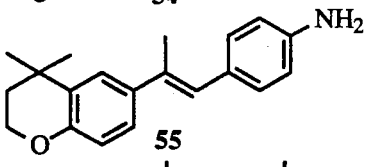
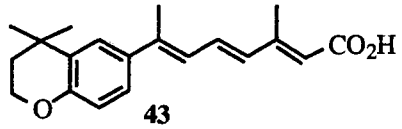
In the HL-60 assay, cell differentiation is involved.[11] When a HL-60 cell line is stimulated with TPA, superoxide ions (O_2^- ions produced by an oxidative metabolic pathway as a part of body's defensive mechanism) are produced by the differentiated cells.[9] These differentiated cells can be identified by a color change (yellow to blue) when treated with a test dye, nitroblue tetrazolium (NBT, see page 18 for structure). Normal HL-60 cells do not produce such superoxide ions and thus do not produce a change in color with the test dye.



[TPA]

TABLE III

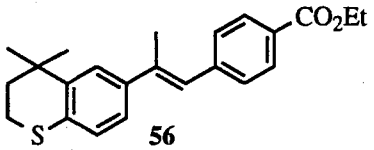
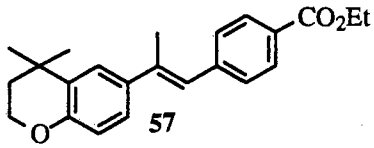
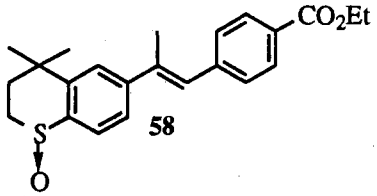
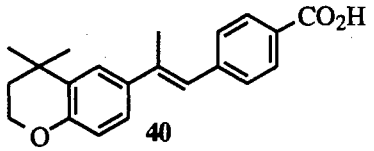
ODC[a] ACTIVITY OF SELECTED HETEROAROTINOIDS[b]

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	34	0.13	87% of Control
 **52**	34	0.062	94
 **41**	34	0.094	91
 **40**	34	0.283	72
 **53**	34	0.085	91
 **54**	34	0.254	75
 **55**	34	0.524	48
 **43**	34	0.246	74

[a]ODC = Ornithine decarboxylase.
[b]References 66 and 74.
[c]% inhibition = [100 x activity (Control)-activity (retinoid)]/activity (Control).

TABLE IV

TOC[a] ACTIVITY OF SELECTED HETEROAROTINOIDS[b]

Test System	Conc., *M*	% Active[c]	ED_{50}, *M*[d]
t-Retinoic Acid	10^{-10}	76.92	2×10^{-11}
 **56**	10^{-10}	53.80	6×10^{-11}
t-Retinoic Acid	10^{-10}	100.0	9×10^{-12}
 **57**	10^{-10}	50.00	100×10^{-12}
t-Retinoic Acid	10^{-10}	83.30	1×10^{-11}
 **58**	10^{-10}	18.60	60×10^{-11}
t-Retinoic Acid	10^{-10}	83.70	1×10^{-11}
 **40**	10^{-10}	57.10	10×10^{-11}

[a]TOC = Tracheal Organ Culture.

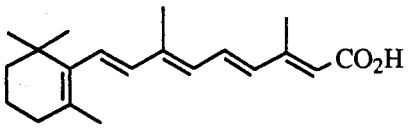
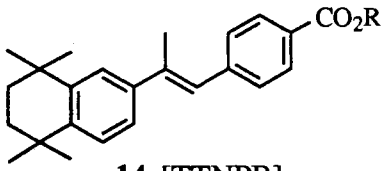
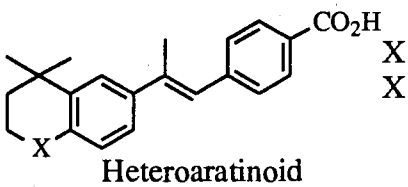
[b]Reference 74.

[c]%Active is based on the ability of retinoids to reverse keratinization of 100% keratinized cells.

[d]ED_{50} is the molarity of the retinoid required to effect reversal of keratinization in 50% of the cultures.

TABLE V

TOXICITY OF RETINOIDS IN SWISS MICE[a]

RETINOID	Dose μmol kg^{-1} day^{-1}	% Survivors Day 15
 **3** [RA]	300 100	0 100
 **14** [TTNPB]	100	0
 X = S X = O Heteroaratinoid **39** [X = S] **40** [X = O]	300 300	80 50

[a]Reference 13.

Regulation of Gene Expression by Retinoids

Many *in vitro* and *in vivo* studies have suggested that retinoids affect gene expression.[17,28,52] The exact mechanism of action and the direct biological responses attributed to this process are still unclear. A broad outlook of the mechanism of action is illustrated in Figure 1. It appears that retinol (**1**) and retinoic acid (**3**) bound to retinol

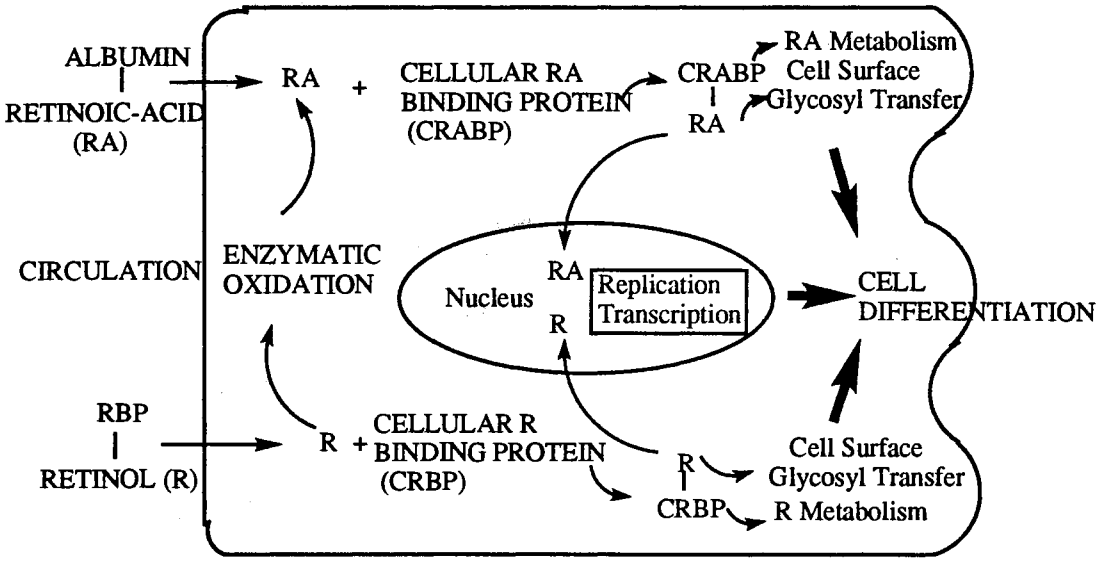
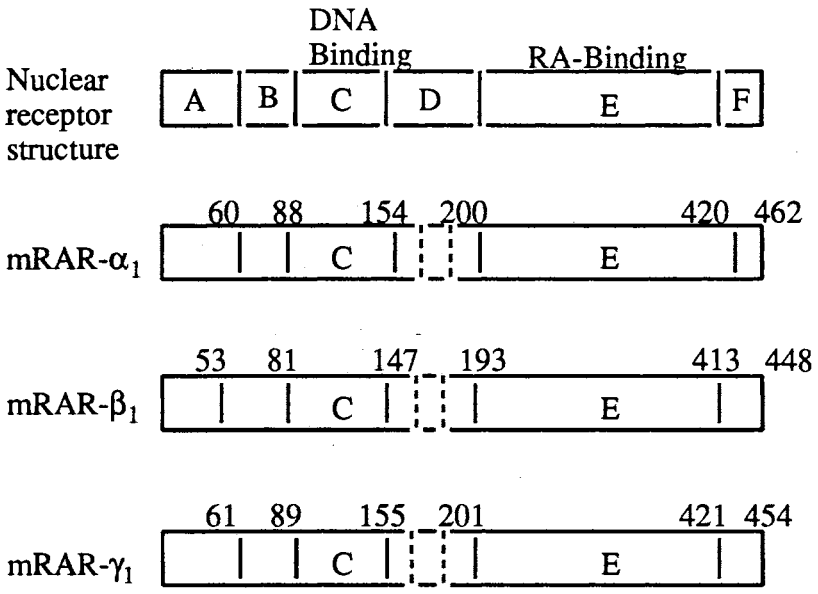


Figure 1. Schematic Representation of Mechanism of Action of Retinoic Acid (**3**)[28]

binding protein (RBP) and albumin, respectively, are transported to the cell. Although the basic structure of RBP is known, the process by which retinol (**2**) and RA (**3**) bind to the protein is not well established [binding is highly regulated and is dependent upon the synthesis and secretion of retinol-binding protein (RBP) by the liver].[51] Inside the cell (the exact mechanisms by which retinol (**1**) and RA (**3**) penetrate a cell membrane have not been established), a complex is formed between cellular retinol binding-protein (CRBP) and retinol. Some of the retinol (**1**) is enzymatically oxidized to RA (**3**) which then binds to cellular retinoic acid binding protein (CRABP). It appears that these complexes play an important role in the activation of gene expression, cell differentiation and proliferation.

Retinoic Acid Receptors. Several types of the nuclear receptors (proteins that act as antennae to detect the presence of certain messengers) have been identified (more than 30).[17,52] Only recently was it discovered that the action of RA (**3**) is also mediated through nuclear receptors.[17,52] Although the structure of various ligands are unrelated, the nuclear receptors seem to have many functional and structural similarities.[17,28,52] In some respects the RA receptors resemble those of steroids.[17,28,52] However, the RA receptors also display certain unique features,[18,52] some of which are discussed below.

Most receptors seem to have a linear arrangement of certain functional elements (namely amino acids), which usually possess six domains [A-F, shown below]. The nuclear receptor is depicted as a linear arrangement of various functional regions (domains, represented by the regions A-F at the top of Figure 2). The DNA and the RA



Percent Homology		A	B	C	D_1	D_2	D_3	E	F
	α_1/β_1	45	79	95	93	50	100	90	20
	α_1/γ_1	29	75	95	93	48	100	85	16
	β_1/γ_1	24	86	95	93	32	100	90	23

Figure 2. Schematic Representation of the Retinoic Acid Receptors (RARs) Family.[52]

binding sites of the nuclear receptor are indicated by the C and E regions. The numbers above the linear depiction of RARs indicate the amino acid sequences which have been aligned on the basis of their identity, and the percent homology of these amino acids between the various subtypes is indicated at the bottom of the figure. The subdomains of region D in the RARs are also indicated. The DNA, the ligand binding regions and the amino acid sequences among the receptors appear to be similar, which might suggest a conservation of basic function although each receptor has its own characteristic identity.[17,52]

Three RA receptors (RARs) [RAR-α,[25] RAR-β,[8] and RAR-γ[44]] have been isolated, identified, and characterized.[52] Although the RAR subtypes [RAR-α, RAR-β, and RAR-γ] seem to possess almost complete structural identity in the DNA binding region (region C, with respect to each other), there are some subtle differences in the amino acid sequences which could be responsible for their functional differences. Figure 4 on the following page illustrates the hypothetical structure of the DNA binding region (region C, of Figure 2) of the human retinoic acid receptor, RAR-α (folded together to contact DNA).[52] The *N*-terminal (amino-), and the *C*-terminal (carboxyl-) ends are indicated on the top of the Figure 4. The 'zinc finger' domains are represented by $\mathbf{C_1}$ and $\mathbf{C_2}$. The dark solid spots represent zinc ions. Amino acid residues containing the asterisks at the bottom of Figure 4 correspond to the adjacent (to C region) D region. The differences in the amino acid residues found in mouse RAR-β and RAR-γ (mβ and mγ, respectively) and zebra fish RAR-γ, (zfγ) are indicated by the arrows. The similarity between region C of zebra fish RAR-γ and the mouse RARs suggest that this sequence has remained essentially unchanged for many years.[52]

Responsive Elements. Specific DNA sequences essential for the action of various nuclear receptors (like the RARs and RXRs) are referred to as 'responsive elements'.[52] Although the action of RA (**3**) seems to be mediated through gene expression, only a few RA-responsive elements (RAREs) have been characterized to date.[52] One of the first

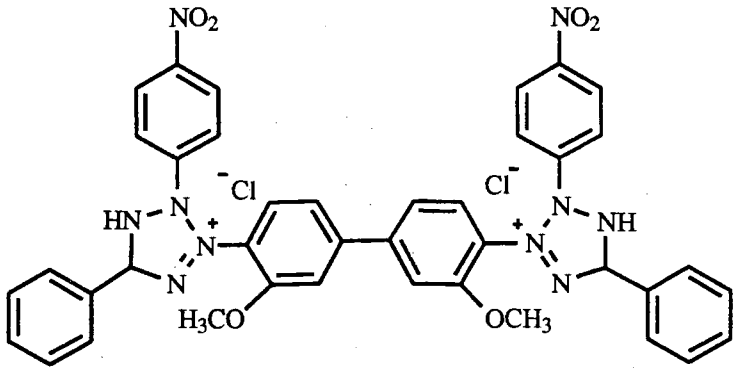


Figure 3. Structure of Nitroblue Tetrazolium dye (NBT, see page 11 of text).[75]

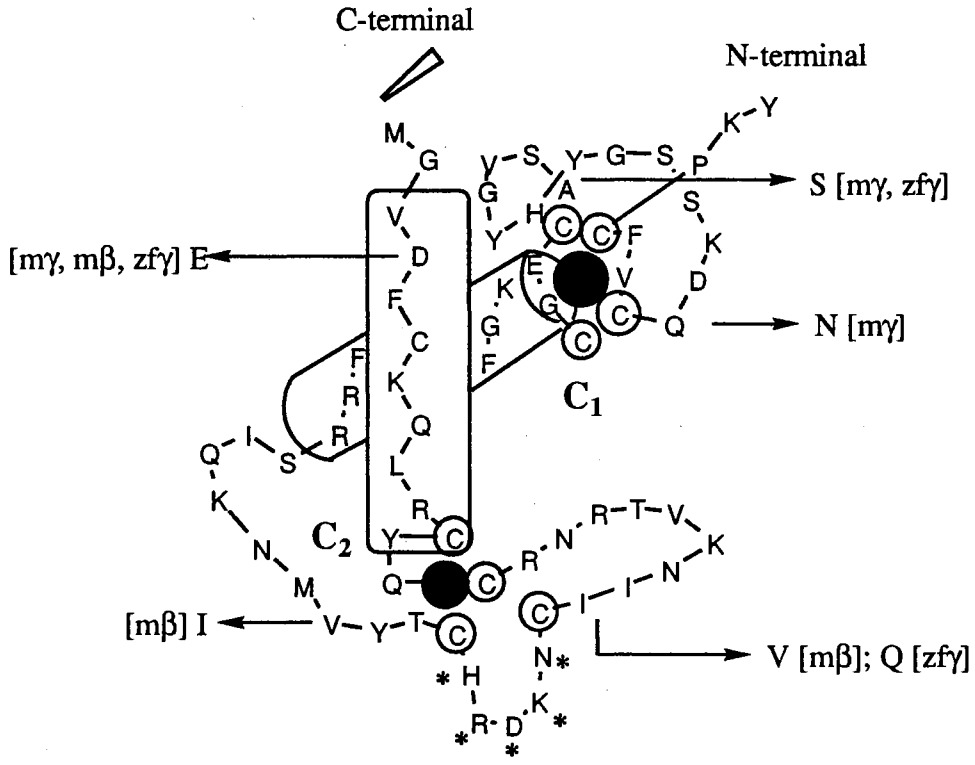


Figure 4. Schematic Representation of the Hypothetical Structure of RAR-α.[52]

RARE to be characterized was a "direct repeat of 'motif GTTCA' separated by a gap of six-nucleotides".[10] This RARE was identified in both the human and mouse RAR-β promoter (region of a particular gene that signals the RNA polymerase binding and the initiation of transcription).[34] A few other types of RAREs have been identified.[52]

The Ligand Binding Domain. Among the various members of the nuclear receptor family, there appears to be moderate similarity in the ligand binding region (same as RA binding region or region E in Figure 2). Ligand-binding, receptor dimerization, and transcription activation are functions ascribed to this domain.[17,28] The amino acid sequence difference between the three RAR subtypes are probably responsible for their functional differences. Only 35 out of the 220 amino acids (in the C-terminal half) that make up the domain were identical when RAR-α, RAR–β, and RAR-γ were compared with each other.[52]

In a recent study by Chambon and co-workers, it was found that the ability of RA (**3**) to bind with the three receptor subtypes could be different.[8] Initially, the affinity of RA (**3**) for human (hRAR-α) and mouse (mRAR-β) were compared. It was found that a much higher concentration of RA (approximately 5-10 fold excess) was required to achieve the same level of activation (reporter gene transcription) with hRAR-α as was achieved with mRAR-β.[8,52] It was also suggested that RA (**3**) could have the greatest affinity for RAR-γ receptor.[24] The other variations of the subtype receptors (α, β, and γ) occur at the A/B region, termed as the 'N-terminal region isoforms' (alternative segment of the gene that carries part of the coding information of a protein, found in the *N*-terminal region).[70] The exact distribution of these isoforms at the tissue level is *not* well established. However, several of these isoforms have been identified at various organs in a restricted manner (RAR-α_1 is expressed ubiquitously, while RAR-α_2 is specifically expressed in trace amounts in lung and intestine tissue; RAR-β_2 is found in the kidney, heart and skeletal muscles, to name a few).[52] Although the regulation of a gene by RA (**3**) in a specific location seems to be restricted to the RAR subtype, many studies suggest

that RAR expression is wide spread throughout various developing tissues and organs.[52] Skin seem to be an important RA target tissue, and *RAR-γ subtype seems to be the predominantly expressed receptor in this organ.* Similarly RAR-γ also seems to be associated with effects of retinoids on bone growth and development.[53]

Retinoid X Receptors (RXRs). A second set of receptors that bind specifically to retinoids have been discovered recently.[49,52] These receptors are called retinoid X receptors (RXRs), and, like the RARs, the RXRs appear to be involved in mediating cellular response to retinoids.[49,52] Drosophilia melanogaster, a closely related receptor to RXRs, was recently identified.[49] However, RA (**3**) did not show any binding affinity to this particular receptor. The RARs and the RXRs differ in their primary structure to a great extent. Moreover, like the RARs, three subtypes of RXRs (α, β, and γ) are known.[49,52] Although the DNA (region C, Figure 5) and ligand binding regions (region E) of the RXR-α and RXR-β resemble each other to a great extent (degree of homology is greater than 85%), relatively higher concentrations of RA are required for the activation of RXR-α than for RARs.[49] The amino acid sequences (homology) of RAR-α

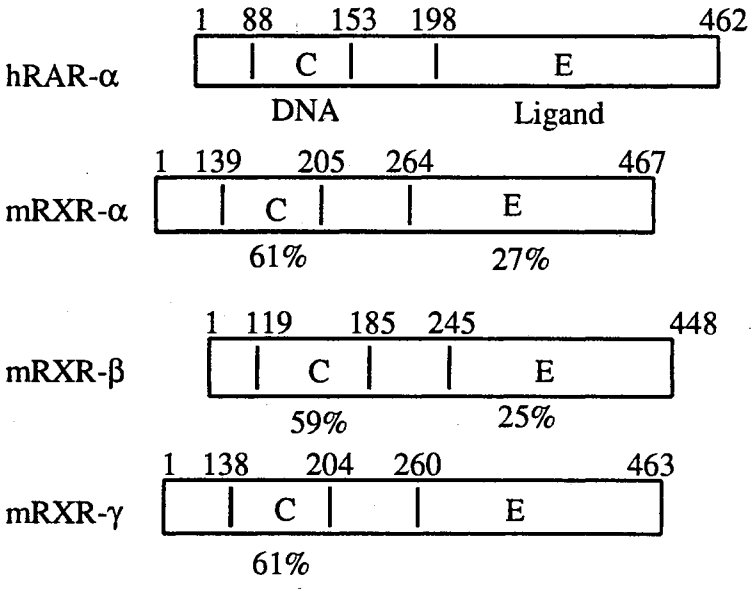


Figure 5 Schematic Representation of the Reinoic Acid Receptor (RXRs) Family.[52]

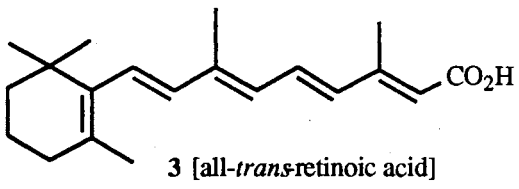
and mouse RXR-α, RXR-β, and RXR-γ are compared in Figure 5. The numbers above the linear depiction of receptors indicate the amino acid sequences which have been aligned on the basis of their identity (only the DNA-binding and the ligand-binding regions of RXRs exhibit significant amino acid identity with RAR-α).[52] Recent transcriptional response studies suggested that the 9-retinoic acid (**11**; an isomer of RA) binds to RXR with a higher affinity (40 times) than does RA (**3**), the metabolite from which **11** is derived.[32,52]

The tissue distribution of the RXRs seems to be quite unique and different from the RARs . Although there is some overlap of the tissue distribution of RXR-α and CRBP in the nervous system, the functional similarity has *not* been established.[52] Relatively high levels of RXR-α expressions have been observed in rat and chicken liver, suggesting a regulatory role for RXR-α in retinoid metabolism and transport.[52] Little is known about the tissue specific expression of RXR-β and RXR-γ.[52] Recently heterodimers of RARs and RXRs have been discovered.[32] These heterodimers appear to be more efficient at binding to the RA-responsive elements (RAREs) than do homodimers of these receptors.[32] Thus different RXR/RAR combinations may exhibit unique specificities in target gene activation. The RXR interaction with other nuclear receptors and their specific biological role remains to be determined.

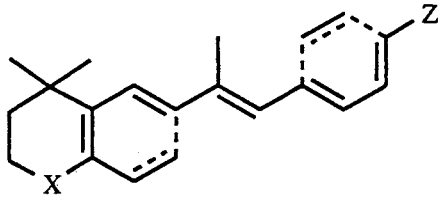
Cellular Retinoic Acid Binding Proteins (CRABPs). Cellular retinoic acid binding proteins (CRABPs) belong to a family of proteins (multigene) that are associated with several types of proteins like the cellular retinol binding proteins (CRBPs I and II), the myelin protein, p2 and the fatty acid binding proteins (FABP).[52,58] These proteins seem to be quite different from any known transcription factors, and they do not exhibit any resemblance to the RAR-ligand binding domains.[52,58] Although the functions of these proteins are not well understood, their binding ability with RA (**3**) is very high and selective.[52] It appears that CRABPs are not directly involved in mediating RA effects.[52] However, they (CRABPs) seem to limit the amount of RA available to the nuclear

receptors, thus controlling RA effects on gene regulation. The maintenance of normal tissue responses may actually require factors such as CRABPs CRBPs RARs, RXRs as well as retinoid-metabolizing enzymes. Isomerization of *t*-RA (**3**) to 9-*cis*-RA (**8**) may be quite important for regulating tissue responses to retinoids.[32] It will be paramount to determine whether heteroarotinoids and other synthetic retinoids are receptor specific.

Structure Activity Correlation of Retinoids. Several modified retinoids based on structure-activity correlations have been reported.[13,40,74] Most of the work focused upon reducing the toxicity and increasing the ability of retinoid to bind with a specific protein (RARs, RXRs, and CRABPs).[24,46,74] Thus, a structural similarity of the synthetic retinoid to the natural retinoid at the cellular level was deemed necessary to obtain an appropriate fit. Such similarities of heteroarotinoids **14** and **15** to RA (**3**) are shown below, suggesting that heteroarotinoids **14** and **15** could be potential mimics of RA (**3**).

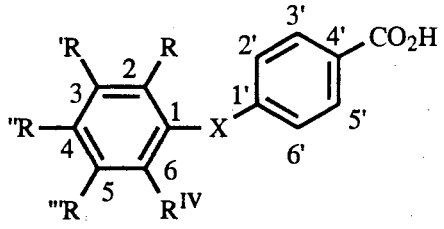


3 [all-*trans*-retinoic acid]



14 [X = O, SO_2, NR, S]
15 [Z = CO_2H, CO_2Et, CO_2Me]

Recently Shudo and co-workers studied a series of compounds termed retinobenzoic acids.[40,41] The generic chemical structure of retinobenzoic acid is represented by compound **18**. R was a large sized alkyl group such as an isopropyl or *t*-butyl group.



18 [R = Medium sized alkyl group
X = -NHC(O)-, -C(O)NH-,
-C(O)C=C-, -N=N-]

The activity of the retinobenzoic acids seemed to be influenced by the position of the alkyl groups on the aromatic ring. For example, when X was an amide group, it was necessary to place an alkyl group at the C-3 position (*meta*-to the amide group) to achieve significant activity.[41] Another required group was a carboxyl function at the C-4' position (*para*-to the amide group) on the aryl ring in the side chain.[40,41] The two aromatic rings were linked (represented by group X in **18**) with different functional groups such as -NHCO- (amides), -CONH- (reverse amides) -COC=C- (chalcone derivatives), and N=N (azo). From the activity (HL-60 assay) data of the amides, the following structural requirements for activity were suggested by Shudo and co-workers:[40,41] (1) a bulky alkyl group at the *meta*-position (C-3) to the linking group X, (2) a carboxyl group at the *para*-position (C-4') of the benzene ring of the side chain, (3) the conformation of the amide group (*trans*-amides **59** and **60** were more active than the *cis*-amide), and (4) the stereochemistry of the Ar-amide single bond (the s-*cis*-form of the amide **59** and the s-*trans*-form of the amide **60** were present as the more preferred

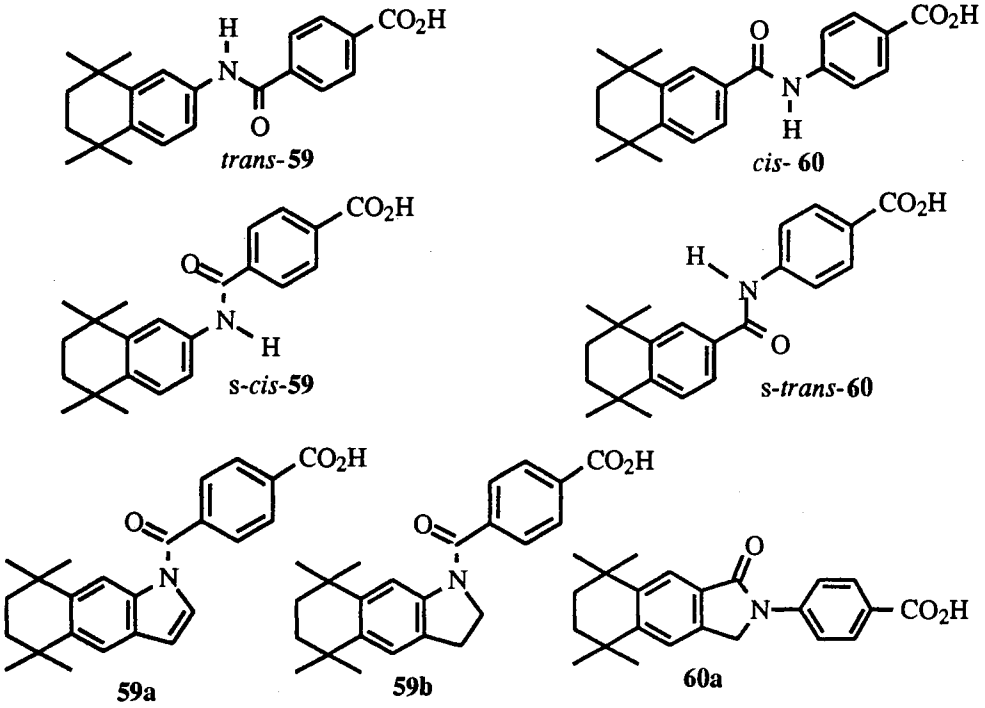
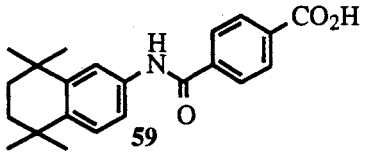
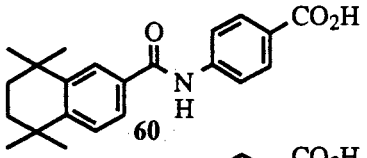
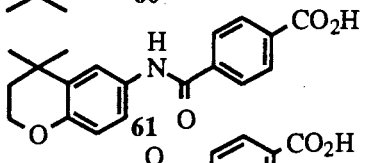
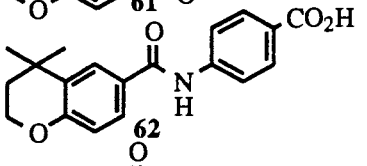
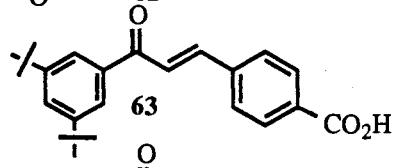
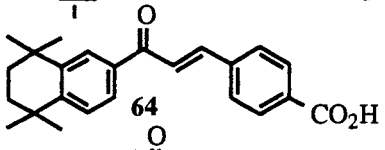
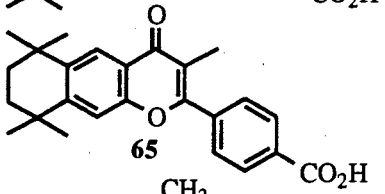
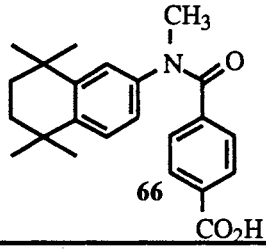


TABLE VI

ACTIVITY OF SELECTED RETINOBENZOIC ACIDS IN THE HL-60 ASSAY[a]

Retinobenzoic Acid	ED_{50}, M[b]	Rel. Activity[c]
3 [RA]	2.4×10^{-9}	1
 59	7.9×10^{-10}	3.5
 60	3.4×10^{-10}	7.2
 61	$> 10^{-10}$	$< 10^{-4}$
 62	8.0×10^{-7}	8.0×10^{-3}
 63	2.1×10^{-10}	6.4
 64	6.4×10^{-10}	2.8
 65	4.8×10^{-10}	8.5
 66	$> 10^{-6}$	$< 10^{-4}$

[a]References 40 and 41.

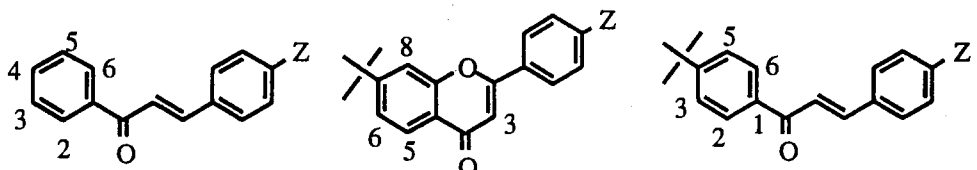
[b]ED_{50} is the molarity of retinoid required to effect cell differentiation in 50% of the cell cultures.

[c]Relative activity is the ratio ED_{50} (RA; **3**) to ED_{50} (a test compound).

conformers). Shudo and co-workers hypothesized that amides **59** and **60** would exist preferably in the s-*cis*-form (s-*cis*-**59**) and the s-*trans*-form (s-*trans*-**60**), respectively.[41] Their hypothesis was based on the HL-60 activity [compared to the activity of RA (**3**)] of amides **59a**, **59b** and **60a** which were structurally similar to amides **59** and **60** but were conformationally restricted.[41] Amides **59a** and **59b** are the conformationally restricted analogues of s-*cis*-**59**, and amide **60a** is the conformationally restricted analog of s-*cis*-**60**. Since the most active compound in the HL-60 assay was **59a**, (**59b** was also more active than **60a**), Shudo and co-workers suggested that s-*cis* form of **59** (s-*cis*-**59**) and s-*trans* form of **60** (s-*trans*-**60**) were the preferred conformations for amides **59** and **60** respectively, to achieve better activity.[41]

N-Methylation of the amide (like in amide **66**; Table VI) reduced the activity to a great extent, probably due to the change in the conformation of the amide group from the *trans*-arrangement to the *cis*-arrangement. A significant reduction in activity resulted when the carboxylic group (of retinobenzoic acids) at the C-4' position was converted to various derivatives like esters, acid chlorides or amides.[41] However, these derivatives could still be considered as useful agents, since the esters and amides could be hydrolyzed (*in vivo*) to the active form (CO_2H groups).

In some chalcone derivatives (generic chemical structure is represented by **18a**), Shudo and co-workers established that a *t*-butyl group induced greater activity if located



18a [Z = CO_2H] **18b** [Z = CO_2H] **18c** [Z = CO_2H]

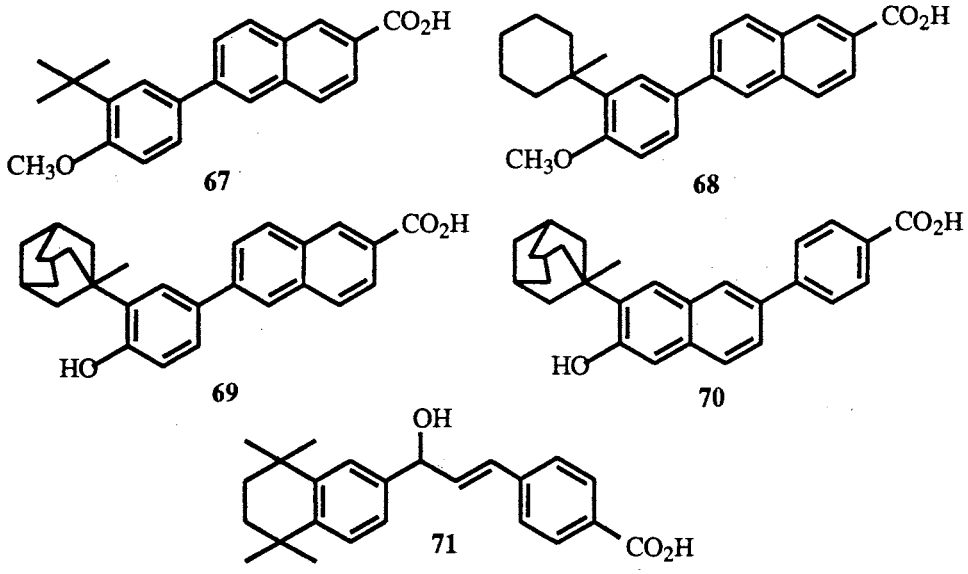
located at the *para* position (C-4) as in **18c**, than at the *meta* position (C-3).[41] This property was marked in the *t*-butyl substituted flavone-type retinoid (**18b**) and was exactly opposite of that found with the amides where *para* substitution decreased the

activity of the amides as discussed earlier.[41] Shudo and co-workers also noted that the effect of *ortho* (C-2) substituents (decrease in activity) on the amide derivatives (like **18**) was much greater than the effect of ortho substituents (C-6) on chalcone derivatives (like **18b**). The authors suggested that the difference in property (between amides and chalcones) could result from different degrees of conformational change in the α,β-unsaturated ketone and the amide groups caused by the *ortho* substituent.[41] In the chalcone skeleton, the two benzene rings were connected by three atoms (α,β-unsaturated ketone) whereas the two benzene rings were linked by two atoms (-NHCO-) in the amides. The chalcone skeleton seems to be more flexible even when an *ortho* substituent is present. Like the amide derivatives, the conformation of the chalcone-4-carboxylic acids also affected the activity. The s-*cis*-form (as amide *cis*-**60**) was the preferred conformation as shown in **64** (Table VI). It is not quite clear as to whether this s-*cis*-form of the chalcone is the most active form *in situ* since the flavone derivative (**65**; similar to the structure of **64**) exists in the s-*trans* form (**65**) and is very active (Table VI).

It was also noted that the chalcone **64** did *not* bind to cellular retinoic acid binding proteins (CRABPs), which suggested that CRABP may not be the crucial specific receptor related to the retinoidal action.[41] This emphasized the importance of other retinoid specific binding proteins, namely the RXRs and RARs. The difference in the binding ability of RA (**3**) to the subtype receptors can be observed with synthetic analogs that exhibit subtype preference.[41] For example, retinobenzoic acid **59** had a higher affinity of RAR-α than for RAR-β.[41] Although retinobenzoic acid **59** had a lower binding ability to CRABP than did RA (**3**), **59** mimics exactly the effects of RA (**3**) in causing duplications in chick wing bud experiments.[41]

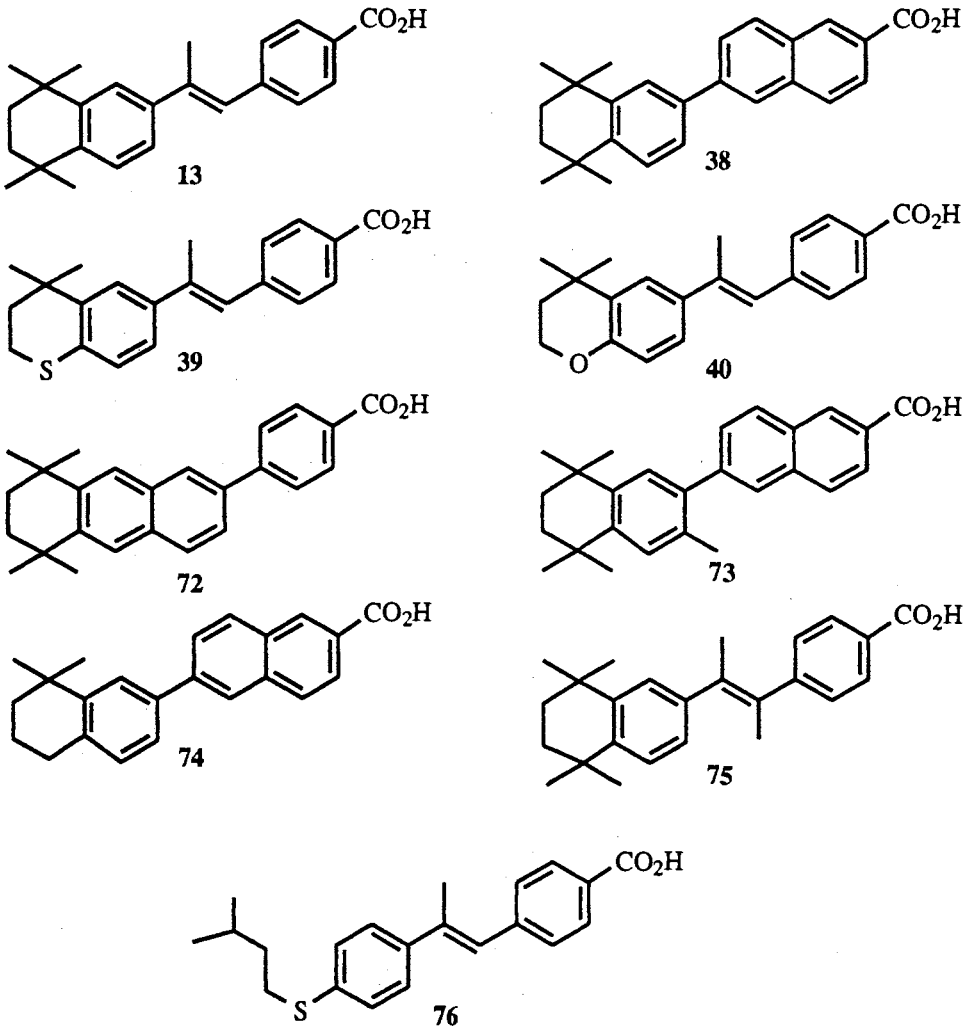
In a recent study (both *in vitro* binding assay and functional transactivation assay) by Bernard and co-workers, it was discovered that retinoids **67** and **68** were RAR-β specific, retinoids **69-71** were RAR-γ specific, and compounds **59** and **60** (Table VI) were RAR-α

specific.[4] The biological significance of the specific receptor binding abilities of these compounds has yet to be determined.



Recently study, Dawson and co-workers illustrated the ability of selected synthetic retinoids (including a few heteroarotinoids) to activate certain types of hybrid receptors.[46] The hybrid receptors were constructed with the ligand binding domain and the carboxy terminal portion of either RAR-α, RAR-β, or RAR–γ, and the DNA-binding domain and amino-terminal portion of the estrogen receptor (ER).[46] The binding ability of the hybrid receptor to RA (**3**) was identical to the binding ability of wild type RARs.[46] The synthetic retinoids tested (**13**, **38-40**, **72-76**) were conformationally restricted [compared to RA (**3**)] by the incorporation of aromatic rings at various positions of the polyene side chains as shown in the structures above.[46]

The synthetic retinoid that activated the receptor the most [100% percent receptor activation represented the activity observed for each receptor in the presence of 10^{-6} *M* RA (**3**)] was **72**, which displayed a high affinity for the RAR-β receptor and a significant affinity for the RAR-α receptor compared to that of RA (**3**). Receptor activation of most of the retinoids seemed to be concentration dependent (greater activation with higher concentration of the test retinoid). However, in the case of heteroarotinoid **39**, the



activation of receptor (RAR-β) was higher at concentrations of 10^{-7} *M* than at 10^{-6} *M*.[46] Heteroarotinoid **39** seemed to be more RAR-β and RAR-γ specific than RAR-α specific at concentrations of 10^{-7} *M* and 10^{-8} *M*.[46] Although heteroarotinoid **40** was RAR-β specific, the receptor activation (RAR-α, RAR–β and RAR–γ) was lower than that of heteroarotinoid **39**. The transcriptional activation activity of retinoids **38** and **73** were highly RAR-β and RAR–γ specific at critical concentrations, but the activation of the α receptor was very poor. Most of the retinoids had similar transcriptional activation activities towards RAR-β and RAR-γ receptors than towards RAR-α receptor. Greater differences in the activation pattern were observed between RAR-α and RAR–γ and RAR-α and RAR-β except in retinoid **74** where the activation of the RAR-γ receptor was

similar to the activation of RAR-α receptor (than to the RAR-β receptor). TTNPB (**13**) was found to be highly RAR-β specific (70% as active as RA for the receptor RAR-β). Retinoids **75** and **76** did not exhibit gene activation for any of the three receptors. Dawson and co-workers also noted that RAR-α receptor was more influenced by slight structural modifications of the retinoids than were RAR-β and RAR-γ receptors.[46] Several synthetic retinoids seem to be strong activators of the RAR-β and RAR-γ receptors and poor activators of the RAR-α receptor. The differential receptor activation of synthetic retinoids could aid as a tool in synthesizing receptor-specific retinoids.[46]

Retinoids and Transglutaminase Activity

Transglutaminase (TGase) is an enzyme that catalyzes a calcium-dependent acyl transfer reaction between the γ-carboxamide group of a peptide bound glutamine residue and the primary amino group of either a peptide-bound lysine or a polyamine as illustrated in Figure 6.[29] Binding of calcium ions and exposure of the active site cysteine (of the enzyme) are essential for enzyme activity.[29] The active site [cysteine] in Tgase

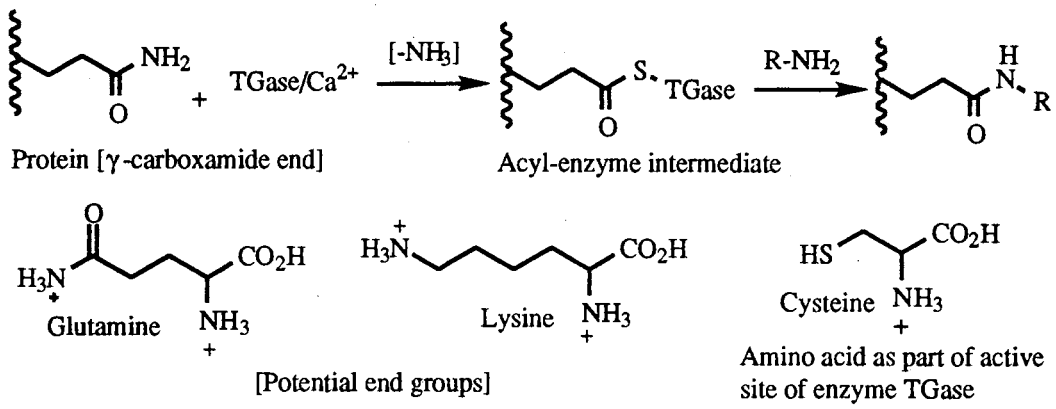


Figure 6. Transamidation in the Presence of Transglutaminase (TG).[29]

reacts with the γ-carboxamide end of the glutamine moiety of a protein forming a γ-glutamyl thioester and releasing ammonia.[29] The transient, acyl-enzyme intermediate then reacts with any nucleophilic primary amine, yielding either an isopeptide bond or a

γ-glutamyl polyamine bond. When an amine is not available, the acyl-enzyme intermediate reacts with water to yield a glutamic acid residue.[29]

Transglutaminases are classified under four major groups, namely plasma (factor-XIIIa), tissue (TG_c), keratenocytes (TG_k) and epidermal (TG_e).[29] Each of these types is believed to have a specific biological function. For example, the plasma transglutaminase is formed at sites of blood coagulation and impedes blood loss by stabilizing the fibrin clot.[29] The squamous epithelium constitutes a protective callus layer of skin and is formed by the action of keratinocyte transglutaminase (TG_k) and epidermal transglutaminase (TG_e).[29] The tissue transglutaminase (TG_c) is a cytoplasmic enzyme present in many cells including those in the blood vessel wall. Tissue transglutaminase (TG_c) function is unknown, although it could stabilize intra- and extracellular molecules in a wide variety of physiological or pathologic process.[29] There has been some evidence that the tissue transglutaminase (TG_c) is involved in cell differentiation.[68]

Regulation of human transglutaminase has been extensively studied in the promyelocytic leukemia cell line, HL-60.[12] It was shown that the retinoic acid-induced differentiation of HL-60 cell line was coupled to a specific induction of the transglutaminase gene.[68] In a recent study, it was also shown that the differentiation of HEL (human erythroleukemia) cells by RA (**3**) was accompanied by an increase in tissue concentration of transglutaminase.[68] RA (**3**; 10 μM) stimulated differentiation in HEL cells as judged by a four-fold increase in hemoglobin content, reduction in cell proliferation and a simultaneous nine-fold increase in transglutaminase activity.[68] Thus, it appears that transglutaminase can be used to predict the response of human myeloid leukemia cells to RA (**3**). Transglutaminase activity is measured by the incorporation of radioactive (^{14}C) putrescine into *N,N*-dimethylcasein.[68] One unit of enzyme activity is defined as 1 nmol of putrescine incorporated in 20 min/mg of protein at 37°C.[68]

CHAPTER II

RESULTS AND DISCUSSION

Modified Heteroarotinoids

The structure of the heteroarotinoids have been modified in our lab in order to achieve a better 'fit' (of the heteroarotinoids) at the receptor site and thus enhance the ligand binding specificity while decreasing the toxicity. Twenty-two new heteroarotinoids (**77-98**), which could be classified under four major categories, were synthesized:

1) heteroarotinoids with modified aryl rings (**77-85**),

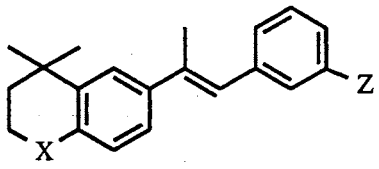
2) heteroarotinoids with an amide group (**86-91**),

3) heteroarotinoids with a reversed amide group and (**92-97**), and a

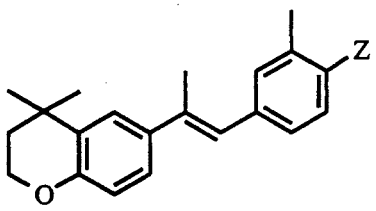
4) chalcone type heteroarotinoid (**98**)

Three different types of modifications of the aryl ring (on the side chain) of the heteroarotinoids **77-85** have been effected. In heteroarotinoids **77** and **78**, the functional group Z (CO_2H or CO_2Et) was introduced into the *meta*-position instead of a *para*-substitution on the aryl group in the side chain. Heteroarotinoids **79-84** contain a methyl group *ortho* to the functional group Z (CO_2H or CO_2Et), while in heteroarotinoid **85** the methyl group was incorporated into the *meta*-position to the functional group Z. Structural modifications of the aromatic ring could influence the orientation of the aryl side chain, or the molecule itself, at the receptor site. Thus, such changes could affect the binding ability of the heteroarotinoid to the ligand. A better 'fit' of the heteroarotinoid at the molecular level could enhance the affinity of the compound to a specific receptor for inducement of a specific response.

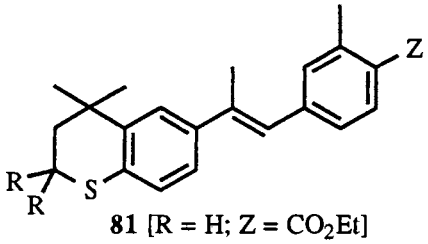
Heteroarotinoids With Modified Aryl Ring



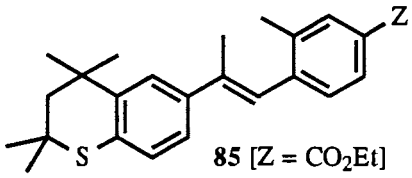
77 [X = S; Z = CO_2Et]
78 [X = S; Z = CO_2H]



79 [Z = CO_2Et]
80 [Z = CO_2H]

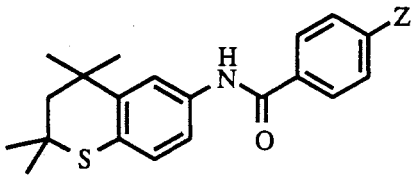


81 [R = H; Z = CO_2Et]
82 [R = H; Z = CO_2H]
83 [R = CH_3; Z = CO_2Et]
84 [R = CH_3; Z = CO_2H]

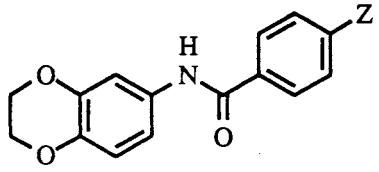


85 [Z = CO_2Et]

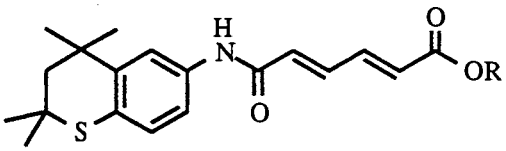
Heteroarotinoids With Amide-Group



86 [Z = CO_2Me]
87 [Z = CO_2H]

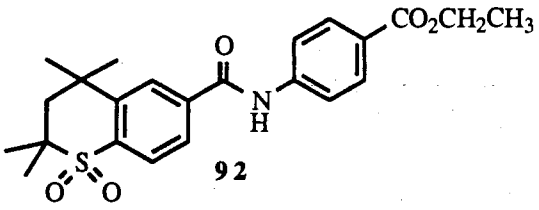


88 [Z = CO_2Me]
89 [Z = CO_2H]

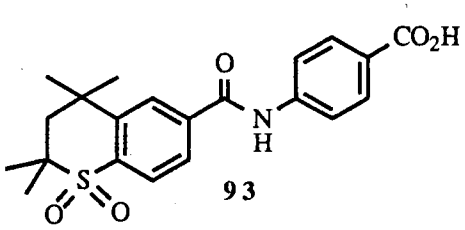


90 [R = CO_2Me]
91 [R = CO_2H]

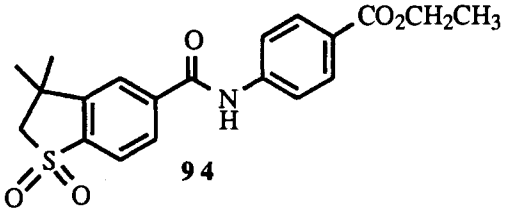
Heteroarotinoids With Reversed-Amide Group



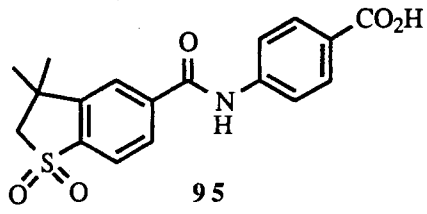
92



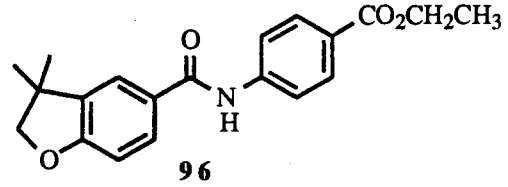
93



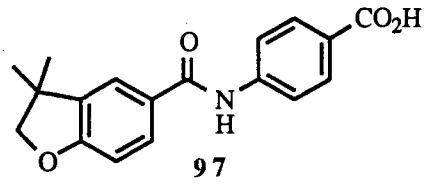
94



95

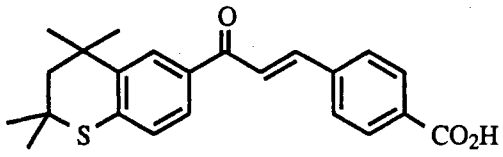


96



97

Chalcone-Type Heteroarotinoid



98

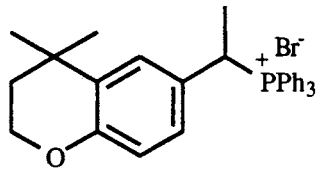
Heteroarotinoids **86-91** contain an amide function [-NHC(O)-] linking the aromatic ring and the side chain (aryl or polyene). Compounds **86** and **87** are six-membered, sulfur-containing derivatives (aryl side chain) with two geminal methyl groups on the six-membered ring. Compounds **88** and **89** are 1,4-benzodioxan derivatives with the amide group linking the aryl rings. Heteroarotinoids **90** and **91** are six-membered, sulfur-containing derivatives (two geminal methyl groups) which have the amide group linking the

aromatic ring and a polyene side chain. The amide groups were introduced as a spacer unit in order to provide the heteroarotinoids with a greater degree of flexibility at the binding site.

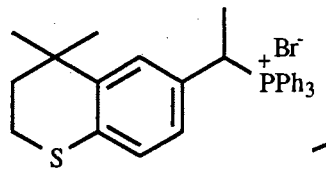
An α,β-unsaturated function [-CH=CHC(O)-] was introduced as the linking unit in heteroarotinoid **97** (chalcone type). The spacer unit in this system contains an additional carbon atom which could make the structure of the heteroarotinoid more flexible at the receptor site. All new heteroarotinoids were tested for their ability to bind to specific retinoic acid receptors (proteins that act as antennae to detect the presence of certain messengers like RA), namely receptors designated as RAR-α, RAR-β and RAR-γ and as RXR-α, RXR-β and RXR-γ. The ability of the new heteroarotinoids to increase the activity of the enzyme transglutaminase was also measured, and the results will be discussed in terms of biological activity.

Synthesis of Heteroarotinoids With Modified Aryl Ring

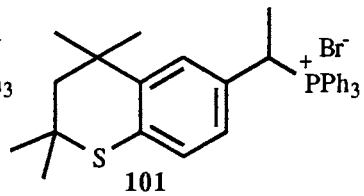
Phosphonium salts **99**, **100** and **101** are essential intermediates for the synthesis of modified heteroarotinoids, **77-85**. Synthetic methodology for the preparation of the salts



99



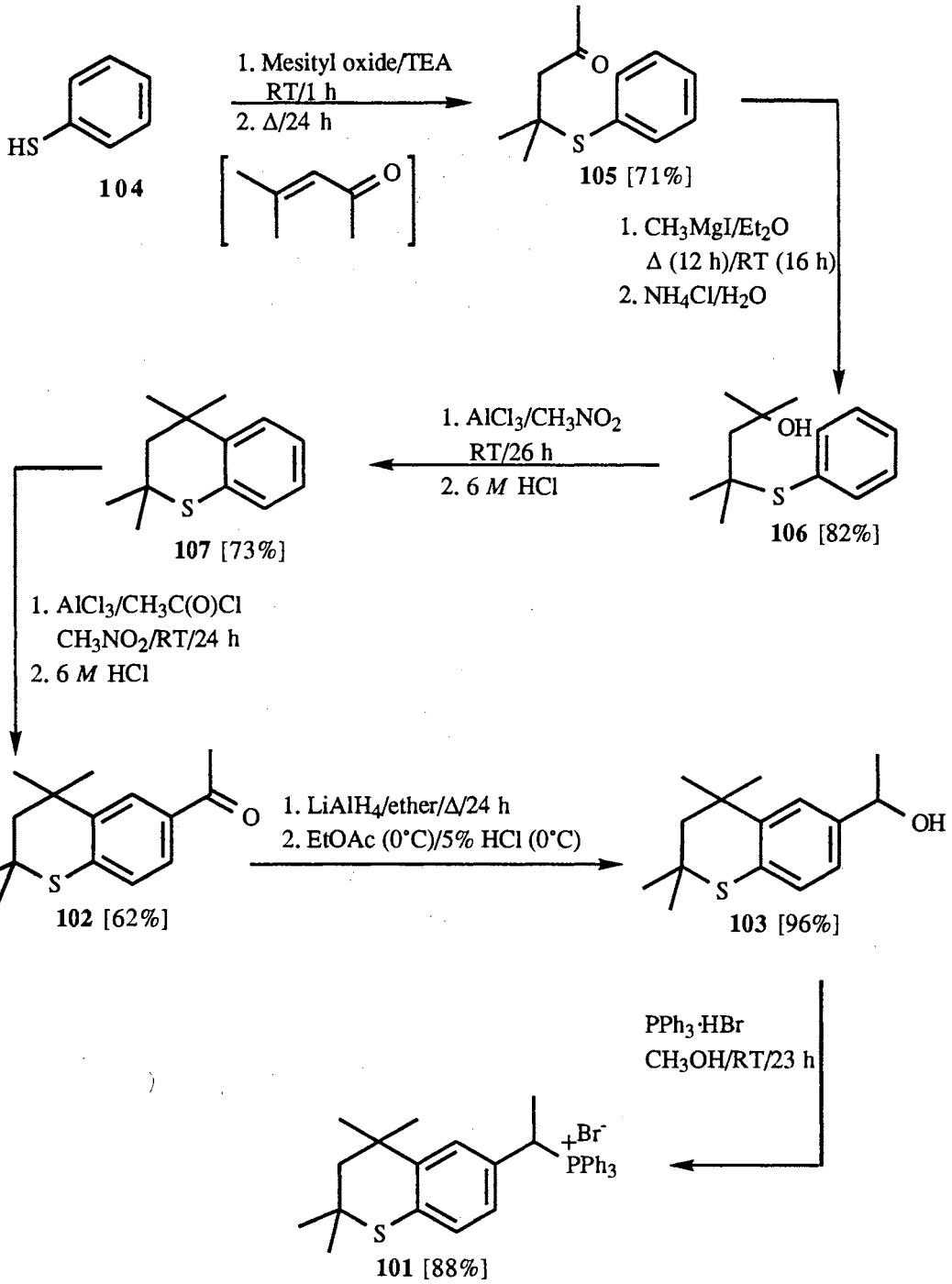
100



101

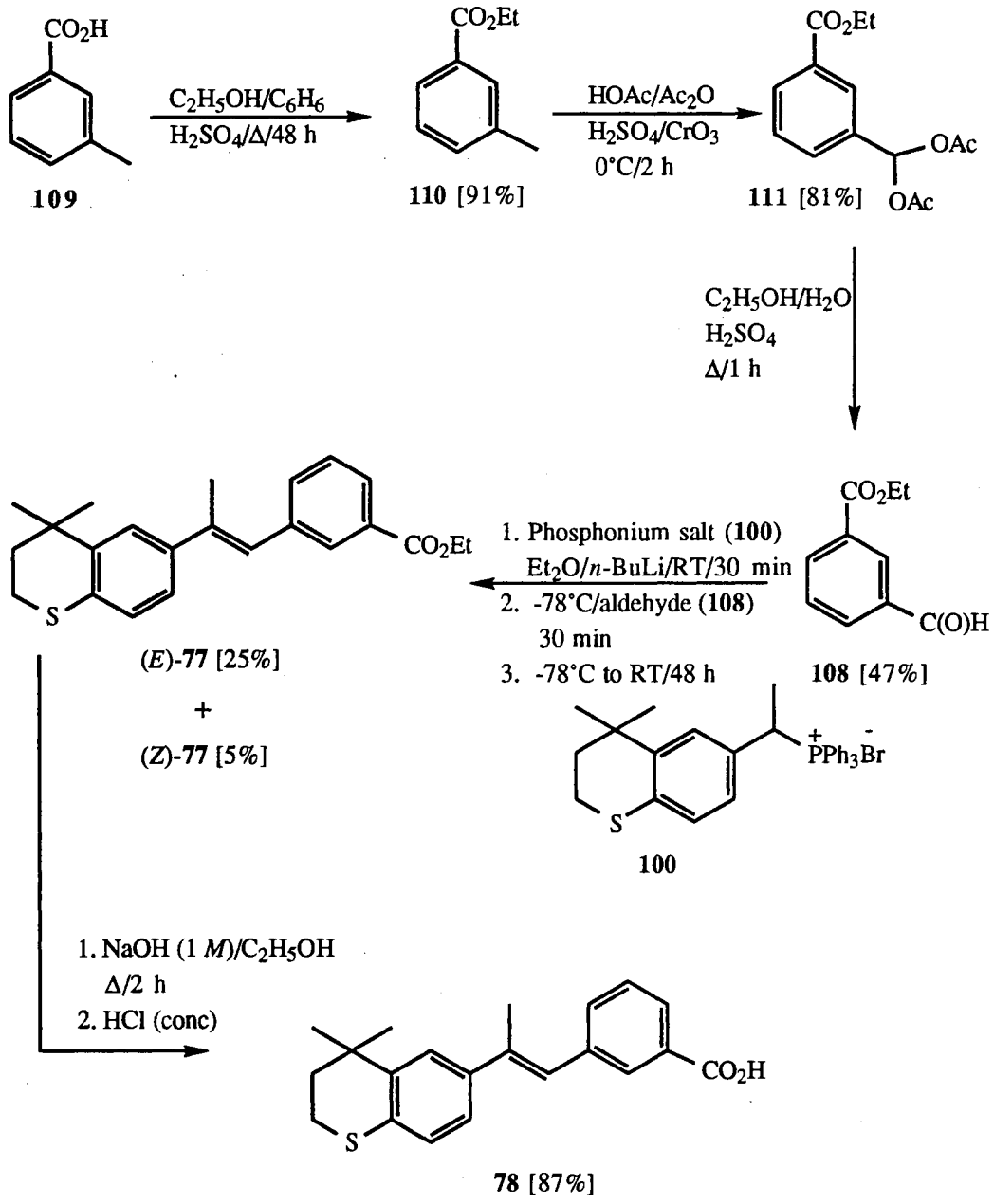
99 and **100** was already established in our lab.[65,55] Phosphonium salt **101** had not been reported, and thus the synthesis of the key synthon ketone **102** was required.[65] The procedure for the synthesis [Scheme I; **104**→**105**→**106**→**107**→**102**][65] of salt **101** was similar to that for phosphonium salts **99** and **100**. Ketone **102** was reduced to the corresponding alcohol **103** using $LiAlH_4$. Alcohol **103** was treated with triphenylphosphine hydrobromide to obtain phosphonium salt **101**.

SCHEME I



In order to obtain modified heteroarotinoids **77** and **78**, with the functional group Z in the *meta*-position, it was essential to prepare aldehyde **108**. The starting material selected was *m*-toluic acid (**109**), a readily available compound, which was esterified under the

SCHEME II



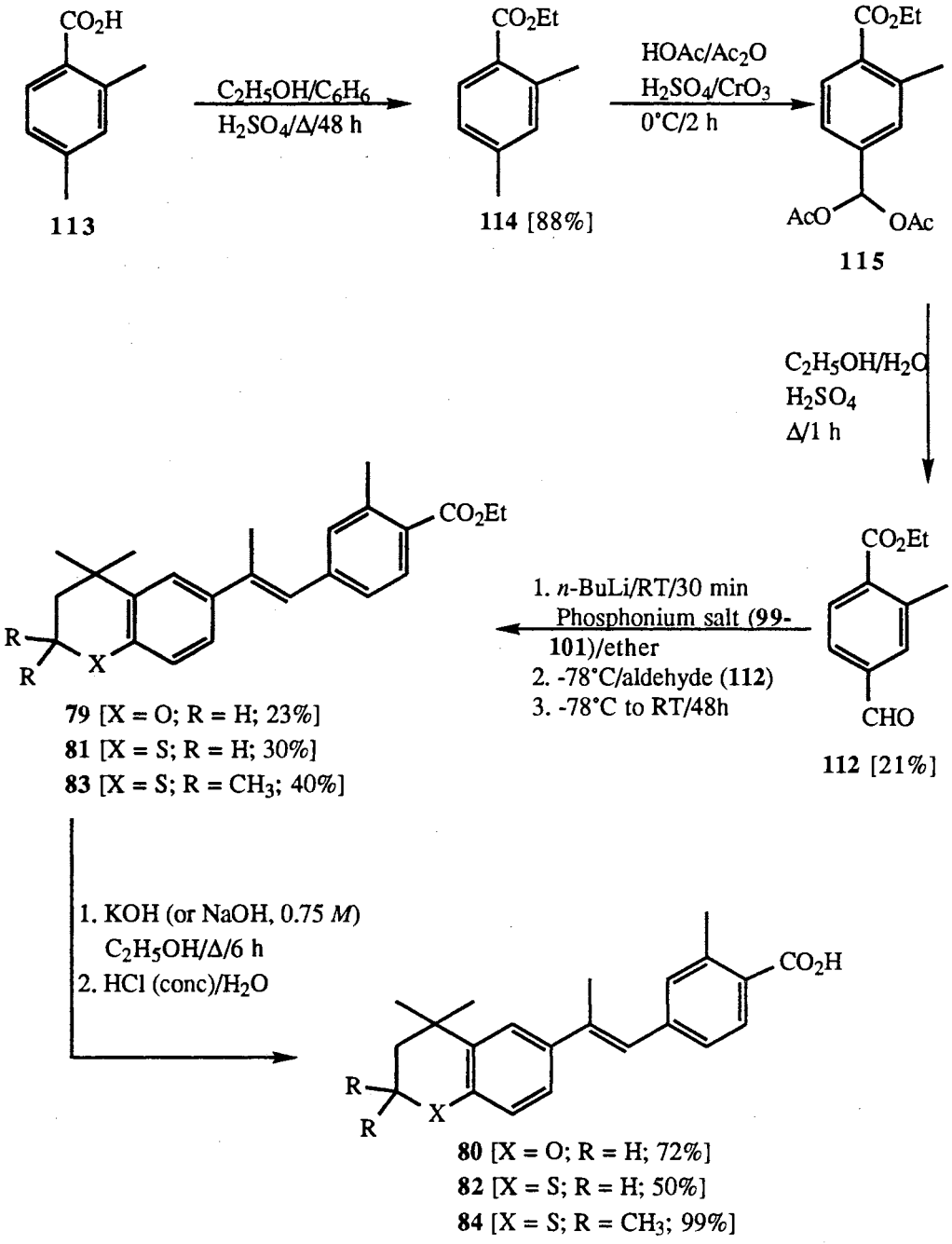
usual conditions (azeotropic removal of water with benzene) to the corresponding ethyl ester **110** (Scheme II). Oxidation of the methyl group on the aryl ring was effected by addition of small increments of CrO_3 at 0°C in the presence of $AcOH/Ac_2O$.[74] The intermediate acetal **111** was then hydrolyzed to aldehyde **108** upon demand. Due to rapid air oxidation, aldehyde **108** was treated immediately with the Wittig reagent from

phosphonium salt **100** (-78°C) in dry ether. The resulting oil was subjected to chromatography with a Chromatotron from which (*E*)-**77** was isolated and recrystallized. Saponification of ester (*E*)-**77** under mild conditions with a slight excess of NaOH gave acid (*E*)-**78**.

To synthesize the new heteroarotinoids **79-84**, it was necessary to devise synthetic strategies to obtain aldehyde **112**, a key intermediate obtained from commercial 2,4-dimethylbenzoic acid (**113**) and ester **114**. Esterification of acid **113** was achieved by boiling the acid under usual conditions (40 h with ethanol and a catalytic amount of sulfuric acid). A benzene-azeotropic process with a Dean Stark apparatus removed the water. Standard workup yielded ester **114** which was used without further purification. Since selective oxidation of the methyl group *para* to the CO_2Et group was required, a bulky oxidizing agent, namely chromyl acetate (generated *in-situ* by dissolving CrO_3 in Ac_2O/AcOH catalyzed by sulfuric acid)[74] was used (Scheme III). The crude diacetate **115** obtained was hydrolyzed to aldehyde **112**. The methyl group in the *ortho* position of **115** also underwent partial oxidation as shown by ^1H NMR analysis and this reduced the yield of **112**.

Aldehyde **112** was purified via chromatography on a Chromatotron. The final yield obtained was 21%. Due to the susceptibility of aldehyde **112** to autooxidation, it was immediately used in a Wittig reaction. To the suspension of phosphonium salt **99** in ether (stirred under N_2 at RT) was added dropwise *n*-BuLi (10 *M*), using a syringe. The reaction mixture turned red (ylide formation), and it was then cooled to -78°C. Aldehyde **42**, dissolved in ether, was then added to the cold reaction mixture [to minimize the formation of (*Z*)-**79**], and the new reaction mixture was allowed to warm to RT. Filtration of the suspension and evaporation of the solvent from the filtrate gave ester (*E*)-**79** as a mixture of isomers (*E*:*Z* = 3:1). The mixture was a clear viscous oil which was partially purified on a Chromatotron using hexane:ether (98:2); the overall yield of **79** was 23%

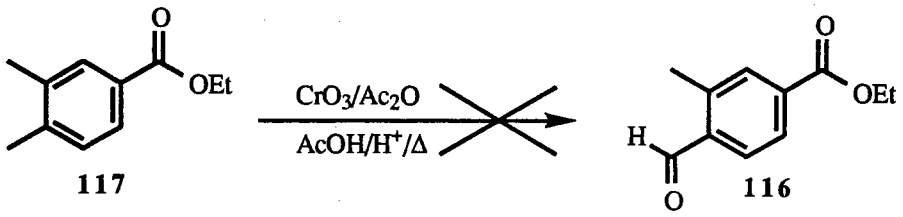
SCHEME III



(*E*:*Z* = 8:2). Attempts to solidify the oil (deep freeze with dry ice, freeze-thaw, and scratching the side of the flask with a few drops of EtOH) failed and ester **79** remained an oil. Saponification of slightly crude ester (*E*)-**79** required much more rigorous conditions than saponification of ester (*E*)-**77** without the methyl group in the *ortho*-position. A large

excess of NaOH was used, and the reaction had to be boiled at least for six hours. Acidification of this mixture with conc HCl and, upon cooling, did **not** yield acid (*E*)-**80** in solid form. Instead, an oil separated out from the aqueous phase, which on freezing (in the freezer 12 h) solidified. Acid (*E*)-**80**, thus obtained as a white solid was recrystallized (95% ethanol) to give colorless, crystals. Although esters (*E*)-**81** and (*E*)-**83** were prepared by similar procedures, purification and freezing of the oils produced the solid form immediately [unlike (*E*)-**79**]. Heteroarotinoids (*E*)-**81** and (*E*)-**83** were recrystallized (EtOH) to give colorless flaky [(*E*)-**81**] and needle-like crystals [(*E*)-**83**]. Saponification of esters (*E*)-**81** and (*E*)-**83** was effected under similar vigorous conditions. However, acidification of the reaction mixture resulted in solid formation at RT, and the acid (*E*)-**82** and (*E*)-**84** were then recrystallized.

The intermediate required for the synthesis of modified heteroarotinoid **85** (the methyl group was incorporated at the *meta*-position to the functional group Z) was aldehyde **116**. Initially, the starting material selected was ethyl 3,4-dimethylbenzoate (**117**). All attempts to oxidize the methyl groups under the usual conditions ($CrO_3/AcOH/Ac_2O$)[74] failed,

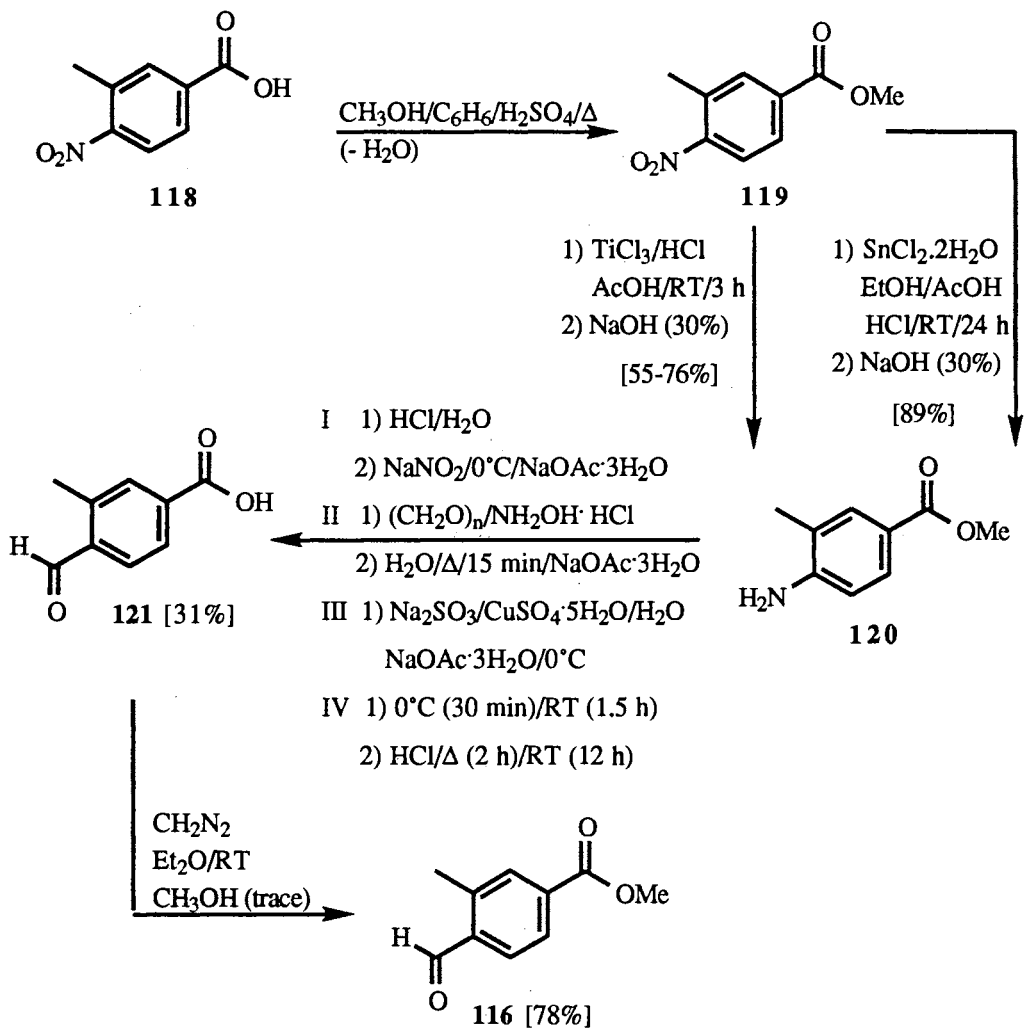


117 116

probably due to the difficulty in formation of the bulky acetal intermediate (the methyl groups of ester **117** were adjacent to each other). The starting ester **117** was recovered from different reaction conditions, including an attempted oxidation with cerric ammonium nitrate (CAN).[69] A multi-step reaction sequence was required for the synthesis of aldehyde **116**, starting from the commercially available acid **118** as illustrated in Scheme IV.

Esterification of acid **118** (acid-catalyzed and azeotropic removal of water using a Dean-Stark apparatus) gave the corresponding ester **119** as a solid by the usual conditions. Reduction of ester **119** was easily performed under very mild conditions (RT/3 h) using

SCHEME IV



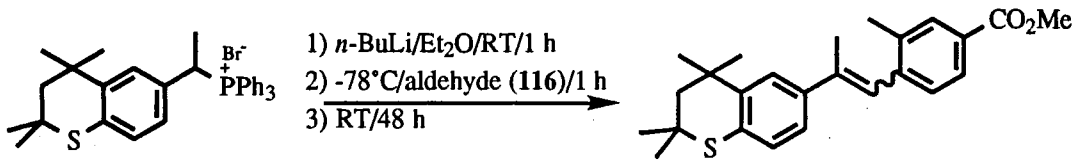
$TiCl_3/HCl$.[60] The mild conditions were favorable for small scale reductions (5 g), although large amounts of the reagent were required and the loss of the amine **120** was high due to a large volume of solvent required in the aqueous workup.

Reduction of **119** with $SnCl_2 \cdot H_2O$[22] required long times but was easily applied to a large scale operation (30 g). The yield of amine **120** was high in this reduction although the solvent volume for workup was again large. Aldehyde **121** was obtained from amine **120** by initially converting amine **120** *in situ* to a diazo derivative and then allowing the diazo derivative to react with an oxime complex (paraformaldehyde and hydroxylamine

complexed with $CuSO_4 \cdot 5H_2O$).[2,37] In the conversion of **120** to **121**, hydrolysis of the resulting complex gave the aldehyde in modest yield (31%). The ester group in **120** hydrolyzed simultaneously *in situ* to the corresponding carboxyl group under strong acidic conditions. It was important to maintain the pH close to 7 during the addition of the diazo derivative of the amine to the reaction mixture. Constant stirring with a large stirring bar was also required due to the separation of a gummy mass upon addition of the diazo derivative to the paraformaldehyde/hydroxylamine complex.[2,37]

Acid-aldehyde **121** was converted to the ester aldehyde **116** by addition of diazomethane. The reaction required only traces of methanol, and the aldehyde had to be very dry to avoid attack on the aldehyde carbonyl. The reaction was monitored by TLC, and the disappearance of the starting material at the base line of the TLC plate was taken as an indication for the completion of the reaction. Aldehyde **116** was purified by chromatography on a Chromatotron plate using dichloromethane as the solvent.

The Wittig reaction involving aldehyde **116** and the anion of phosphonium salt **101** was performed under the usual conditions using *n*-BuLi as the base. Due to possible steric hindrance by the *ortho*-methyl group next to the aldehyde function in **116**, an initial mixture of isomers (*E*:*Z* = 3:2) of the heteroarotinoid **85** was obtained. Separation of the



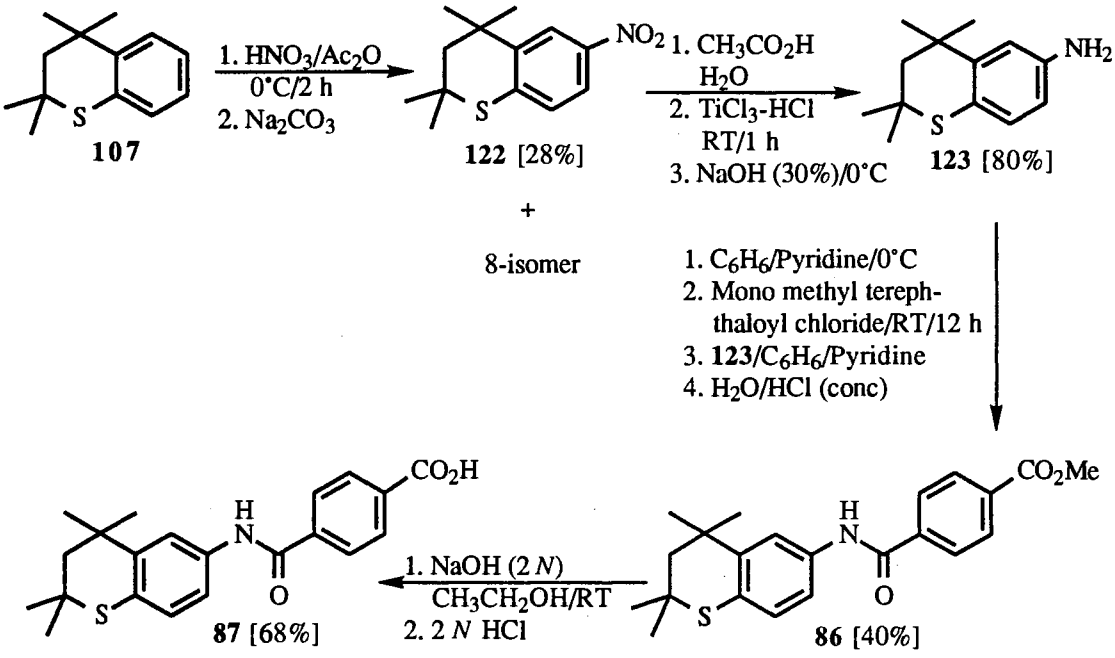
101 **85** [E:Z = 4:1, 40%]

isomers by chromatography on a Chromatotron has not yet been totally effective when 98:2 hexane:ether has been used. Various solvent systems [hexane, hexane:ether (100:1), and CH_2Cl_2] were tried in an effort to separate (*E*)-**85** from the isomer (*Z*)-**85**. The maximum separation achieved to date has been with the solvent system hexane:ether (100:1) which gave a 4:1 (*E*:*Z*) mixture of **85** which exists as a clear oil.

Synthesis of Heteroarotinoids With Amide-Group

The synthesis of retinobenzoic acids **86** and **87** (heteroarotinoids with an amide linkage) is illustrated in Scheme V. In the first step, nitration of the thio-ether **107**[40] with HNO_3 and Ac_2O at 0°C gave two solid isomers (6-isomer, and 8-isomer) of a nitro compound in almost in equal amounts. Although the isomers had identical R_f values in a

SCHEME V



wide range of solvent systems [$HCCl_3$:MeOH (3:1) and ether:hexanes (1:1)], a slight separation was achieved with H_2CCl_2 on a TLC plate. This separation was inadequate on a silica gel column or a regular Chromatotron plate. However, separation of the two compounds was effected by chromatography via the Chromatotron (silica gel) by extremely careful (*very slow development of the plate*) use of H_2CCl_2 as the solvent. The first band (R_f 0.3) containing the 6-isomer **122** was narrow and concentrated while the band for the 8-isomer (R_f of 0.1 to 0.3) was dispersed over a wide area. Thus, it was necessary to collect small fractions and analyze each via TLC methodology to separate single spots for

each compound. The slightly crude 6-nitro isomer **122** was finally isolated (10:1, 6-isomer:8-isomer, 30%) by this approach.

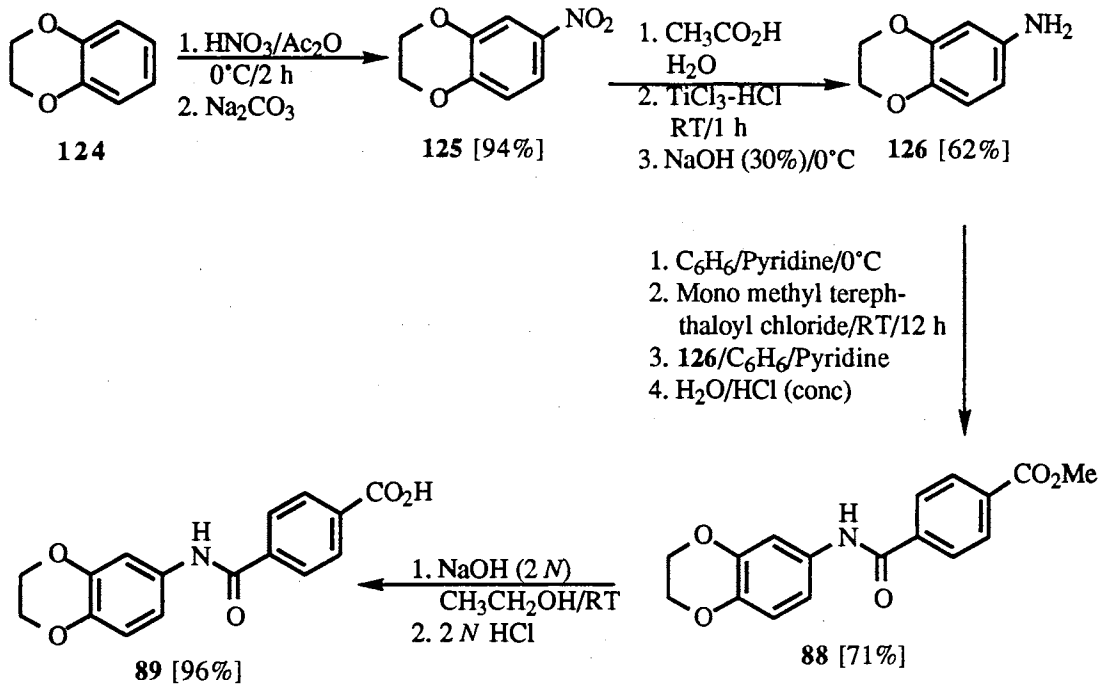
Reduction of the NO_2 group in the mixture of 6-isomer **122** and the 8-isomer to an NH_2 group in **123** was performed under mild conditions by using $TiCl_3$ /HCl at RT (1.5 h)[60] (Scheme V). Amine **123** was separated from the starting mixture by chromatography on a Chromatotron (silica gel and CH_2Cl_2:EtOAc, 50:1 system). The last fraction (R_f 0.8) collected from the plate contained the desired pure amine **123**.

Mono methyl terephthalate was stirred with an excess of thionyl chloride and DMF (few drops) at 0°C for 3 h and then at RT for 3 h. Evaporation of the excess thionyl chloride gave a white solid which was immediately transferred to a flask containing the amine **123** dissolved in benzene and pyridine. The resulting yellow solution was stirred overnight and decomposed with water. The crude yellow ester-amide **86** was purified by chromatography on a Chromatotron (H_2CCl_2; silica gel), and the solid **86** obtained after evaporation of the solvent was recrystallized (hexane:EtOAc, 3:1). Saponification of the ester **86** was effected under very mild conditions (RT) to avoid cleavage of the amide group with an excess of NaOH. After neutralization of the solution, acid-amide **87** was obtained as a white solid which was recrystallized.

The starting material for the synthesis of heteroarotinoids **88** and **89** was the readily available 1,4-benzodioxan (**124**, Scheme VI). Nitration of this compound (Ac_2O/HNO_3) gave the desired isomer **125** as the only product (94%). Reduction of the nitro compound, **125** to the amine **126** was carried out using the above mentioned $TiCl_3$-HCl reagent,[60] and **126** was condensed with mono methyl terephthaloyl chloride to give heteroarotinoid **88**. Saponification of the ester-amide was performed under mild conditions due to the vulnerability of amide group for hydrolysis. A high yield (96%) of acid **89** was obtained.

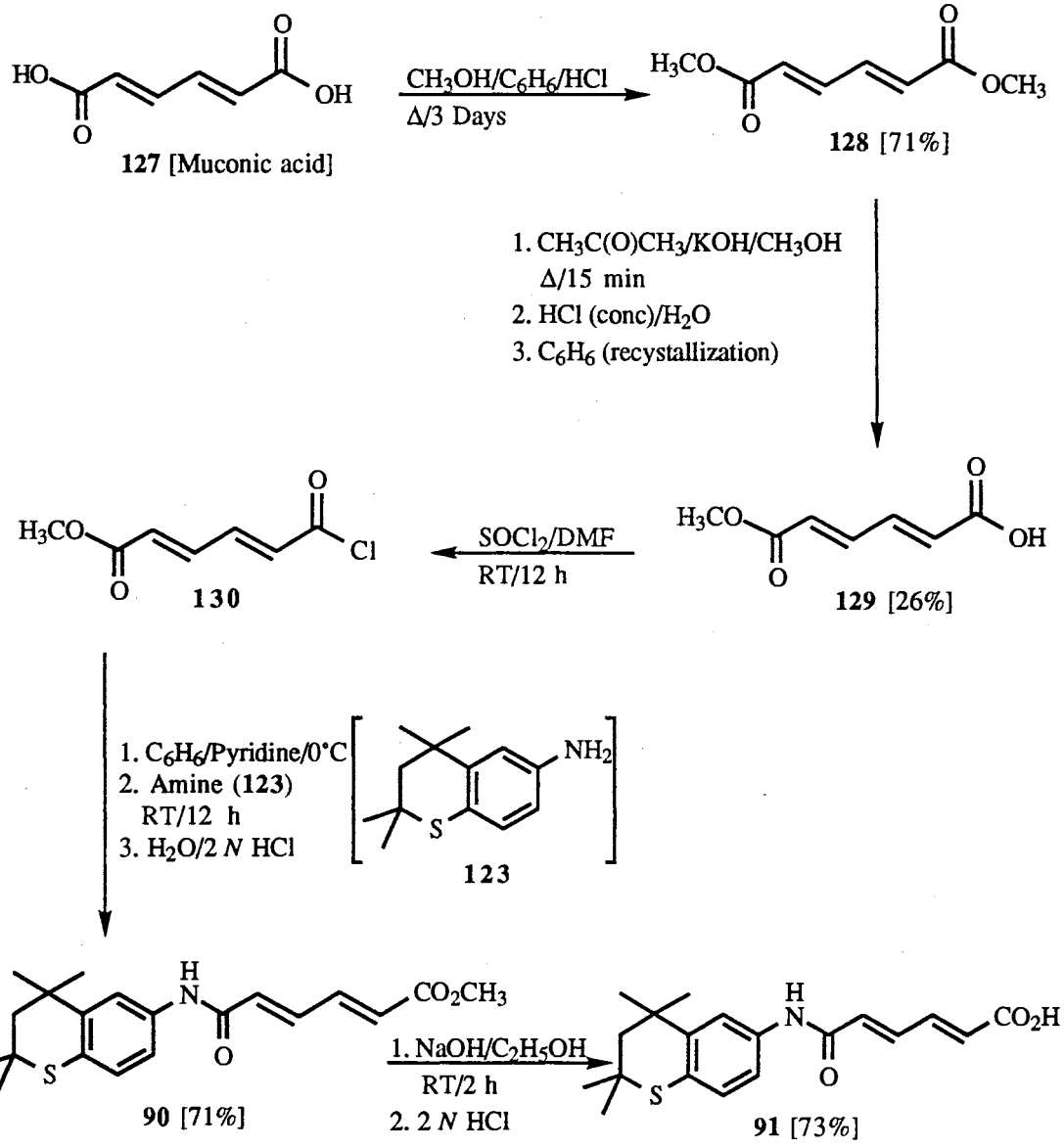
In order to synthesize heteroarotinoids **90** and **91**, which contain an amide group and a polyene side chain, muconic acid (**127**) was selected as the starting material. Esterification was effected by treating acid **127** with a large excess of methanol in the

SCHEME VI



presence of HCl and boiling the reaction mixture for 3 days (azeotropic removal of water Scheme VII).[16,42] The diester **128** separated from the reaction mixture upon cooling and was recrystallized (long needles) in benzene. When diester **128** was boiled with one equivalent of methanolic KOH, three products were obtained, namely muconic acid (**127**), the diester **128**, and the mono ester **129**. Muconic acid was sparingly soluble in methanol and was separated from the mixture by simple filtration. Mono methyl muconate (**129**) was then separated from the diester by extracting the mixture several times with hot benzene. Ester **129** was obtained as colorless, flaky crystals. Acid chloride **130** was prepared by the usual conditions from **129** using a large excess of thionyl chloride at RT. Amine **123** was condensed with acid chloride **130** (in a pyridine/benzene solvent system), and bright yellow crystals of ester-amide **90** were obtained after purification by chromatography on a Chromatotron. Saponification of ester **90** was performed under the usual mild conditions, and acid **91** was obtained as bright orange crystals.

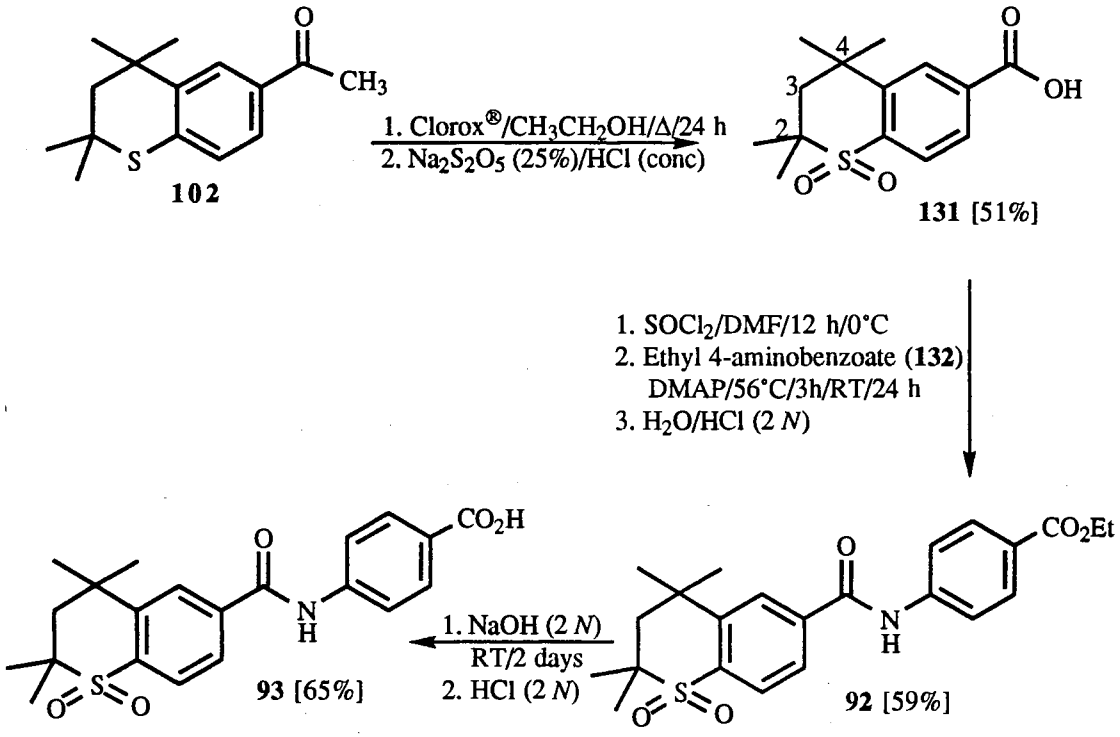
SCHEME VII


HO
O
OH
O
127 [Muconic acid]
CH3OH/C6H6/HCl
Δ/3 Days
H3CO
O
O
OCH3
128 [71%]
1. CH3C(O)CH3/KOH/CH3OH
Δ/15 min
2. HCl (conc)/H2O
3. C6H6 (recystallization)
H3CO
O
O
OH
129 [26%]
SOCl2/DMF
RT/12 h
H3CO
O
O
Cl
130
1. C6H6/Pyridine/0°C
2. Amine (123)
RT/12 h
3. H2O/2 N HCl
NH2
S
123
H
N
O
S
CO2CH3
90 [71%]
1. NaOH/C2H5OH
RT/2 h
2. 2 N HCl
H
N
O
S
CO2H
91 [73%]

Synthesis of Heteroarotinoids With Reversed Amide-Group

One report indicated that reversing the amide linkage could provide a retinoid which retained the activity of the retinobenzoic acids **86-89**.[40] Heteroarotinoids **92-97** contain reverse amide groups. In the first step of the synthesis of heteroarotinoid **92**, conversion of ketone **102** (synthesized by known procedures, Scheme I)[65] to the corresponding acid **131** involved a slightly modified haloform reaction (Scheme VIII). The ketone was dissol-

SCHEME VIII

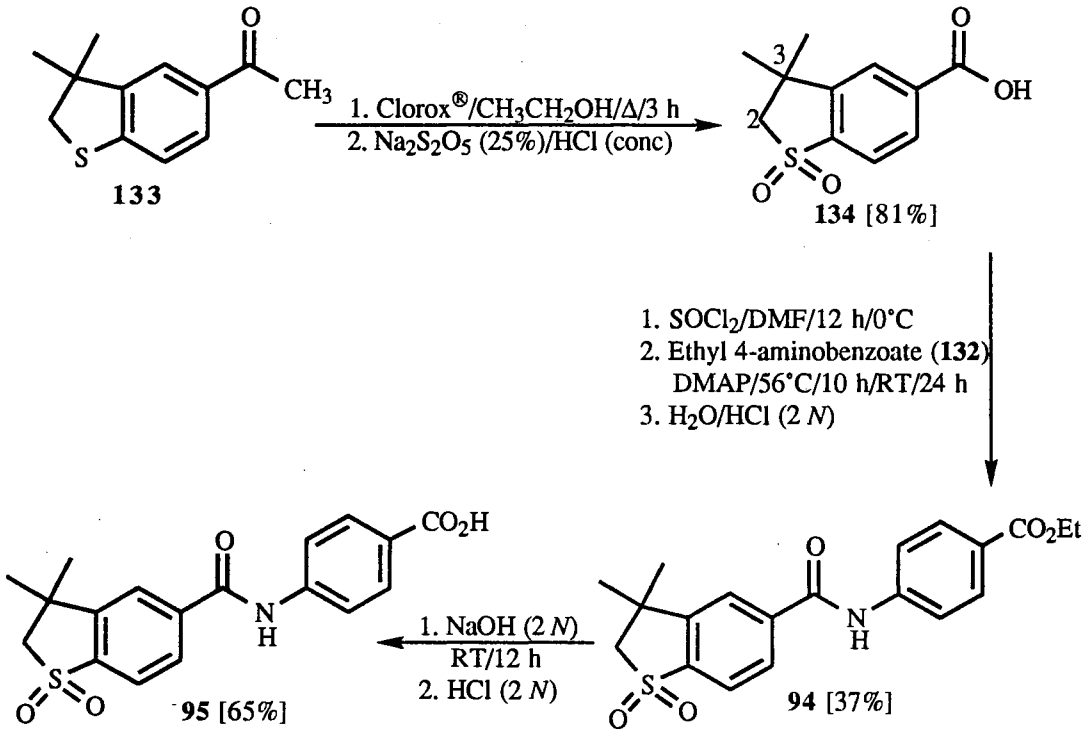


ved in ethanol and boiled with excess commercially available Clorox® (containing 5.25% NaOCl).[21] An acidic workup, after destruction of excess Clorox® by addition of sodium metabisulfite, gave acid **131**. A downfield shift in the ^{13}C NMR signal pattern for the C(2) adjacent to the sulfur atom in **131** (48 ppm) suggested that the sulfur atom in **102** (42 ppm) had been oxidized to a sulfone group in **131**. Acid **131** was recrystallized (EtOH, 95%) and then stirred at 0°C with excess thionyl chloride and a few drops of DMF. Acid-

sulfone **131** slowly dissolved in the thionyl chloride (~3 h), and the resulting clear solution was stirred overnight. Excess thionyl chloride was then removed and the solid acid chloride obtained was dissolved in pyridine and treated with ethyl 4-aminobenzoate (**132**) in the presence of a catalytic amount (5-10 mg) of DMAP. The reaction mixture was heated at a constant temperature (~56°C) and then stirred at RT. An aqueous workup of the reaction yielded the crude amide **92** which was partially purified by chromatography on a Chromatotron followed by recrystallization (EtOH, 95%). From analysis of the downfield shift of the ^{13}C NMR signal of C(2) adjacent to the sulfur atom (48 ppm in **131** compared to 42 ppm in **102**), it was clear that the product was a sulfone-amide **92** [48.6 ppm for the ^{13}C NMR signal for C(2)]. Hydrolysis of the ester group in **92** with 10 equivalents of NaOH under mild conditions yielded the retinobenzoic acid **93** as a white solid. The acid was purified by recrystallization.

Synthesis of the five-membered, fused heterobenzoic acids **94** and **95** (Scheme IX)

SCHEME IX

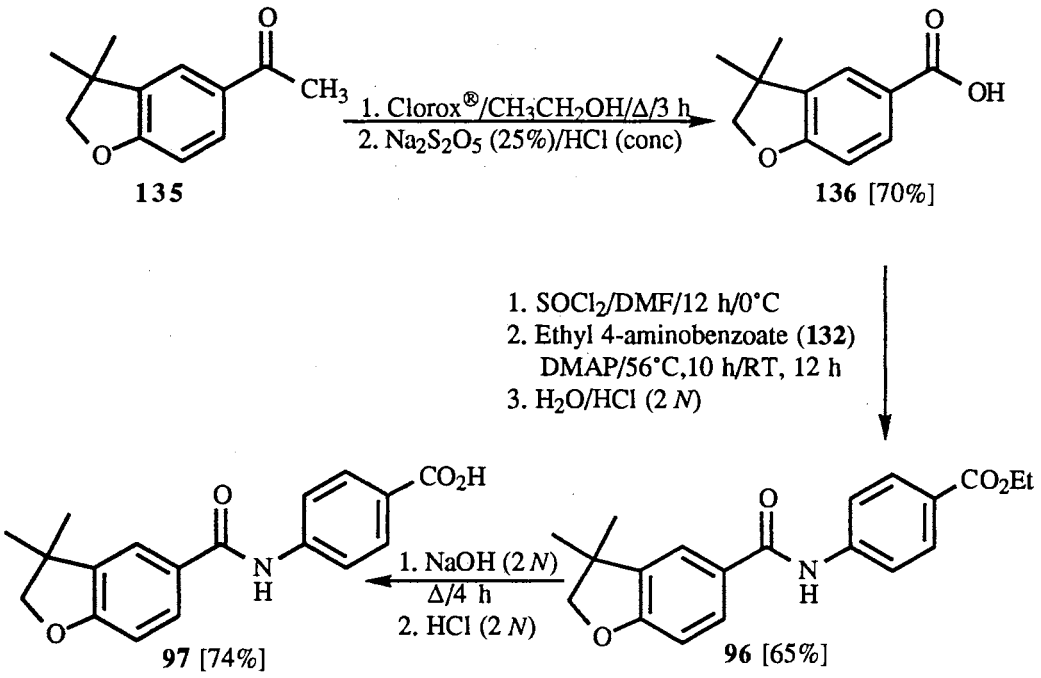


with related amide linkages involved a similar sequence of reactions as that used for the six-membered, fused heterobenzoic acid systems. Oxidation of S to SO_2 occurred in the first haloform reaction of **133** to yield **134** which was converted to the amid-ester **94**, also a sulfone. The downfield shift (~20 ppm) of the ^{13}C NMR signal of C(2) adjacent to the sulfur atom in **94** (61.0 ppm) was more prominent with the five membered ring system (62 ppm in **134** compared to 42 ppm in **133**). Hydrolysis of **94** (described for **92→93**) gave acid-amide **95**. Heteroarotinod **94** was purified by chromatography on a Chromatotron using a polar solvent system ($HCCl_3$:CH_3OH, 3:1). It was necessary to perform a gradient elution ($HCCl_3$ to CH_3OH) to elute the compounds from the plate. The ester-amide **94** was readily recrystallized, but the acid-amide **95** required a large excess of EtOH.

The reduction of the sulfone group of acid **131** and acid **134** to the corresponding sulfide groups reportedly requires vigorous conditions (LAH and high temperatures)[7] that would destroy the carboxylic acid function. Mild conditions (Zn/HCl)[7] that would not affect the carboxylic acid function were not effective in reducing the sulfone to sulfide for these systems in our hands.

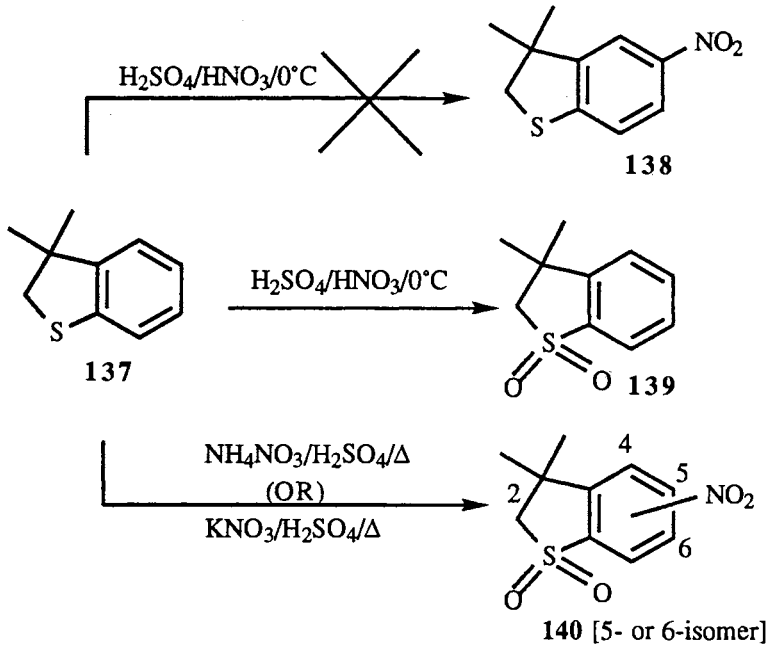
To obtain heteroarotinoids **96** and **97** with reverse amide groups and without an oxidized heteroatom, ketone **135** was selected as the starting material. Ketone **135** contains an oxygen atom in a five-membered ring (with geminal dimethyl groups) that is fused to an aromatic ring (Scheme X). The procedure is similar to that of Schemes VIII and IX. Oxidation of ketone **135** with commercially available Chlorox gave acid **136** which was converted to the acid chloride and which was then condensed with ethyl 4-aminobenzoate to give ester-amide **96**. Ester-amide **96** was purified several times by chromatography (on a Chromatotron) with various solvent systems. However, due to its hygroscopic nature, heteroarotinoid **96** remained a sticky solid and had a broad melting range. Hydrolysis of ester-amide **96** required heating for a few hours with 2 *N* NaOH, and the acid-amide **97** thus obtained solidified upon freezing. Acid **97** was purified by recrystallization (hexane:EtOAc, 2:1).

SCHEME X

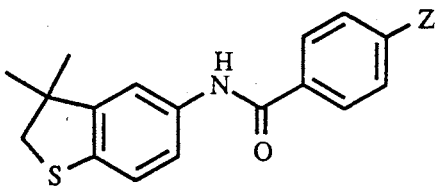


Oxidation of the sulfur atom of heterocycle **137**[22] to the corresponding sulfone **139** readily occurred in all attempts to synthesize nitro compound **138**, a key intermediate in the

SCHEME IX



proposed synthesis of heteroarotinoid **141**. Scheme IX illustrates the different reaction



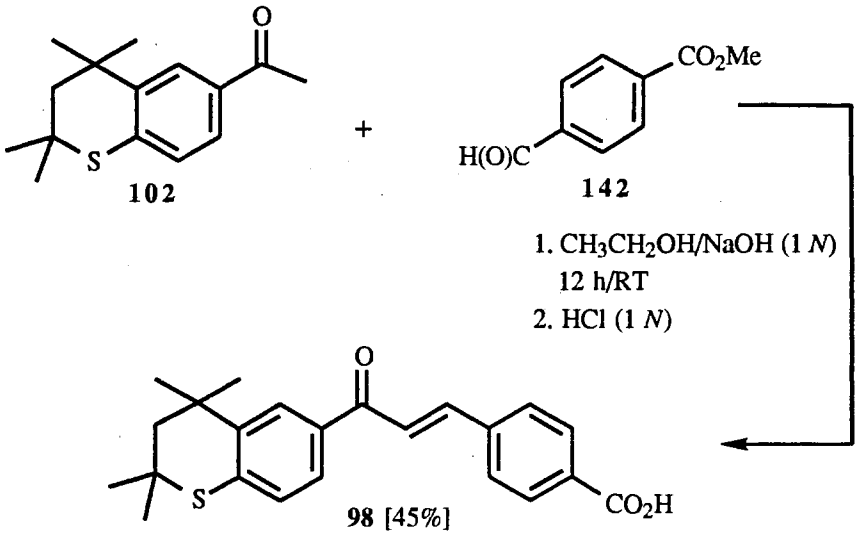
141 [Z = CO_2Et, CO_2H]

conditions examined. Deactivation of the aromatic ring probably results from rapid formation of sulfone **139** and thus severe conditions are required for nitration of the aromatic ring. Numanov and co-workers reported the formation of 5- and 6-isomers of the nitro compound **140** with the simultaneous oxidation of sulfur to the sulfone (*Caution*: reaction with ammonium or potassium nitrate in sulfuric was highly exothermic) when a compound similar to **137** was treated with ammonium or potassium nitrate in the presence of sulfuric acid.[38,45] Other methods must be found to obtain heteroarotinoid **141**.

Synthesis of Chalcone-Type Heteroarotinoids

Chalcone derivatives like compounds **63** and **64** (Table VI, page 24) possess an extra carbon between the aromatic rings, and yet some have exhibited activity in the differentiation-induction HL-60 assay.[41] This observation may be due to the skeletal structure of the chalcone derivatives which may be more flexible at the receptor site and thereby promoting improved binding. Ketone **102**[65] and aldehyde **142**[55] (both synthesized by reported procedures)[55,65] were dissolved in EtOH and NaOH (2 *N*) was added. The resulting solution was stirred for 12 h (Scheme XII). An acidic workup gave the crude acid as a yellow solid which was purified by chromatography on a Chromatotron ($HCCl_3$:H_3COH, 3:1). The solid obtained was recrystallized (hexane) to give the pure yellow, crystalline chalcone derivative **98**.

SCHEME XII



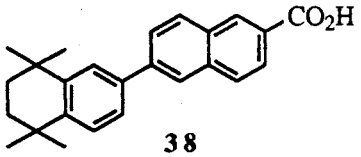
Biological Activities of Modified Heteroarotinoids

Understanding the structure-activity correlation of retinoids has been one major focus of current research. Although there are numerous assays to measure the activity of retinoids, it is very difficult to predict the exact structural requirement for maximum activity based only on selected assay results. With the discovery of specific nuclear receptors for retinoids,[52] the binding ability of natural and synthetic retinoids can be tested. Such data serve as a valuable tool to understand the influence of the structure on a biological function.[52]

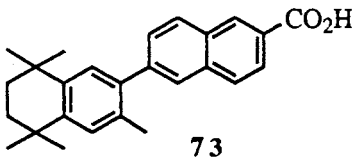
There are several factors that govern the activity of retinoids. The oxidation state of retinoid seems to be a vital factor for retinoid activity. For example, it has been hypothesized that RA (**3**) is the active form of vitamin A (**1**) and that RA (**3**) is responsible for controlling cell differentiation.[56] Retinol (**1**) and retinal (**2**) are in lower oxidation states compared to RA (**3**) and need to be oxidized to the acid function to elicit cell differentiation. The presence of a terminal carboxylic acid moiety seems to be essential for activity even among the synthetic retinoids[56] reported and in our present work.

It is known that the metabolism of retinoids could influence the activity of retinoids to

a great extent. While metabolic activation (oxidation of CHO to CO_2H) could increase the biological activity, metabolic deactivation (oxidation of S to SO_2) could decrease the activity of retinoids.[18] Physical properties like structure, solubility and transport properties could alter the biological activity of retinoids to a considerable extent. For example, poly-aromatic retinoids (like **38** and **73**) have low solubility in culture medium to which they are



38



73

added while dissolved in DMSO. Their low solubility and moderate affinity for proteins, such as serum albumin, may influence results [decreased ODC activities of **38** (ID_{50} = 2.2 n*M*) and **73** (ID_{50} = 0.5 n*M*), compared to that of RA (**3**, ID_{50} = 0.04 n*M*), were observed].[56] Transport into tracheal epithelial cells can also be affected by retinoid structure and lipophilicity.[56]

In a recent study, Dawson and co-workers established that out of thirty-six synthetic retinoids tested, 13 retinoids exhibited some degree of activity in the TOC assay.[56] Eight retinoids (out of the 13 retinoids) possessed or were capable of 12,13-'cisoid' side chain topography, but a CO_2H end group also seemed to be required for cell differentiation. Current research appears to be targeted on the identification of retinoids (both natural and synthetic) with specific receptor-subtypes and related biological activities.[56]

All of the new modified hetroarotinoids, **77-98** and several other heteroarotinoids synthesized previously in our lab were tested for activity in two different assays: (1) transglutaminase (TGase) activity (Noble Foundation, Ardmore, OK), and (2) binding potency (Ligand Pharmaceuticals, San Diego, CA) to specific receptors. Table VII illustrates the effect of forty different heteroarotinoids on the TGase activity (decreasing order), and Table VIII lists the binding potency of fourteen heteroarotinoids (decreasing order) with human retinoic acid receptors RAR-α, RAR-β and RAR-γ. The relation- ship of receptor binding to specific biological responses will be discussed shortly.

TABLE VII

EFFECT OF HETEROAROTINOIDS ON TGase ACTIVITY[a]

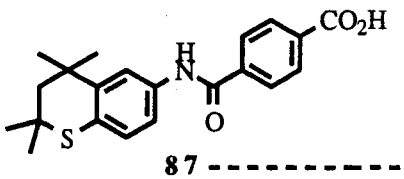
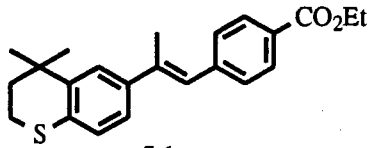
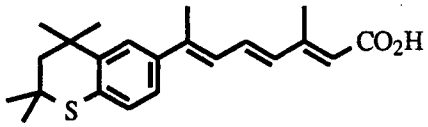
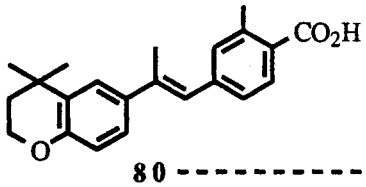
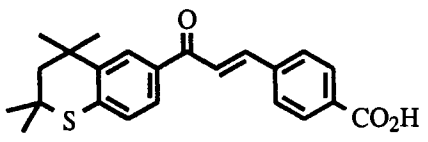
HETEROAROTINOIDS	Ratio Sp. Activity[b]	R[c]
3 [RA]	3.0	1.0
 **87**	2.3	0.76
3 [RA]	5.1	1.0
 **56**	3.4	0.67
3 [RA]	4.9	1.0
 **46**	3.1	0.63
3 [RA]	5.7	1.0
 **80**	3.5	0.61
3 [RA]	3.0	1.0
 **98**	1.8	0.60

TABLE VII (Continued)

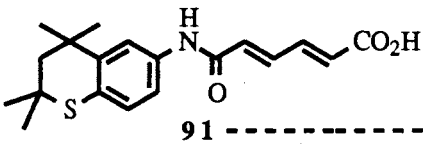
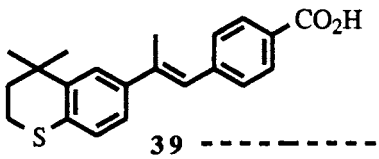
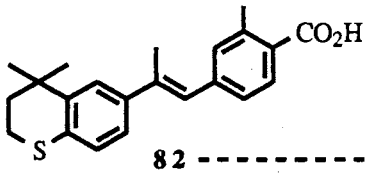
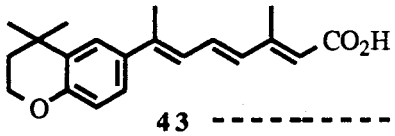
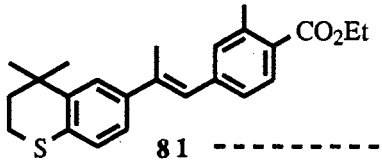
HETEROAROTINOIDS	Ratio Sp. Activity[b]	R[c]
3 [RA]	4.1	1.0
 **91**	2.4	0.60
3 [RA]	3.2	1.0
 **39**	1.9	0.59
3 [RA]	4.8	1.0
 **82**	2.8	0.58
3 [RA]	5.1	1.0
 **43**	3.0	0.58
3 [RA]	4.8	1.0
 **81**	2.7	0.56

TABLE VII (Continued)

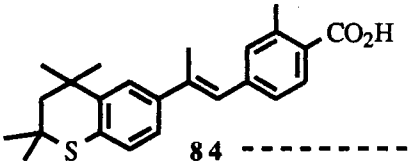
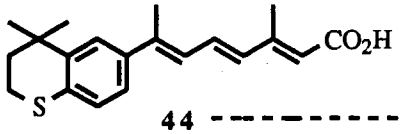
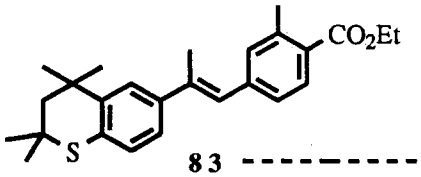
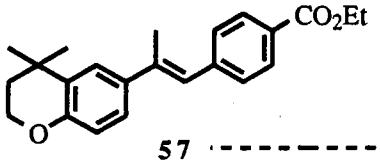
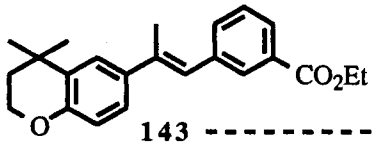
HETEROAROTINOIDS	Ratio Sp. Activity[b]	R[c]
3 [RA]	3.2	1.0
 **84**	1.8	0.56
3 [RA]	4.9	1.0
 **44**	2.7	0.55
3 [RA]	4.8	1.0
 **83**	2.5	0.52
3 [RA]	5.1	1.0
 **57**	2.6	0.51
3 [RA]	4.9	1.0
 **143**	2.5	0.51

TABLE VII (Continued)

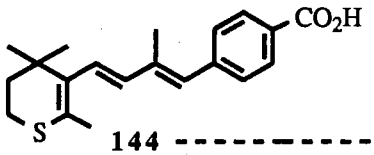
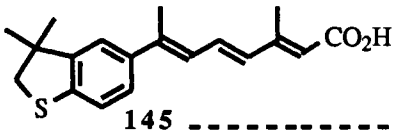
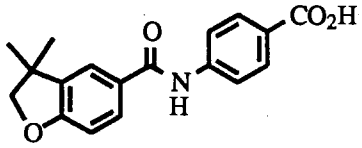
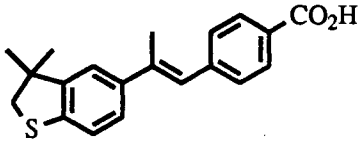
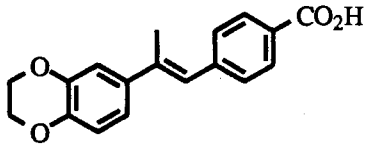
HETEROAROTINOIDS	Ratio Sp. Activity[b]	R[c]
3 [RA]	4.9	1.0
 **144**	2.4	0.49
3 [RA]	4.9	1.0
 **145**	2.4	0.49
3 [RA]	4.1	1.0
 **97**	1.9	0.47
3 [RA]	4.9	1.0
 **41**	2.3	0.47
3 [RA]	3.0	1.0
 **146**	1.4	0.47

TABLE VII (Continued)

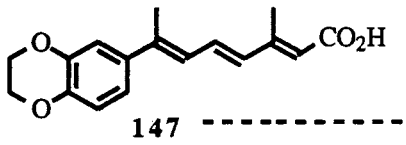
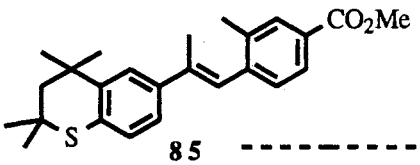
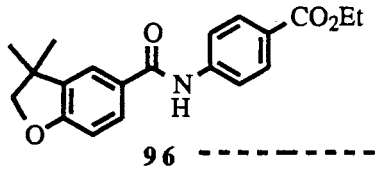
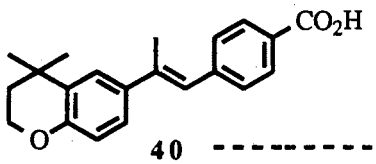
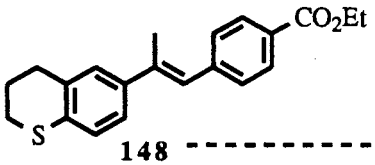
HETEROAROTINOIDS	Ratio Sp. Activity[b]	R[c]
3 [RA]	3.0	1.0
 **147**	1.4	0.47
3 [RA]	3.2	1.0
 **85**	1.4	0.44
3 [RA]	4.1	1.0
 **96**	1.8	0.44
3 [RA]	5.1	1.0
 **40**	2.2	0.43
3 [RA]	3.3	1.0
 **148**	1.4	0.42

TABLE VII (Continued)

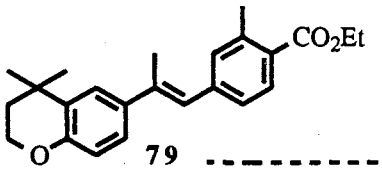
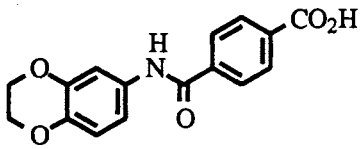
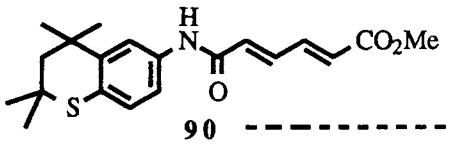
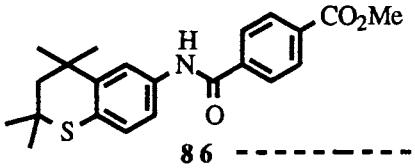
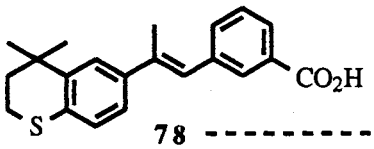
HETEROAROTINOIDS	Ratio Sp. Activity[b]	R[c]
3 [RA]	5.7	1.0
 **79**	2.4	0.42
3 [RA]	3.2	1.0
 **89**	1.3	0.41
3 [RA]	4.1	1.0
 **90**	1.6	0.40
3 [RA]	3.0	1.0
 **86**	1.2	0.40
3 [RA]	4.8	1.0
 **78**	1.9	0.40

TABLE VII (Continued)

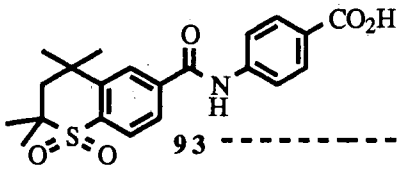
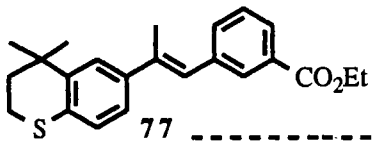
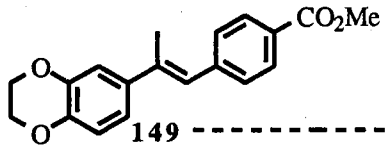
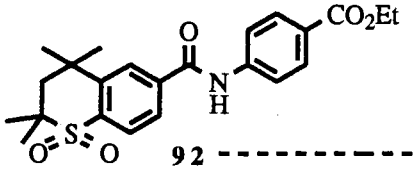
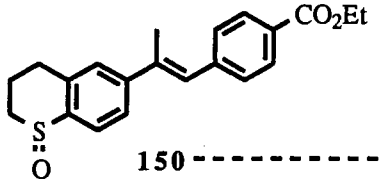
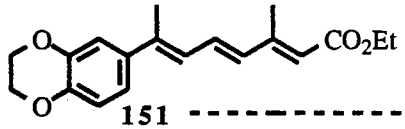
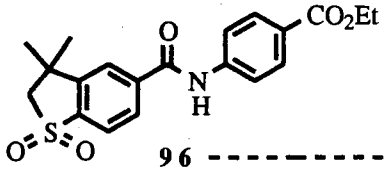
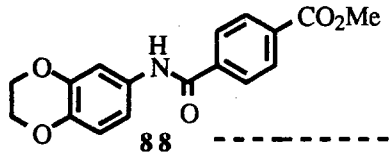
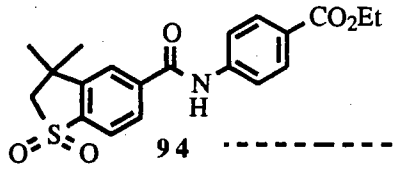
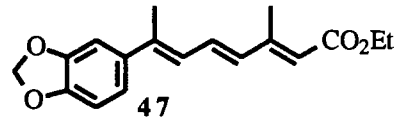
HETEROAROTINOIDS	Ratio Sp. Activity[b]	R[c]
3 [RA]	3.3	1.0
 **93**	1.3	0.39
3 [RA]	5.7	1.0
 **77**	2.0	0.35
3 [RA]	3.0	1.0
 **149**	1.0	0.33
3 [RA]	3.3	1.0
 **92**	1.0	0.30
3 [RA]	3.3	1.0
 **150**	1.0	0.30

TABLE VII (Continued)

HETEROAROTINOIDS	Ratio Sp. Activity[b]	R[c]
3 [RA]	3.0	1.0
 **151**	0.9	0.30
3 [RA]	3.3	1.0
 **96**	0.9	0.27
3 [RA]	3.3	1.0
 **88**	0.9	0.27
3 [RA]	3.3	1.0
 **94**	0.7	0.21
3 [RA]	5.1	1.0
 **47**	1.2	0.23

[a]Reference 68

[b]Activity ratio = Specific activity (dpm/mg/hr) of test compound/Specific activity (dpm/mg/hr) of control RA (3). [Dpm = Disintegration/min].

[c]Activity ratio of test heteroarotinoid/activity ratio of RA (3).

TABLE VIII

HETEROAROTINOIDS: DECREASING BINDING POTENCY WITH SPECIFIC HUMAN RETINOIC ACID RECEPTORS[a]

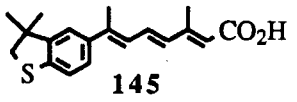
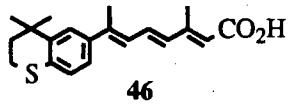
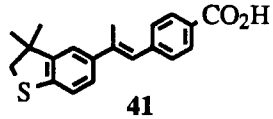
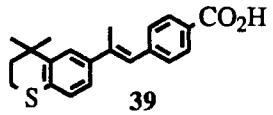
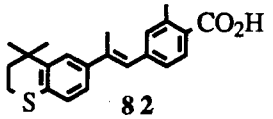
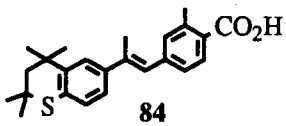
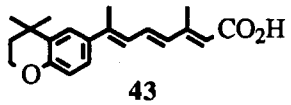
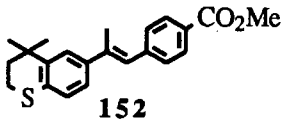
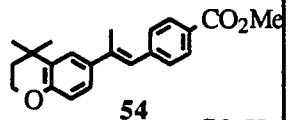
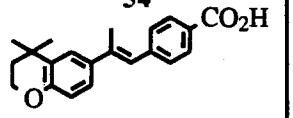
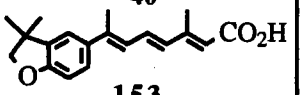
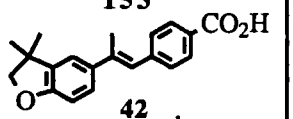
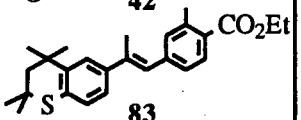
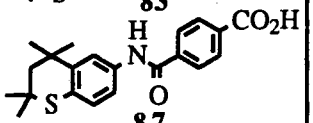
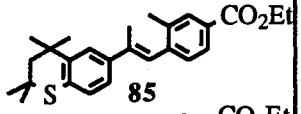
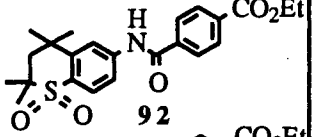
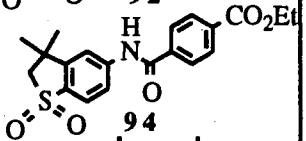
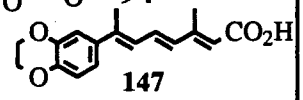
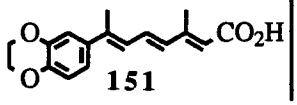
Heteroarotinoids	Efficacy %[b]	Potency (n*M*)[c]	9-*cis*-RA (**8**) Potency (n*M*)	Receptor[d]
 **145**	66 77 89	7.5 1200 1000	37 74 210	γ β α
 **46**	106 99 103	12 43 1400	28 47 340	γ β α
 **41**	34 44 22	18 30 420	47 28 340	β γ α
 **39**	53 60 46	21 57 220	83 17 320	β γ α
 **82**	65 96 64	23 60 210	50 53 200	β γ α
 **84**	47 68 141	33 43 640	320 83 17	α β γ
 **43**	111 107 92	39 72 740	28 47 340	γ β α
 **152**	65 45 62	84 110 620	53 50 200	γ β α

TABLE VIII (Continued)

Heteroarotinoids	Efficacy %[b]	Potency (n*M*)[c]	9-*cis*-RA (**8**) Potency (n*M*)	Receptor[d]
 **54**	72	140	50	β
	71	330	53	γ
	73	1100	200	α
 **40**	88	190	50	β
	76	200	53	γ
	76	1100	200	α
 **153**	93	230	37	γ
	77	770	74	β
	96	800	210	α
 **42**	50	320	47	β
	40	460	28	γ
	32	2300	340	α
 **83**	52	300	50	β
	76	420	53	γ
	33	890	200	α
 **87**	25	400	320	α
	29	870	83	β
	29	960	17	γ
 **85**	01	--	320	α
	42	2300	83	β
	36	390	17	γ
 **92**	02	–	67	α
	01	–	87	β
	06	–	107	γ
 **94**	32	1600	170	α
	15	--	730	β
	35	2000	160	γ
 **147**	02	--	170	α
	04	--	730	β
	06	--	160	γ
 **151**	02	--	170	α
	01	--	730	β
	23	32	160	γ

[a]Reference 3.

[b]Maximal response observed relative to that of RA (**3**) at 10^{-5} *M*.

[c]Potency [EC_{50} = concentration of heteroarotinoid to prduce 50% of the maximal observed response of RA (**3**)] values for both reference compound [9-*cis*-RA (**11**)] and heteroaortinoids were calculated.

[d]Human retinoic acid receptors RAR–α, RAR–β and RAR-γ.

Evaluation of the Effect of Heteroarotinoids on TGase activity

In a recent study it was shown that the differentiation of HEL (human erythroleukemia) cells by RA was accompanied by an increase in tissue concentration of a certain enzyme transglutaminase (see Historical for details).[68] RA (**3**; 10 μM) stimulated differentiation of the HEL-60 cell line, and this was accompanied by a simultaneous *nine-fold* increase in transglutaminase activity.[68] Thus, it appears that transglutaminase can be used to predict the response of human myeloid leukemia cells to RA (**3**). In a series of tests performed in our work using heteroarotinoids, it was shown that heteroarotinoids, like RA (**3**), increased TGase activity. The results of the TGase assay are shown in Table VII with the effect of each heteroarotinoid being compared to that of all RA (**3**).

In the transglutaminase assay, HEL cells were grown in McCoy's medium 5a supplemented with 10% bovine serum to a density of 1 x 10^6 cells/mL and then split to either 1 or 2 x 10^5 cell/mL, respectively. RA (**3**) or the test heteroarotinoids were added 24 h after subculture from a 10 mM stock solution prepared in 100% ethanol, stored at -20°C.[68] The cultures were covered from aluminum foil to protect from the light and were treated again on days 3, 4, 5, 6, and 7 with medium. The concentration of RA (**3**) was 10 μ*M*. The HEL cells were then pelleted by centrifugation for ten minutes at 5000 x g, washed once with Tris-HCl (20 m*M*), NaCl (150 m*M*), and EDTA (1 m*M*) at a pH of 7.5. The cells were resuspended in the same solution containing 0.5 m*M* phenylmethylsulfonyl flouride, disrupted by a 10-s sonication, and the cytosol fraction was prepared by centrifugation 100,000 x g for 1 h.[68] TGase activity was measured by the incorporation of radioactive (^{14}C) peutrescine into *N,N*-dimethylcasein.[68] One unit of enzyme activity was defined as 1 nmol of peutrescine incorporated in 20 min/mg of protein at 37°C.[68]

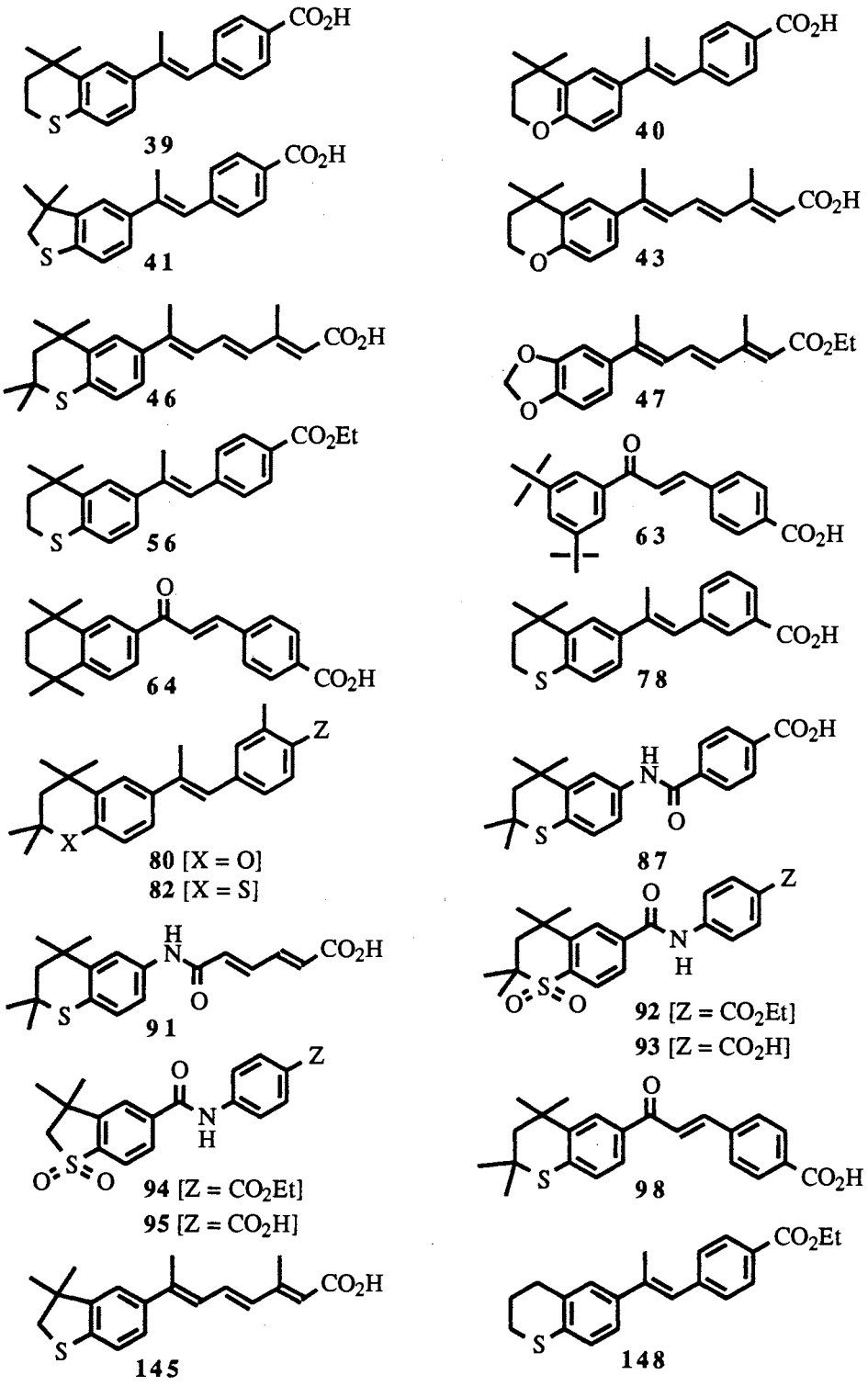
The most active heteroarotinoid was amide **87** [76% of RA (**3**) effect, page 53]. The amide may exist primarily in the *trans* form, which has been suggested[41] to be the most active form of heteroarotinoids with amide groups. The compound contained an *amide function [-NHC(O)-]*, two sets of $(H_3C)_2C$ groups on the six membered ring and a

carboxylic acid group on the aromatic ring (see Table VII, page 53). It is possible that the orientation and the distance between the dimethyl group on the benzylic carbon and the acid function on the aryl group in the side chain may be critical for the activity of retinobenzoic acids possessing amide linking groups.[41] Interestingly, when the acid function of amide **87** was esterified (**86**, Table VII), the TGase activity *decreased* to 40% of the activity of RA (**3**). Possibly there is a lack of esterases in the TGase preparations making conversion of ester to acid real slow.

Among the ten most active heteroarotinoids (in TGase assay, Table VII), eight heteroarotinoids were sulfur-containing heterocycles (**39**, **46**, **56**, **81**, **82**, **87**, **91**, and **98**) out of which four heteroarotinoids (**46**,**87**, **91**, and **98**) had two sets of $(H_3C)_2C$ groups as part of the six-membered ring. Most of the heteroarotinoids have a carboxylic acid group on the terminal end and only two heteroarotinoids (**56** and **81**) contained an ester function. Seven heteroarotinoids (**39**, **56**, **80-82**, **87**, and **98**) had aromatic rings in the side chain, and three heteroarotinoids (**46**, **43**, and **91**) had polyunsaturated side chains. Only two oxygen-containing heteroarotinoids (**43** and **80**) had TGase activity greater than 55% of the activity of RA (**3**). The results support the theory that sulfur-containing heteroarotinoids (especially acids) may be better potential mimics of RA (**3**) in terms of some biological activity in this assay.

Although most of the active heteroarotinoids have carboxylic acid functions, heteroarotinoid **56** was an exception (67% active) in that an ethyl ester function was present. Ester **56** was more active than the corresponding acid **39** (59% active) which was somewhat unusual. Modification of the aryl ring of heteroarotinoid **40** with a methyl group on the ortho position (to the CO_2H), as in **80**, increased the TGase activity from 43% to 61%.

In the sulfur analog **39** (59% activity), the TGase activity remained nearly constant when a methyl group at the *ortho* position [**82** (58% activity)] was present. Characterization of heteroarotinoids **41**[23] and **143**[67] (Table VII) via X-ray crystal

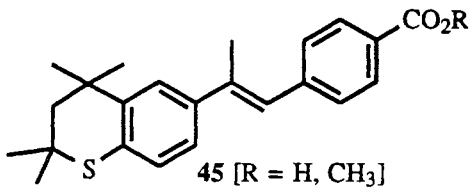


analysis revealed that the aryl rings of the heteroarotinoids were twisted out of plane and lacked coplanarity with the central double bond in the solid state. Thus the presence of a

methyl group could influence the orientation of aromatic ring (force the aryl ring into a plane with the central double bond) of the heteroarotinoid at the receptor binding site and thus allow the heteroarotinoid to mimic RA (**3**). This concept might be supported by comparing the crystal structure of heteroarotinoid **80** or **82** with that of RA (**3**). It is planned to obtain crystallographic data for **80** and **82** and see if such 'fits' the structure of RA (**3**) via molecular modeling techniques. Changing the position of the CO_2H group from the *para*-position (as in heteroarotinoid **39**) to the meta position (as in **78**) decreased the TGase activity from 59% to 40%. Introduction of the methyl group at the *meta*-position to the CO_2H group, as in **85**, also decreased the activity (44%) compared to **56** (67%).

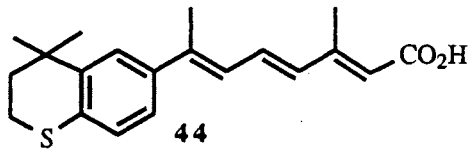
The polyene side chain derivatives **43**, **46**, and **91** had moderate activities (58%, 63% and 60%, respectively). Heteroarotinoid **91** (60% active) contained an amide group linking the aromatic ring and a polyene side chain. Inclusion of the amide group (spacer unit that could give more flexibility at the receptor site) allowed retention of activity compared to **46** which contains a polyene side chain without an amide group. Unlike in the RAR binding studies (discussed later) where many five-membered heteroarotinoids (like **41** and **145**) possess high affinity for receptors, TGase activity of the five-membered heteroarotinoids are low (**41**-47% activity and **145**-49% activity). Oxidation of the heteroatom, namely sulfides to the sulfones, destroyed the activity of the heteroarotinoids in both five- and six-membered amides **92-95** as shown in Table VII.

Previous activity studies on chalcone derivatives (like **63** and **64**, Table VI) revealed that the presence of a heteroatom in the six-membered ring greatly reduced retinoidal properties.[41] However, judging from the effect of the chalcone derivative **98** (Table VII) on transglutaminase activity [60% of RA (**3**) activity], it is possible that the *two* sets of $(H_3C)_2C$ groups on the six-membered ring cause a significant increase in hydrophobicity, and this might be key for the enhanced effect of the compound. The additional methyl groups may also exert a steric factor for improved binding to a receptor site. For example, it has been previously observed that the relative **45** was very active in the ODC assay.[66]

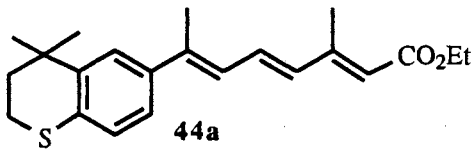


45 [R = H, CH_3]

Three other heteroarotinoids with two sets of dimethyl groups on the six-membered ring namely, **87** (amide, 76%), **91** (amide with a polyene side chain, 60%), and **46** (polyene side chain, 63%) also exhibited an effect on TGase activity. Thus, in heteroarotinoids with less rigid structures, as in **46**, **87**, **91** and **98** (Table VII), it appears that the presence of sulfur and two methyl groups on the carbon alpha to sulfur may be significant structural features for maximum activity. When the five- of six-membered rings were devoid of any methyl groups, as in **47** [the $(CH_3)_2C$ groups in a five-membered ring were replaced by two oxygen atoms; 23% activity), **148** (the dimethyl groups in a six-membered ring were replaced by hydrogens; 30% activity) and **149** [the $(CH_3)_2C$ groups in a six-membered ring were replaced by two oxygen atoms; 33% activity), the TGase activity *diminished*. Heteroarotinoid **44** [56% TGase activity of RA (**3**)] and the corresponding ethyl ester derivative **44a** (yet to be tested for TGase activty) were extremely



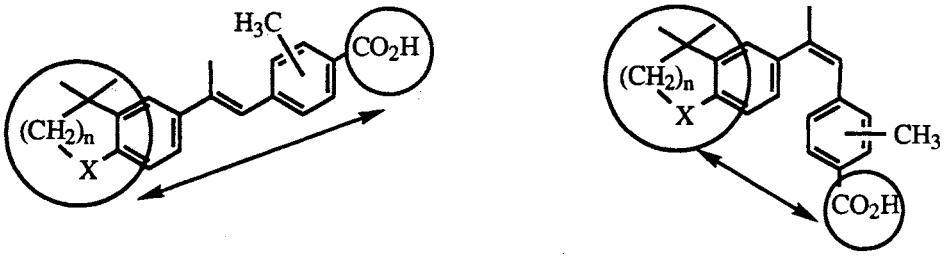
44



44a

active in the ODC assay.[74] Heteroarotinoid **44a** was 100% active in the ODC assay compared to 11-cis-RA (**6**) which was 89% active, and heteroarotinoid **44** was 89% active in the ODC assay [compared to 11-cis-RA (**6**); 89% active]. Although ester **44a** was more active than the corresponding acid **44**, both the heteroarotinoids had six-membered sulfur-containing fused rings with geminal dimethyl groups and a polyene side chain. This also suggests that the presence of a geminal dimethyl group on the five- or six-membered ring is also essential for activity.

In summary, two of the biologically active isomeric heteroarotinoid systems shown below have have special structural features. For example, the partially saturated ring containing a heteroatom is encircled as is the carboxyl group, since these groups appear important. The distances between between the geminal dimethyl groups and the carboxyl group or between the heteroatom and carboxyl group may also be very relevant for useful



activity. Increasing the flexibility of the heteroarotinoids by the introduction of the spacer units like *-NHC(O)-* and *-CH=CHC(O)-* increased the activity of the heteroarotinoids and may be quite important for activity. Taken together, data from such assays as ODC, TOC, HL-60 and TGase measurements could provide insight as to structural requirements for very active synthetic retinoids.

Evaluation of the Heteroarotinoids in Receptor Capability

Several heteroarotinoids were tested for both agonist (capacity to bind with receptors and produce an effect) and antagonist (possess affinity for receptors without intrinsic activity or efficacy) activity. Agonist activity was examined in the presence of the test compound alone. Antagonist activity was determined by incubating the test heteroarotinoid in the presence of the appropriate reference agonist, RA [**3**, EC_{50} concentration (EC_{50} = concentration of heteroarotinoid to prduce 50% of the maximal observed response of RA (**3**)]. If the sample possessed agonist properties, it enhanced gene expression.[3] If the sample contained antagonist activity, the response was lower than the appropriate control (agonist alone).[3] CV-1 African green monkey cells were cultured in Dulbecco's modified

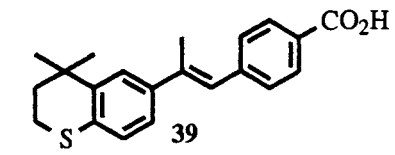
Eagle's medium (DMEM) supplemented with 10% charcoal resin-stripped fetal bovine serum and then transferred to 96-well microtiter plates one day prior to transfection. Heteroarotinoid activity was normalized relative to that of RA (**3**) and was expressed as potency (EC_{50}) which was the concentration of heteroarotinoid required to produce 50% of the maximal observed response of RA (**3**). The agonist efficacy was based upon maximal activation compared to the reference compound RA (**3**). The antagonist efficacy (%) was a function of the ability of the heteroarotinoid to cause maximal inhibition [maximal response observed relative to that of RA (**3**)]. A control curve using 9-*cis*-RA (**8**) was included for each compound by Ligand Pharmaceuticals Incorporated.[3]

The fourteen most active heteroarotinoids [**39-43**, **46**, **54**, **82-84**, **87**, **145**, **152**, and **153**, with respect to human receptor binding, in comparison with 9-*cis*-retinoic acid (9-*cis*-RA) tested by Ligand] are listed in Table VIII. Among theese active compounds, eleven (**39-43**, **46**, **82**, **84**, **87**, **145**, and **153**) were acids (a carboxylic acid group in the aryl side chain) and nine were heteroarotinoids (**39**, **41**, **46**, **82-84**, **87**, **145**, and **152**) with sulfur-containing rings (five- or six-membered). Twelve out of the fourteen heteroarotinoids were RAR-β (**39-42**, **54**, **82**, and **83**) or RAR-γ (**43**, **46**, **145**, **152**, and **153**) specific, and only two heteroarotinoids (**84** and **87**) were RAR-α specific. With the identification of RAR-γ receptors, transcription activation studies suggested that of the three receptors, RAR-γ might have the highest affinity for RA (**3**), and RAR-α might have the lowest affinity for RA (**3**) in the system examined.[52]

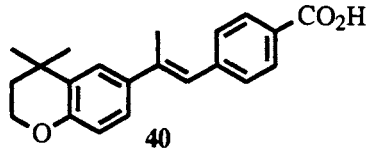
Heteroarotinoid **145** (Table VIII) with a five-membered, sulfur-containing ring and a long unsaturated side chain with acid functionality *exhibited very high specificity for RAR-γ receptor*. The *second* most active compound in terms of receptor binding was **46** (Table VIII) which was similar to **145** in structure (unsaturated long chain with acid functionality). However, a sulfur atom is present in a six-membered ring in **46** while **144** had a five-membered ring. Heteroarotinoid **46** was also somewhat RAR-γ receptor specific and also exhibited an affinity for RAR-β receptor greater than **145**.

Heteroarotinoid **41** also had good β-binding specificity and, to some degree like **145**, exhibited some RAR-γ specificity. However, **41** differs from **145** in that the former contained a five-membered ring with a sulfur atom as well as a carboxylic acid group in the *aryl* side chain. Heteroarotinoids **39** and **82** contain six-membered fused ring systems and an aryl group with a CO_2H function on the *para*-position. The aryl ring on the side chain of heteroarotinoid **39** was modified to give **82** by incorporating a methyl group on the *ortho*-position to functional group (CO_2H). Both heteroarotinoids **39** and **82** displayed strong trancriptional activity (stimulated RNA synthesis that occurs on DNA template) for the RAR-β receptor.

From the receptor binding data, it appears that heteroarotinoids with a *long unsaturated side chain* (**41**, **43**, **52** and **153**) exhibit (Table VIII) a *marked affinity towards RAR-γ receptor binding* [similar to RA (**3**)]. *Heteroarotinoids with an aromatic side chain (**39-41**, **54**, **82**, **83** and **152**) exhibit specificity towards RAR-β receptors.* Heteroarotinoid **84** seemed to be an exception to the above generalization. Heteroarotinoid **84**, with a six-membered sulfur-containing heterocycle with two geminal dimethyl groups and a modified aryl side chain (with CO_2H function), was surprisingly RAR-α specific unlike the other heteroarotinoids (such as **39-41**, **54**, **82**, **83** and **152**) with similar structures. Even the ester analog **83** of acid **84** was RAR-β specific (although to a moderate extent) and *not* RAR-α specific. When oxygen was incorporated into the ring instead of sulfur, the activity of the heteroarotinoids *decreased.* For example, although heteroarotinoid **43** (oxygen analog of **46**) was RAR-γ specific (just like the sulfur analog, **46**), the affinity of **46** for the RAR-γ receptor was much higher (approximately three orders of magnitudes) compared to the affinity of **43** for RAR-γ. The activity of **145** dropped drastically when the sulfur atom was replaced by an oxygen atom as in **153**. However, the receptor subtype specificity (RAR-γ) remained the same for **145** and **153**. A similar decrease in potency was observed when the sulfur-containing **39** was replaced by an oxygen-containing **40** (higher concentration of **40** was required to achieve maximum binding).



39



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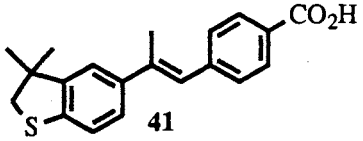

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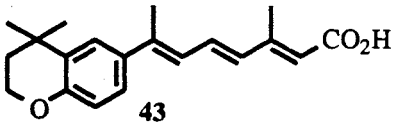

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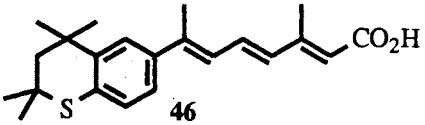

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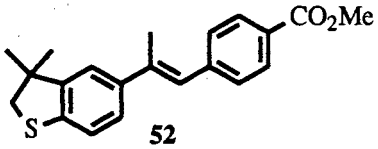

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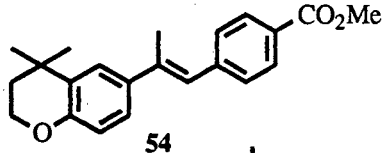
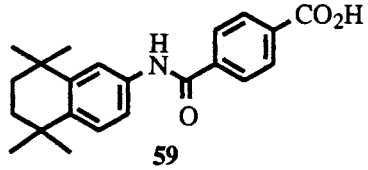
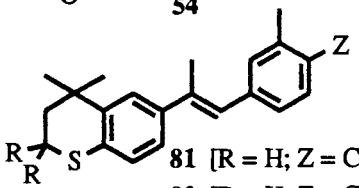


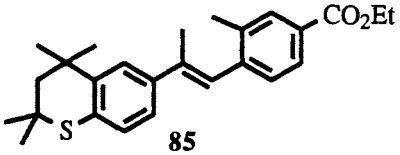
54



59



81 [R = H; Z = CO_2Et];
82 [R = H; Z = CO_2H]
83 [R = CH_3; Z = CO_2Et];
84 [R = CH_3; Z = CO_2H]



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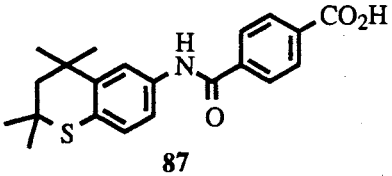

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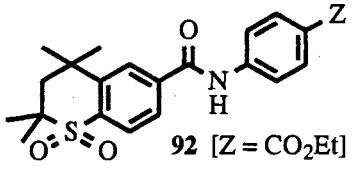
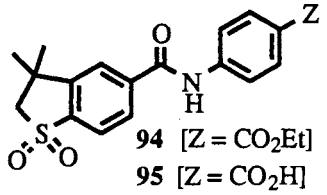
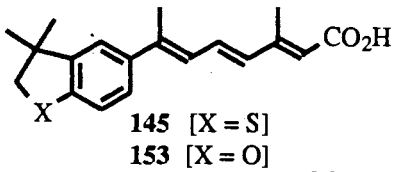


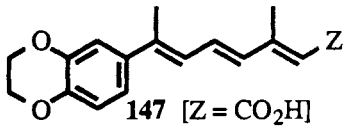
92 [Z = CO_2Et]
93 [Z = CO_2H]



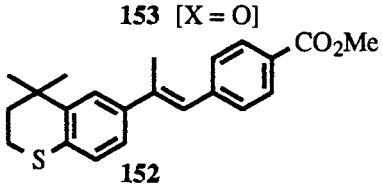
94 [Z = CO_2Et]
95 [Z = CO_2H]



145 [X = S]
153 [X = O]



147 [Z = CO_2H]
151 [Z = CO_2Et]



152

The acid form of the heteroarotinoids was the most active form for all except heteroarotinoid **40**. The affinity of heteroarotinoid **54**, the methyl ester of acid **40**, for the

RAR-β receptor, was slightly higher than that of acid **40**. However, acid **40** displayed a greater binding abilities for the RAR-γ receptor than did ester **54**.

Previous studies[4] revealed that arotinoid **59**[40] (Table VI) with amide bonds between the aryl rings (like in **87** Table VIII) was RAR-α receptor specific. Although retinobenzoic acid **87** did not exhibit high binding affinity towards any of the receptors, the affinity for RAR-α (consistent with the reported observation)[4] was much higher than that for RAR-β and RAR-γ. The presence of the geminal dimethyl groups seemed to be required for activity. Replacing the $(H_3C)_2C$ groups with a oxygen can destroy the binding activity, as was true for **88** (not included in Table VIII). Likewise, heteroarotinoids **147** and **151** also did not exhibit any binding ability.

Incorporation of the methyl groups on the aromatic ring of the side chain resulted in varying binding abilities. For example, both heteroarotinoids **82** and **84** have a methyl group incorporated on the *ortho*-position (to CO_2H function). As discussed above, **82** was RAR-β specific and **84** was RAR-α specific, and the *binding potency* was comparable to that of acid **39** (RAR-γ specific) which had exhibited lower toxicity and cell differentiation abilities in other test assays.[13,74] Introduction of the methyl group in the *meta*-position (ester **85**) decreased the activity or the affinity for the RARs, although it may be due to the presence of a very small amount of the Z-isomer of **85** in the sample.

Introduction of a spacer unit influenced binding ability of heteroarotinoids. Unlike most of the heteroarotinoids, amide **87**, containing the -NHC(O)- spacer unit, was RAR-α specific. Oxidation of the heteroatom (as in sulfone **92**) resulted in the inactivation of the heteroarotinoids towards receptor binding. The binding specificity studies of the heteroarotinoids **96** and **97** (reverse amide group) and that of amides **90** and **91** (long chain containing amides) are yet to be completed.

Receptor Specificity and Biological Activity

The ability of retinoids to prevent carcinomas (of head and neck) in patients and to induce complete remission of acute leukemia with RA (**3**) were demonstrated in two recent studies.[35,36] Although some retinoids are potent anticancer drugs, the undesirable toxicity associated with the long term treatment and high doses of retinoids have caused a serious problem in retinoid therapy.[46] With the identification receptor-specific retinoids and tissue distribution of the receptors, retinoid therapy can potentially target specific tissue disorders efficiently and possibly induce a correction of an abnormality with a minimum of undesirable side effects.

The exact tissue distribution of the RARs and RXR is still under investigation.[52] However, it has been reported that in adult rodents and humans RAR-α is ubiquitous,[52] RAR-β is found in heart, lung and spleen,[52] and RAR-γ is confined to lung and skin.[52] The biological effects associated with the binding of retinoids to specific receptors are not yet established although it appears that the agent binding with RAR-γ could target skin disorders, bone growth and development. In developing vertebrate limb, RAR-γ expression appears to be restricted to precartilagenous condensations that could eventually form bone models. Retinoid effects on tracheal epithelium (and other epithelial tissues) seems to be mediated through RAR-β expression. Recently it was speculated that RAR-α might be involved in the pathogenesis of acute promyelocytic leukemia, and thus retinoid differentiation of promyelocytes can be mediated through RAR-α expression.[52] The exact retinoidal effects mediated through the RXR subtype receptor is not known. However, the discovery that RXR-α specifically binds to 9-*cis* RA (**8**) indicates that retinoid isomerization may be vital for regulating certain target tissue responses by retinoids.[32]

Suggestion for Future Work

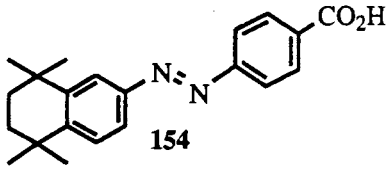
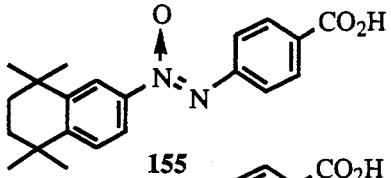
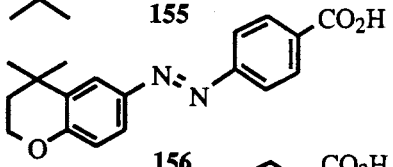
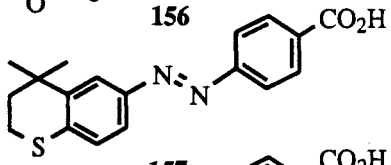
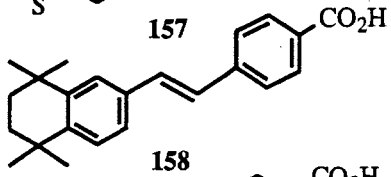
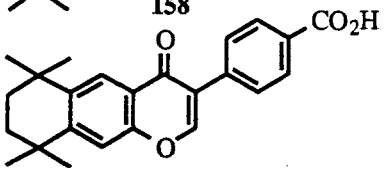
Retinoids **154-159** shown in Table IX exhibited high differentiation inducing activities [compared to RA (**3**), in HL-60 cell line].[39] Retinoid **155**, which is the N-oxidized version of retinoid **154**, was close to 2000 times more active than RA (**3**). Introduction of the heteroatom in a six-membered ring of the azo-retinoid *decreased* the activity of the resulting hetero-aza-retinoids (**156** and **157**) relative to RA (**3**) activity.[39] The loss in hydrophobicity due to the substitution of a carbon atom by a heteroatom and the resulting change in tissue distribution could be factors in the decrease in activity. Thus hereroarotinoids like **160** with an azo spacer unit and with two geminal dimethyl groups on the fused ring system could be potential targets.

Heteroarotinoid **160** is suggested for future work since it contains a geminal dimethyl group on the carbon adjacent to the heteroatom (X = O or S), and this could compensate for the loss of hydrophobicity [as observed by the TGase activity of modified chalcone derivative **98** (60%, Table VII)]. Similarly imine **161** could be a potential target. Shudo and co-workers noted that the presence of the vinyl methyl group was not required for activty as shown by stilbene derivative **158** which was 260 times more active than RA (**3**).[39] Imine **161** is a partial combination of azaretinoid **157** (contains the a double bonded nitrogen atom) and stilbene-retinoid **158** (contains a double bonded carbon atom without the methyl group on it). The presence of the heteroatom in imine **161** [X = O, S, etc] could result in increased hydrophilicity and thus reduced toxicity. Imine **161** could also be a potential anticancer agent.

Recently it was discovered that RXR-α specifically binds 9-*cis*-RA (**8**).[49] Thus, retinoid isomerization may be vital for regulating target tissue responses for retinoids. The importance of the 'cisoid' form for activity has led to the development of synthetic retinoids (like flavone dervative, **159**) that bear structural resemblance to 9-*cis* -RA (**8**). Retinoid **159** was highly active in the HL-60 assay [27 times more active than RA (**3**)].[41] Shudo and coworkers suggested that the 'cisoid' conformation of the molecule could have

TABLE IX

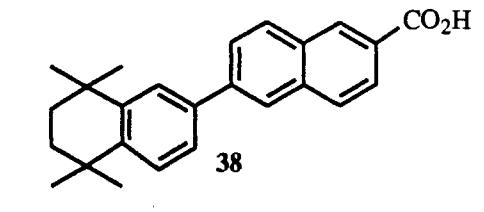
HL-60 DIFFERENTIATION-INDUCING ACTIVITIES OF SELECTED RETINOIDS[a]

Retinoid	ED_{50}, M[b]	Relative[c] Activity
3 [RA]	2.4×10^{-9}	100
 **154**	1.7×10^{-9}	130
 **155**	2.5×10^{-10}	1960
 **156**	1.3×10^{-7}	3.5
 **157**	2.1×10^{-7}	2.1
 **158**	5.0×10^{-10}	260
 **159**	4.6×10^{-11}	127

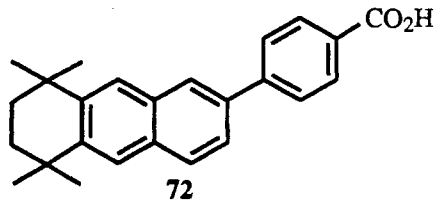
[a]Reference 39

[b]ED_{50} is the molarity of retinoid required to effect cell differentiation in 50% of the cell cultures.

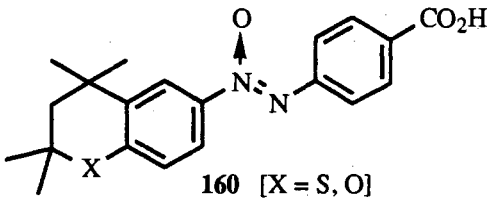
[c]The ratio of ED_{50} of RA (**3**)/ED_{50} of retinoid.



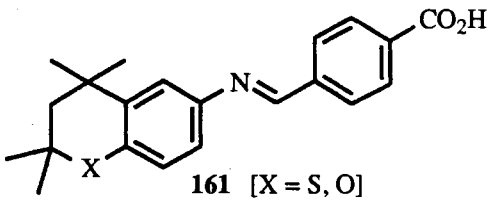
38



72



160 [X = S, O]



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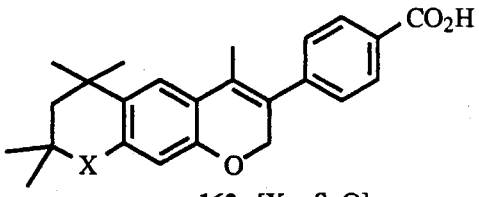
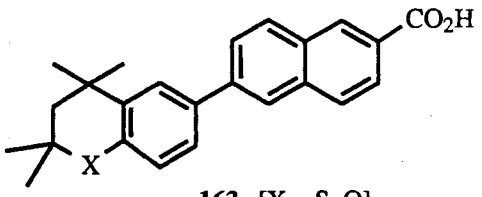
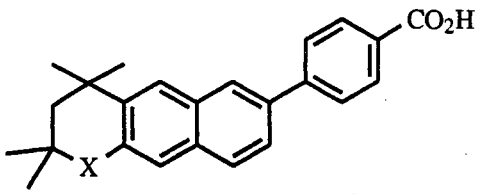


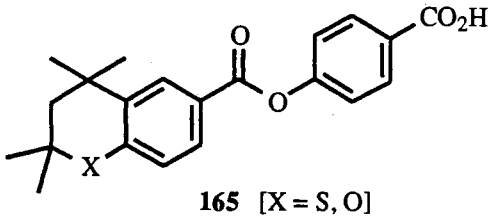
162 [X = S, O]



163 [X = S, O]



164 [X = S, O]



165 [X = S, O]

contributed to the high activity of **159**.[41] Heteroarotinoid **162** (suggested for future work) is locked in the 'cisoid' form and contains a heteroatom that would increase the hydrophilicity. Polyaromatic heteroarotinoids, **163** and **164** could also be potential targets on the basis of the 'cisoid' conformation. Retinoids **38** and **72,** similar to the proposed heteroarotinoids **163** and **164** but without the heteroatom, were found to be more active than RA (**3**) in the ODC assay.[56]

Since the introduction of the spacer unit [-NHC(O)- and CH=CHC(O)-] appeared to have an effect on the TGase activity, incorporation of an ester group [-C(O)O-] as the linking unit is worthy of consideration. Heteroarotinoid **165**, for example with the ester unit possesses a degree of conjugation and possibly a certain amount of flexibility at the receptor site and thus could be a potential target.

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 information was obtained on solutions ($DCCl_3$ or DMSO-d_6) using a Varian XL-300 spectrometer with ^1H and ^{13}C data being collected at 299.99 MHz and 75.4 MHz, and on a Varian XL-400 BB spectrometer with ^1H and ^{13}C data being taken at 399.99 MHz and 100.5 MHz. References were to TMS in δ values or ppm, respectively. Data are reported as follows: chemical shifts (in δ value or ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and bs = broad singlet), coupling constants (in Hz), and assignments. IR spectra were recorded on a Perkin-Elmer 681 spectrophotometer as films or in KBr pellets while UV data were obtained on samples in 95% ethanol on a Varian DMS 200 UV-Visible spectrophotometer [equipped with an Epson LX-800 professional computer printer]. Mass spectral data were recorded on a VG analytical instrument model, ZAB-2SE. Melting points were determined with a Fischer-Johns melting point apparatus and a Thomas-Hoover melting point apparatus and are uncorrected. High temperature melting points were obtained on a Electrothermal Series IA 9100 Digital melting point apparatus and are uncorrected.

Reagent grade solvents were used without further purification and chromatography was performed with the aid of Chromatotron (Harrison Research, model 7924) and using silica gel (pF 254 containing gypsum, EM science) plates (2 mm and 4 mm thick). All elemental analyses were performed by Galbraith laboratories, Knoxville, TN 37921. The

following reagents were obtained commercially, and all liquid reagents were freshly distilled prior to use: 2, 4-dimethylbenzoic (mp 124-126°C, Aldrich), acetic anhydride (bp 138-1406°C, Aldrich), acetic acid (glacial, Fisher), *n*-butyl lithium (10 *M*, Aldrich), lithium aluminum hydride (mp 125°C dec., 95% +, Aldrich), $TiCl_3$/HCl (12 wt. % $TiCl_3$ solution in 21 wt. % HCl, Aldrich), *mono*-methyl terephthalate (mp 221-222°C, Aldrich), thionyl chloride (bp 79°C, Eastman) 1,4-benzodioxan (bp 103°C/6 mm, Aldrich), acetyl chloride (bp 52°C, Aldrich), aluminum chloride (Fisher), Clorox® (sodium hypochlorite 5.25%), ethyl 4-aminobenzoate (mp 88-90°C, Aldrich), DMAP (mp 108-110°C, Aldrich), DMF (bp 74°C/35 mm, EM Science), *p*-toluic acid [mp 180-182°C, Aldrich], *m*-toluic acid (mp 108-110°C, Aldrich), chromium (VI) oxide (mp 196°C (dec.), 99% +, Aldrich), 3-methyl-4-nitrobenzoic acid (mp 216-218°C, Aldrich), stannous (II) chloride dihydrate (Fisher), sodium nitrite (Fisher), sodium acetate trihydrate (Mallinckrodt), paraformaldehyde (Aldrich), hydroxylamine hydrochloride (mp 151-152°C, Sigma), sodium sulfite (anhydrous, Eastman), copper sulfate pentahydrate (Fisher), diazald (99%, Aldrich), and muconic acid [mp 290°C (dec.), Lancaster]. TLC analyses were performed using Kodak Chromagram-13181 silica gel sheets with fluorescent indicator.

Ethyl (*E*)-3-[(2,3-Dihydro-4,4-dimethyl-2*H*-1-benzothiopyran-6-yl)-1-propenyl] benzoate [(*E*)-77]. A solution of *n*-butyllithium in hexane (10 M, 0.72 mL, 7.25 mmol) was added dropwise (5 min, N_2) to a stirred suspension of the white phosphonium salt **100** (3.3 g, 6.03 mmol) in ether (20 mL dried over sodium ribbon) in a 100-mL, three-necked, round-bottomed flask fitted with an addition funnel, spiral condenser, and a magnetic stirrer. The resulting reddish-brown mixture was cooled to -78°C, (dry ice, acetone, 10 min), and a solution of ethyl 3-formylbenzoate (**108**, 0.98 g, 5.50 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 (triphenylphosphine oxide) was washed with ether (100 mL). The filtrate was dried (Na_2SO_4, 4 h), 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. The solvent system used to separate the starting materials from (*E*)-**77** and (*Z*)-**77** isomers was hexane:ether [8:2]. The last fraction obtained was concentrated to give 1.01 g (2.76 mmol, 50 %) of a mixture of esters [10:1, (*E*)-**77**:(*Z*)-**77**] which was then treated with boiling ethanol (95%, 3 mL). 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 0.45 g (1.23 mmol, 25%) of needle-like crystals of ester (*E*)-**77**; mp 72.5-74°C. IR (KBr) 1725 (C=O), cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.39 [s, 6 H, $C(CH_3)_2$], 1.40 [t, 3 H, OCH_2CH_3], 1.97 [t, 2 H, SCH_2CH_2], 2.26 [s, 3 H, $CH=CCH_3$], 3.05 [t, 2 H, SCH_2CH_2-], 4.40 [q, 2 H, OCH_2CH_3], 6.80[s, 1 H, vinyl-H], 7.11 [d, 1 H, Ar-*H*], 7.21 [d, 1 H, Ar-*H*], 7.43 [m, 2 H, Ar-*H*], 7.52 [d, 1 H, Ar-*H*], 7.92 [d, 1 H, Ar-*H*], 8.04 [s, 1 H, Ar-*H*]. ^{13}C NMR ($DCCl_3$) ppm 14.33 [OCH_2CH_3], 17.30 [$CH=CCH_3$], 23.01 [CH_2], 30.22 [$C(CH_3)_2$], 30.70 [$C(CH_3)_2$], 37.71 [SCH_2], 60.87 [OCH_2CH_3], 123.70, 124.02, 125.58, 126.43, 127.38, 128.14, 130.18, 130.42,

131.16, 138.41, 138.63, 139.39, 141.78 [Ar*C* and viny-*C*] and 166.66 [*C*O_2Et]. Mass spectral (EI) data Calcd for $C_{23}H_{26}O_2S$ m/z (M^+): 366.16534; Found: 366.1660. Anal.for $C_{23}H_{26}O_2S$: C, 75.37; H, 7.15. Found: C, 75.13; H, 7.20.

(*E*)-3-[(2,3-Dihydro-4,4-dimethyl-2*H*-1benzothiopyran-6-yl)-1-propenyl] benzoic Acid [(*E*)-78]. In a 2-necked, 50-mL, round-bottomed flask (N_2) fitted with a spiral condenser and a magnetic stirrer was placed ester (*E*)-**77** (mp 72.5-74°C, 0.25 g, 0.54 mmol), ethanol (95%, 10 mL), water (10 mL), and NaOH (0.76 g, 1.9 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 (5 mL, 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, 85°C/0.2 mm Hg) to give acid (*E*)-**78** (0.66 g, 0.47 mmol, 87%) as a dry white powder; mp 217-218°C. IR (KBr), 3440 [C(O)O-H], 1690 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.34 [s, 6 H, C(C*H*$_3$)$_2$], 1.91 [t, 2 H, SCH$_2$C*H*$_2$], 2.22 [s, 3 H, CH=CC*H*$_3$], 3.03 [t, 2 H, SC*H*$_2$CH$_2$-], 6.93[s, 1 H, vinyl-*H*], 7.05[d, 1 H, Ar-*H*], 7.28 [d, 1 H, Ar-*H*], 7.53 [m, 1 H, Ar-*H*], 7.62 [m, 2 H, Ar-*H*], 7.82 [d, 1 H, Ar-*H*], 7.99 [s, 1 H, Ar-*H*]; ^{13}C NMR (DMSO-d_6) ppm 16.94 [CH=C*C*H$_3$], 22.22 [*C*H$_2$], 29.86 [C(*C*H$_3$)$_2$], 32.70 [*C*(CH$_3$)$_2$], 37.18 [S*C*H$_2$], 123.54, 123.94, 125.07, 125.98, 127.14, 128.42, 129.73, 130.65, 133.21, 137.37, 138.10, 138.57, 139.10, 141.62 [Ar*C* and viny-*C*] and 167.23 [*C*O_2H]. Mass spectral (EI) data Calcd for $C_{21}H_{22}O_3$ m/z (M^+): 338.1340; Found: 338.1343. Anal for $C_{21}H_{22}O_3$: C, 78.01; H, 6.91. Found: C, 78.23, H, 6.88.

Ethyl (*E*)-4-[(2,3-Dihydro-4,4-dimethyl-2*H*-1-benzopyran-6-yl)-1-propenyl]-2-methylbenzoate [(*E*)-79]. A solution of *n*-butyllithium in hexane (10 *M*, 0.11 mL, 1.25 mmol) was added dropwise (10 min, N_2) to a stirred suspension of the white phosphonium salt **99** (0.60 g, 1.25 mmol) in ether (25 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 2-methyl-4-formylbenzoate (**112**, 0.22 g, 1.14 mmol) in ether (20 mL) was added dropwise (20 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 (triphenylphosphine oxide) was washed with ether (100 mL). The filtrate was dried (Na_2SO_4, 12 h), and the solvent was evaporated [rotovap, followed by high vacuum (40°C/0.25 mm Hg), 10 min]. The resulting yellow oil was subjected to chromatography on a 4 mm thick plate of silica gel (silica gel pF 254 containing gypsum) with the aid of the Chromatotron. The solvent system used to separate the starting materials from the (*E*)-**79** and (*Z*)-**79** isomers was composed of hexane:ether [9:1]. The last fraction obtained was concentrated to give 0.097 g (0.26 mmol, 23%) of a mixture of esters [9:1, (*E*)-**79**:(*Z*)-**79**] as a clear viscous oil. IR (neat) 1720 (C=O), cm^{-1}. ^1H NMR ($DCCl_3$) δ 1.39 [s, 6 H, $C(CH_3)_2$], 1.40 [t, 3 H, CH_2CH_3], 1.86 [t, 2 H, OCH_2CH_2-], 2.26 [S, 3 H, $CH{=}CCH_3$], 2.64 [s, 3 H, Ar-CH_3], 4.24 [t, 2 H, OCH_2CH_2-], 4.35 [q, 2 H, OCH_2CH_3], 6.75 [d, 1 H, vinyl-H], and 6.80 [d, 1 H, Ar-*H*], 7.22 [m, 3 H, Ar-*H*], 7.41 [s, 1 H, Ar-*H*] and 7.93 [d, 1 H, Ar-*H*]. ^{13}C NMR ($DCCl_3$) ppm 14.37 [OCH_2CH_3], 17.72 [$CH{=}CCH_3$], 21.96 [Ar-CH_3], 30.70 [$C(CH_3)_2$], 31.07 [$C(CH_3)_2$], 37.64 [OCH_2CH_2-], 60.10 [OCH_2CH_2-], 60.72 [CH_3CH_2O], 116.79, 124.48, 124.87, 126.28, 130.56, 131.28, 132.40, 135.76, 139.13, 140.03, 142.30 [vinyl-C and Ar-C], and 166.48 [CO_2R]. Mass spectral (EI) data Calcd for $C_{24}H_{28}O_3$ m/z (M^+): 369.2038;

Found: 369.2049. Anal. Calcd for $C_{24}H_{28}O_3$: C, 79.08; H, 7.74. Found: C, 78.74; H, 7.81.

(*E*)-4-[(2,3-Dihydro-4,4-dimethyl-2*H*-1-benzopyran-6-yl)-1-propenyl]-2-methyl benzoic Acid [(*E*)-80]. In a single-necked, 50-mL, round-bottomed flask (N_2) fitted with a spiral condenser and a magnetic stirrer was placed a mixture of the esters [(*E*)-**79**:(*Z*)-**79**, 9:1; 0.50 g, 1.37 mmol], ethanol (95%, 20 mL), water (5 mL), and KOH (0.76 g, 13.7 mmol). The resulting clear solution was boiled (12 h), cooled slowly to RT (30 min), and then chilled (0°C) with an ice bath. Dropwise addition of conc HCl (20 mL, pH 2) resulted in the formation of a white solid. This white solid was acid (*E*)-**80** (0.34 g, 72%) which was recrystallized (95% ethanol) to give colorless crystals; mp 167-169°C. IR (KBr) 3500 [C(O)O-H], 1725 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.34 [s, 6 H, C(C*H*$_3$)$_2$], 1.79 [t, 2 H, OCH$_2$C*H*$_2$-], 2.23 [S, 3 H, CH=CC*H*$_3$], 2.56 [s, 3 H, Ar-CH$_3$], 4.16 [t, 2 H, OC*H*$_2$CH$_2$-], 6.79 [bs, 1 H, vinyl-H], 6.72 [d, 1 H, Ar-*H*], 7.25 [m, 3 H, Ar-*H*] 7.51 [s, 1 H, Ar-*H*] and 7.86 [d, 1 H, Ar-*H*]. ^{13}C NMR ($DCCl_3$) ppm 17.31 [CH=C*C*H$_3$], 21.40 [Ar-*C*H$_3$], 30.27 [*C*(CH$_3$)$_2$], 30.61 [C(*C*H$_3$)$_2$], 36.99 [OCH$_2$*C*H$_2$-], 62.42 [O*C*H$_2$CH$_2$-], 116.36, 124.36, 124.57, 126.17, 127.66, 130.33, 131.21, 132.11, 134.90, 138.32, 139.12, 141.92, 152.92 [vinyl-C and Ar-C], and 168.27 Mass spectral (EI) data Calcd for $C_{22}H_{24}O_3$ m/z (M^+): 336.1725; Found: 336.1729. Anal. Calcd for $C_{22}H_{24}O_3$: C, 78.54, H, 7.19. Found: C, 78.45, H, 7.30.

Ethyl (*E*)-4-[(2,3-Dihydro-4,4-dimethyl-2*H*-1-benzothiopyran-6-yl)-1-propenyl]-2-methylbenzoate [(*E*)-81]. A solution of *n*-butyllithium in hexane (10 *M*, 0.22 mL, 2.26 mmol) was added dropwise (10 min, N_2) to a stirred suspension of the white phosphonium salt **100** (1.17 g, 2.13 mmol) in ether (25 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, 30 min), and a solution of ethyl 2-methyl-4-formylbenzoate (**112**, 0.37 g, 1.92 mmol) in ether (25 mL) was added dropwise (10 min, vigorous stirring). 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 (triphenylphosphine oxide) was washed with ether (100 mL). The filtrate was dried (Na_2SO_4, 6 h), and the solvent was evaporated [rotovap, followed by high vacuum (40°C/0.25 mm Hg), 10 min]. The resulting yellow oil was subjected to chromatography on a 4 mm thick plate of silica gel (silica gel pF 254 containing gypsum) with the aid of the Chromatotron. The solvent system used to separate the starting materials from the (*E*)-**81** and (*Z*)-**81** isomers was hexane:ether [98:2]. The last fraction obtained was concentrated to give of a mixture of esters [8:1, (*E*)-**81**:(*Z*)-**81**] as a clear, viscous oil (0.27 g, 0.68 mmol, 35%) which was then treated with boiling ethanol (95%, 3 mL, 5 min). The resulting solution was chilled (dry ice bath) for 24 h, and the oil solidified. The white solid was recrystallized (95% ethanol) to give colorless, needle-like crystals of (*E*)-**81** (0.22 g, 0.58 mmol 30%); mp 60-62°C. IR (KBr) 1710 (C=O), cm^{-1}. ^1H NMR ($DCCl_3$) δ 1.39 [s, 6 H, $C(CH_3)_2$], 1.43 [t, 3 H, CH_3CH_2O], 1.99 [t, 2 H, SCH_2CH_2-], 2.27 [s, 3 H, $CH{=}CCH_3$], 2.64 [s, 3 H, Ar-CH_3], 3.05 [t, 2 H, SCH_2CH_2], 4.38 [q, 2 H, CH_3CH_2O], 6.75 [d, 1 H, vinyl-*H*], 7.08 [d, 1 H, Ar-*H*], 7.23 [m, 3 H, Ar-*H*], 7.51 [s, 1 H, Ar-*H*] and 7.93 [d, 1 H, Ar-*H*]. ^{13}C NMR ($DCCl_3$) ppm 14.29 [CH_3CH_2O], 17.52 [$CH{=}CCH_3$], 21.85 [Ar-CH_3], 23.03 [SCH_2CH_2-], 30.14 [$C(CH_3)_2$], 33.18 [$C(CH_3)_2$], 37.64 [SCH_2CH_2-], 60.63 [CH_3CH_2O], 123.75, 124.07, 125.74, 126.32, 126.49, 127.55, 130.59. 131.34, 132.43, 139.14, 139.45, 140.06, 141.83, 142.12 [vinyl-C and Ar-C], and 167.34 [CO_2R]; Mass spectral (EI) data Calcd for $C_{24}H_{28}O_2S$ m/z (M^+): 380.1809; Found: 380.1809. Anal. Calcd for $C_{24}H_{28}O_2S$: C, 75.75; H, 7.41. Found: C, 75.46; H, 7.48.

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 stirred at RT (1 h) and cooled to -78°C (dry ice, acetone, 10 min). A solution of ethyl 2-methyl-4-formylbenzoate (**112**, 0.25 g, 1.3 mmol) in ether (20 mL) was added dropwise (15 min). This solution was stirred (45 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 (triphenylphosphine oxide) was washed with ether (100 mL). The filtrate was dried (Na_2SO_4, 12 h), and the solvent was evaporated [rotovap, followed by high vacuum (40°C/0.25 mm Hg), 10 min]. The resulting yellow oil was subjected to chromatography on a 4 mm thick plate of silica gel (silica gel pF 254 containing gypsum) with the aid of the Chromatotron. The solvent system used to separate the starting materials from the (*E*)-**83** and (*Z*)-**83** isomers was hexane:ether [98:2]. The last fraction obtained was concentrated to give 0.25 g (0.62 mmol, 48%) of a mixture of esters [8:1, (*E*)-**83**:(*Z*)-**83**] as clear viscous oil which was then treated with boiling ethanol (95%, 2 mL). The resulting solution was chilled (dry ice bath) for 24 h and the oil solidified. The white solid was recrystallized (95% ethanol) to give colorless, flaky crystals (0.21 g, 0.51 mmol, 40%) of ester (*E*) **83**; mp 77-79°C. IR (KBr) 1705 (C=O), cm^{-1}. ^1H NMR ($DCCl_3$) δ 1.44 [s, 12 H, $C(CH_3)_2$, $SC(CH_3)_2$], 1.40 [t, 3 H, OCH_2CH_3], 1.98 [s, 2 H, CH_2], 2.28 [s, 3 H, $CH{=}CCH_3$], 2.63 [s, 3 H, Ar-CH_3], 4.36 [q, 2 H, OCH_2CH_3], 6.77 [bs, 1 H, vinyl-H], and 7.11 [d, 1 H, Ar-*H*], 7.25 [m, 3 H, Ar-*H*], 7.53 [s, 1 H, Ar-*H*] and 7.93 [d, 1 H, Ar-*H*]. ^{13}C NMR ($DCCl_3$) ppm 14.38 [CH_2CH_3], 17.62 [$CH{=}CCH_3$], 21.93 [Ar-CH_3], 31.67 [$C(CH_3)_2$], 32.65 [$C(CH_3)_2$], 35.68 [$C(CH3)_2$], 42.13 [$SC(CH3)_2$], 54.47 [CH_2-], 60.61 [CH_3CH_2O], 123.70, 124.40, 125.91, 126.31, 127.92, 130.58, 130.65, 132.42, 139.14, 140.05, 140.24, 142.09, 142.49 [vinyl-C and Ar-C], and 167.50 [CO_2R]. Mass spectral (EI) data Calcd for $C_{26}H_{32}O_2S$ m/z (M^+): 408.2123; Found: 408.2114. Anal. Calcd for $C_{26}H_{32}O_2S$: C, 76.43; H, 7.89. Found: C, 76.32; H, 7.96.

(*E*)-4-[(2,3-Dihydro-2,2,4,4-tetramethyl-2*H*-1-benzothiopyran-6-yl)-1-propenyl]-2-methylbenzoic Acid [(*E*)-84]. In a single-necked, 50-mL, round-bottomed flask (N_2) fitted with a spiral condenser and magnetic stirrer was placed ester (*E*)-**83** (0.32 g, 0.80 mmol], ethanol (95%, 20 mL), water (5 mL), and KOH (0.45 g, 8.03 mmol). The resulting solution was boiled (2 h), stirred at RT (12 h), and then chilled (0°C) with an ice bath. Dropwise addition of HCl (2 *N*, 20 mL) resulted in the formation of acid (*E*)-**84** as a white solid (0.30 g, 0.79 mmol, 99%). Acid (*E*)-**84** was recrystallized (95% ethanol) to give colorless crystals; mp 178-180°C. IR (KBr) 3450 [C(O)O-H], 1690 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.39 [s, 12 H, $C(CH_3)_2$, $SC(CH_3)_2$], 1.95 [s, 2 H, CH_2], 2.24 [s, 3 H, CH=CC*H*$_3$], 2.55 [s, 3 H, Ar-CH_3], 6.87 [bs, 1 H, vinyl-H], 7.06 [d, 1 H, Ar-*H*], 7.31 [m, 3 H, Ar-*H*], 7.64 [s, 1 H, Ar-*H*] and 7.86 [d, 1 H, Ar-*H*]. ^{13}C NMR (DMSO) ppm 17.17 [CH=C*C*H$_3$], 21.37 [Ar-*C*H$_3$], 31.17 [C(*C*H$_3$)$_2$], 32.30 [C(*C*H$_3$)$_2$], 35.13 [*C*(CH$_3$)$_2$], 41.86 [S*C*(*C*H$_3$)$_2$], 53.60 [*C*H$_2$-], 123.63, 124.31, 125.41, 126.23, 127.31, 127.85, 130.30, 131.33, 132.15, 138.17, 139.10, 139.54, 141.25, 142.03 [vinyl-C and Ar-C], and 168.23 [*C*O$_2$R]. Mass spectral (EI) data Calcd for $C_{24}H_{28}O_2S$ m/z (M^+): 380.1812; Found: 380.1810. Anal. Calcd for $C_{24}H_{28}O_2S$: C, 75.75; H, 7.42. Found: C, 75.47; H, 7.49

Methyl (*E*)-4-[(2,3-Dihydro-2,2,4,4-tetramethyl-2*H*-1-benzothiopyran-6-yl)-1-propenyl]-3-methylbenzoate [(*E*)-85]. A solution of *n*-butyllithium in hexane (10 *M*, 0.18 mL, 1.79 mmol) was added dropwise (5 min, N_2) to a stirred suspension of the white phosphonium salt **101** (3.29 g, 5.72 mmol) in ether (25 mL dried over sodium ribbon) in a 100-mL, three-necked, round-bottomed flask fitted with an addition funnel, spiral condenser and a magnetic stirrer. The resulting reddish-brown mixture was cooled to -78°C (dry ice, acetone, 10 min), and a solution of methyl 3-methyl-4-formylbenzoate (**116**, 0.85 g, 4.76 mmol) in ether (20 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 (triphenylphosphine oxide) was washed with ether (100 mL). The combined filtrate and washing was dried (Na_2SO_4, 12 h), and the solvent was evaporated [rotovap, followed by high vacuum (40°C/0.25 mm Hg), 10 min]. The resulting yellow oil was subjected to chromatography (three times) on a 4 mm thick plate of silica gel (silica gel pF 254 containing gypsum) with the aid of the Chromatotron. The solvent system used to separate the starting materials and the (*E*)-**85** and (*Z*)-**85** isomers (1:1) was hexane:ether [98:2 and 100:1]. The last fraction obtained was concentrated to give 0.75 g (1.91 mmol, 40%) of a mixture of esters [4:1, (*E*)-**85**:(*Z*)-**85**] as a clear viscous oil. IR neat) 1725 (C=O), cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.45 [s, 12 H, $C(CH_3)_2$, $SC(CH_3)_2$], 1.98 [s, 2 H, CH_2], 2.11 [s, 3 H, $CH{=}CCH_3$], 2.34 [s, 3 H, $Ar\text{-}CH_3$], 3.92 [s, 3 H, OCH_3], 6.80 [bs, 1 H, vinyl-*H*], and 7.13-7.90 [m, 6 H, Ar-*H*]. ^{13}C NMR ($DCCl_3$) ppm 17.18 [$CH{=}CCH_3$], 19.99 [$Ar\text{-}CH_3$], 31.67 [$C(CH_3)_2$], 32.59 [$C(CH_3)_2$], 35.64 [$C(CH3)_2$], 42.10 [$SC(CH3)_2$], 51.97 [CH_2-], 54.39 [OCH_3], 123.61, 124.29, 124.83, 126.69, 127.90, 129.31, 129.94, 130.91, 132.30, 136.95, 138.51, 139.54, 142.45, 142.59 [vinyl-*C* and Ar-*C*], and 167.16 [CO_2R] Mass spectral (EI) data Calcd for $C_{25}H_{30}O_2S$ m/z (M^+): 394.1966; Found: 394.1962. Anal. Calcd for $C_{25}H_{30}O_2S$: C, 76.10; H, 7.66. Found: C, 76.39; H, 7.75.

Methyl 4-[(2,3-Dihydro-2,2,4,4-tetramethyl-2*H*-1-benzothiopyran-6-yl)carbamoyl]benzoate (86). In a 50 mL, three necked, round bottomed flask (N_2) fitted with a condenser and a magnetic stirrer, amine **123** (0.4 g 1.8 mmol) was dissolved (vigorous stirring) in benzene (40 mL) and pyridine (2.3 mL) at RT. To the stirred reaction mixture was added mono methyl terephthaloyl chloride (0.39 g, 2.16 mmol), and the solution was stirred (RT, 12 h). The reaction mixture was poured into water (100 mL) and the resulting mixture was extracted with EtOAc (4 x 50 mL). The combined organics were washed with HCl (2 *N*, 4 x 50 mL), H_2O (3 x 50 mL), $NaHCO_3$ (2 x 50 mL) water (50

mL) and brine (50 mL). The organic layer was dried (Na_2SO_4, 12 h) and then evaporated (rotovap) to give crude ester **86** as a yellow solid (0.42 g, 1.09 mmol, 60%) which was purified by chromatography on a Chromatotron (4 mm plate, with $HCCl_2$ as the solvent) Yellow solid **86** was recrystallized (hexane:EtOAc, 3:1, 0.28 g, 40%); mp 162-164°C. IR (KBr) 3395-3390 [NH], 1725 (C=O), 1690-1680 (NHC=O) cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.41 [s, 6 H, $C(CH_3)_2$], 1.42 [s, 6 H, $SC(CH_3)_2$], 1.96 [s, 2 H, CH_2], 3.95 [s, 3 H, CH_3], 7.11 [d, 1 H, Ar-*H*], 7.33 [d, 1 H, Ar-*H*], 7.76 [s, 1 H, Ar-*H*], 7.91 [d, 2 H, Ar-*H*], 7.93 [bs, 1 H, Ar-*H*], 8.12 [d, 2 H, Ar-*H*] and 8.13 [s, 1 H, NH]. ^{13}C NMR ($DCCl_3$) ppm 31.51 [$C(CH_3)_2$], 32.48 [$SC(CH_3)_2$], 35.94 [$C(CH_3)$], 42.17 [$SC(CH_3)_2$], 52.43 [CH_2], 54.33 [OCH_3], 118.61, 119.14, 127.09, 128.58, 129.12, 129.92, 132.81, 134.90, 138.91, 143.66 [Ar-C], 164.72 [NHC=O], and 166.21 [CO_2H]. Mass spectral (EI) data Calcd for $C_{22}H_{25}O_3SN$ m/z (M^+): 383.1555. Found: 383.1552. Anal. Calcd for $C_{22}H_{25}O_3SN$: C, 68.90; H, 6.57; N,3.65. Found: C, 68.88; H, 6.58; N, 3.64.

4-[(2,3-Dihydro-2,2,4,4-tetramethyl-2*H*-1-benzothiopyran-6-yl)carbamoyl]benzoic Acid (87). In a 25-mL, single-necked, round-bottomed flask with a condenser and magnetic stirrer was added dropwise NaOH (2 *N*, 10 eq, 3.9 mmol) to the ester **86** (0.15 g, 0.39 mmol) in EtOH (95%, 7 mL). After stirring the yellow solution at RT (4 h), the mixture was acidified with HCl (2 *N*, 30 mL), and the white solid formed was filtered, washed (H_2O, 100 mL), dried (80°C/0.2 mm, 12 h), and recrystallized (EtOAc:hexane, 2:1). Flaky crystals of acid-amide **87** (0.1 g, 68%) formed in the solution were stored at 0-5°C in the freezer. The crystals were dried in an Abderhalden (12 h, under vacuum, benzene as the heating solvent); mp 208-209.5°C. IR (KBr) 3320 [NH], 3500 [CO_2H], 1700 (C=O), 1650 (NHC=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.36 [s, 6 H, $C(CH_3)_2$], 1.37 [s, 6 H, $SC(CH_3)_2$], 1.93 [s, 2 H, CH_2], 7.04 [d, 1 H, Ar-*H*], 7.60 [d, 1 H, Ar-*H*], 7.88 [d, 1 H, Ar-*H*], 8.09 [s, 4 H, Ar-*H*] and 10.32 [s, 1 H, N*H*]. ^{13}C NMR (DMSO-d_6) ppm 30.03 [$C(CH_3)_2$], 31.07 [$SC(CH_3)_2$], 35.18 [$C(CH_3)$], 41.46

[S*C*(CH_3)$_2$], 53.40 [CH_2], 118.67, 118.71, 119.17, 119.24, 126.97, 127.57, 127.72, 129.25, 133.07, 136.01, 138.55, 142.71 [Ar-C], 164.72 [NHC=O], and 166.21 [C(O)]. Mass spectral (EI) data Calcd for $C_{21}H_{23}O_3SN$ m/z (M^+): 369.1398. Found: 369.1384. Anal. Calcd for $C_{21}H_{23}O_3SN{\cdot}0.5\ H_2O$: C, 66.64; H, 6.39; N, 3.70. Found: C, 66.57; H, 6.47; N, 3.68.

Methyl 4-[(2,3-Dihydro-1,4-benzodioxan-6-yl)carbamoyl]benzoate (88). In a 50 mL, three-necked, round-bottomed flask (N_2) fitted with a condenser and a magnetic stirrer was placed amine **126** (1.19 g, 12.63 mmol) dissolved in benzene (150 mL) and pyridine (12 mL) at RT. To the stirred reaction mixture was added mono methyl terephthaloyl chloride (2.8 g, 14.43 mmol), and the white, thick suspension formed was stirred (RT, 12 h). The reaction mixture was poured into water (100 mL) and the resulting mixture was extracted with EtOAc (4 x 50 mL). The combined organics were washed with HCl (2 *N*, 4 x 200 mL), H_2O (3 x 50 mL), $NaHCO_3$ (3 x 50 mL) water (50 mL) and brine (2 x 50 mL). The organic solvent was dried with $MgSO_4$ (12 h) and then evaporated (rotovap) to give crude ester **88** as an off-white solid (3.7 g, 81%) which was purified by recrystallization (EtOH:$HCCl_3$, 2:1; 2.8 g, 8.90 mmol, 71%); mp 203-205°C. IR (KBr) 3300 [NH], 1720 (C=O), 1650 (NHC=O) cm^{-1}. ^1H NMR (DMSO) δ 4.24 [s, 4 H, O*C*H_2*C*H_2O], 3.90 [s, 3 H O*C*H3], 6.83 [d, 1 H, Ar-*H*], 7.23 [d, 1 H, Ar-*H*], 7.41 [s, 1 H, Ar-*H*], 8.10 [m, 4 H, Ar-*H*] and 10.30 [s, 1 H, N*H*]. ^{13}C NMR (DMSO) ppm 63.91 [O*C*H_2], 64.12 [O*C*H_2], 55.32 [O*C*H_3], 109.47, 113.61, 116.61, 127.89, 129.07, 131.82, 132.44, 139.02, 139.80, 142.79 [Ar-*C*], 164.17 [*C*(O)NH], and 165.62 [*C*O_2H]. Mass spectral (EI) data Calcd for $C_{17}H_{25}O_5SN$ m/z (M^+): 313.0947. Found: 313.0950. Anal. Calcd for $C_{17}H_{25}O_5SN$: C, 65.17; H, 4.83; N, 4.47. Anal. Calcd for $C_{17}H_{25}O_5SN{\cdot}0.25\ H_2O$: C, 64.24; H, 4.91; N, 4.40. Found: C, 64.28; H, 4.75; N, 4.09.

4-[(2,3-Dihydro-1,4-benzodioxan-6-yl)carbamoyl]benzoic Acid (89). In a 25-mL, single-necked, round-bottomed flask (N_2) fitted with a condenser and a magnetic stirrer was added dropwise NaOH (2 *N*, 10 eq, 15.9 mmol) to the ester **88** (0.5 g, 1.59 mmol) in EtOH (95 %, 20 mL). After stirring the yellow solution at RT (4 h), the mixture was acidified with HCl (2 *N*, 30 mL), and the white solid which formed (0.462, 1.4 mmol, 96%) was filtered, washed (H_2O, 100 mL), dried (12 h/0.2 mm , 80°C), and recrystallized (EtOH, 100%). Flaky crystals of acid-amide **89** formed in the solution were stored at 0-5°C in the freezer. The crystals were dried in an Abderhalden [12 h, under vacuum (0.2 mm), benzene as the heating solvent]; mp 289-291°C. IR (KBr) 3600 [CO_2H], 3340 [N*H*], 1690 (C=O), 1690 (N*H*C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.23 [bs, 4 H, OCH_2], 6.83 [d, 1 H, Ar-*H*], 7.22 [d, 1 H, Ar-H], 7.40 [d, 1 H, Ar-H], 8.05 [m, 4 H, Ar-*H*], 10.27 [s, 1 H, N*H*]. ^{13}C NMR (DMSO-d_6) ppm 63.87, 64.07 [OCH_2], 109.38, 113.53, 116.58, 127.70, 129.16, 132.45, 133.02, 138.65, 139.72, 142.74 [Ar-*C*], 164.28 [NHC=O], and 166.63 [C(O)]. Mass spectral (EI) data Calcd for $C_{16}H_{13}O_5N$ m/z (M^+): 299.0794. Found: 299.0796. Anal. Calcd for $C_{16}H_{13}O_5N$: C, 64.21; H, 4.38; N, 4.68. Found: C, 63.89; H, 4.59; N, 4.69.

Methyl 4-[(2,2,4,4-Tetramethylthiochromanyl)carbamoyl]muconate (90). In a 100 mL, three necked, round-bottomed flask (N_2) fitted with a condenser and a magnetic stirrer, amine **123** (0.64 g 2.86 mmol) was dissolved in benzene (50 mL), and pyridine (2.5 mL) at RT. To the stirred reaction mixture was added mono methyl muconyl chloride (0.55 g, 3.15 mmol), [prepared by stirring monomethyl muconate (**128**, 0.50 g, 3.15 mmol) in excess $SOCl_2$ (20 ml, RT, 12 h)], and the resulting solution was stirred (RT, 14 h). The mixture was poured into water (150 mL) and the aqueous layer was extracted with EtOAc (4 x 40 mL) and CH_2Cl_2 (2 x 25 mL). The organic layers were combined and washed with HCl (2 *N*, 4 x 50 mL), H_2O (2 x 50 mL), $NaHCO_3$ (2 x 50 mL) water (50 mL) and brine (50 mL). The organic solution was dried (Na_2SO_4, 12 h)

and then evaporated (rotovap) to give crude ester **90** as a yellow solid (0.86 g, 2.41 mmol, 83%) which was purified by chromatography on a Chromatotron (4 mm plate, with $HCCl_2$ as the solvent). The yellow solid obtained was recrystallized (hexane:EtOAc, 2:1, 0.74 g, 71%); mp 167-168°C. IR (KBr) 3380 [NH], 1700 (C=O), 1680 (NHC=O) cm^{-1}. ^1H NMR ($DCCl_3$) δ 1.37 [s, 6 H, $C(CH_3)_2$], 1.40 [s, 6 H, $SC(CH_3)_2$], 1.93 [s, 2 H, CH_2], 3.79 [s, 3 H, OCH_3], 6.17 [d, 1 H, MeO(O)CC*H*=CH], 6.32 [d, 1 H, HN(O)CC*H*=CH] 7.06-7.76 [m, 3 H, Ar-*H*], and 7,82 [N*H*]. ^{13}C NMR ($DCCl_3$) ppm 31.51 [$C(CH_3)_2$], 32.45 [$SC(CH_3)_2$], 35.75 [$C(CH_3)_2$], 42.17 [$SC(CH_3)_2$], 51.94 [CH_2], 52.26 [OCH_3], 118.19, 118.80, 127.19, 128.51, 129.00, 131.46, 134.92, 138.22, 141.25, 143.62 [Ar-*C* and vinyl-*C*], 162.66 [NH*C*=O], and 166.73 [CO_2Me]. Mass spectral (EI) data Calcd for $C_{20}H_{25}O_3SN$ m/z (M^+): 359.1555. Found: 359.1555. Anal. Calcd for $C_{20}H_{25}O_3SN$: C, 66.82; H, 7.00; N,3.89. Found: C, 66.78; H, 7.20; N, 3.80.

4-[(2,2,4,4-Tetramethylthiochromanyl)carbamoyl]muconic Acid (91). In a 50-mL, single-necked, round-bottomed flask (N_2) fitted with a condenser and a magnetic stirrer, was added dropwise NaOH (2 *N*, 12 eq, 9.96 mmol) to the ester **90** (0.30 g, 0.83 mmol) in EtOH (100%, 20 mL). After stirring the orange solution at RT (3 h), the mixture was acidified with HCl (2 *N*, 100 mL, 0°C), and the orange solid formed was filtered, washed (H_2O, 100 mL), dried (80°C/0.2 mm, 12 h), and recrystallized (EtOAc:hexane, 1:2). Bright orange crystals of acid-amide **91** (0.21 g, 0.61 mmol, 73%) formed in the solution were stored at 0-5°C in the freezer. The crystals were dried in an Abderhalden (12 h, under vacuum, benzene as the heating solvent); mp 225-227.5C. IR (KBr) 3350 [NH], 3300 [CO_2H], 1710 (C=O), 1640 (NHC=O) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.33 [s, 6 H, $C(CH_3)_2$], 1.34 [s, 6 H, $SC(CH_3)_2$], 1.91 [s, 2 H, CH_2], 6.27 [d, 1 H, HO(O)CC*H*=CH], 6.28 [d, 1 H, HN(O)CC*H*=CH], 7.01 [d, 1 H, Ar-*H*], 7.45 [d, 1 H, Ar-*H*],.7.75 [s, 1 H, Ar-*H*], 7.29 [m, 2 H, (-O(O)CCH=C*H*-)$_2$] and 10.28 [s, 1 H,

N*H*] ^{13}C NMR (DMSO-d_6) ppm , 31.60 [C(CH_3)$_2$], 32.12 [SC(CH_3)$_2$], 35.20 [*C*(CH_3)], 41.85 [S*C*(CH_3)$_2$], 53.40 [*C*H_2], 117.60, 117.87, 126.58, 127.72, 128.11, 132.05, 136.31, 137.09, 141.12, 142.75, [vinyl-*C* and Ar-*C*], 162.45 [NH*C*=O], and 166.92 [*C*(O)OH]. Mass spectral (EI) data Calcd for $C_{19}H_{23}O_3SN$ m/z (M^+): 345.1399 Found: 345.1399. Anal. Calcd for $C_{19}H_{23}O_3SN$: C, 66.06; H, 6.71, N, 4.05. Found: C, 66.02; H, 6.99; N, 3.92.

Ethyl 4-[(2,3-Dihydro-2,2,4,4-tetramethyl-1,1-dioxy-2*H*-1-benzothiopyran-6-yl)carboxamido]benzoate (92). In a 50-mL, single-necked round-bottomed flask fitted with a spiral condenser and a magnetic stirrer (N_2), was placed acid **131** (1.1 g, 3.89 mmol), thionyl chloride (excess, 25 mL) and DMF (4 drops), and the mixture was stirred under N_2 at 0°C for 12 h. Acid **131** slowly dissolved in the thionyl chloride to form a pale yellow solution. Excess thionyl chloride was removed under reduced pressure (water aspirator), and the white solid formed was dried under vacuum (RT) for additional 6 h until no detectable odor of thionyl chloride remained in the flask. Pyridine (35 mL) was added to this solid, and the resulting solution was quickly transferred to a jacketed flask (50 mL) containing acetone as the heating solvent. Ethyl 4-aminobenzoate (**132**, 0.76 g, 4.6 mmol, Aldrich) and DMAP (Aldrich, catalytic amount, ~10 mg) were added, and the resulting brown solution was heated to 56°C for 3 h and then stirred at RT for 24 h. Water (125 mL) was added and the resulting mixture was extracted with EtOAc (4 x 35 mL). The combined organic layers were washed with HCl (2 *N*, 4 x 50 mL), sat. $NaHCO_3$ (2 x 50 mL), water (50 mL), and brine (50 mL); it was dried over Na_2SO_4 (1 h). Evaporating the solvent (rotovap) gave a white solid which was dissolved in a minimum amount of $HCCl_3$ (3 mL), and the solution was subjected to chromatography on a Chromatotron (4 mm thick silica gel plate, $HCCl_3$:MeOH, 50:1). The resulting white solid was recrystallized (95% EtOH) to give colorless needles of amide-ester **92** (0.98 g, 2.28 mmol, 59%); mp 245-247°C. IR (KBr) 3360 [NH], 1715 (C=O), 1675 (NHC=O)

cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.32-1.45 [m, 15 H, CH_2CH_3, $C(CH_3)_2$, $SC(CH_3)_2$], 2.29 [s, 2 H, CH_2], 4.37 [q, 2 H, OCH_2CH_3], 7.85 [m, 4 H, Ar-*H*], 8.05 [m, 4 H, Ar-*H*] and 8.95 [s, 1 H, NH]. ^{13}C NMR ($DCCl_3$) ppm 14.30 [OCH_2CH_3], 21.60 [$C(CH_3)_2$], 34.02 [$SC(CH_3)_2$], 34.08 [$C(CH_3)$], 48.66 [$SC(CH_3)_2$], 54.81 [CH_2], 60.93 [OCH_2CH_3], 119.55, 125.18, 125.53, 126.45, 128.74, 130.82, 136.73, 139.16, 142.12, and 146.55 [Ar-*C*], 164.82 [NHC=O], and 164.19 [C(O)]. Mass spectral (EI) data Calcd for $C_{23}H_{27}O_5SN$ m/z (M^+): 429.1610. Found: 429.1619. Anal. Calcd for $C_{23}H_{27}O_5SN$: C, 64.31; H, 6.39; N, 3.20. Found: C, 64.45; H, 6.69; N, 3.31.

4-[(2,3-Dihydro-2,2,4,4-tetramethyl-1,1-dioxy-2*H*-1-benzothiopyran-6-yl)carboxamido]benzoic Acid (93). In a 25-mL, single-necked, round-bottomed flask with a magnetic stirrer was added NaOH (2 *N*, 10 eq) dropwise to the ester **92** (0.4 g, 1.0 mmol) in EtOH (95%, 5 mL). The solution was boiled for 1 h and stirred at RT for 2 days. The resulting mixture was acidified with HCl (2 *N*, 50 mL), and the white solid formed was filtered, washed (H_2O, 100 mL), dried (12 h/0.2 mm, 80°C), and recrystallized twice (95% EtOH, large excess). Flaky crystals of acid-amide **93** (0.26 g, 0.65 mmol, 65%) formed in the solution were stored at 0-5°C in the freezer. These crystals were dried in an Abderhalden (12 h, under vacuum, benzene as the heating solvent); mp 331-331.8°C. IR (KBr) 3380 [NH] 3350 [CO_2H], 1700 (C=O), 1680 (NHC=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.37 [s, 6 H, $C(CH_3)_2$, 1.46 [s, 6 H, $SC(CH_3)_2$], 2.31 [s, 2 H, CH_2], 7.88-8.12 [m, 7 H, Ar-H], 10.68 [s, 1 H, NH]. ^{13}C NMR (DMSO-d_6) ppm 20.94 [$C(CH_3)_2$], 33.45 [$SC(CH_3)_2$], 3.67 [$C(CH_3)_2$], 47.54 [$SC(CH_3)_2$], 53.98 [CH_2], 119.73, 123.98, 125.83, 126.52, 127.83, 130.14, 136.18, 138.66, 142.52, 145.87 [Ar-*C*], 164.45 [NHC=O], and 166.17 [CO_2H]. Mass spectral (EI) data Calcd for $C_{21}H_{23}O_5SN$ m/z (M^+): 401.1296; Found: 401.1296. Anal. Calcd for $C_{21}H_{23}O_5SN$: C, 62.82; H, 5.77; N, 3.49. Found: C, 62.88; H, 5.94; N, 3.50.

Ethyl 4-[(2,3-Dihydro-3,3-dimethyl-1,1-dioxybenzo[*b*]thien-5-yl)carboxamido]benzoate (94). In a 50-mL, single-necked flask (with a magnetic stirrer) were mixed and stirred acid **134** (0.87 g, 3.62 mmol), thionyl chloride (excess, 25 mL) and DMF (5 drops) under N_2 at 0°C for 12 h. The acid slowly dissolved in the thionyl chloride to form a clear solution. When the reaction was completed excess thionyl chloride was removed (reduced pressure, water aspirator), and the white solid formed was dried (vacuum, RT) for an additional 6 h to remove traces of thionyl chloride. Pyridine (35 mL) was added to this solid, and the resulting solution was quickly transferred to a jacketed flask (50 mL) containing acetone as the heating solvent. Ethyl 4-aminobenzoate (**132**, 0.75 g, 4.59 mmol, Aldrich) and DMAP (Aldrich, catalytic amount, ~10 mg) were added, and the resulting brown solution was heated at 56°C for 3 h and then stirred at RT for 24 h. Water (150 mL) was added, and the resulting mixture was extracted with EtOAc (4 x 35 mL). The combined organic layers were washed with HCl (2 *N*, 4 x 50 mL), sat. $NaHCO_3$ (2 x 50 mL), water (50 mL), and brine (50 mL); it was then dried (Na_2SO_4, 1 h). Evaporation of the solvent (rotovap) gave a white solid which was dissolved in a minimum amount (~ 2 mL) of $HCCl_3$. The resulting solution was subjected to chromatography on a Chromatotron (4 mm thick silica gel plate, 100:1, $HCCl_3$:MeOH). Several fractions (5-15) that contained only crude **94** were combined, and the solvent was evaporated under reduced pressure (rotovap). The resulting pink-colored solid was dissolved in hot EtOH (95 %), and this solution was treated with activated charcoal, and the resulting mixture was filtered. Upon cooling to RT, crystals formed. Colorless needles of the amide-ester **94** were obtained (0.6 g, 1.55 mmol, 37%); mp 172.3-174°C. IR (KBr) 3340 [NH], 1710 (C=O), 1680 (NHC=O) cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.40 [t, 3 H, CH_2CH_3], 1.52 [s, 6 H, $C(CH_3)_2$], 3.37 [s, 2 H, SCH_2], 4.37 [q, 2 H, OCH_2CH_3], 7.53 [d, 1 H, Ar-*H*], 7.79 [d, 2 H, Ar-*H*], 7.89 [d, 1 H, Ar-*H*], 8.03 [m, 3 H, Ar-*H*] and 8.71 [s, 1 H, N*H*]. ^{13}C NMR ($DCCl_3$) ppm 14.31 [OCH_2CH_3], 29.23 [$C(CH_3)_2$], 39.42 [$C(CH_3)_2$], 61.02 [SCH_2], 63.77 [OCH_2CH_3], 119.54, 121.51, 124.07, 126.56, 127.36, 130.76, 130.37, 140.48,

141.79, 147.46 [Ar-*C*], 165.55 [NHC=O], and 166.13 [C(O)]. Mass spectral (EI) data Calcd for $C_{20}H_{21}O_5SN$ m/z (M^+): 387.1140; Found: 387.1140. Anal. Calcd for $C_{20}H_{21}O_5SN$: C, 61.99; H, 5.46; N, 3.61 Anal. Calcd for $C_{20}H_{21}O_5SN{\cdot}0{\cdot}25\ H_2O$: C, 61.28; H, 5.52; N, 3.61. Found: C, 61.40; H, 5.60; N, 3.51.

4-[(2,3-Dihydro-3,3-dimethyl-1,1-dioxybenzo[*b*]thien-5-yl)carboxamido]benzoic Acid (95). In a 25-mL, single-necked, round-bottomed flask with a magnetic stirrer containing ester **94** (0.2 g, 0.51 mmol) in EtOH (100%, 8 mL) was added dropwise NaOH (2 *N*, 10 eq, 5.1 mmol), and the solution was stirred at RT for 12 h. The mixture was acidified with HCl (2 *N*, 50 mL), and a white solid formed which was filtered, washed (H_2O), dried (12 h/0.2mm, 80°C), and recrystallized twice (95% EtOH, large excess). Flaky crystals of acid-amide **95** (0.12 g, 0.33 mmol, 65.1%) formed at 0-5°C in the freezer. The crystals were dried in an Abderhalden (12 h, under vacuum, benzene as the heating solvent); mp 303.5-304.5°C. IR (KBr) 3390 [NH], 3500 [CO_2H], 1700, 1690 (C=O, NH*C*=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.53 [s, 6 H, C(CH_3)$_2$], 3.60 [s, 2 H, SCH_2], 7.88-8.20 [m, 7 H, Ar-*H*], 10.72 [s, 1 H, N*H*]. ^{13}C NMR (DMSO-d_6) ppm 18.52 [C(CH_3)$_2$], 33.67 [*C*(CH_3)$_2$], 62.71 [SCH_2], 119.69, 120.90, 124.09, 125.96, 128.50, 130.23, 139.95, 140.33, 142.78, 147.22 [Ar-*C*], 164.82 [NH*C*=O], and 166.87 [CO_2H]. Mass spectral (EI) data Calcd for $C_{18}H_{17}O_5SN$ m/z (M^+): 359.0827. Found: 359.0811. Anal. Calcd for $C_{18}H_{17}O_5SN$: C, 60.15; H, 4.76; N, 3.89. Found: C, 60.00; H, 4.85; N, 3.78.

Ethyl 4-[(2,3-Dihydro-3,3-dimethyl-5-benzofuranyl) carboxamido]-benzoate (96). In a 50-mL, single-necked round-bottomed flask fitted with a condenser and a magnetic stirrer were mixed and stirred acid **136** (0.71 g, 3.69 mmol), thionyl chloride (excess, 20 mL) and DMF (4 drops) under N_2 at 0°C for 12 h. The acid slowly dissolved in the thionyl chloride to form a clear solution. Excess thionyl chloride was

removed under reduced pressure (water aspirator), and the white solid formed was further dried under vacuum (~2 mm Hg) for an additional 3 h to remove traces of thionyl chloride. Pyridine (35 mL) was added to this solid, and the resulting solution was quickly transferred to a jacketed flask (50 mL) containing acetone as the heating solvent. Ethyl 4-aminobenzoate (**132**, 0.67 g, 4.06 mmol, Aldrich) and DMAP (catalytic amount, ~10 mg) were added, and the resulting brown solution was heated at 56°C for 3 h and then stirred (RT, 24 h). Water (100 mL) was added, and the resulting mixture was extracted with EtOAc (4 x 50 mL). The combined organic layers were washed with HCl (2 *N*, 4 x 50 mL), sat. $NaHCO_3$ (2 x 50 mL), water (50 mL), and brine (50 mL); it was then dried (Na_2SO_4, 1 h). Evaporation of the solvent (rotovap) gave a sticky yellow solid which was dissolved in a minimum amount (~2 mL) of H_2CCl_2. The resulting solution was subjected to chromatography (three times) on a Chromatotron (4 mm thick silica gel plate, H_2CCl_2:EtOAc, 3:1; H_2CCl_2; H_2CCl_2:EtOAc, 100:1). Several fractions that contained only crude **96** (from TLC) were combined, and the solvent was evaporated under reduced pressure (rotovap). A white foamy solid **96** was obtained (0.82 g, 2.42 mmol, 65%); mp 51-55°C. IR (KBr) 3340 [NH], 1725 (C=O), 1660 (NHC=O) cm^{-1}. ^1H NMR ($DCCl_3$) δ 1.31 [s, 6 H, $C(CH_3)_2$], 1.38 [t, 3 H, CH_2CH_3], 4.29 [s, 2 H, OCH_2], 4.33 [q, 2 H, OCH_2CH_3], 7.78 [m, 4 H, Ar-*H*], 8.01[d, 2 H, Ar-*H*], 8.61 [s, 1 H, N*H*]. ^{13}C NMR ($DCCl_3$) ppm 14.24 [OCH_2CH_3], 27.41 [$C(CH_3)_2$], 41.52 [$C(CH_3)_2$], 60.78 [OCH_2CH_3], 85.21 [OCH_2], 109.36, 113.61, 119.21, 122.54, 126.99, 127.92, 130.60, 131.43, 137.46, 142.59, 162.54 [Ar-*C*], 165.90 [NH*C*=O], and 166.21 [CO_2H]. Mass spectral (EI) data Calcd for $C_{20}H_{21}O_4N$ m/z (M^+): 339.1470; Found: 339.1470. Anal. Calcd for $C_{20}H_{21}O_4N$: C, 70.78; H, 6.24; Anal. Calcd for $C_{20}H_{21}O_4N \cdot 0{\cdot}1\ H_2O$: C, 70.41; H, 6.26. Found: C, 70.35; H, 6.34;

4-[(2,3-Dihydro-3,3-dimethyl-5-benzofuranyl)carboxamido]benzoic Acid (97). In a 50-mL, single-necked, round-bottomed flask fitted with a condenser and a magnaetic stirrer, containing ester **96** (0.56 g, 1.61 mmol) in EtOH (100%, 20 mL) was added dropwise NaOH (2 *N*, 10 eq, 16.1 mmol), and the solution was boiled (4 h). The mixture was acidified with HCl (2 *N*, 0°C, 50 mL), and the resulting aqueous mixture was stored in the freezer (12 h). The white solid formed was filtered, washed (H_2O), dried (12 h/0.5 mm, 80°C), and recrystallized (hexane:EtOAc, 2:1). Flaky crystals of acid-amide **97** (0.38 g, 1.22 mmol, 74%) formed at 0-5°C in a freezer. The crystals were dried in an Abderhalden (12 h, under vacuum, benzene as the heating solvent); mp 219-220°C. IR (KBr) 3500 [O-H], 3340 [NH], 1700, 1660 (C=O, NH*C*=O) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.36 [s, 6 H, C(C*H*$_3$)$_2$], 4.30 [s, 2 H, OC*H*$_2$], 6.89 [d, 1 H, Ar-*H*], 7.96 [m, 6 H, Ar-*H*] and 10.30 [s, 1 H, N*H*]. ^{13}C NMR (DMSO-d_6) ppm 27.17 [C(*C*H$_3$)$_2$], 41.13 [*C*(CH$_3$)$_2$], 84.48 [S*C*H$_2$], 108.88, 119.33, 122.70, 125.04, 126.97, 128.85, 140.08, 136.99, 143.48, 161.75 [Ar-*C*], 165.31 [NH*C*=O], and 166.84 [*C*O$_2$H]. Mass spectral (EI) data Calcd for $C_{18}H_{17}O_4N$ m/z (M^+): 311.1157. Found: 311.1157. Anal. Calcd for $C_{18}H_{17}O_4N$: C, 69.44; H, 5.50; N, 4.50. Anal. Calcd for $C_{18}H_{17}O_4N \cdot 0.1\ H_2O$: C, 69.04; H, 5.54; N, 4.47. Found: C, 68.98; H, 5.74; N, 4.31.

(*E*)-4-[(2,3-Dihydro-2,2,4,4-tetramethyl-2*H*-1-benzothiopyran)-3-oxo-1-propenyl]-benzoic Acid (98). In a 100-mL, single-necked, round-bottomed flask, fitted with an addition funnel, condenser and magnetic stirrer (charged with N_2) was placed ketone **102** (0.92 g, 3.53 mmol) and terephthaldehydic acid methyl ester (**142**, 0.61 g, 3.71 mmol) dissolved in MeOH (30 mL). To the stirred solution was added NaOH (1 *N*, 22 mL) dropwise over a period of 15 min, and the resulting turbid solution was stirred at RT for 12 h. The solution became clear in a few hours (~5 h). At the end of the reaction time, the mixture was poured slowly into HCl (1 *N*, 100 mL) whereupon a yellow solid formed. Ethyl acetate (4 x 35 mL) was used to extract the compound from the aqueous

layer. The combined organics were washed with water (2 x 50 mL) and brine (50 mL) and then dried (Na_2SO_4, 4 h). The yellow solid obtained previously was purified by chromatography on a Chromatotron (separation on a 4 mm thick silica gel plate using gradient elution, 3:1 $HCCl_3$:MeOH; 1:1 $HCCl_3$:MeOH; MeOH). The fractions (8-15) containing crude acid **98** were combined, and solvent was evaporated to give a yellow solid which was recystallized (hexane) to give yellow crystals (0.61 g, 45%) of **98**; mp 198-199°C. IR (KBr) 3500 [C(O)O-H], 1690 [C=O(OH)], 1680 [C=O] cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.45 [s, 12 H, $C(CH_3)_2$, $SC(CH_3)_2$], 2.01 [s, 2 H, CH_2], 7.23 [d, 1 H CH=C*H*-R, J = 8 Hz], 7.61-8.10 [m, 7 H, Ar-*H*], 8.74 [d, 1 H C*H*=CH-R, J = 8 Hz]. ^{13}C NMR ($DCCl_3$) ppm 31.72 [$C(CH_3)_2$], 32.63 [$SC(CH_3)_2$], 35.64 [$C(CH_3)_2$], 42.67 [$SC(CH_3)_2$], 53.86 [CH_2], 124.45, 126.05, 127.20, 127.82, 128.25, 130.76, 134.41, 140.08, 140.63, 142.50, 142.94 [Ar-*C* and vinyl *C*], 171.3 [*C*(O)OH] and 189.12 [CO_2H]. Mass spectral (EI) data Calcd for $C_{23}H_{24}O_3S$ m/z (M^+): 380.1446; Found: 380.1454. Anal. Calcd for $C_{23}H_{24}O_3S$: 73.60; H, 6.35. Found: C, 74.02; H, 6.22.

1-[(4,4-Dimethylchroman-6-yl)ethyl]triphenylphosphonium Bromide (101). A solution of alcohol **103** (5.5 g 21.96 mmol) and triphenylphosphonium hydrobromide (8.9 g 26.4 mmol) in methanol (125 mL) was stirred at RT (N_2, 24 h), in a 250-mL, three-necked, round-bottomed flask fitted with a spiral condenser and a magnetic stirrer. The pale yellow solvent was then evaporated (rotovap), and the resulting clear oil was triturated repeatedly with dry ether (100 mL) until solidification occurred. The resulting pale yellow solid was suspended with stirring in dry ether at RT (N_2, 4 h). After filtration, a yellow solid **101** was obtained which was dried (110°C/2 mm Hg) and weighed (11.1 g, 19.28 mmol, 88%, not a reported compound); melting range 140-150°C. Compound **101** was used without further purification to prepare **79**. ^1H NMR ($DCCl_3$) δ 1.12 [s, 3 H, CH_3], 1.23 [s, 3 H, CH_3], 1.37 [s, 6 H, $C(CH_3)_2$], 1.38-1.85 [m, 2 H, CH_2], 1.87 [d, 3 H, $CHCH_3$], 6.48-6.54 [m, 1 H, $CHCH_3$], and 6.60 [m, 2 H, Ar-*H*],

6.89 [d, 1 H, Ar-*H*] and 7.77 [m, 15 H, Ar-*H*]. ^{13}C NMR ($DCCl_3$) ppm 16.96 [$CHCH_3$], 31.96, 31.49 [$C(CH_3)_2$], 32.54, 32.77 [$SC(CH_3)_2$], 35.53 [$C(CH_3)_2$], 54.10 [CH_2], 70.37 [$CHCH_3$], and 117.23, 118.32, 126.94, 128.04, 129.87, 130.08, 130.18, 130.24, 130.40, 130.58, 132.00, 133.21, 133.35, 134. 55, 134.61, 134.73, 135.15 and 135.19 [Ar-C].

6-Acetyl-2,2,4,4-tetramethylthiochroman (102). A solution of 7.8 g (37.3 mmol) of 2,2,4,4-tetramethylthiochroman (**107**) and 2.9 g (37.3 mmol) of acetyl chloride in 50 mL of freshly distilled nitromethane was added dropwise over a 45-min period to a stirred suspension of $AlCl_3$ (9.96 g, 74.6 mmol in 60 mL of nitromethane, N_2, magnetic stirrer) 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), 80 mL of ice water was added slowly over a period of 20 min to a chilled (0°C) reaction mixture. The resulting mixture was stirred (10 min) and then 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 water (50 mL) and brine (50 mL). After drying (Na_2SO_4, 12 h, stirring) the solution, the solvent was evaporated [rotovap and high vacuum (0.3 mm Hg), at 50-60°C (water bath), 10 min] to give a thick, reddish-brown oil which was distilled (high vacuum, bp 180-210°C/3.5 mm Hg) to give 5.82 g (62%) of ketone **102** as a light yellow oil. The IR, ^1H and ^{13}C NMR spectra matched those of the reported compound.[65] Ketone **102** was used in the following reaction without further purification. IR (neat) 1680 (C=O) cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.42, 1.43 [s, 12 H, $C(CH_3)_2$, $SC(CH_3)_2$], 1.97 [s, 2 H, CH_2], 2.55 [d, 3 H, $CHCH_3$], 7.17 [d, 1 H, Ar-*H*], 7.58 [d, 1 H, Ar-*H*] and 8.01 [s, 1 H, Ar-*H*]. ^{13}C NMR ($DCCl_3$) ppm 26.34 [CH_3], 31.60 [$C(CH_3)_2$], 32.42 [$SC(CH_3)_2$], 35.47 [$C(CH_3)_2$], 42.47 [$SC(CH_3)_2$], 53.80 [CH_2], 125.92, 126.52, 127.66, 133.80, 140.11, 142.51 [Ar-*C*], and 197.36 [$C(O)CH_3$].

2,2,4,4-Tetramethylthiochroman-6-methanol (103). A solution of the ketone **102** [(5.8 g, 23.3 mmol) in anhydrous ether (25 ml) was added (15 min, N_2)] to a stirred suspension of $LiAlH_4$ (1.42 g, 37.36 mmol) in dry ether (15 mL) in a 100-mL, 3-necked, round-bottomed flask with the usual setup. The mixture, a grey suspension, was heated at reflux for 24 h. After cooling the suspension to RT (1 h), ethyl acetate (10 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%, 60 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 (1 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 Hg, at 50-55°C, water bath), 15min]. Alcohol **103** was a thick, yellow oil (5.6 g, 22.36 mmol, 96%, not a reported compound) which was used without further purification to prepare **101**. IR (neat) 3350 (O-H) cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.41 [s, 6 H, $C(CH_3)_2$], 1.43 [s, 6 H, $SC(CH_3)_2$], 1.53 [d, 3 H, $CHCH_3$], 1.96 [s, 2 H, CH_2], 4.84 [q, 1 H, $CHCH_3$], 7.15 [m, 2 H, Ar-*H*] and 7.42 [s, 1 H, Ar-*H*]. ^{13}C NMR ($DCCl_3$) ppm 24.98 [CH_3], 31.64 [$C(CH_3)_2$], 32.52 [$SC(CH_3)_2$], 35.62 [$C(CH_3)_2$], 41.97 [$SC(CH_3)_2$], 54.46 [CH_2], 70.37 [$CHCH_3$], and 123.14, 123.93, 124.92, 128.01, 128.08 and 142.74 [Ar-C].

Ethyl 3-Formylbenzoate (108). In a 200-mL, single-necked, round-bottomed flask (N_2) fitted with a condenser was placed ethyl 3-methylbenzoate (**110**, 5.0 g, 30.44 mmol), glacial acetic acid (50 mL), and 50 mL of freshly distilled acetic anhydride containing concentrated H_2SO_4 (2.0 mL). After stirring at RT for 15 min, 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 (**111**, 7.5 g, 81%) as a white solid. To ester **111** in a 100-mL, single-necked, round-bottomed flask fitted with a condenser and a magnetic stirrer, was added (RT, stir, 10 min), EtOH (30 mL), water (30 mL), concentrated H_2SO_4 (2.5 mL) and the reaction mixture was boiled (2 h). After cooling to RT, water (80 mL) was added, the organic phase separated, and the aqueous phase was extracted with ether (3 x 40 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 **108** [2.59 g, 14.53 mmol, 47% (lit[59] bp 162-164°C/1 atm)] as a golden yellow liquid. The ester was used without purification to prepare **77**. IR ($DCCl_3$) 2720 (C(O)-H), 1740, 1720 [C=O(H), C=O(OEt)] cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.43 [t, 3 H, CH_3], 4.41 [q, 2 H,CH_2], 7.63 [t, 1 H, Ar-*H*], 8.08 [d, 1 H, Ar-*H*], 8.28 [d, 1 H, Ar-*H*], 8.50 [s, 1 H, Ar-*H*] and 10.1 [*C*(O)H]; ^{13}C NMR ($DCCl_3$) ppm 14.3 [CH_3], 61.4 [CH_2], 129.18, 131.04, 131.48, 133.08, 135.06, 136.72 [Ar-*C*], 165.62 [CO_2Et] and 191.3 [*C*(O)H].

Ethyl 3-Toluate (110). 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 (**109**, 10 g, 73.4 mmol) in absolute ethanol (30 mL) and benzene (100 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). Then the combined extacts were 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 10.9 g (66.8 mmol, 91%) of ester **110** as a colorless liquid (strong and sweet odor), bp 72-74°C/0.250 mm Hg [lit[54] 105.6-105.9°C/11 mm Hg]. IR (neat) 1725 (C=O) cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.39 [t, 3 H, OCH_2CH_3], 2.37 [s, 3 H, *m*-Ar-CH_3], 4.35 [q, 2 H, OCH_2CH_3], 7.31 [m, 2 H, Ar-*H*] and 7.86 [m, 2 H, Ar-*H*]. ^{13}C NMR ($DCCl_3$) ppm 14.35 [OCH_2CH_3], 21.26 [Ar-CH_3], 60.87 [CH_3CH_2O], 126.69, 128.21, 130.08, 130.45, 133.56, 136.08 [Ar-*C*], and 166.80 [CO_2Et].

4-Carboethoxy-3-methylbenzaldehyde (112). In a 300-mL, three-necked, round-bottomed flask (N_2) fitted with a condenser and a magnetic stirrer was placed ethyl 2,3-dimethylbenzoate (**114**, 1.6 g, 8.9 mmol), glacial acetic acid (20 mL), and 20 mL of freshly distilled acetic anhydride with H_2SO_4 (1.5 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 (1.6 g, 16.8 mmol) was added in small portions (30 min). After stirring on a cool water bath (40 h), CrO_3 (0.5 g, 5 mmol) was added, and the reaction mixture was stirred at RT for an additional 8 h. TLC analysis (hexane:ether, 8:2) showed four distinct spots, but the starting material was *absent*. The dark green reaction mixture was treated **carefully** with ice water (150 mL) and then ether (40 mL). The organic phase separated, and the aqueous phase was extracted [$HCCl_3$ (3 x 50 mL) and then ether (1 x 50 mL)]. The combined organic phases were washed with saturated $NaHCO_3$ (3 x 40 mL), water (1 x 50 mL), and then brine (1 x 50 mL). After drying (Na_2SO_4, 12 h), the solvent was evaporated (rotovap, followed by high vacuum at 0.25 mm Hg, 45°C) to give the diacetate **115** as an orange oil. To ester **115** (1.9 g, 76.8%), dissolved in ethanol (15 mL) in a 100-mL, single-necked, round-bottomed flask was added dropwise (RT, stir, 10 min) water (10 mL) and concentrated H_2SO_4 (0.7 mL); the mixture was then boiled for 5.5 h. After cooling to RT, water (150 mL) was added to the solution; the organic phase

separated, and the aqueous phase was extracted with $HCCl_3$ (5 x 40 mL). The combined organic phases were washed with saturated $NaHCO_3$ (4 x 35 mL), water (50 mL), and brine (50 ml). After drying the solution (Na_2SO_4, 4 h), the solvent was evaporated (rotovap, followed by high vacuum at 0.2 mm Hg) to give a brown liquid. The crude aldehyde **112** was purified by chromatography with a Chromatotron (4 mm thick silica gel plate; the solvent system used was hexane:ether, 8.5:1.5). A pale yellow oil (0.36 g, 1.88 mmol, 21%) of **112**[14] that solidified at 0°C and remelted at RT was obtained.[53] IR (neat) 2720 [C(O)-H], 1725 (CO_2Et) 1710 [C=O(H)] cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.39 [t, 3 H, CH_3], 2.63 [s, 3 H, CH_3], 4.36 [q, 2 H, CH_2], 7.70 [bs, 2 H, Ar-*H*], 8.01 [s, 1 H, Ar-*H*], 10.04 [C(O)H]. ^{13}C NMR ($DCCl_3$) ppm 14.18 [CH_2CH_3], 21.36 [Ar-CH_3], 61.23 [CH_3CH_2O], 126.55, 130.86, 132.49, 135.23, 137.97, 140.50 [Ar-*C*], 166.77 [CO_2R], and 191.77 [*C*(O)H].[14]

Ethyl 2,4-Dimethylbenzoate (114). In a 300-mL, single-necked, round-bottomed flask equipped with a Dean-Stark apparatus, a spiral condenser, and a magnetic stirrer was placed 2,4-dimethylbenzoic acid (**113**, 6 g, 39.95 mmol) in absolute ethanol (80 ml) and benzene (150 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 (100 mL) was added, and the aqueous phase was separated and extracted (ether, 3 x 40 mL). The combined extracts were washed with saturated $NaHCO_3$ (3 x 40 ml), water (2 x 50 mL) and then 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] to give 6.3 g (35.34 mmol, 88%, crude) of ester **114** as a yellow oil which was used without further purification [lit[1] NMR spectral data: ^1H NMR values (1.35 CH_2CH_3, 2.3 Ar-CH_3, 2.5 Ar-CH_3)]. IR (neat) 1710 (C=O) cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.39 [t, 3 H, CH_2CH_3], 2.31 [s, 3 H, *p*-Ar-CH_3], 2.58 [s, 3 H, *o*-Ar-CH_3], 7.05 [bs, 2 H, Ar-*H*], 7.85 [d, 1 H, Ar-*H*]. ^{13}C NMR ($DCCl_3$) ppm 14.30 [CH_2CH_3],

21.30 [Ar-CH_3], 21.72 [Ar-CH_3], 60.41 [CH_3CH_2O], 126.34, 126.94, 130.69, 132.41, 140.15, 142.24 [Ar-*C*], and 167.57 [CO_2R].

Methyl 4-Formyl-3-methylbenzoate (116). In a 100 mL, single-necked (ground-glass joint), round-bottomed flask fitted with a condenser and a magnetic stirrer was suspended dry aldehyde-acid **121** (1 g, 6.19 mmol) in ether (10 mL) with MeOH (0.5 mL). To this suspension, CH_2N_2 [generated by adding a solution of diazald (4 g in 50 mL of ether, Aldrich) to a solution of KOH (8 g, in 15 mL of TEG and 5 mL of water) at 30°C; *Caution*: CH_2N_2 is explosive and hazardous to health] was added dropwise over a period of 40 min [until the suspension became clear and TLC (silica gel, 9:1, CH_2Cl_2:EtOAc) did not show acid in the base line)]. Excess diazomethane was removed by passing N_2 into the solution (25 min). The ether was evaporated, and the resulting oil was subjected to chromatography on a Chromatotron (4 mm thick silica gel plate) using dichloromethane as the solvent. The first band contained the aldehyde ester **116** (0.84 g, 4.71 mmol, 78.21%); mp 35-37°C [lit[37] 36-38°C]. IR (KBr) 2740 (CHO) 1745, 1690 [(CHO) and (CO_2Me)] cm^{-1}; ^1H NMR ($DCCl_3$) δ 2.72 [s, 3 H, Ar-CH_3], 3.95[s, 3 H, OCH_3], 7.85 [d, 1 H, Ar-*H*], 7.92 [s, 1 H, Ar-*H*], 7.99 [d, 1 H, Ar-*H*], 10.35[s, 1 H, C(O)*H*]. ^{13}C NMR ($DCCl_3$) ppm 19.38 [Ar-CH_3], 52.48 [OCH_3], 127.27, 131.55, 132.80, 134.05, 136.96, 140.55 [Ar-*C*], 166.16 [CO_2Me], 192.12 [*C*(O)H].

Methyl 4-Nitro-3-methylbenzoate (119). In a 300 mL, three-necked, round-bottomed flask, fitted with a Dean-Stark apparatus, a condenser, an addition funnel and a magnetic stirrer was placed 4-nitro-3-methylbenzoic acid (**118**, 5 g, 27.6 mmol, Aldrich) dissolved in MeOH (60 mL) and benzene with a little sulfuric acid (2 mL). The reaction was boiled for 36 h, cooled to RT and quenched with water (200 mL). The organic layer was separated, and the aqueous layer was extracted with ether (4 x 45 mL). The combined organics were washed with $NaHCO_3$ (2 x 50 mL), water (4 x 45) and brine (50 mL) and

then dried with Na_2SO_4 (3 h). Evaporating the solvent (rotovap) gave ester **119** as a pale yellow, sweet-smelling solid (4.41 g, 22.6 mmol, 82%); mp 80-83°C [lit[37] 82-84°C]. IR (KBr) 1735 (C=O), 1540, 1365 (NO_2) cm^{-1}; ^1H NMR ($DCCl_3$) δ 2.1 [s, 3 H, Ar-C*H*$_3$], 3.81 [s, 3 H, OC*H*$_3$], 6.45 [d, 1 H, Ar-*H*], 7.6 [m, 2 H, Ar-*H*]. ^{13}C NMR ($DCCl_3$) ppm 17.1 [Ar-*C*H$_3$], 51.6 [O*C*H$_3$], 124.52 128.02, 133.74, 133.81, 133.94, 151.61 [Ar-*C*], 161.63 [*C*O$_2$Me].

Methyl 4-Amino-3-methylbenzoate (120). (Method **A**). A single-necked, 500-ml, round-bottomed flask with a magnetic stirrer was charged with N_2 and nitro compound **119** (5 g, 25.61 mmol) which was dissolved in acetic acid (75 mL) and water (5 mL). To this solution, $TiCl_3$/HCl (230 g, 1.49 mol, Aldrich, weighed and not measured) was added dropwise, and the purple reaction mixture was stirred (3 h). The new mixture was cooled (0°C), and chilled NaOH (30%, 200 mL, pH 12) was added. The aqueous layer was extracted with ether (5 x 75 mL) and $HCCl_3$ (2 x 50 mL). The combined extracts and organic layer were washed with water (2 x 50 mL) and dried with Na_2SO_4 (3 h). Evaporating the solvent under reduced pressure gave amine **120** as a yellow solid (3.25 g, 19.6 mmol, 76%). Crude amine **120** was recrystallized (MeOH); mp 119-121°C [lit[55] 120-122°C]. Method **B**. In a 1-L, three-necked, round-bottomed flask fitted with an addition funnel, a condenser and a magnetic stirrer, was placed nitro compound **119** (30 g, 0.153 moles) which was dispersed in EtOH (250 mL) and acetic acid (300 mL) at 0°C. To this mixture was added HCl (conc, 145 mL) followed by a white, emulsion-like solution of $SnCl_2 \cdot 2\ H_2O$ (108 g, 0.478 moles) in EtOH (150 mL) which was added dropwise over a period of 1 h. The reaction mixture was stirred at RT (24 h) with occasional gentle warming with a heat gun. It was then neutralized with NaOH (30%, pH 12, 0°C). The white precipitate formed was dissolved in water (150 mL), and the aqueous layer was extracted with ether (5 x 70 mL). The combined organics was washed with water (2 x 60 mL) and brine (1 x 75 mL) and then dried (Na_2SO_4, 3 h).

Evaporating the solvent under reduced pressure (rotovap) gave amine **120** as a yellow solid (22.6 g, 0.136 mol, 89%). Amine **120** was used in the following reaction without further purification; mp 119-121°C (lit[37] 120-122°C). IR (KBr) 3490, 3390 (NH_2), 1700 (C=O) cm^{-1}; ^1H NMR ($DCCl_3$) δ 2.15 [s, 3 H, Ar-C*H*$_3$], 3.83 [s, 3 H, OC*H*$_3$] 3.9 [bs, 2 H N*H*$_2$], 6.52 [d, 1 H, Ar-*H*], 7.5 [m, 2 H, Ar-*H*]. ^{13}C NMR ($DCCl_3$) ppm 17.1 [Ar(CH_3)], 51.6 [OCH_3], 113.5, 119.55, 120.97, 129.25, 132.19, 149.11 [Ar-*C*] and 167.35 [CO_2H].

3-Methyl-4-Formylbenzoic Acid (121). (a) In a 100 mL, round-bottomed flask with a condenser and a magnetic stirrer, was placed pulverized amine **120** (3.3 g, 19.97 mmol) to which was added slowly with HCl (4.5 mL), water (4.0 mL) and ice (8 g). The slurry formed was cooled to 0°C, and a solution of $NaNO_2$ [1.4 g, 20.28 mmol, in water (2 mL)] was added dropwise. To the yellow orange solution formed was added a solution of NaOAc·3 H_2O (6.1 g, 44.82 mmol, in 3 mL water). The mixture was stored at 0°C (pH 6-7).

(b) A 50-mL, singled-necked, round-bottomed flask containing a suspension of paraformaldehyde (0.92 g, 30.63 mmol) and hydroxylamine hydrochloride (2.1 g, 30.22 mmol) in 13 mL of water was gently warmed (15 min) with stirring which was continued until the solution was clear. To this solution was added NaOAc·3H_2O (4.1 g, 30.13 mmol) and the resulting solution was boiled (15 min). After cooling to RT, the reaction mixture was stored at 0°C.[2]

(c) In a 50-mL, Erlenmeyer flask was placed Na_2SO_3 (0.08 g, 0.63 mmol) dissolved in water (14.5 mL) and to this was added $CuSO_4$ (0.5 g, 2 mmol). The resulting blue solution was treated with NaOAc·3 H_2O (14 g, 0.10 mol) and cooled (0°C).[2]

In a 300-mL, three-necked, round-bottomed flask (N_2) fitted with a condenser and a magnetic stirrer were placed solutions from (*b*) and (*c*) above which were mixed at 0°C (a dark solution resulted). To this solution was added mixture *a* with vigorous stirring over a

period of 40 min, and a dark gummy mass separated as the stirring continued. The reaction mixture was warmed to RT (1.5 h), and conc HCl (10 mL) was added to acidify the reaction mixture. An additional amount of conc HCl (20 mL) was added, and the reaction mixture was boiled (2 h). A yellow solid separated as the solution was allowed to cool to RT (12 h). The solid was filtered, washed with water (200 mL) and dried in an Abderhalden [12 h, under vacuum (0.2 mm), benzene as the heating solvent]. The dry solid was recrystallized using a mixture of acetone:benzene (1:2). Aldehyde-acid **121** was obtained as yellow, flaky crystals (1.01 g, 6.19 mmol, 31%); mp 216-220°C [lit[37] 220-222°C]. IR (KBr) 3500 [C(O)O-H], 1710 and 1690 [C=O(H), C=O(OH)] cm^{-1}; ^1H NMR ($DCCl_3$) δ 2.69 [s, 3 H, Ar-C*H*$_3$], 7.89 [m, 3 H, Ar-*H*], 10.32 [s, 1 H, C(O)H]. ^{13}C NMR ($DCCl_3$) ppm 18.65 [Ar-C*H*$_3$], 126.95, 130.82, 132.26, 134.63, 136.55, 140.31 [Ar-*C*], 166.50 [*C*O$_2$H], and 192.98 [C(O)H]

2,2,4,4-Tetramethyl-6-nitrothiochroman (122). In a 50 mL, singled necked, round bottomed flask, fitted with a condenser and magnetic stirrer, ether **107** (4.35 g, 21.08 mmol), was dissolved in Ac_2O (8 mL) at 0°C. A mixture of cold concentrated HNO_3 (3 mL) and Ac_2O (9 mL) was added dropwise to the reaction mixture (0°C, 10 min) which was then stirred (1 h). The mixture was then poured into a solution of saturated $NaHCO_3$ (100 mL), and the resulting mixture was extracted with H_2CCl_2 (3 x 40 mL). The organic layer was washed with water (50 mL) and brine (50 mL) and then dried with Na_2SO_4 (4 h). The solvent was evaporated (rotovap), and the crude solid (brown in color) was purified by chromatography on a Chromatotron (H_2CCl_2, 4 mm thick silica gel plate) to give 6-isomer **122** (10:1, 6-isomer:8-isomer; 1.5 gm, 5.96 mmol, 28%; not a reported compound) as a yellow solid which was used without further purification to prepare **123**; mp 106-109°C. IR (KBr) 1545, 1360 (NO_2) cm^{-1}. ^1H NMR ($DCCl_3$) δ 1.10 [s, 3 H, C(C*H*$_3$)$_2$], 1.37 [s, 3 H, C(C*H*$_3$)$_2$], 1.52 [C(C*H*$_3$)$_2$], 1.56 [s, 3 H, SC(C*H*$_3$)$_2$], 2.03 [m, 3 H, C*H*$_2$], 8.01 [d, 1 H, Ar-*H*] and 8.24 [d, 2 H, Ar-*H*]. ^{13}C NMR ($DCCl_3$) ppm 18.55

[C(CH_3)$_2$], 26.16 [C(CH_3)$_2$], 32.01 [SC(CH_3)$_2$], 34.57 [SC(CH_3)$_2$], 34.75 [C(CH_3)$_2$], 46.37 [CH_2], 54.45 [SC(CH_3)$_2$], and 121.61, 121.83, 127.94, 128.34, 146.03, 146.56 [Ar-C].

2,2,4,4-Tetramethyl-6-aminothiochroman (123). In a 250 mL single-necked round bottomed flask (N_2) fitted with a spiral condenser and a magnetic stirrer, was added nitro compound **122** (0.69 g, 2.7 mmol) dissolved in acetic acid (25 mL, vigorous stirring) and water (5 mL). Then $TiCl_3$/HCl (28.06 g. 18 mmol, Aldrich, weighed rather than measuring the volume) was added dropwise, and the resulting purple reaction mixture was stirred (2 h, RT). The new mixture was cooled (0°C) and NaOH (30%, 110 mL) was added slowly. The aqueous layer was extracted with EtOAc (4 x 35 mL) and H_2CCl_2 (2 x 40 mL), and the combined organic layers were washed with water (2 x 50 mL) and saturated $NaHCO_3$ (2 x 50 mL). The solution was then dried with Na_2SO_4 (2 h). Purification of the crude amine by chromatography on a Chromatotron (H_2CCl_2:EtOAc, 50:1) plate (4 mm thick silica gel) gave amine **123** as a brown oil (0.48 g, 80.4%; not a reported compound) which was without further purification to prepare **86**. IR (KBr) 3450, 3360 (NH_2) cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.36 [s, 6 H, C(CH_3)$_2$], 1.39 [s, 6 H, SC(CH_3)$_2$], 1.90 [s, 2 H, CH_2], 3.50 [bs, 2 H, NH_2], 6.44 [d, 1 H, Ar-H], 6.75 [s, 1 H, Ar-H] and 9.92 [d, 1 H, Ar-H]. ^{13}C NMR ($DCCl_3$) ppm 31.64 [C(CH_3)$_2$], 32.04 [SC(CH_3)$_2$], 35.72 [C(CH_3)$_2$], 41.89 [SC(CH_3)$_2$], 54.76 [CH_2], and 113.79, 113.83, 121.26, 129.12, 143. 91 and 144.15 [Ar-C].

6-Nitro-2,3-dihydro-1,4-benzodioxan (125). In a 50-mL, singled-necked, round-bottomed flask fitted with a condenser and a magnetic stirrer was placed 1,4-benzodioxan (**124**, 4.35 g, 31.95 mmol) dissolved in Ac_2O (8 mL) at 0°C. A mixture of cold concentrated HNO_3 (3 mL) and Ac_2O (9 mL) was added dropwise to the reaction mixture (0°C, 10 min) which turned into a thick, yellow suspension. After stirring the

suspension at RT (4 h), it was poured into a solution of saturated $NaHCO_3$ (250 mL), and the mixture was extracted with H_2CCl_2 (3 x 40 mL). The organic layer was washed with water (50 mL) and brine (50 mL) and then dried with $MgSO_4$ (4 h). The solvent was evaporated (rotovap) to give crude 6-nitro-1,4-benzodioxan (**125**, 5.5 gm, 94%) as a yellow solid which was used without further purification to prepare **126**; mp 114-117°C. IR (KBr) 1530-1520, 1350 (NO_2) cm^{-1}; ^1H NMR ($DCCl_3$) δ 4.30-4.41 [m, 4 H, OCH_2CH_2O], 6.91 [d, 1 H, Ar-*H*], 7.41 [s, 1 H, Ar-*H*], 7.77 [m, 1 H, Ar-*H*]. ^{13}C NMR ($DCCl_3$) ppm 64.03 [OCH_2], 64.64 [OCH_2], and 113.37, 114.42, 117.21, 117.52, 143.14 and 149.34 [Ar-C].

6-Amino-2,3-dihydro-1,4-benzodioxan (126). In a 500-mL, three-necked, round-bottomed flask, fitted with a magnetic stirrer and charged with N_2 was placed nitro compound **125** (4.0 g, 22.08 mmol) dissolved in acetic acid (110 mL) and water (5 mL). Then $TiCl_3$/HCl (198.6 g. 15.46 mmol, Aldrich, weighed rather than measuring the volume) was added dropwise, and the resulting purple reaction mixture was stirred (12 h, RT). The new mixture was cooled (0°C), and NaOH (30%, 500 mL) was added. The aqueous layer was extracted with EtOAc (4 x 75 mL) and $HCCl_3$ (2 x 40 mL), and the combined organic layers were washed with water (3 x 75 mL) and saturated $NaHCO_3$ (2 x 50 mL); the new solution was dried ($MgSO_4$, 2 h). Purification of the crude amine by chromatography on a Chromatotron (H_2CCl_2:EtOAc, 3:1) plate (4 mm thick silica gel) gave amine **126** as a brown oil (2.08 g, 13.75 mmol, 62%) which was used without further purification to prepare **88**. ^1H NMR ($DCCl_3$) δ 3.36 [bs, 2 H, NH_2], 4.12-4.18 [m, 4 H, OCH_2CH_2O], 6.17 [m, 2 H, Ar-*H*] and 6.64 [d, 1 H, Ar-*H*]. ^{13}C NMR ($DCCl_3$) ppm 63.94 [OCH_2], 64.45 [OCH_2], and 103.93, 108.49, 117.41, 136.17, 140.71 and 143.72 [Ar-*C*].

Dimethyl *trans*, *trans*-Muconate (128). In a 500-mL, single-necked round-bottomed flask (N_2) equipped with a Dean-Stark apparatus, a spiral condenser, and a magnetic stirrer was placed muconic acid [**127**, *trans*, *trans*-1,3-butadiene-1,4-dicarboxylic acid (Aldrich, 10 g, 70.30 mmol)] in absolute methanol (150 mL) and benzene (100 mL) with HCl (conc 10 mL). The solution was heated at reflux (3 days), and then it was allowed to cool to RT (3 h). Colorless needles separated from the brown colored solution at RT. These were filtered, washed and recrystallized (benzene) to give long, colorless needle-like crystals of dimethyl muconate (**128**, 8.5 g, 49.90 mmol, 71%); mp 156-158°C [lit[16] 156-157°C]. IR (neat) 1770 (C=O) cm^{-1}; ^1H NMR ($DCCl_3$) δ 3.79 [s, 6 H, OCH_3], 6.19 [d, 2 H, $(MeO(O)CCH{=}CH)_2$], and 7.34 [d, 2 H, $(MeO(O)CCH{=}CH)_2$]. ^{13}C NMR ($DCCl_3$) ppm 51.91 [OCH_3], 127.99 [$(MeO(O)CCH{=}CH)_2$], 140.93 [$(MeO(O)CCH{=}CH)_2$], and 166.26 [CO_2H].

Muconic Acid Mono Methyl Ester (129). To a stirred solution of dimethyl muconate (**128**, 8 g 47.01 mmol) in acetone (anhydrous, 370 mL) in a 1000-mL, single-necked, round-bottomed flask fitted with a condenser (N_2 and magnetic stirrer) was added boiling methanol (absolute, 150 mL). The resulting solution was treated, in one portion, with KOH (2.6 g, 47.01 mmol) dissolved in methanol (absolute, 85 mL). This reaction mixture was then boiled (15 min), acidified with HCl [6 *N* (methanol), 10 mL] and filtered. To the resulting filtrate was added water (75 mL), and then the solvents were evaporated to dryness (under vacuum) to give a white residue. The dry residue (mostly dimethyl muconate) was boiled several times with methanol (400 mL), and the mixture was filtered hot. The filtrate was then evaporated to dryness (rotovap), and the residue was boiled with benzene (100 mL) and filtered hot. The undissolved solid was boiled three times with benzene (300 mL, 200 mL, and 200 mL) and filtered. Colorless flaky crystals of the monoester **129** (1.9 g, 12.61 mmol, 26%) were obtained upon cooling (RT); mp 162-163°C [lit[42] 161-162°C]. IR (KBr) 3500 [C(O)O-H], 1735 [C=O(OMe)], 1680

[C=O(OH)] cm^{-1}. ^1H NMR ($DCCl_3$) δ 3.70 [s, 3 H, OCH_3], 6.34-6.46 [2 d, 2 H, (-O(O)CC*H*=CH-)$_2$], and 7.29-7.43 [m, 2 H, (-O(O)CCH=C*H*-)$_2$]. ^{13}C NMR ($DCCl_3$) ppm 51.57 [O*C*H_3], 127.42 [MeO(O)*C*CH=CH], 129.68 [HO(O)*C*CH=CH], 140.53 [MeO(O)CCH=*C*H], 141.42 [HO(O)CCH=*C*H], 165.82 [*C*(O)OMe], and 166.68 [*C*(O)OH].

4-[(2,2,4,4-Tetramethyl-1,1-dioxythiochromanyl)]benzoic Acid (131). In a 250-mL, single-necked, round-bottomed flask fitted with a condenser and a magnetic stirrer was placed ketone **102** (2 g, 8.0 mmol) in ethanol (95%, 20 mL). To the stirred solution was added commercial Clorox® (150 mL), and the turbid reaction mixture was boiled for 24 h. The clear solution was cooled to 0°C, and a solution of sodium metabisulfite (25%, 100 mL) was added dropwise (*Caution*: pungent fumes formed) followed by slow addition of conc HCl (30 mL). A white solid formed and was filtered and dried (12 h, Abderhalden, benzene as drying solvent). Slightly crude acid **131** was recrystallized (ethanol, 95%). Colorless crystals (1.1 g, 51%, not a reported compound) of acid **131** thus obtained were used without any further purification to prepare **92**; mp 256-259°C. IR (KBr) 3500 [C(O)O-H], 1710 (C=O) cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.45 [s, 6 H, (C*H*$_3$)$_2$], 1.50 [s, 6 H, SC(C*H*$_3$)$_2$], 2.36 [s, 2 H, C*H*$_2$], 8.12 [s, 2 H, Ar-*H*], 8.20 [s, 1 H, Ar-*H*]. ^{13}C NMR ($DCCl_3$) ppm 21.76 [C(*C*H$_3$)$_2$], 34.05 [*C*(CH$_3$)$_2$], 34.18 [SC(*C*H$_3$)$_2$], 48.74 [S*C*(CH$_3$)$_2$], 54.80 [*C*H$_2$], and 120.12, 125.22, 128.57, 130.22, 133.00, 139.05, 145.95 [Ar-*C*] and 170.38 [*C*O$_2$H].

2,3-Dihydro-3,3-dimethyl-1,1-dioxybenzo[*b*]thiophen-5-carboxylic Acid (134). In a 250-mL, single-necked, round-bottomed flask fitted with a condenser and a magnetic stirrer was placed ketone **133** [1.0 g, 6.1 mmol, prepared by reported procedure[22] (mp lit[22] 20.1-21.4°C); the IR, ^1H and ^{13}C NMR spectra matched those of the reported compound[22]] dissolved in 35 mL of ethanol (95%). To the stirred solution was

added commercial Chlorox (140 mL), and the turbid reaction mixture was boiled for 4 h. The clear solution was cooled to 0°C, and a solution of sodium metabisulfite (25%, 30 mL) was added dropwise (*Caution*: pungent fumes formed) followed by the *slow* addition of conc HCl (50 mL). Another 70 mL of $Na_2S_2O_8$ (25%) was added *after* a white solid formed. The solid was filtered and dried in the Abderhalden (80°C/2 mm Hg, 12 h). Crude acid **134** was recrystallized (95% ethanol) to yield colorless crystals (0.9, 81%, not a reported compound) of **134** which were used without further purification to prepare compound **94**; mp 285.5-286.4°C. IR (KBr) 3500 [C(O)O-H], 1710 (C=O) cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.50 [s, 6 H, $C(CH_3)_2$], 3.58 [s, 2 H, CH_2], 7.82 [d, 1 H, Ar-*H*], 8.08 [d, 1 H, Ar-*H*], 8.17 [s, 1 H, Ar-*H*]. ^{13}C NMR ($DCCl_3$) ppm 28.33 [$C(CH_3)_2$], 34.18 [$C(CH_3)_2$], 64.62 [CH_2], and 121.07, 125.39, 129.77, 136.00, 141.30, 147.39 [Ar-C], and 166.16 [CO_2H].

5-[2,3-Dihydro-3,3-dimethyl-5-benzofuranyl]benzoic Acid (136). In a 100-mL, single-necked, round-bottomed flask fitted with a condenser and a magnetic stirrer was placed ketone **135** (1.0 g, 5.26 mmol, prepared by reported procedure,[22] the IR, ^1H and ^{13}C NMR spectra matched those of the reported compound[22]) dissolved in 17 mL of ethanol (95%). To the stirred solution was added commercial Chlorox (50 mL), and the turbid reaction mixture was boiled (5 h) and stirred (RT, 5 h). The clear solution was cooled to 0°C, and a solution of sodium metabisulfite (25%, 50 mL) was added dropwise (*Caution*: pungent fumes formed) followed by the addition of conc HCl (10 mL). Another 70 mL of $Na_2S_2O_8$ (25%) was added *after* the white solid was formed. The solid was filtered and dried in the Abderhalden (80°C/2 mm Hg, 12 h). Crude acid **136** was recrystallized (95% ethanol) to yield colorless crystals (0.71, 3.69 mmol, 70%, not a reported compound) of **136**, which was used without further purification to prepare compound **96** ; mp 175-176°C. IR (KBr) 3500 [C(O)O-H], 1680 (C=O) cm^{-1}. ^1H NMR ($DCCl_3$) δ 1.38 [s, 6 H, $(CH_3)_2$], 4.34 [s, 2 H, OCH_2], 6.18 [d, 1 H, Ar-*H*], 7.87 [s, 1

H, Ar-*H*], and 7.99 [d, 1 H, Ar-*H*]. ^{13}C NMR ($DCCl_3$) ppm 27.64 [C(CH_3)$_2$], 41.50 [*C*(CH_3)$_2$], 85.51 [*C*H_2], 109.58, 121.97, 125.01, 131.94, 137.18, 164.15 [Ar-*C*] and 172.26 [*C*O_2H].

2,3-Dihydro-3,3-dimethylbenzo[b]thiophene (137). In a 100-mL, three-necked round-bottomed flask (N_2) equipped with an addition funnel, spiral condenser, and a magnetic stirrer, a solution of alcohol **137a**[22] (4.5 g, 52 mmol) in freshly distilled CS_2 (25 mL) was added dropwise to a stirred suspension of $AlCl_3$ (12 g, 0.89 mmol) in CS_2 (25 mL). The reaction mixture was boiled (3 h). After cooling (0°C, 10 min) the mixture was very cautiously quenched with HCl (5%, 50 mL) and diluted with CH_2Cl_2. The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 40 mL). The combined organics were washed with saturated $NaHCO_3$ solution (2 x 50 mL) and brine (50 mL)and then dried (Na_2SO_4, 4 h). Evaporating the solvent under reduced pressure (rotovap) gave **137** as an oil. Vacuum distillation [lit[22] bp 56.3-58.2°C/0.3 mm Hg] gave **137** a pale yellow oil (3.05 g, 70%). ^{1}H NMR ($DCCl_3$) δ 1.35 [s, 6 H, C(C*H*$_3$)$_2$], 3.16 [s, 2 H, SC*H*$_2$], and 7.02-7.22 [m, 4 H, Ar-*H*]; ^{13}C NMR ($DCCl_3$) ppm 27.1 [C(*C*H_3)$_2$], 46.9 [S*C*H_2], 46.9 [*C*(CH_3)$_2$], 122.1, 122.4, 124.1 127.1, 140.2, and 147.6 [Ar-*C*].

Attempted O-Alkylation of Methyl 4-[(2,3-Dihydro-1,4-benzodioxan-6-yl)carbamoyl]benzoate (88). In a 100 mL three-necked, round-bottomed flask (N_2), fitted with a condenser and a magnetic stirrer was dissolved amide **88** (0.2 g, 0.64 mmol) in CH_2Cl_2 (30 mL). To the yellow solution was added a suspension of trimethyloxonium tetrafluoroborate (Aldrich, 0.15 g, 0.75 mmol)[76] in CH_2Cl_2 (15 mL), and the resulting mixture was boiled (12 h). The reaction mixture was cooled (RT) and was poured into a 200 mL three-necked, round-bottomed flask fitted with a condenser and a magnetic stirrer, containing a solution of potassium carbonate (50%, 100 mL). The resulting mixture was

gently warmed (~50°C, 4 h), cooled to RT (1 h) and then extracted with CH_2Cl_2 (4 x 40 mL) and EtOAc (2 x 50 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL) and then dried (Na_2SO_4, 2 h). The solvent was evaporated (rotovap) to give a yellow solid. The ^1H and ^{13}C NMR spectra of the solid was identical to that of the starting material.

Attempted Preparation of Methyl 3-Methyl-4-Formylbenzoate (116) Via Oxidation. In a 300-mL, three-necked, round-bottomed flask (N_2) fitted with a condenser and a magnetic stirrer was placed methyl 3,4-dimethylbenzoate (**117**, 1.g, 5.6 mmol), glacial acetic acid (15 mL), and 15 mL of freshly distilled acetic anhydride with H_2SO_4 (1 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 (0.56 g, 5.61 mmol) was added in small portions (30 min). After stirring on a cool water bath (40 h), CrO_3 (0.5 g, 5 mmol) was added, and the reaction mixture was stirred at RT for an additional 12 h. TLC analysis (hexane:ether, 8:2) showed several spots (5-6), including the starting material. The dark green reaction mixture was treated **carefully** with ice water (150 mL) and then ether (40 mL). The organic phase separated, and the aqueous phase was extracted [$HCCl_3$ (3 x 50 mL) and then ether (1 x 50 mL)]. The combined organic phases were washed with saturated $NaHCO_3$ (3 x 40 mL), water (1 x 50 mL), and then brine (1 x 50 mL). After drying (Na_2SO_4, 12 h), the solvent was evaporated (rotovap, followed by high vacuum at 0.25 mm Hg, 45°C) to give a brown oil. The oil (1.3 g), was dissolved in ethanol (15 ml) in a 100-mL, single-necked, round-bottomed flask, (RT, stir, 10 min) with water (10 mL), and concentrated H_2SO_4 (0.5 mL); the mixture was then boiled for 4 h. After cooling to RT, water (150 mL) was added to the solution; the organic phase separated, and the aqueous phase was extracted with $HCCl_3$ (5 x 40 mL). The combined organic phases were washed with saturated $NaHCO_3$ (4 x 35 mL), water (50 mL), and brine (50 ml). After drying the solution (Na_2SO_4, 4 h), the solvent was evaporated

(rotovap, followed by high vacuum at 0.2 mm Hg) to give a brown liquid. The crude aldehyde **116** was purified by chromatography with a Chromatotron (4 mm thick silica gel plate; the solvent system used was hexane:ether, 8.5:1.5). A pale yellow oil (less than 0.02 g) of the aldehyde was obtained. Most of the starting material was recovered and hence aldehyde **116** had to be prepared by Scheme IV, page 40

Attempted Preparation of Methyl 3-Methyl-4-Formylbenzoate (116) Via Oxidation with CAN. In a 100-mL, three-necked, round-bottomed flask (N_2) fitted with a condenser, an addition funnel, and a magnetic stirrer was placed methyl 2,4-dimethylbenzoate (**117** 0.5.g, 2.8 mmol), in glacial acetic acid (15 mL). A solution of ceric ammonium nitrate (CAN, Aldrich, 6.5 g, 11.22 mmol) in glacial acetic acid (35 mL) was added dropwise (15 min) to the reaction mixture which turned colorless upon boiling (16 h). After cooling the reaction mixture (RT), water (100 mL) was added and the solution was extracted with ether (3 x 30 mL). The combined organic layers were washed with H_2O (50 mL), brine (50 mL) and dried (Na_2SO_4, 2 h). Evaporating the solvent gave only the starting material.

Attempted Reduction of 2,3-Dihydro-3,3-dimethyl-1,1-dioxybenzo[*b*]-thiophen-5-carboxylic Acid (134). In a 100-mL, single-necked, round-bottomed flask fitted with a condenser and a magnetic stirrer was placed acid **134** (1.0 g, 4.16 mmol) dissolved in glacial acetic acid (20 mL) with conc HCl (10 mL). To the stirred solution was added zinc dust (2.4 g, 36.71 mmol), and the resulting reaction mixture was boiled for 8 h. The reaction mixture was cooled (RT) and filtered through a Buchner funnel with a Celite pad. The filtrate was treated with water (150 mL) and extracted with CH_2Cl_2 (4 x 40 mL) and $CHCl_3$ (2 x 50 mL). The combined organic layers were washed with water (2 x 50 mL) brine (50 mL) and dried (Na_2SO_4, 1 h). The solvent was evaporated (rotovap) to give a white solid. From the ^{13}C NMR spectrum of the white

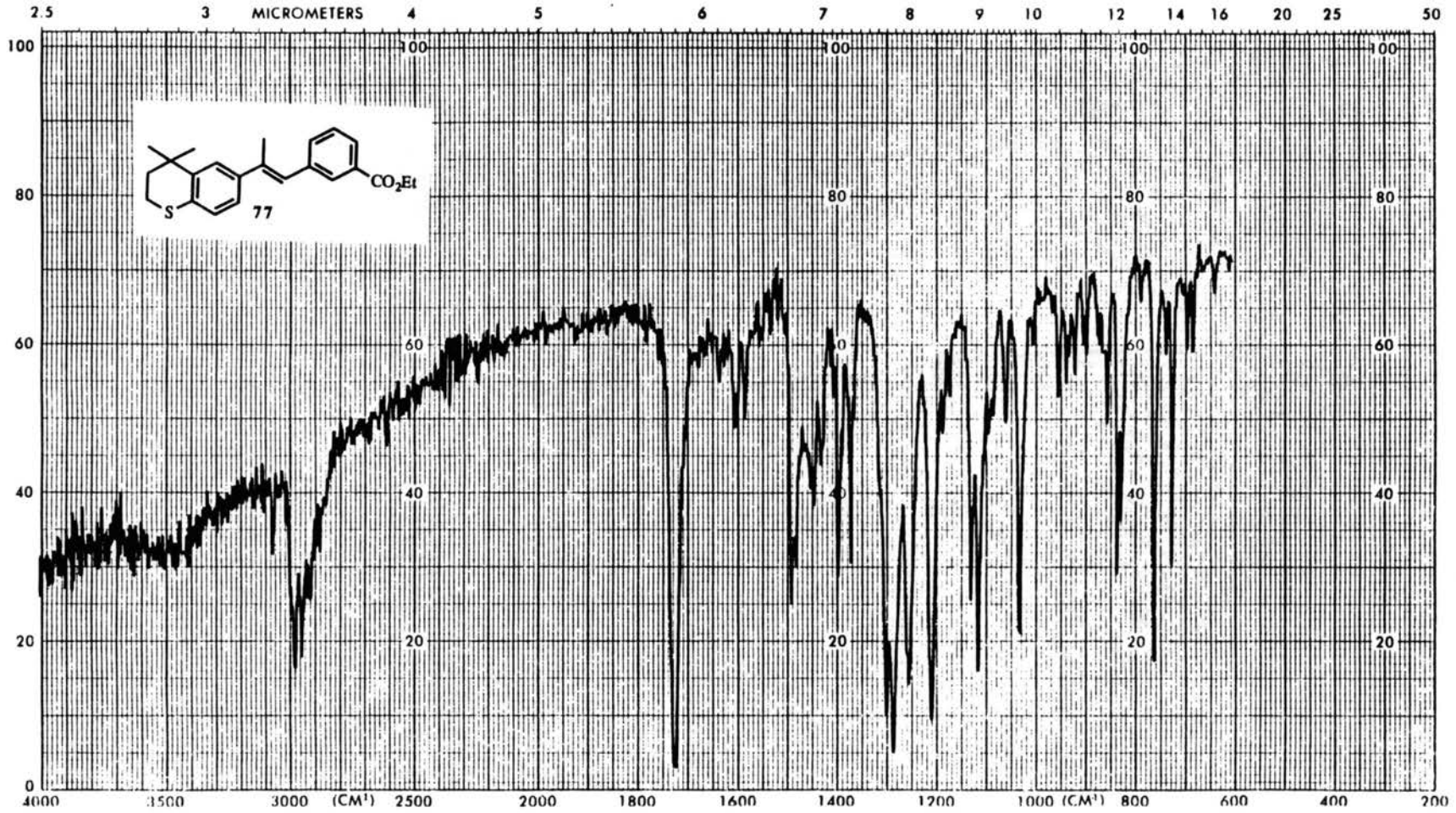
solid, it was clearly evident that the sulfone (**134**) was not reduced to the sulfide [^{13}C NMR value for C(2) was 65.62 ppm (sulfone **134**)] and the starting material was obtained.

Attempted Nitration of 2,3-Dihydro-3,3-dimethylbenzo[*b*]thiophene (137). In a 50 mL, singled necked, round bottomed flask, fitted with a condenser and magnetic stirrer, was added thioether **137**[22] (3 g, 18.26 mmol), dissolved in Ac_2O (6 mL) at 0°C. A mixture of cold concentrated HNO_3 (2 mL) and Ac_2O (7 mL) was added dropwise to the reaction mixture (0°C, 10 min) which was then stirred (several different attempts with time ranging from 2-24 h). The mixture was then poured into a solution of saturated $NaHCO_3$ (100 mL), and the resulting mixture was extracted with H_2CCl_2 (3 x 40 mL). The organic layer was washed with water (50 mL) and brine (50 mL) and then dried with Na_2SO_4 (4 h). The solvent was evaporated (rotovap) to give a yellow oil of the oxidized starting material [sulfone **139**, the ^{13}C NMR value for C(2) was 64.62 ppm instead of 47 ppm, (sulfide **137**)]. Nitration was not effected even on heating the reaction mixture for several hours (4-12 h). ^1H NMR ($DCCl_3$) δ 1.39 [s, 3 H, C*H*$_3$], 1.61 [s, 3 H, C*H*$_3$], 3.12 [d, 1 H, SC*H*] 3.28 [d, 1 H, SC*H*], 7.35 [d, 1 H, Ar-*H*], 7.41 [t, 1 H, Ar-*H*], 7.53 [t, 1 H, Ar-*H*], 7.78 [d, 1 H, Ar-*H*]. ^{13}C NMR ($DCCl_3$) ppm 29.17 [C(*C*H$_3$)], 31.28 [C(*C*H$_3$)], 45.90 [S*C*(CH$_3$)$_2$], 65.77 [*C*(CH$_3$)$_2$], 123.69, 126.53, 128.21, 132.22, 143.14, 150.94 [Ar-*C*].

Attempted Nitration of 2,3-Dihydro-3,3-dimethylbenzo[*b*]thiophene (137) with Ammonium Nitrate or Potassium Nitrate. In a 50 mL, singled necked, round bottomed flask, fitted with a condenser and magnetic stirrer was added thioether **137** (3 g, 18.26 mmol) dissolved in H_2SO_4 (6 mL) at 0°C. To this dark brown reaction mixture was added dropwise a solution of ammonium or potassium nitrate (1.60 or 2.03 g, 0.02 mmol, 0°C, 10 min) in conc H_2SO_4 (10 mL). The reaction mixture was stirred at 0°C (2 h) and then was very slowly warmed to RT [(3 h); *Caution*: the reaction is

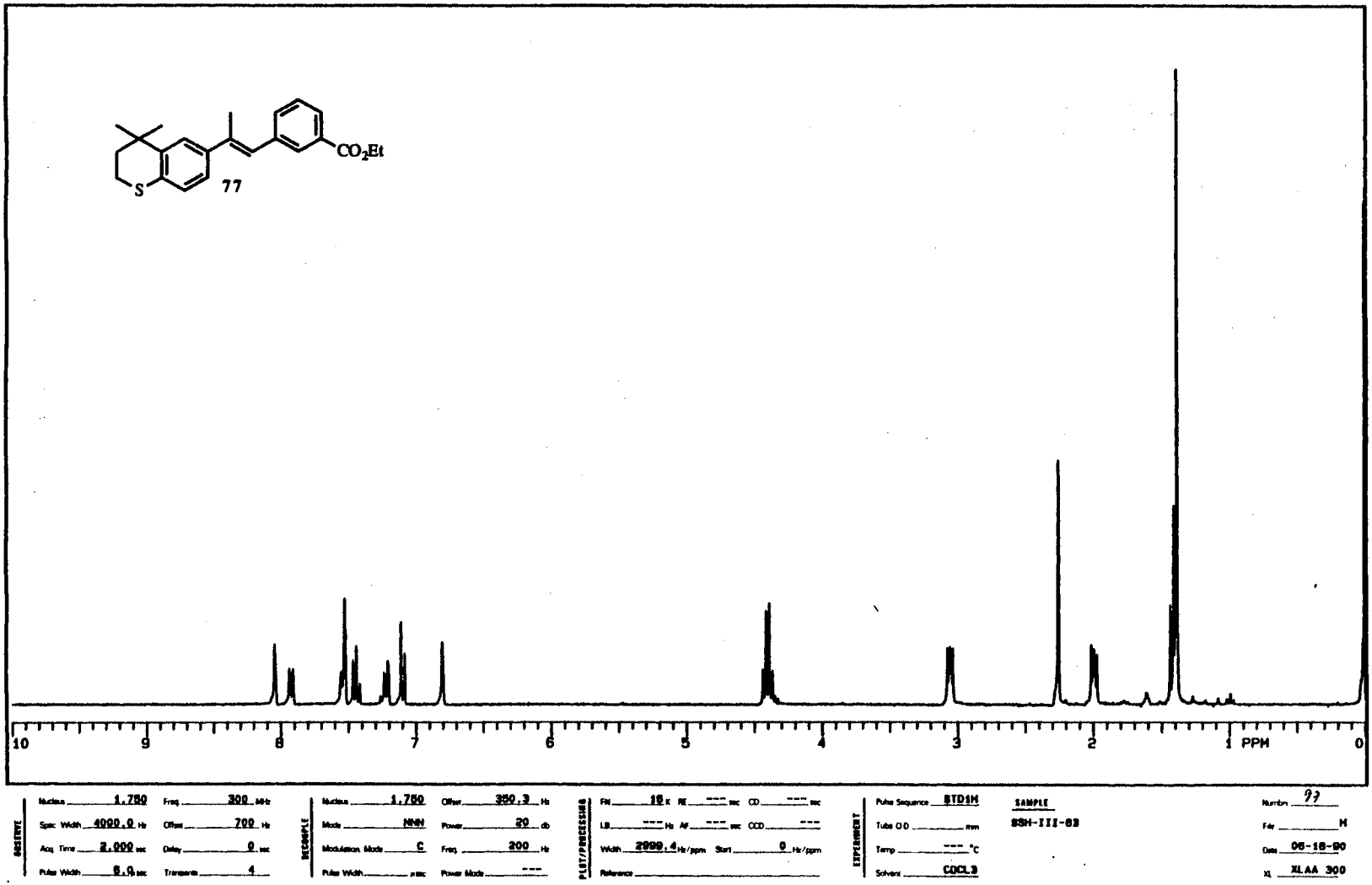
highly exothermic]. The mixture was then poured into a solution of saturated $NaHCO_3$ (0°C, 100 mL), and the resulting mixture was extracted with H_2CCl_2 (3 x 40 mL). The organic layer was washed with water (50 mL) and brine (50 mL) and then dried with Na_2SO_4 (4 h). The solvent was evaporated (rotovap), to give a brown oil of the oxidized starting material [sulfone **139**, ^{13}C NMR value for C(2) was 65 ppm (sulfone) instead of 47 ppm (for sulfide **137**)]. A mixture of the suspected nitro compound **138** and the oxidized starting material sulfone **139** was obtained along with other tarry oil. All attempts of separation of this mixture by chromatography failed and the oil proved intractable.

Plate I



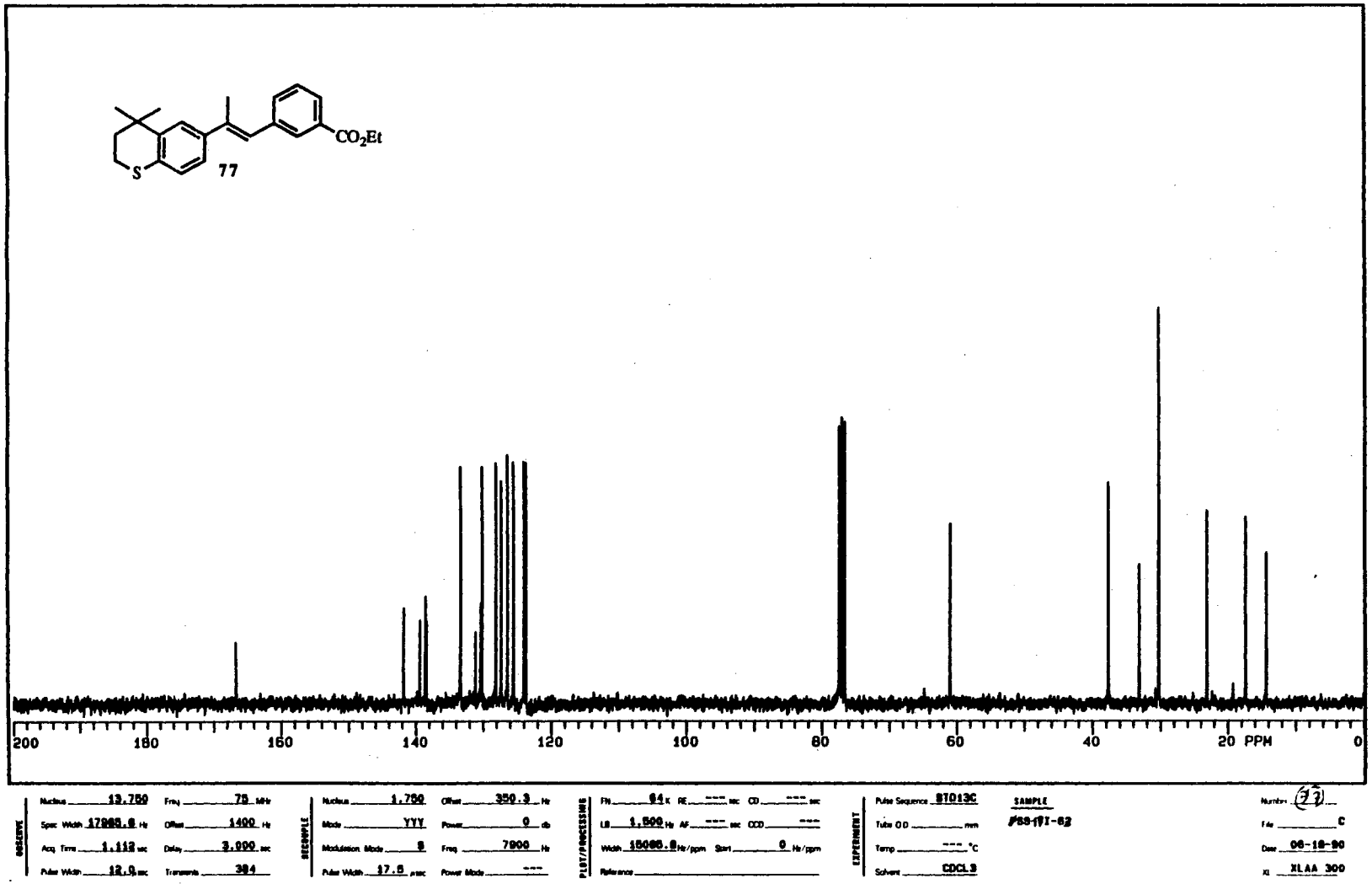
IR Spectrum of **77**

Plate II



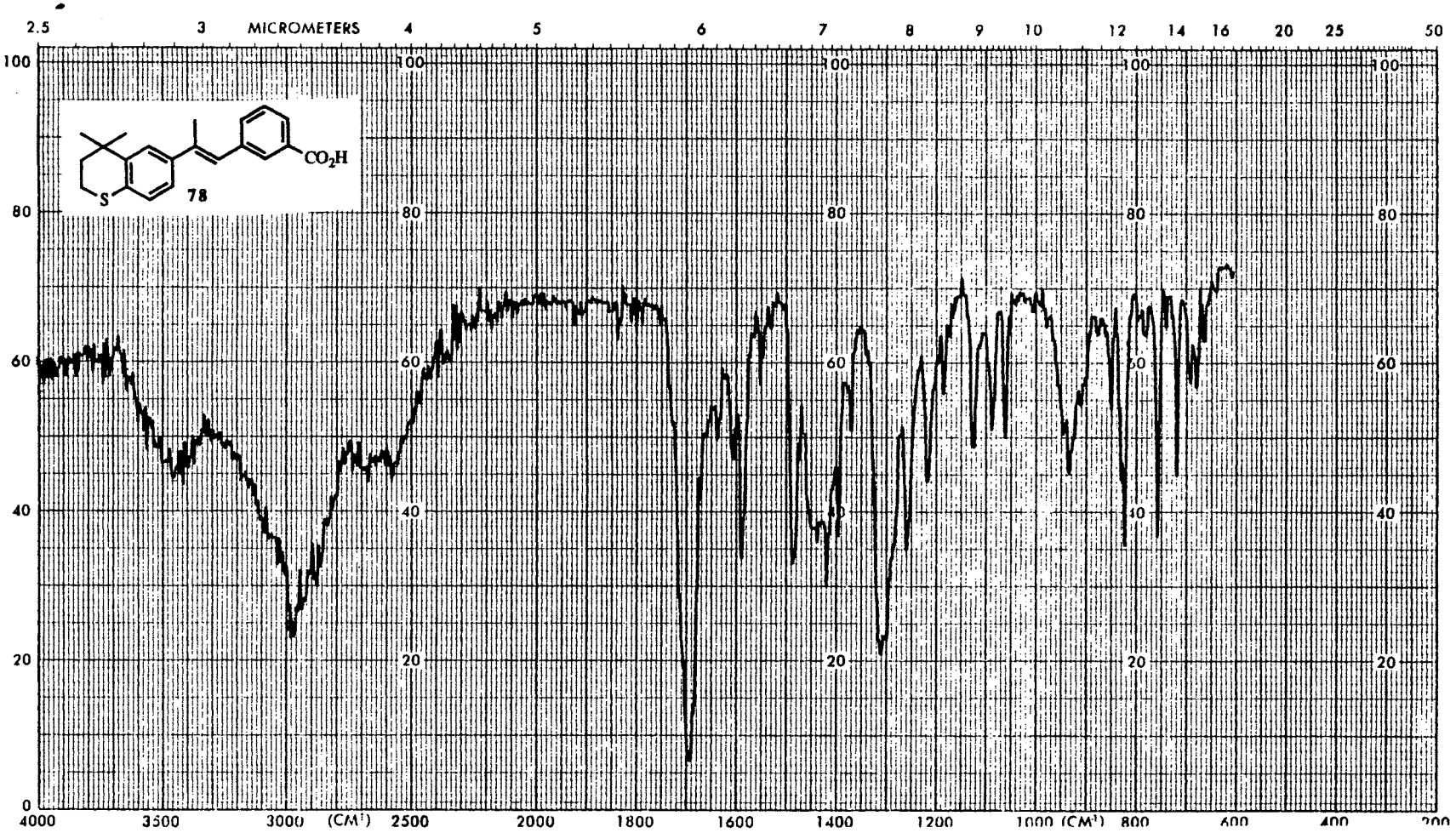
^{1}H NMR Spectrum of **77**

Plate III



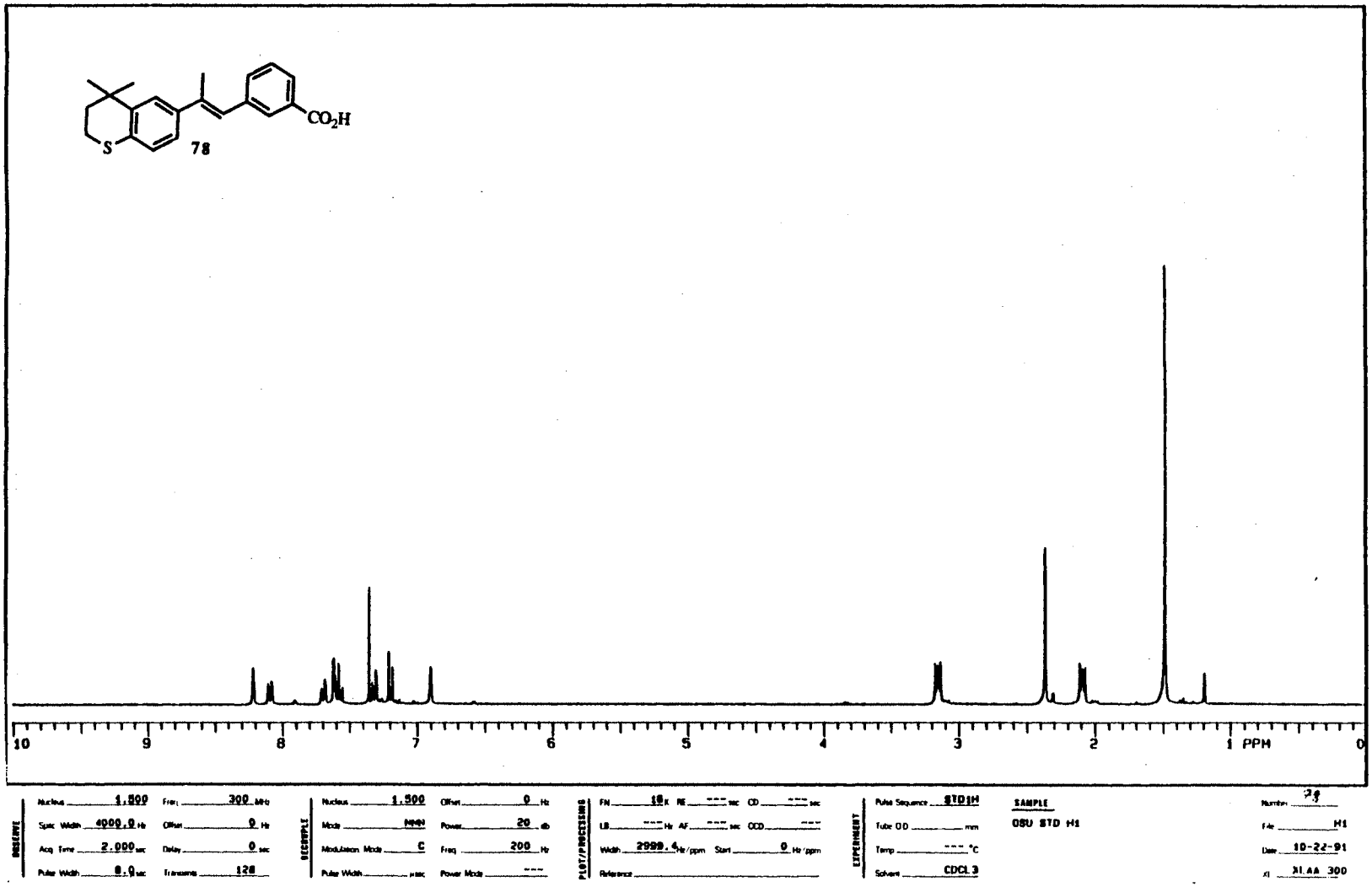
^{13}C NMR Spectrum of **77**

Plate IV



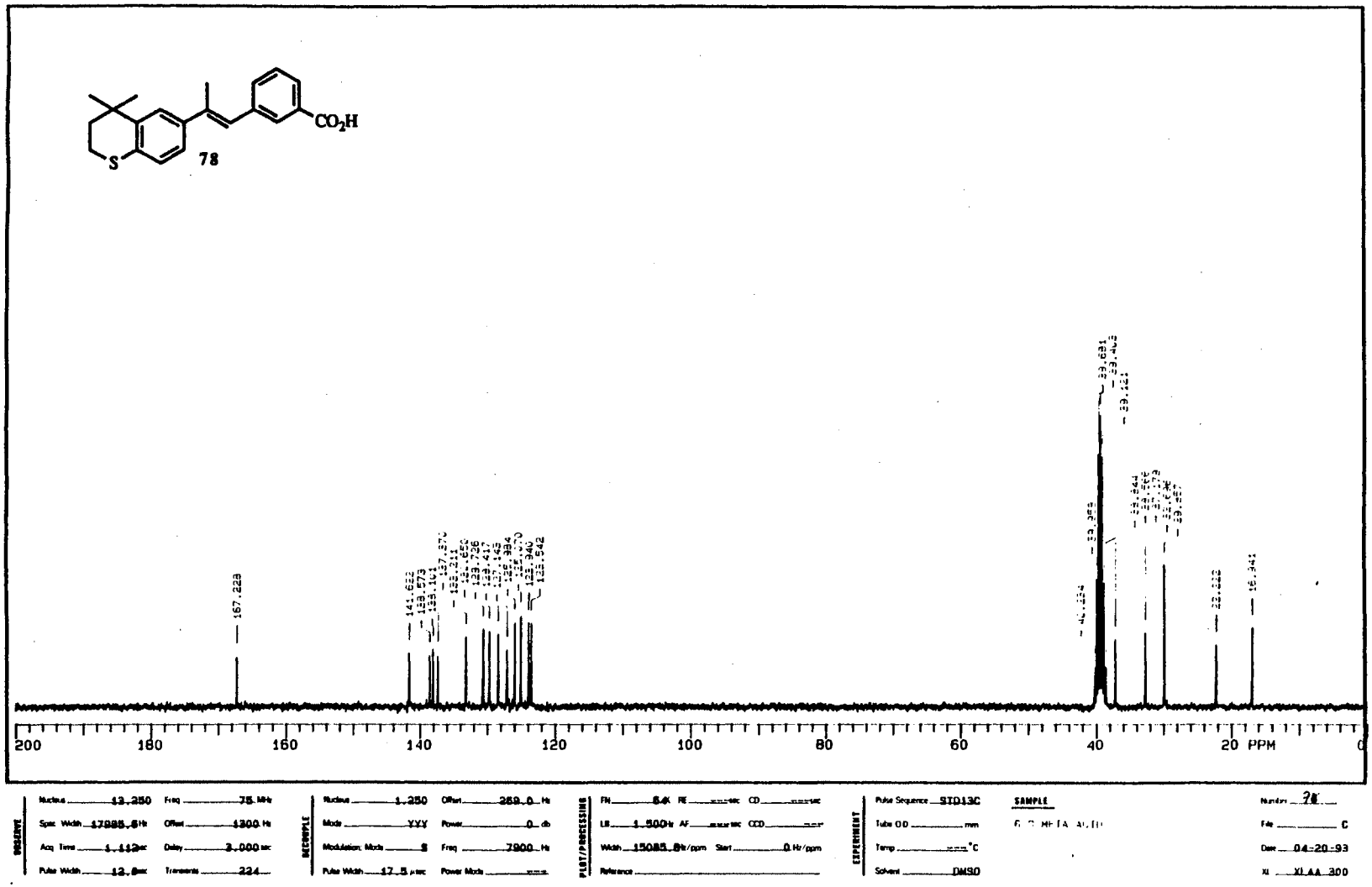
IR Spectrum of **78**

Plate V



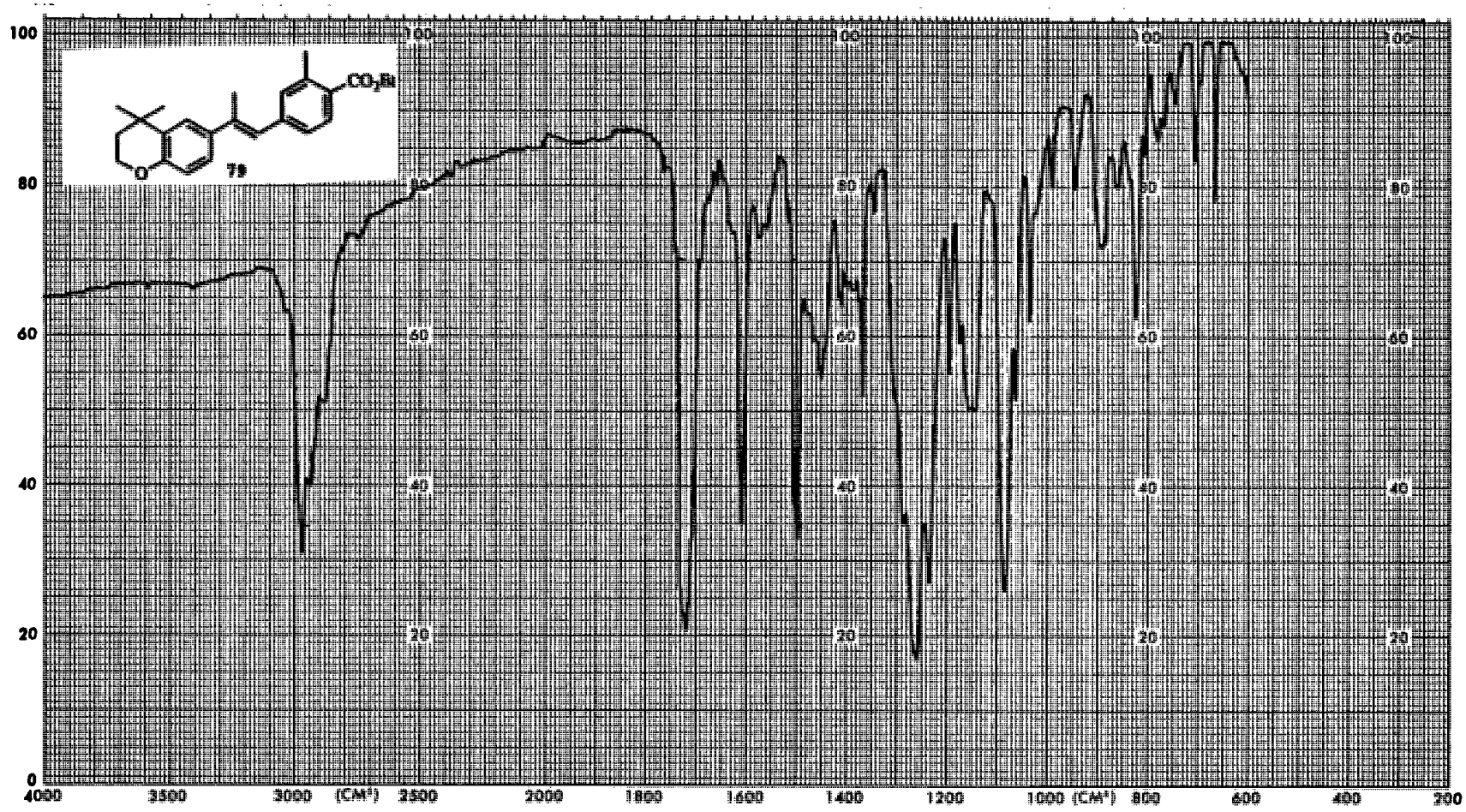
^{1}H NMR Spectrum of **78**

Plate VI



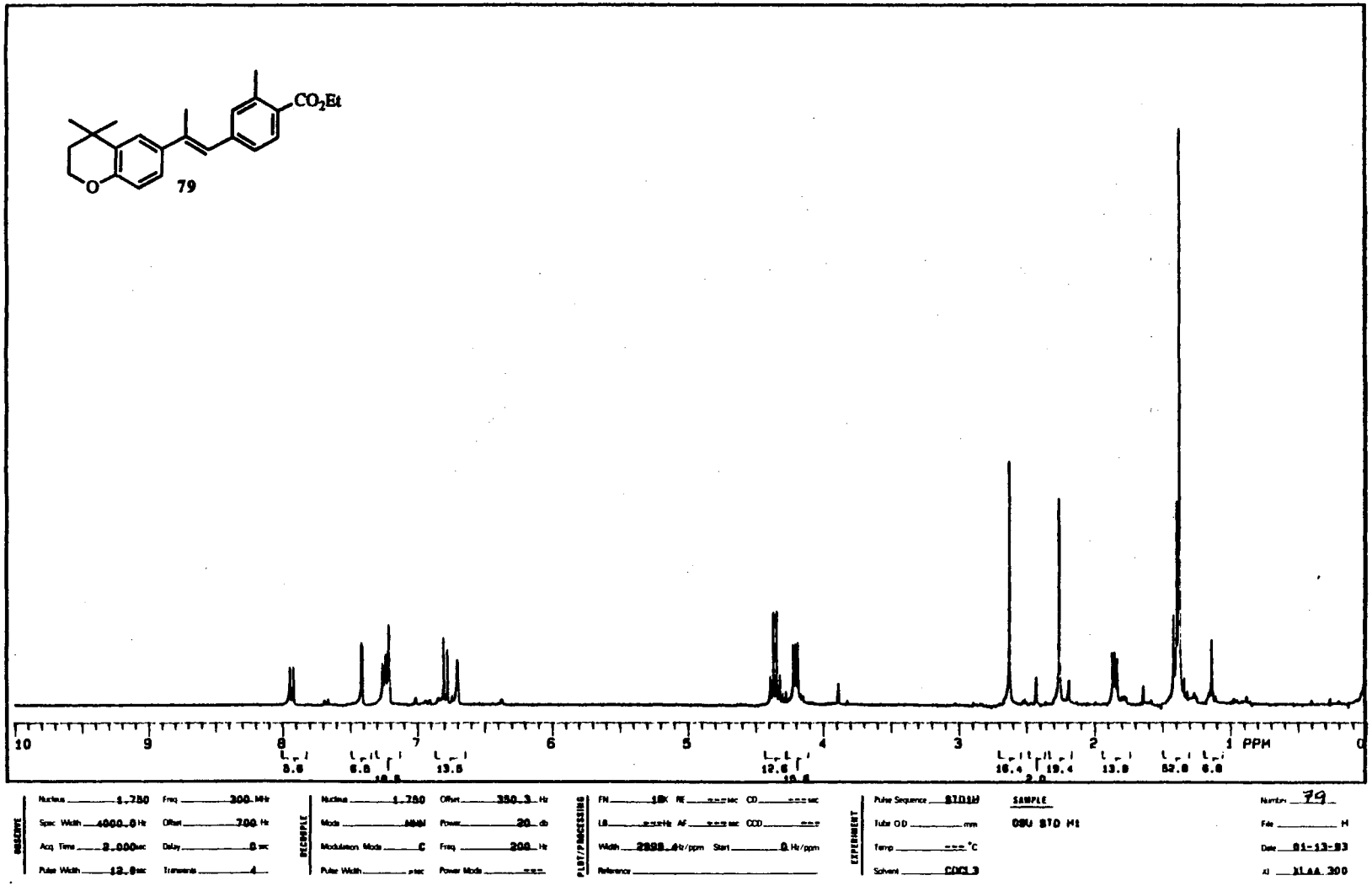
^{13}C NMR Spectrum of **78**

Plate VII



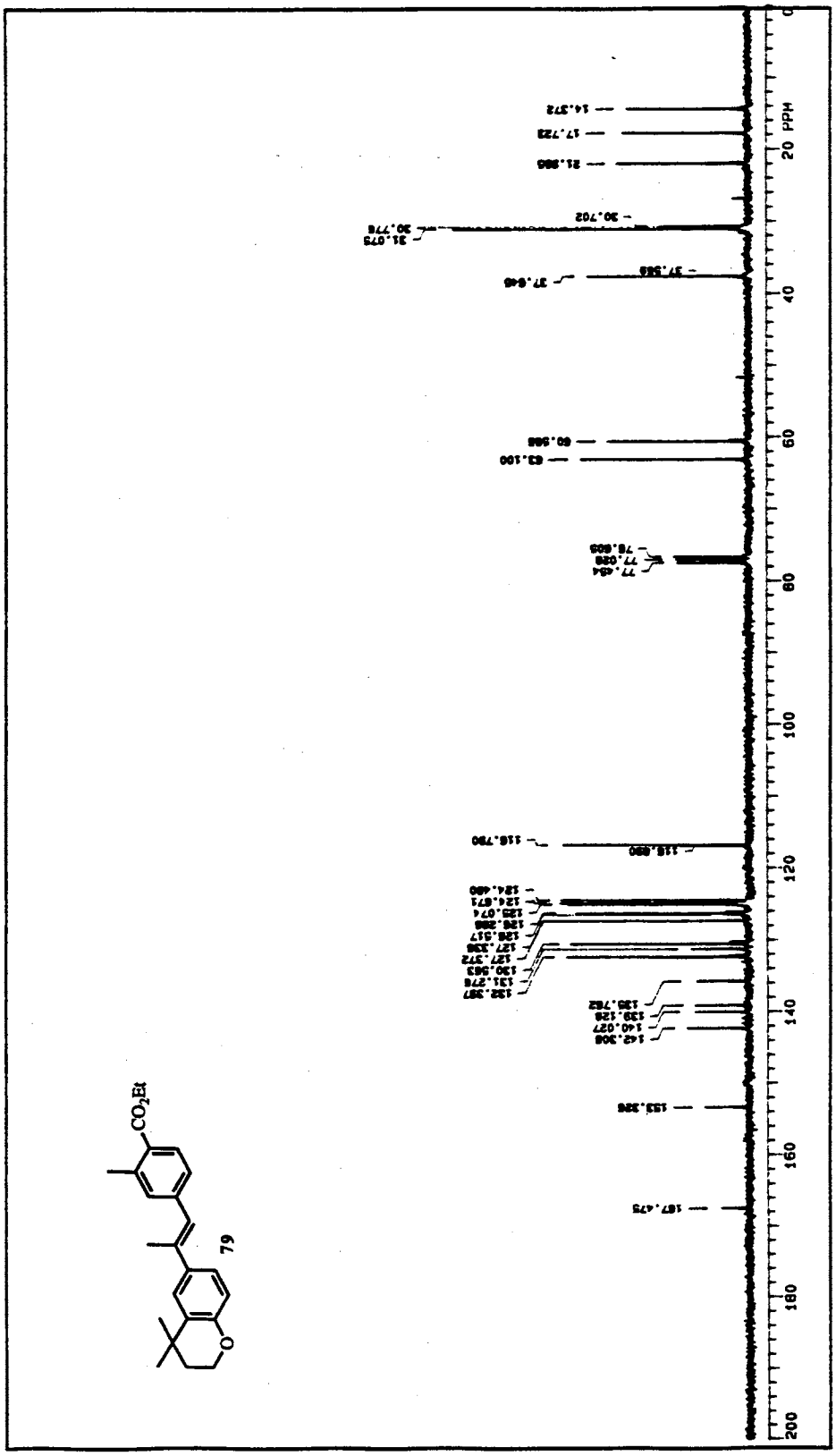
IR Spectrum of **79**

Plate VIII



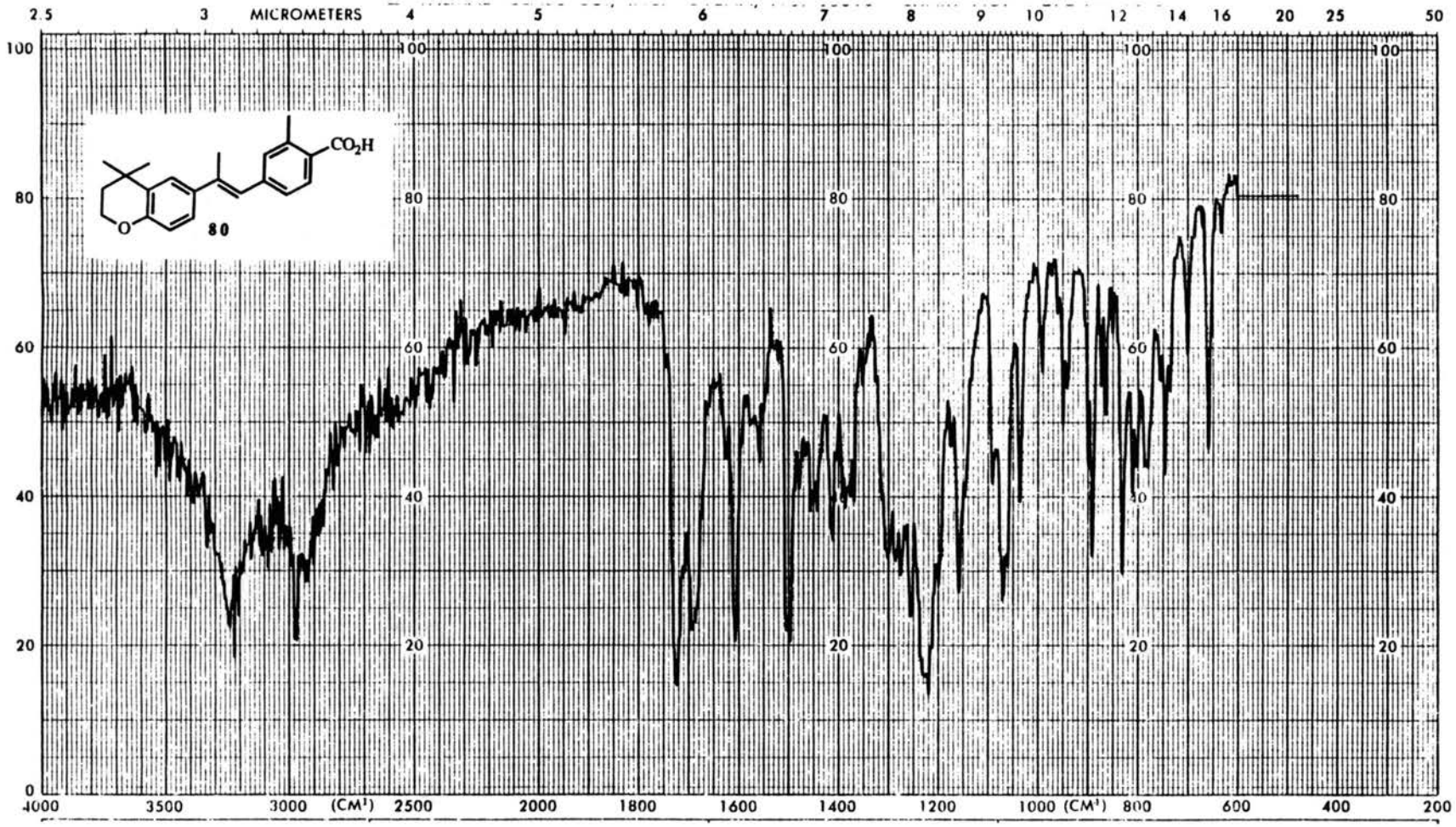
^{1}H NMR Spectrum of **79**

Plate IX



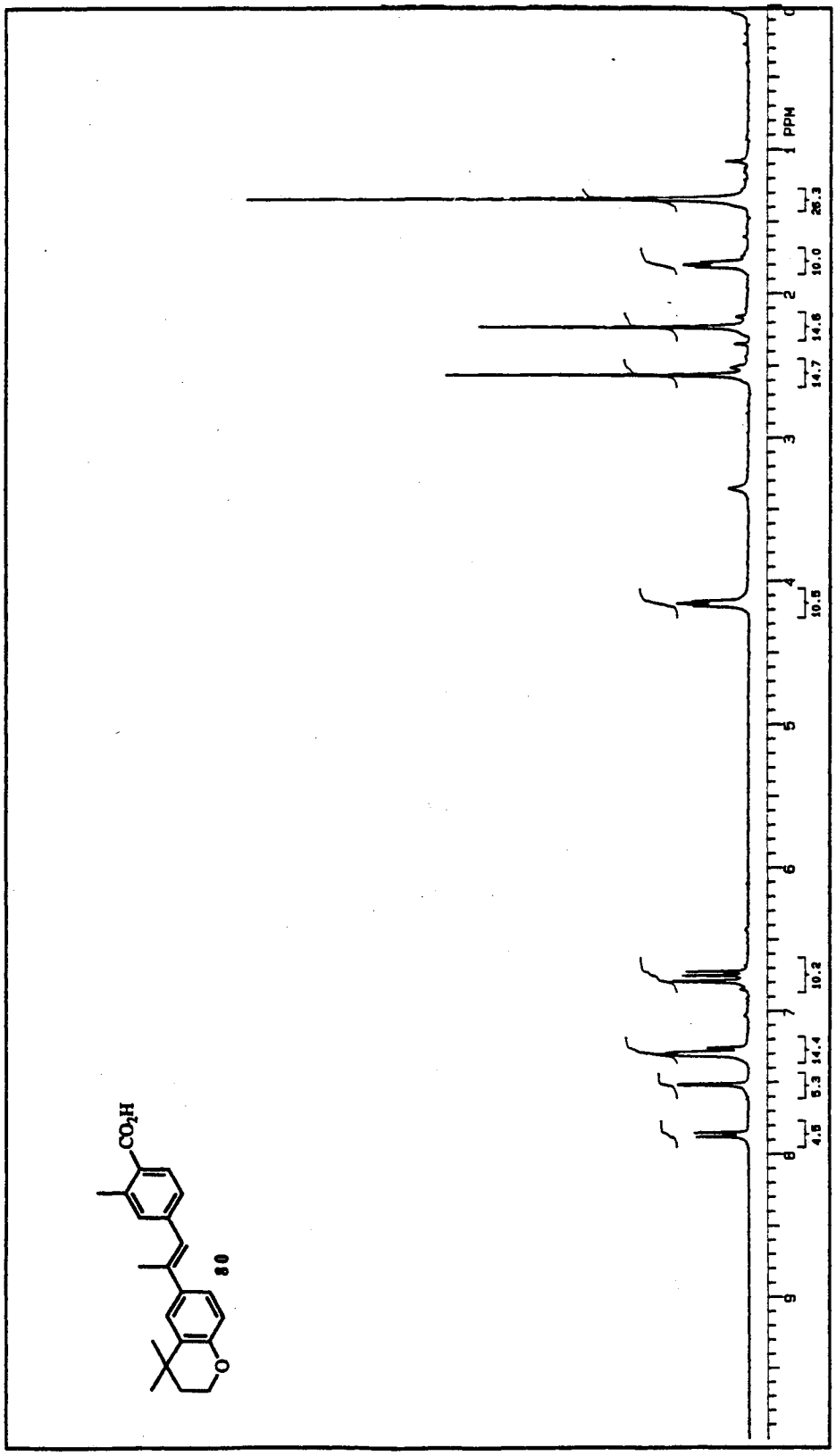
^{13}C NMR Spectrum of **79**

Plate X



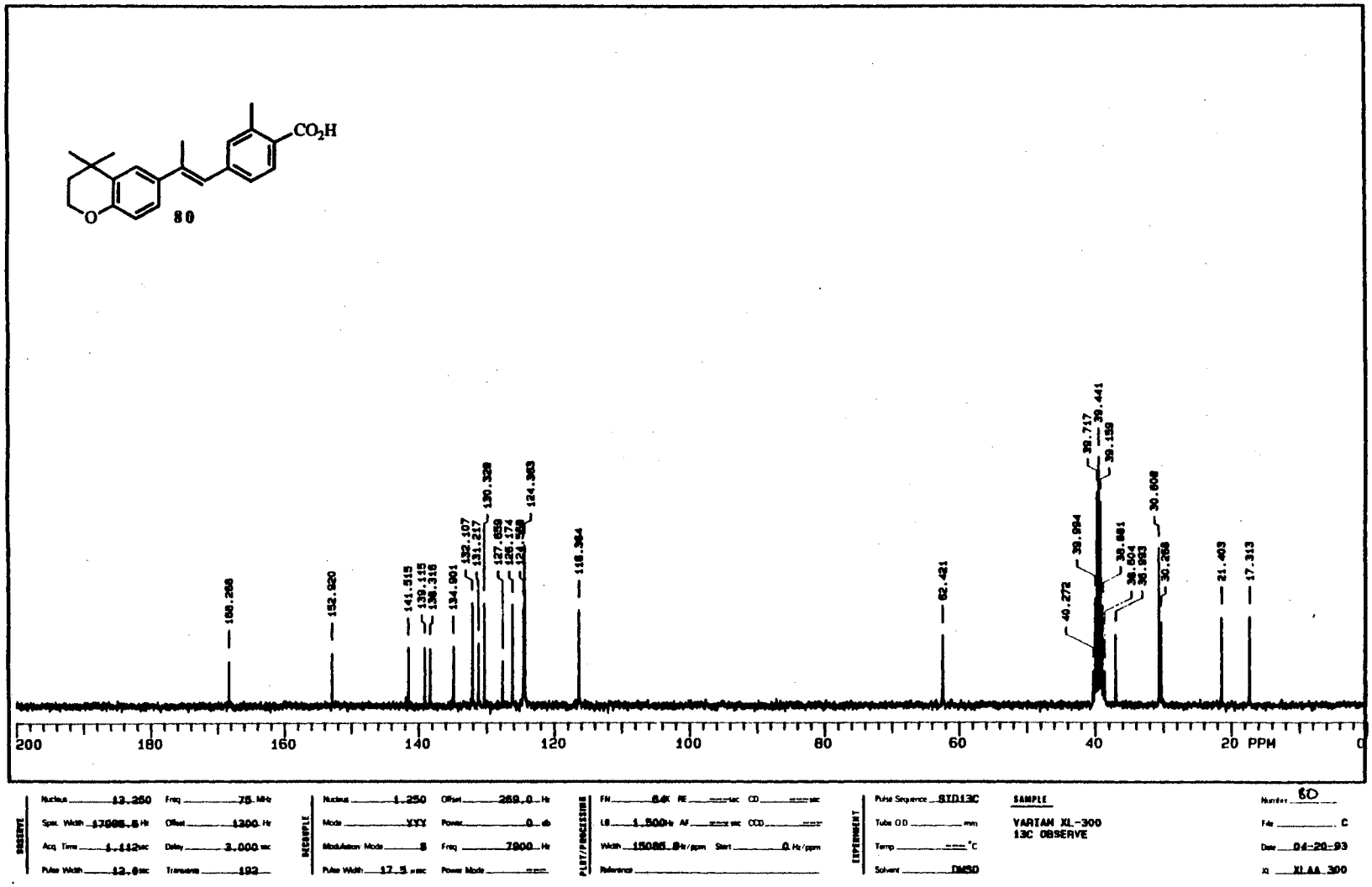
IR Spectrum of **80**

Plate XI



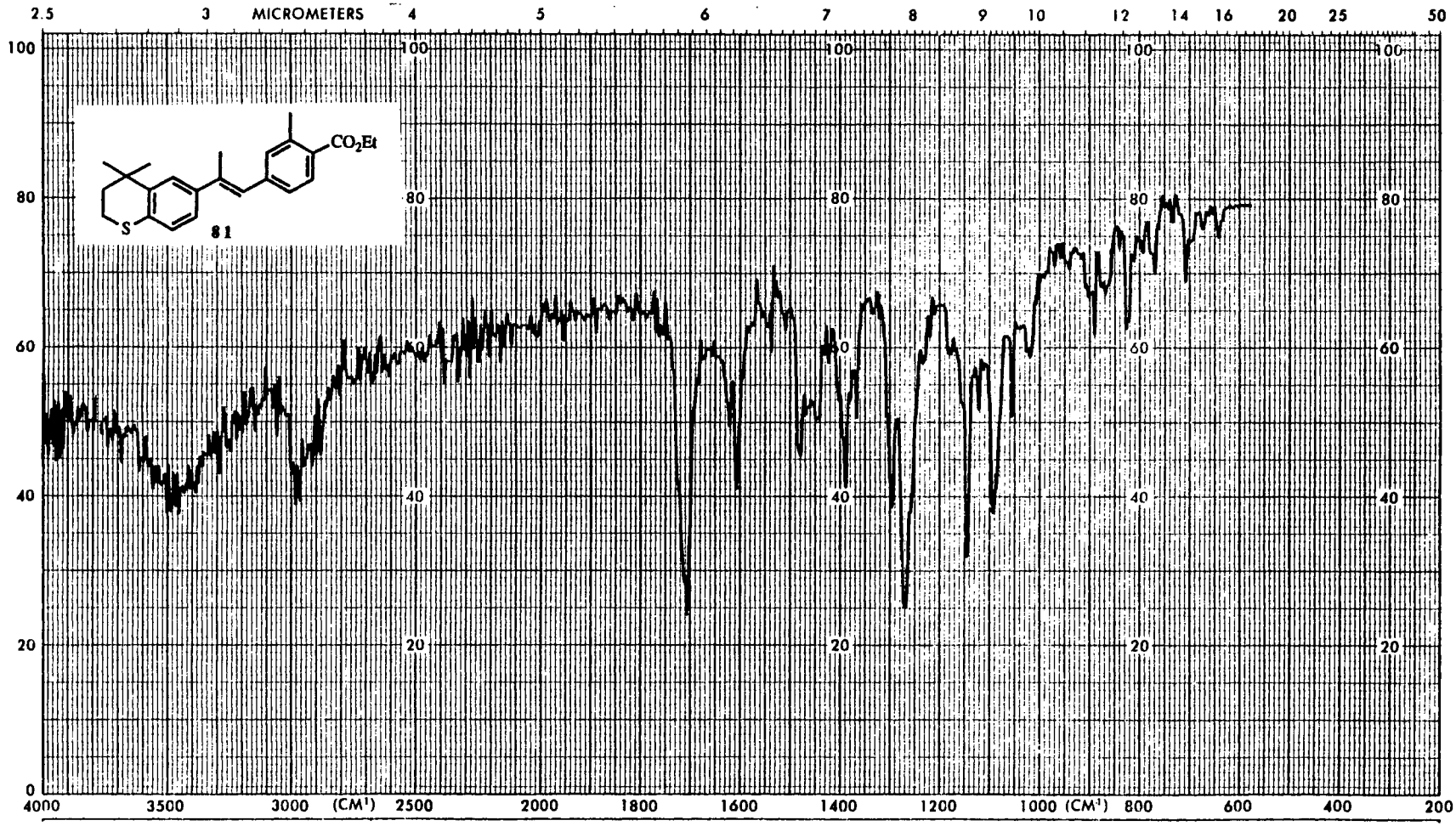
^{1}H NMR Spectrum of **80**

Plate XII



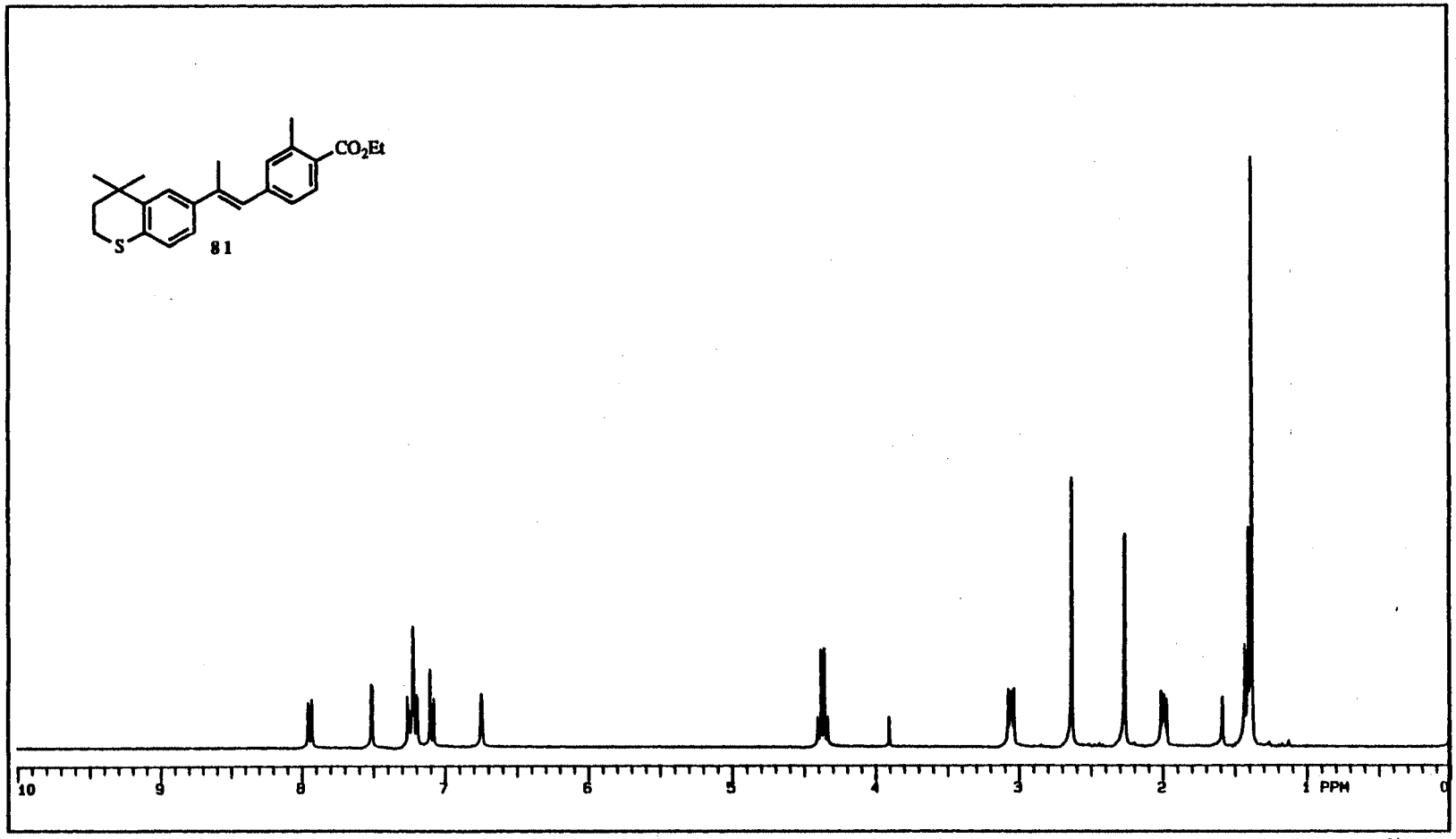
^{13}C NMR Spectrum of **80**

Plate XIII



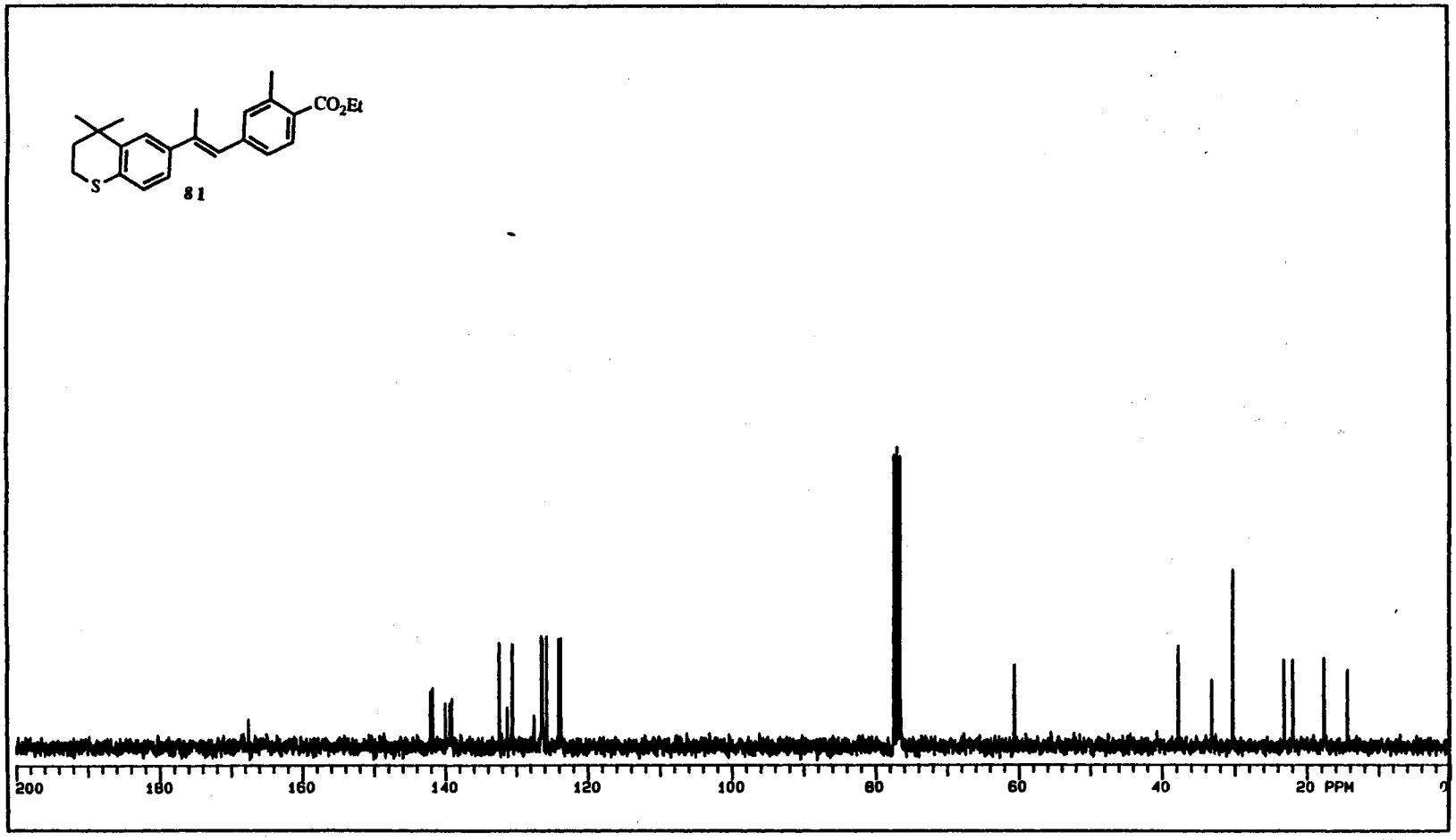
IR Spectrum of **81**

Plate XIV



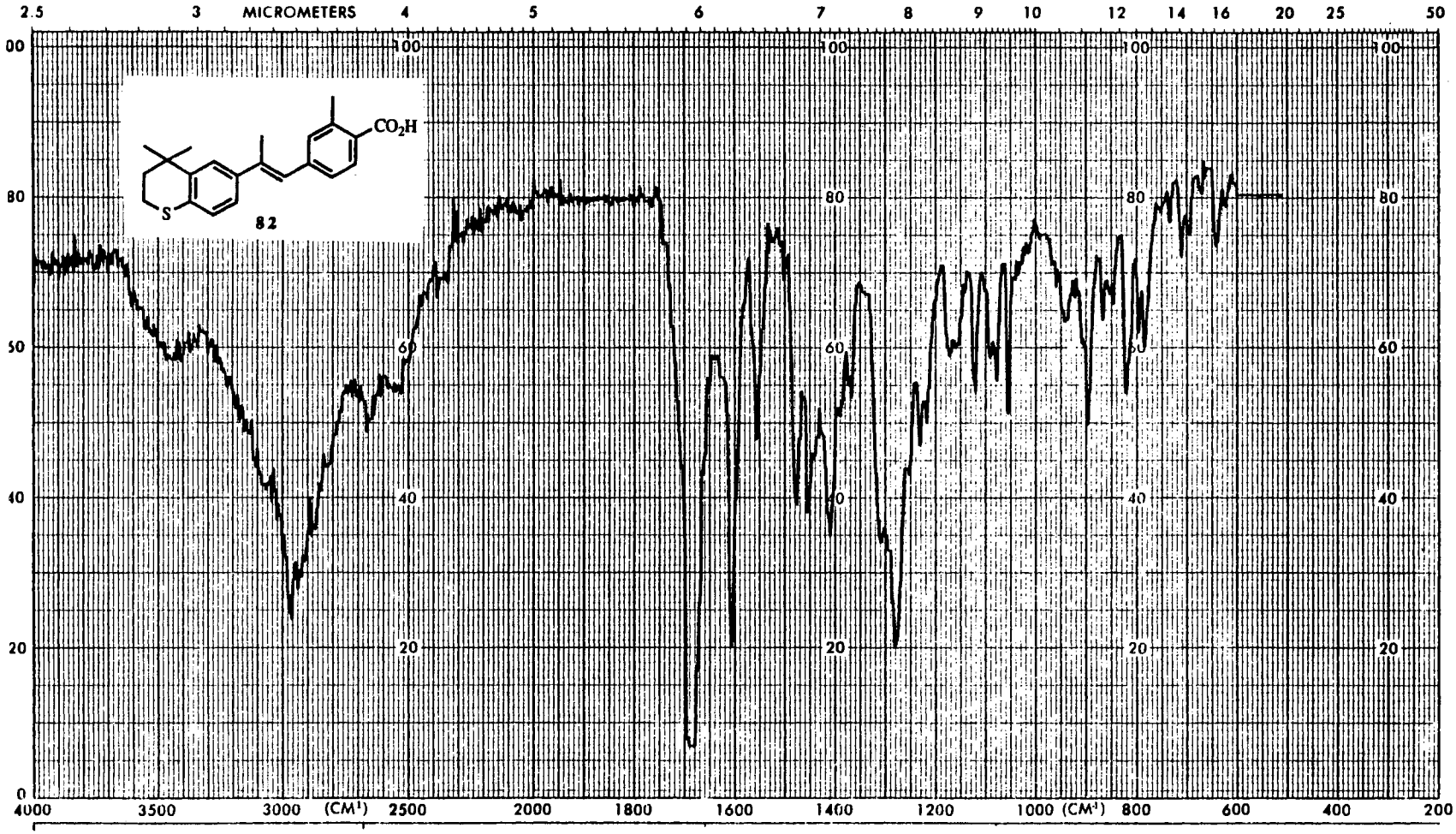
^{1}H NMR Spectrum of **81**

Plate XV



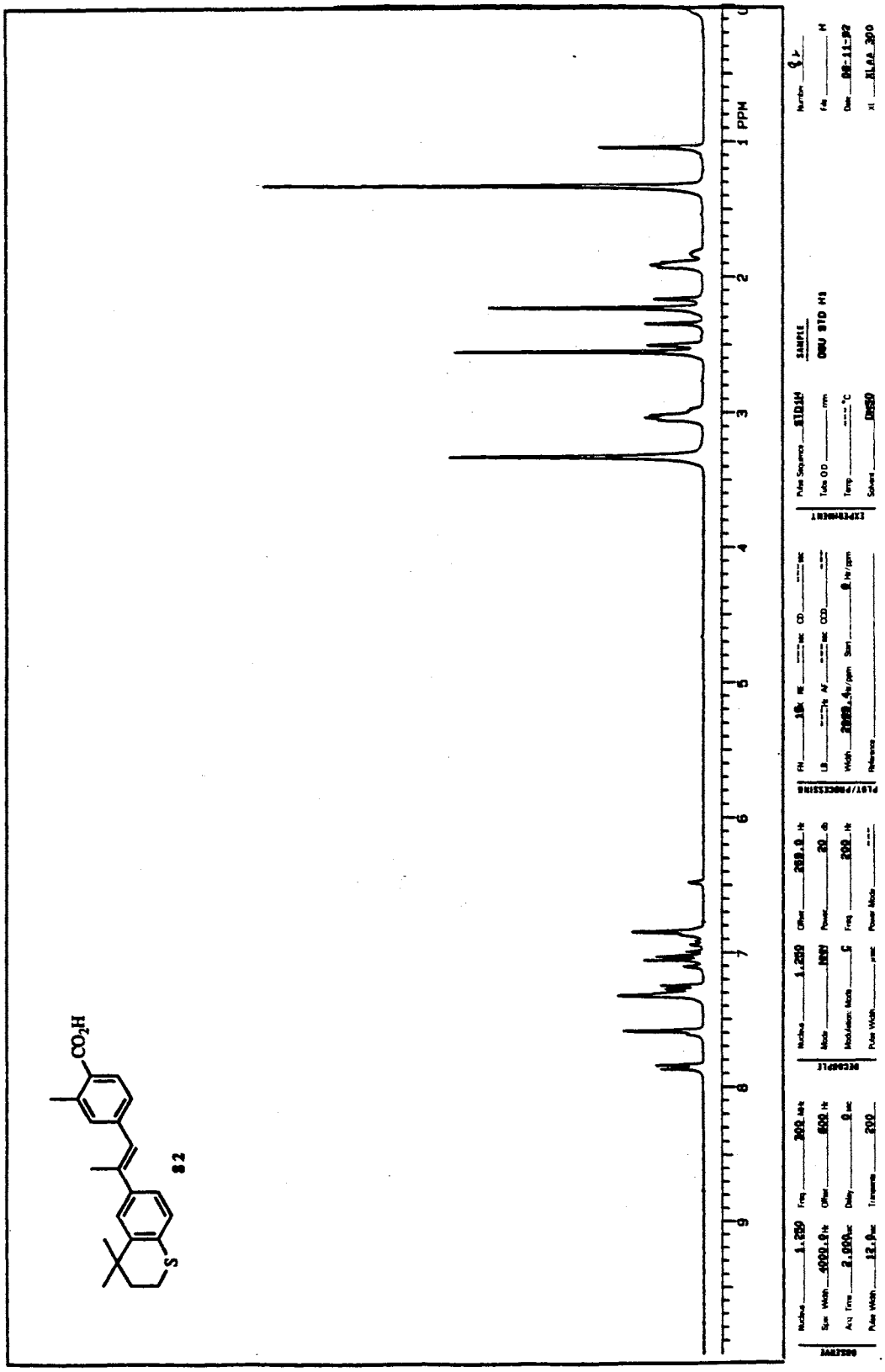
^{13}C NMR Spectrum of **81**

Plate XVI



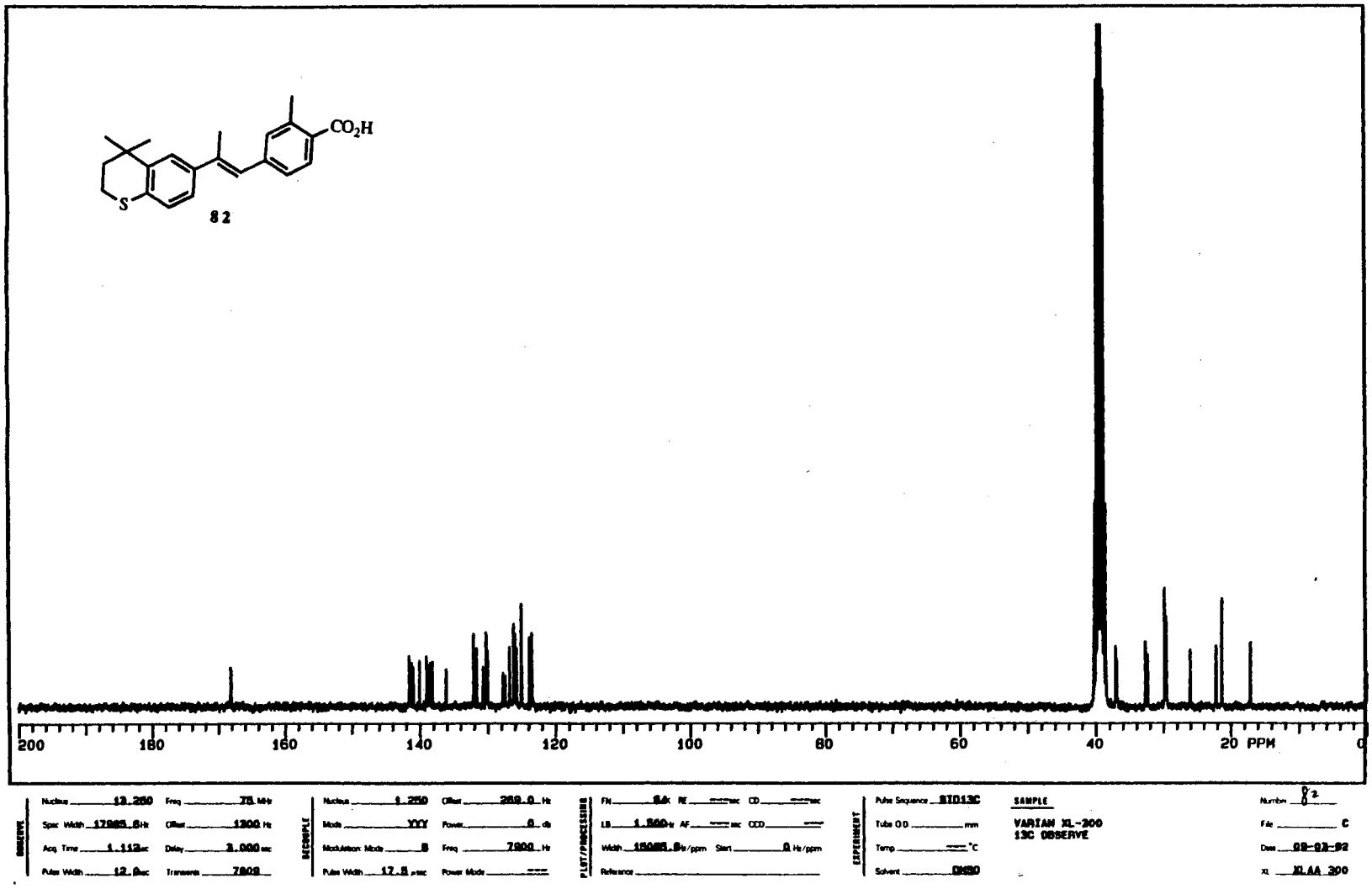
IR Spectrum of **82**

Plate XVII



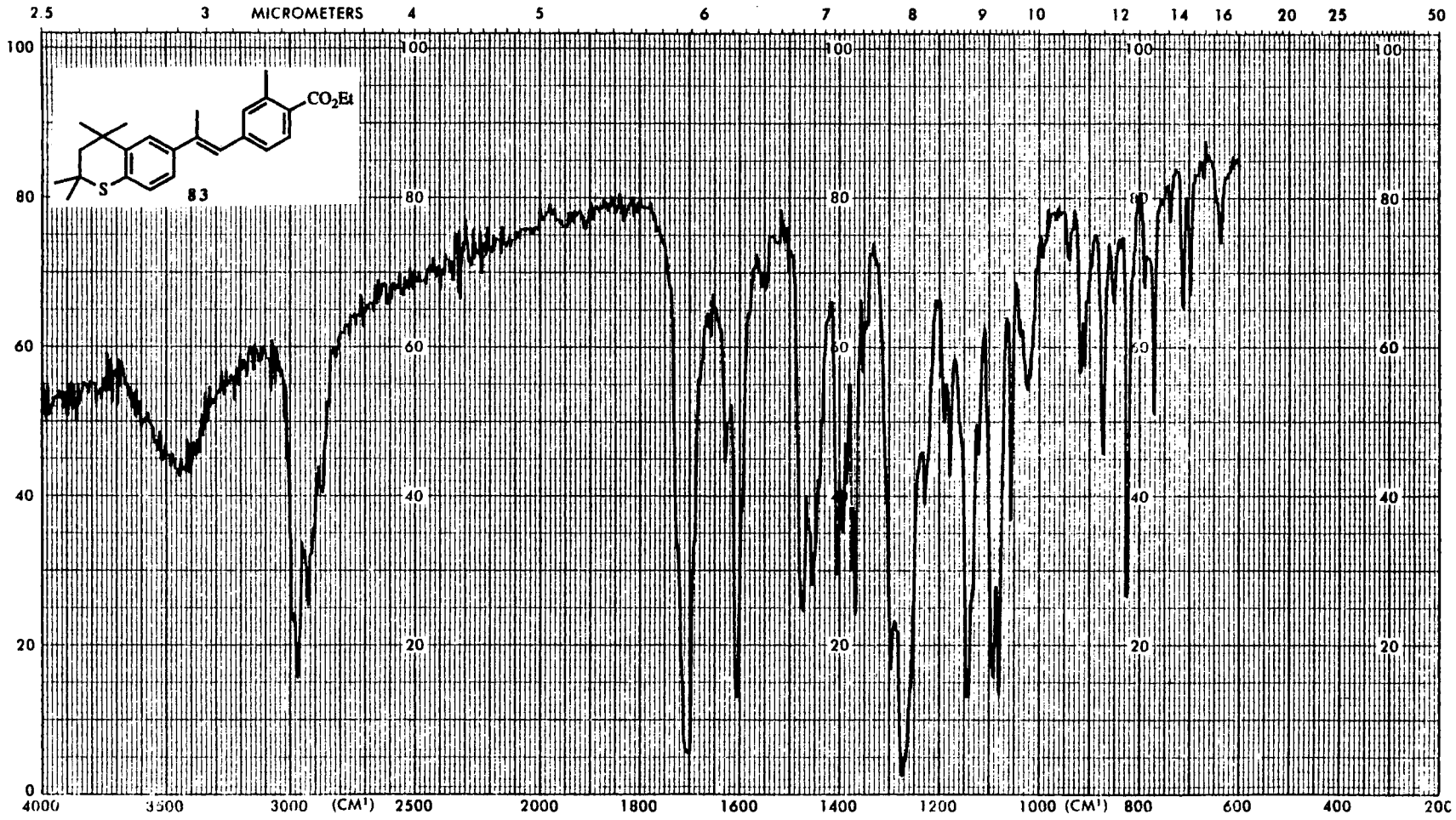
^{1}H NMR Spectrum of **82**

Plate XVIII



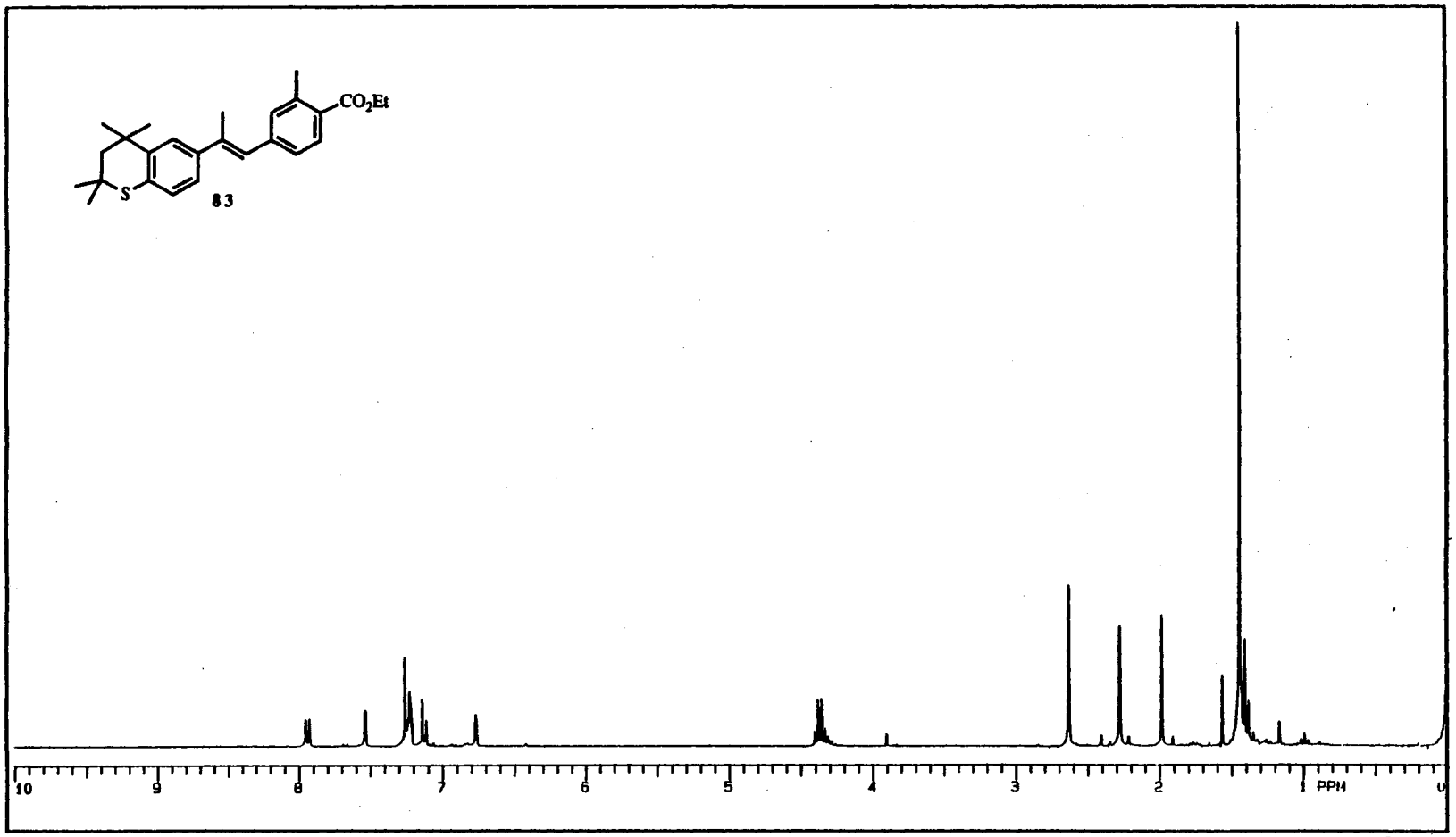
^{13}C NMR Spectrum of **82**

Plate XIX



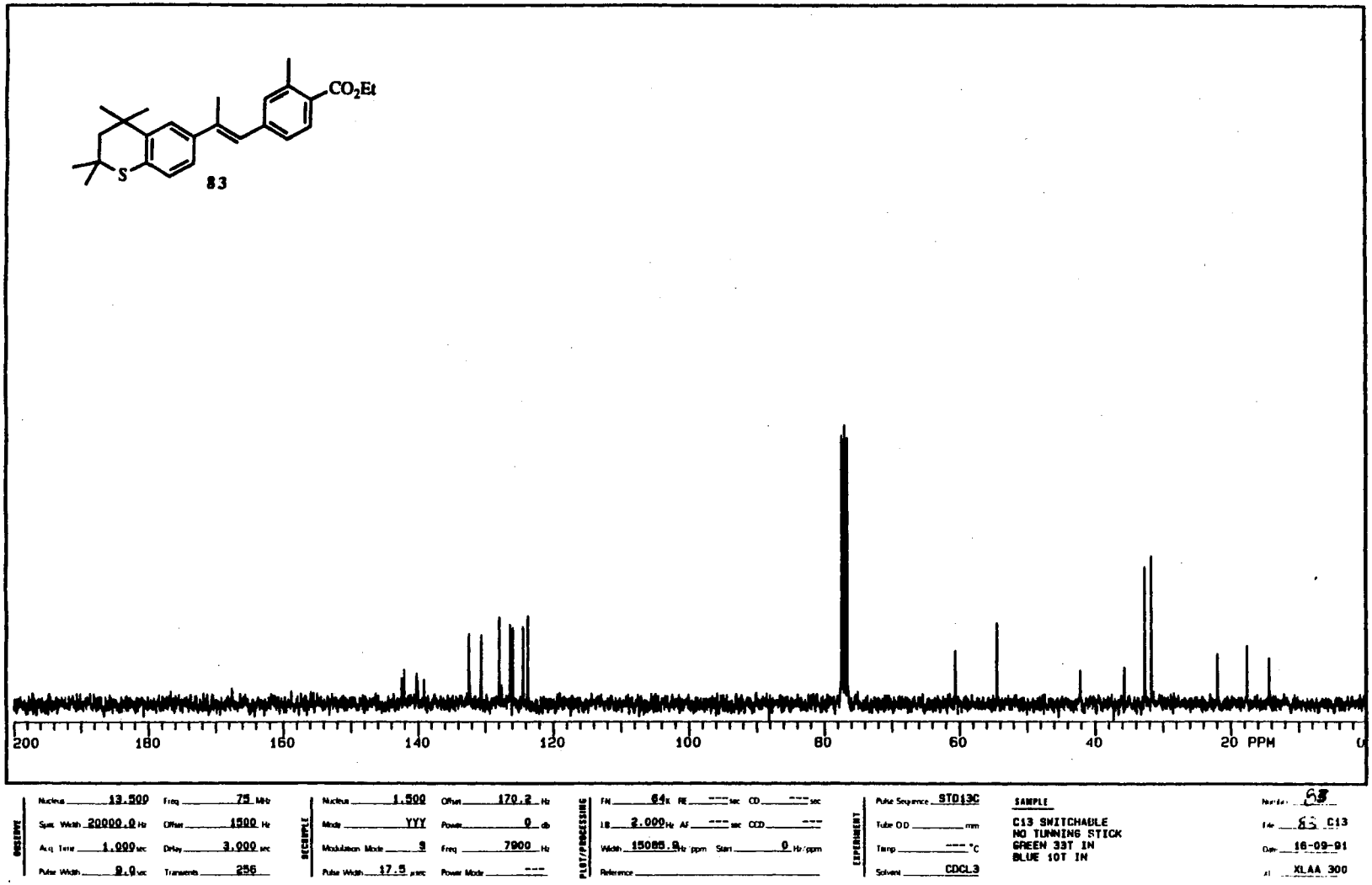
IR Spectrum of **83**

Plate XX



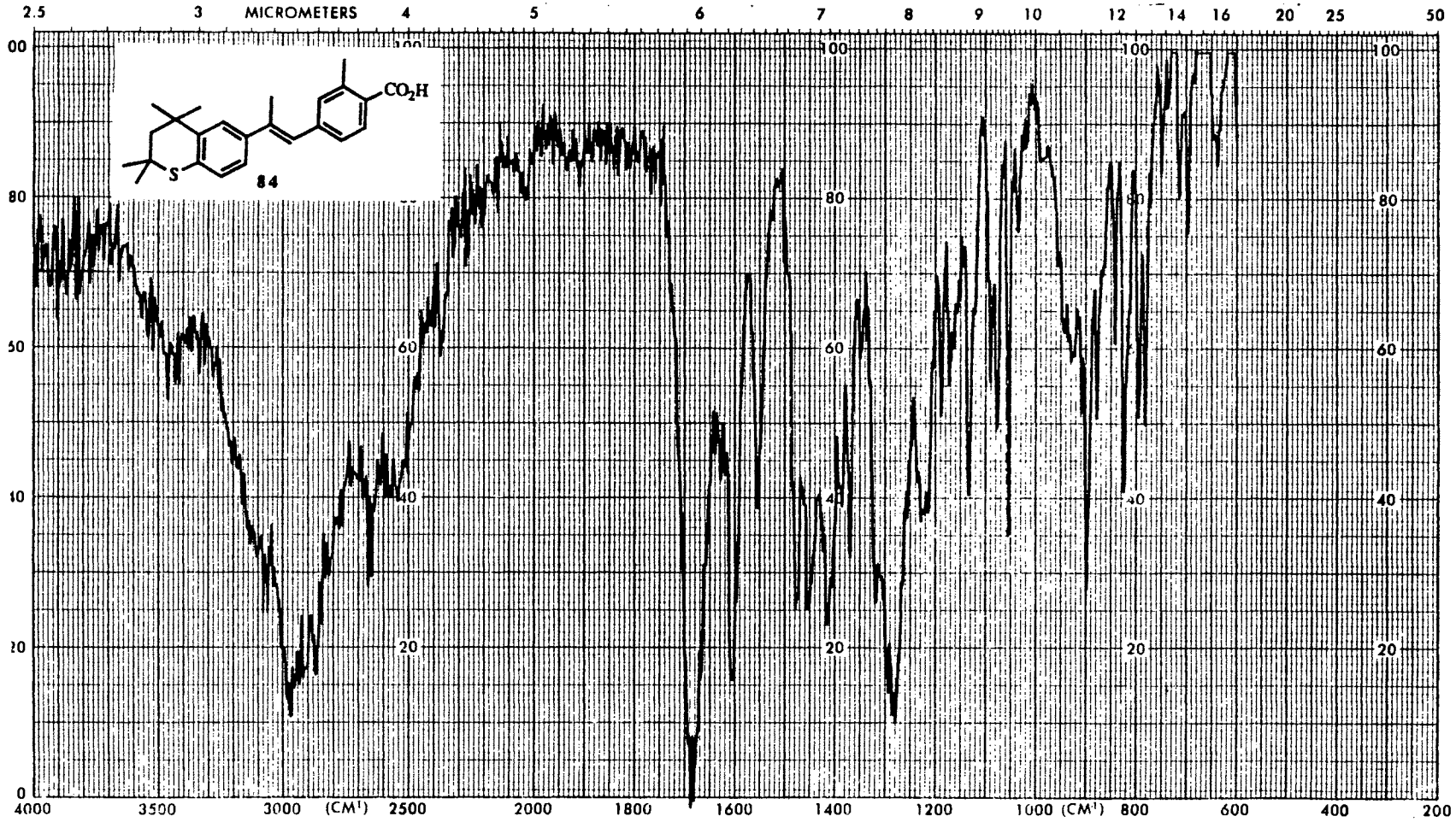
^{1}H NMR Spectrum of **83**

Plate XXI



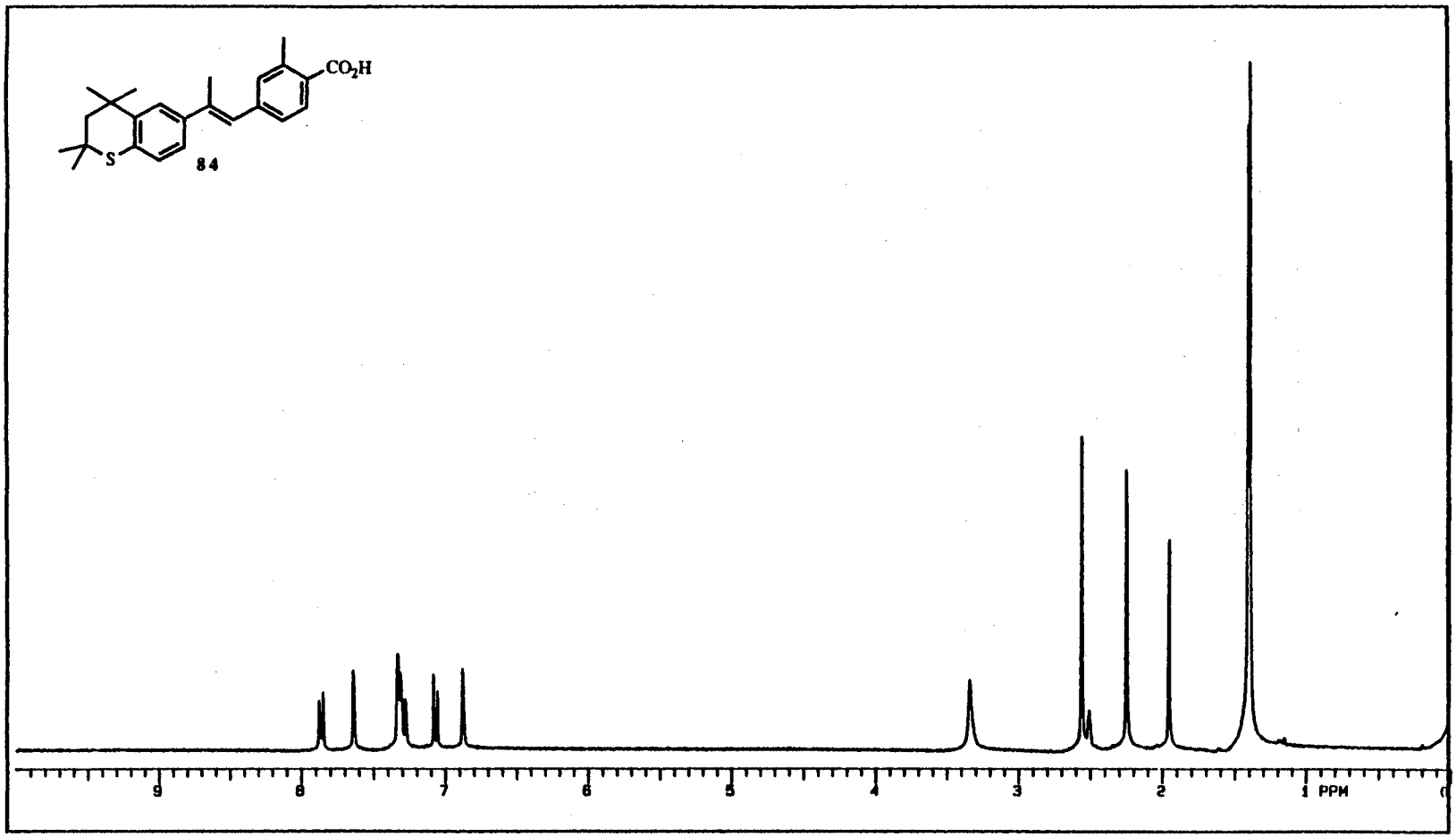
^{13}C NMR Spectrum of **83**

Plate XXII



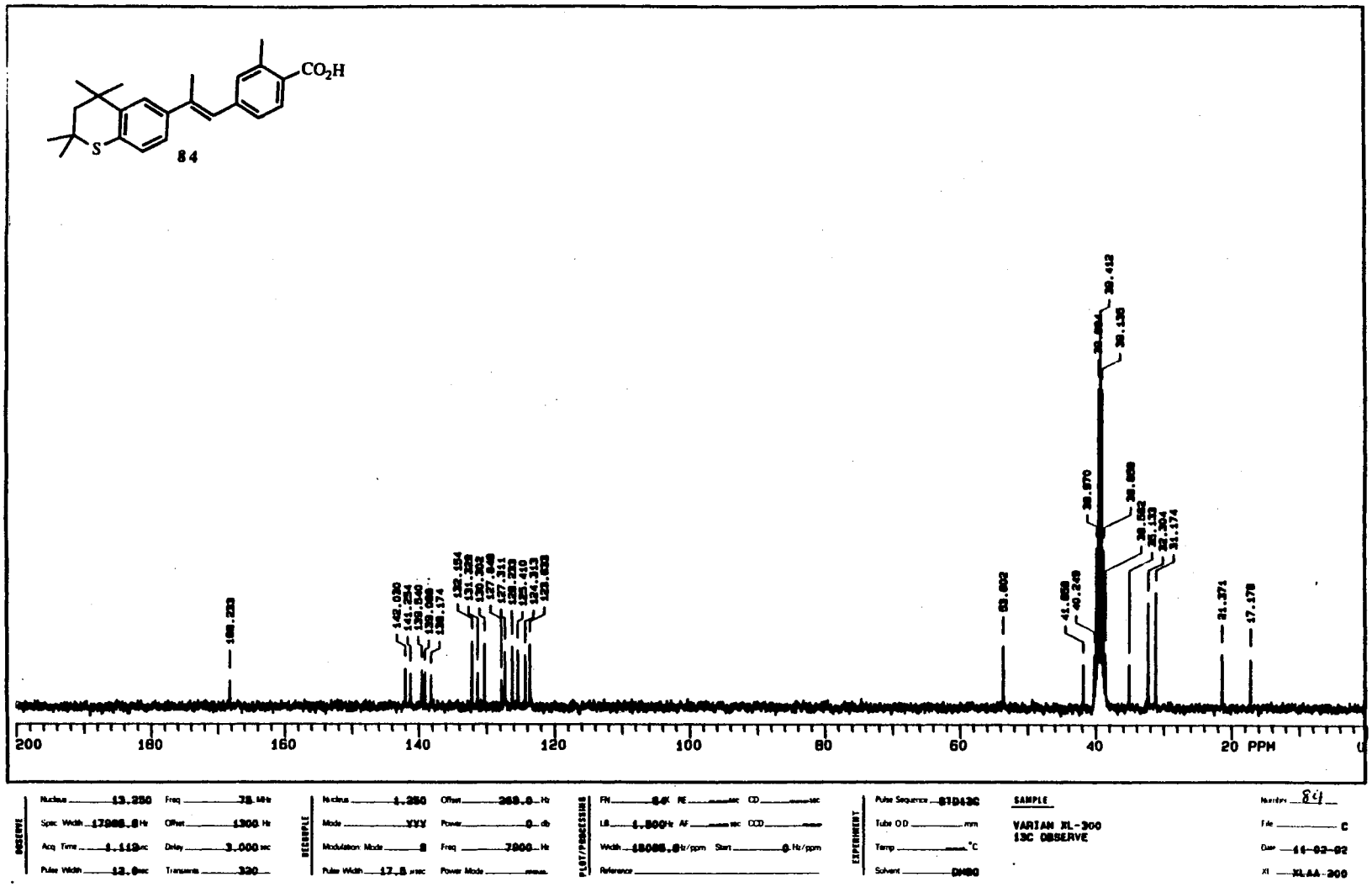
IR Spectrum of **84**

Plate XXIII



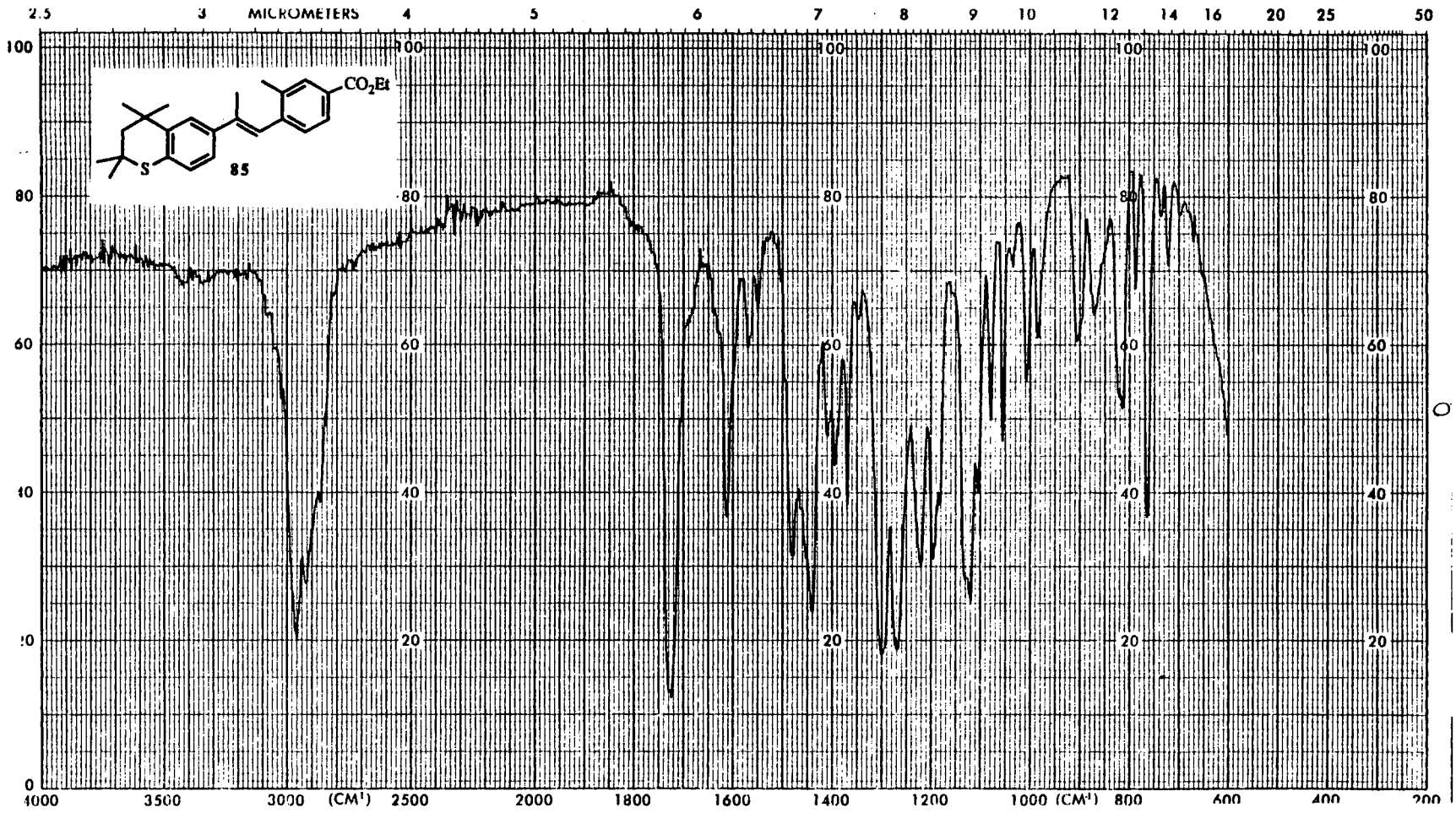
^{1}H NMR Spectrum of **84**

Plate XXIV



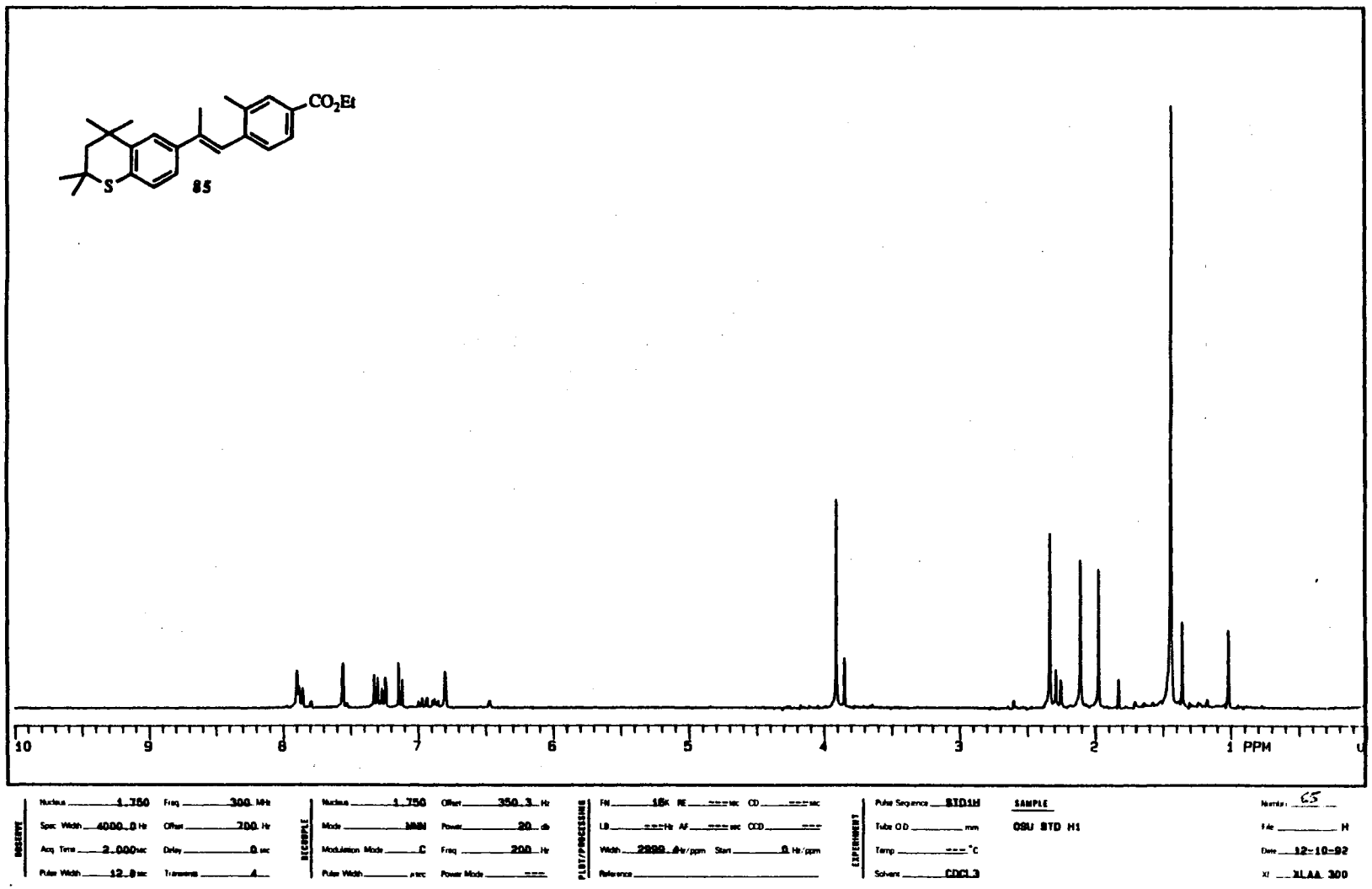
^{13}C NMR Spectrum of **84**

Plate XXV



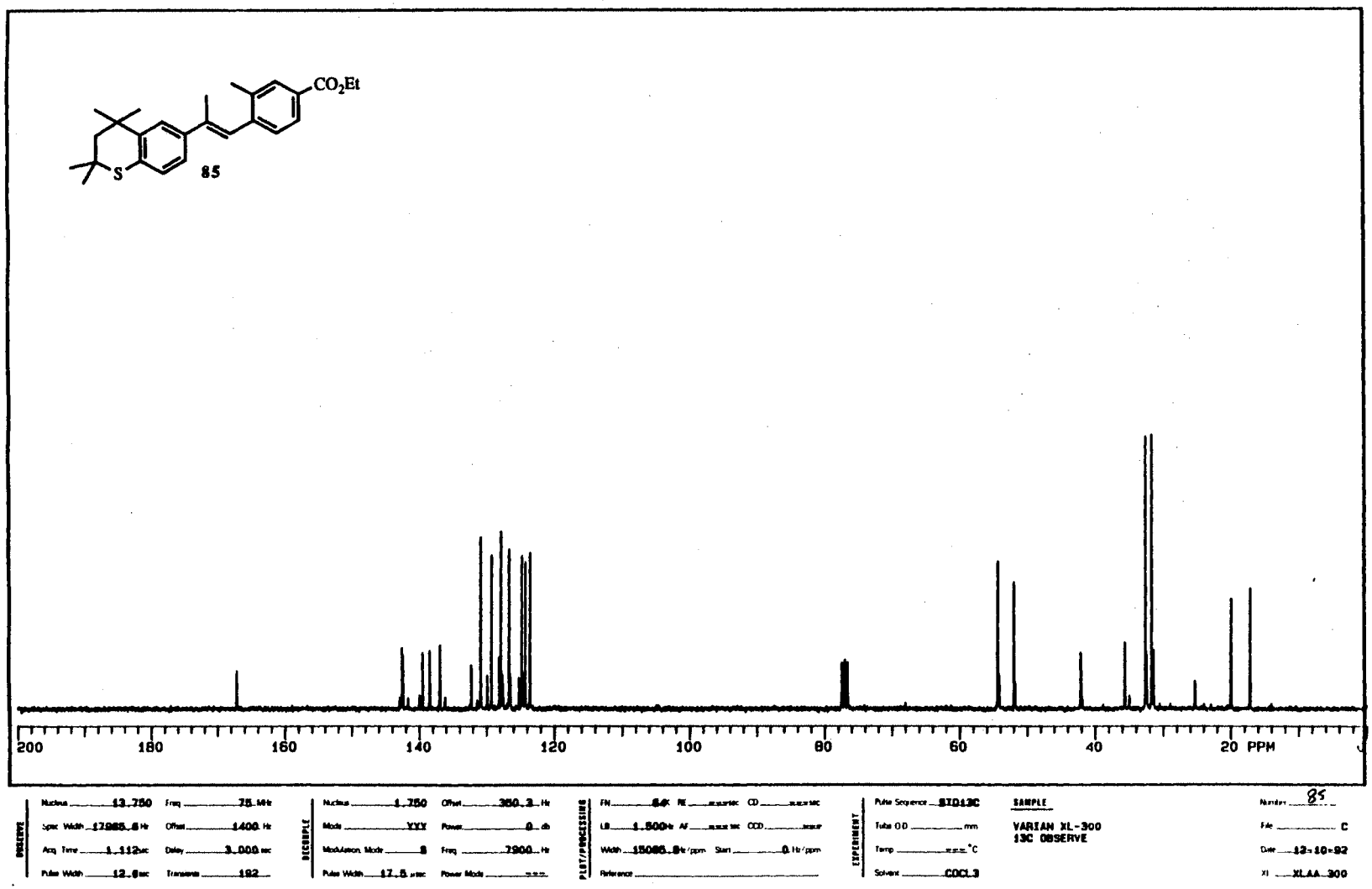
IR Spectrum of **85**

Plate XXVI



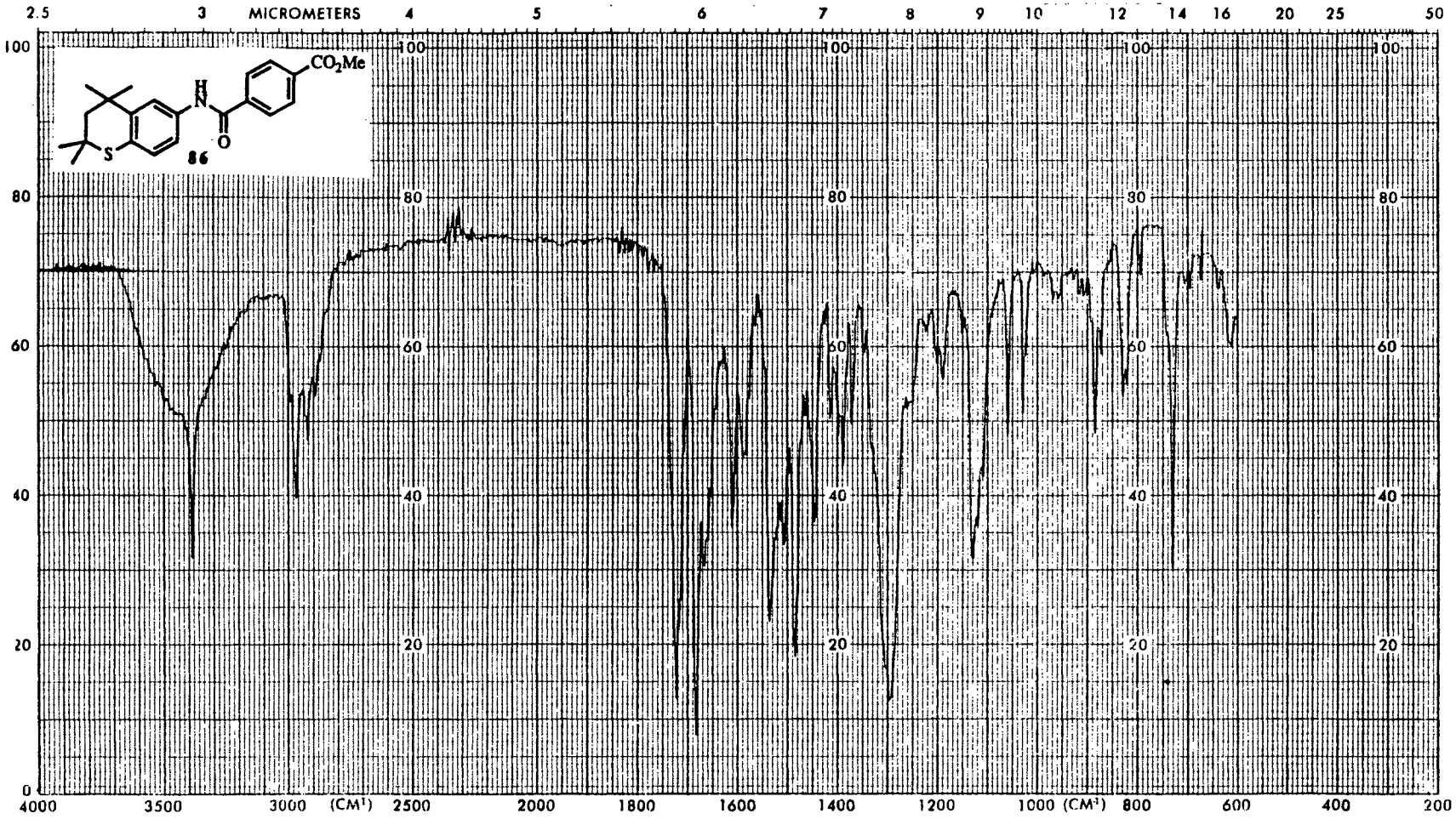
^{1}H NMR Spectrum of **85**

Plate XXVII



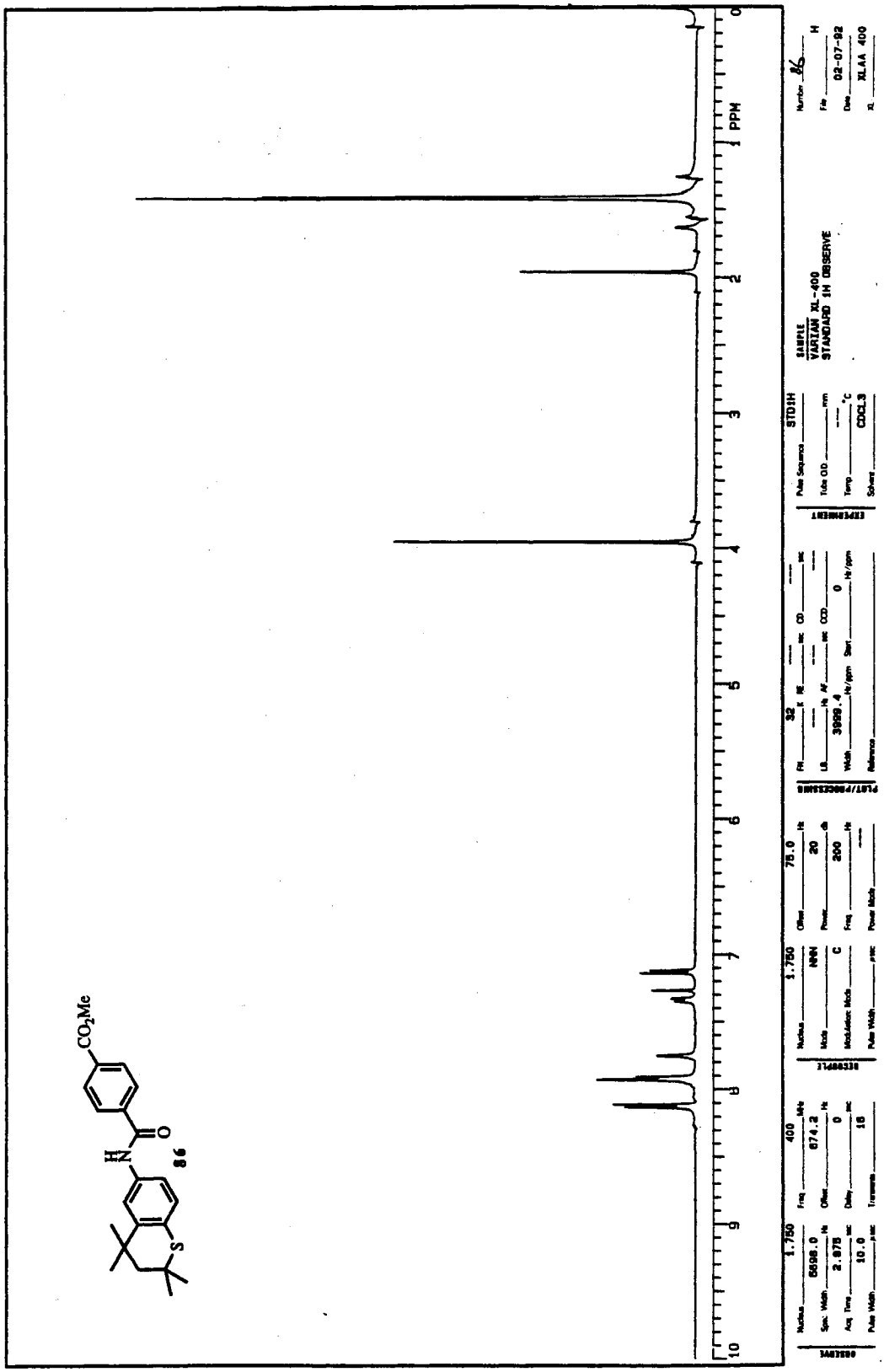
^{13}C NMR Spectrum of **85**

Plate XXVIII



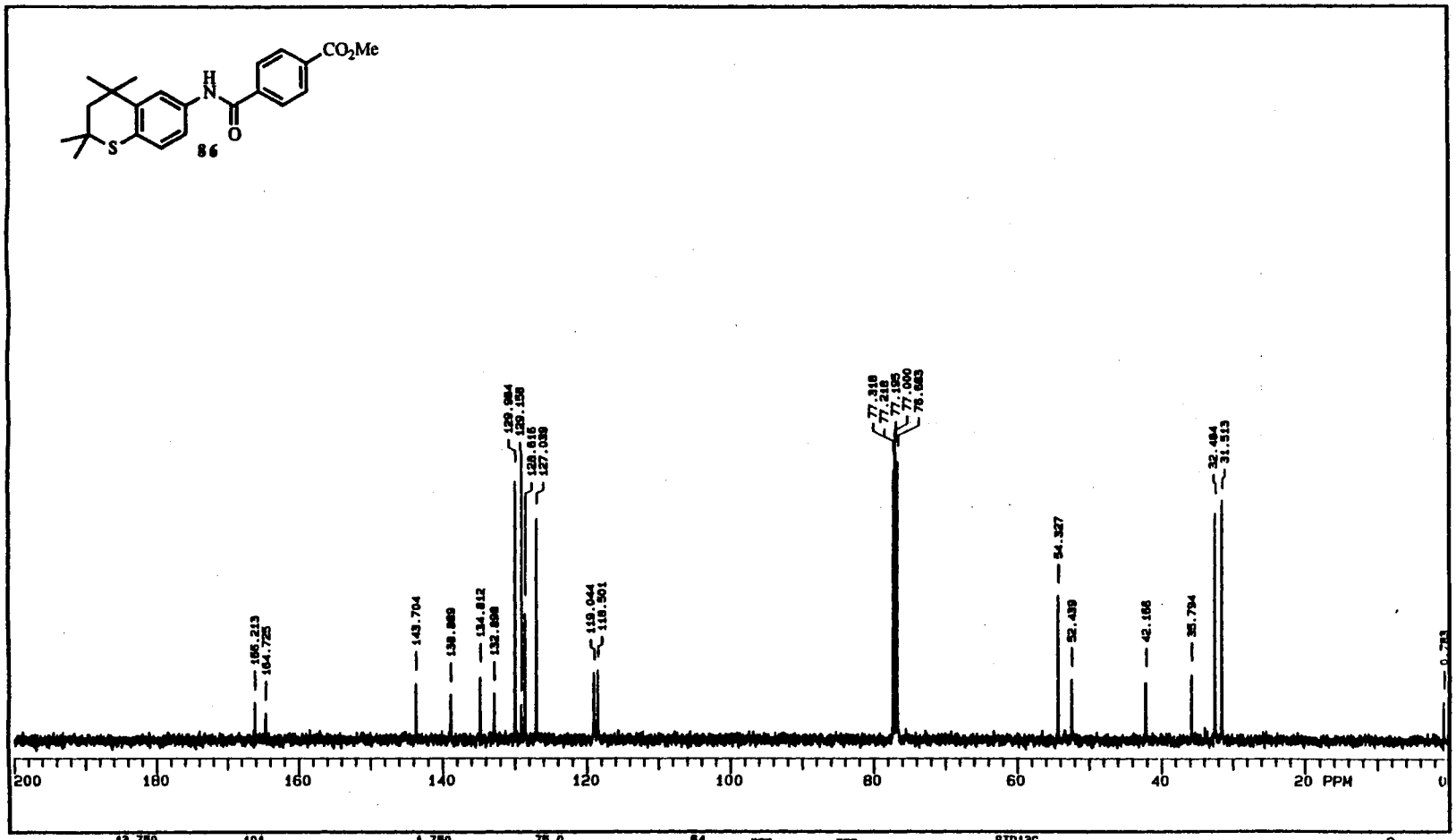
IR Spectrum of **86**

Plate XXIX



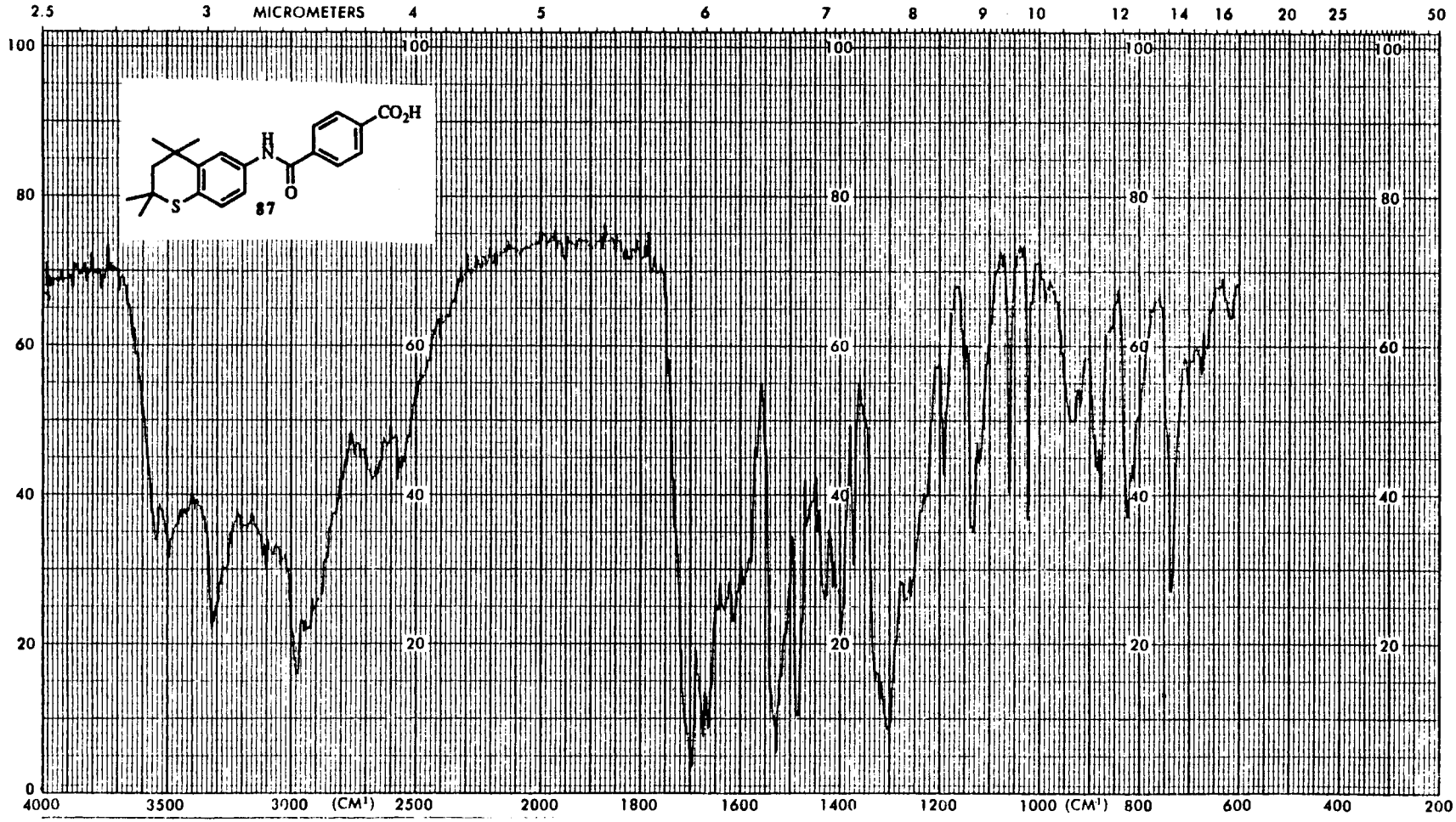
^{1}H NMR Spectrum of **86**

Plate XXX



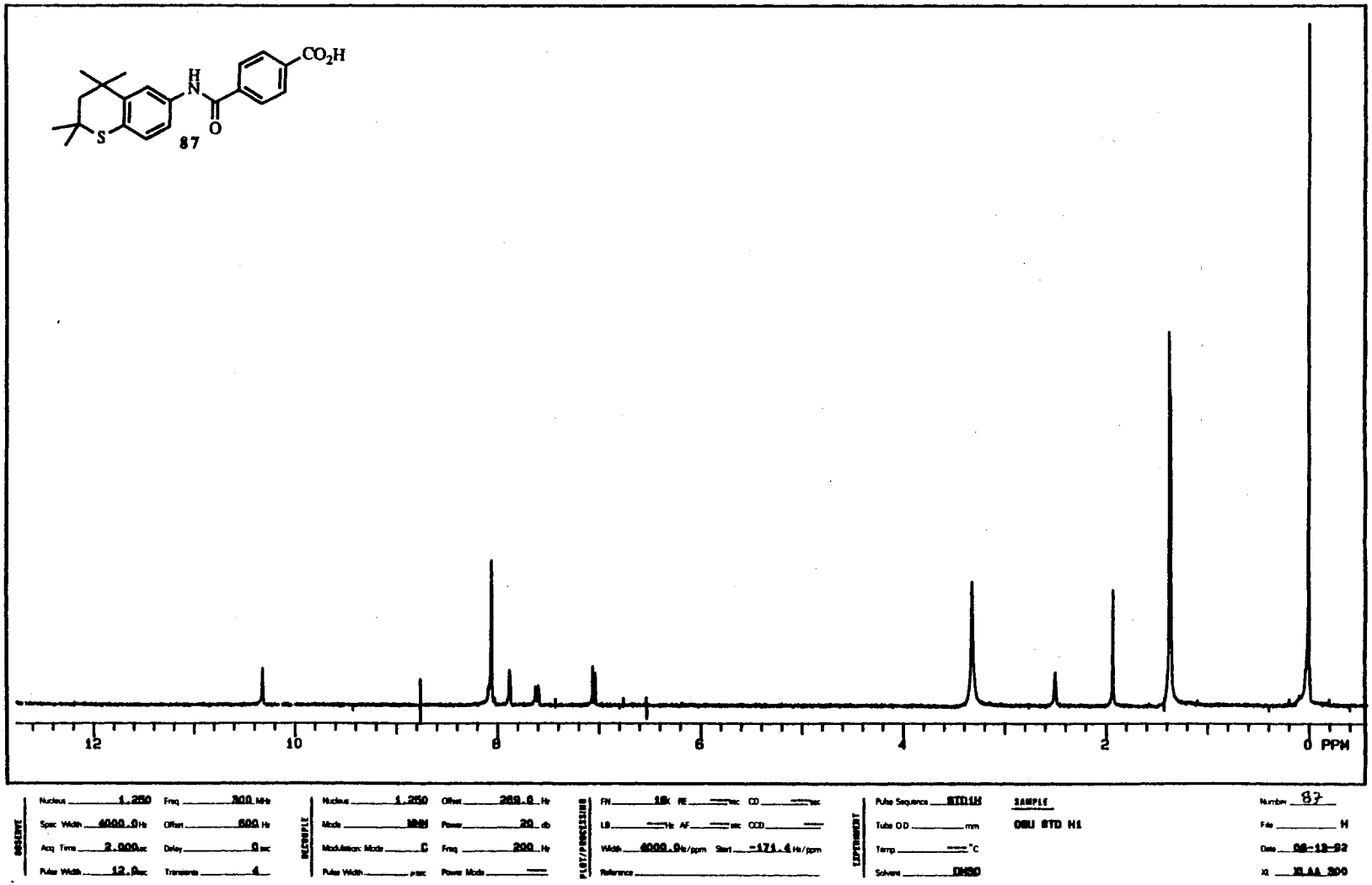
^{13}C NMR Spectrum of **86**

Plate XXXI



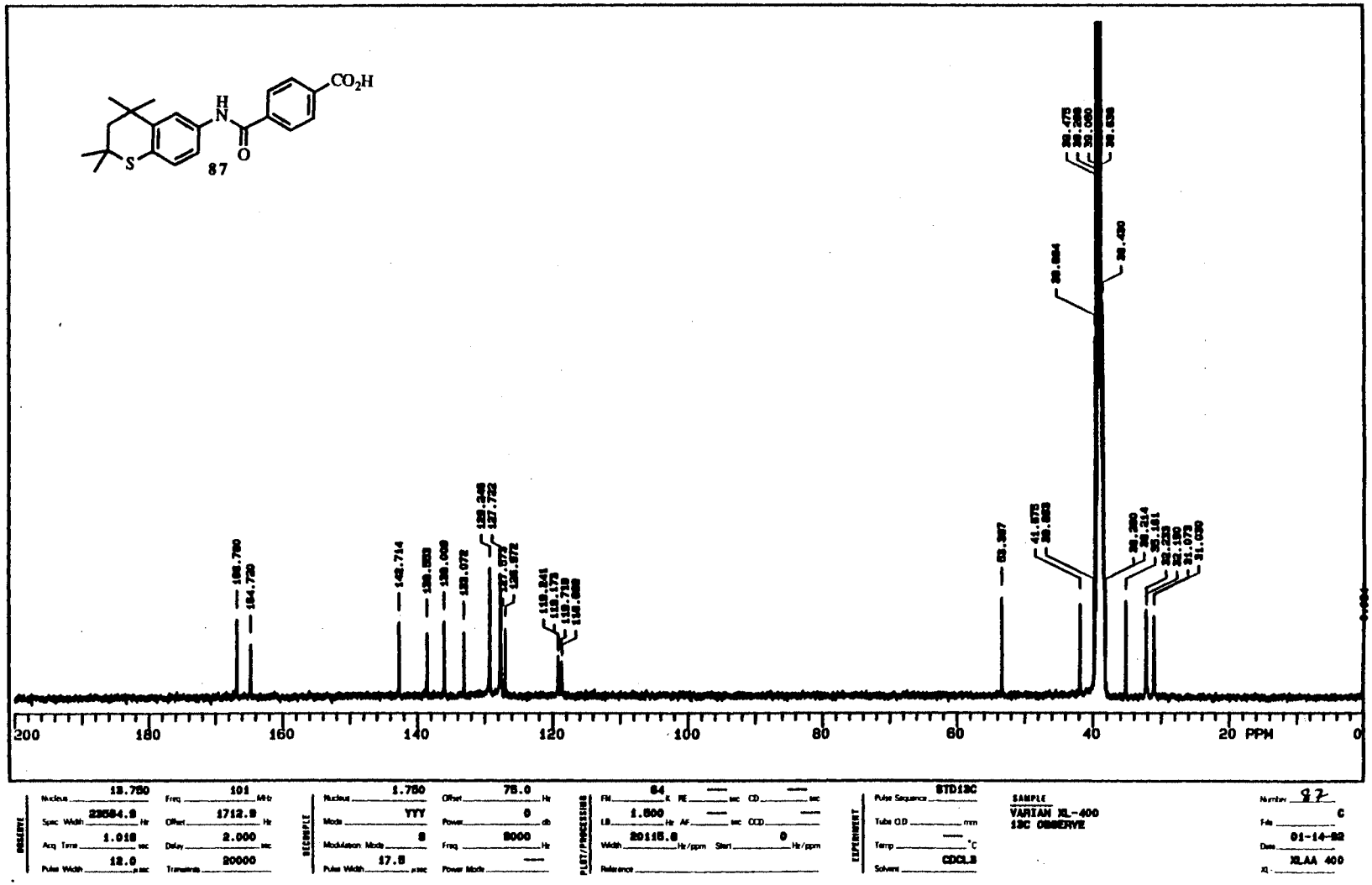
IR Spectrum of **87**

Plate XXXII



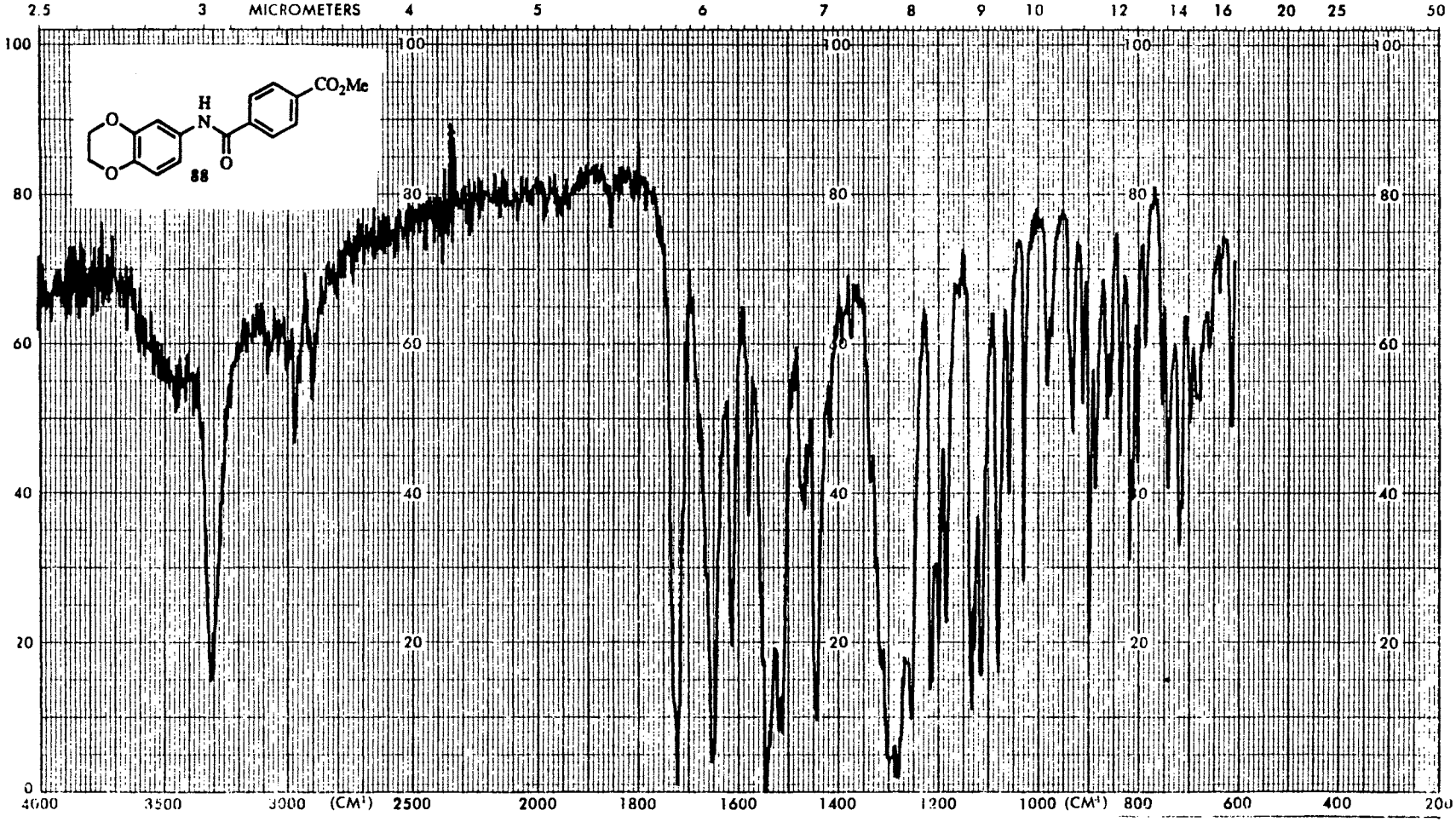
^{1}H NMR Spectrum of **87**

Plate XXXIII



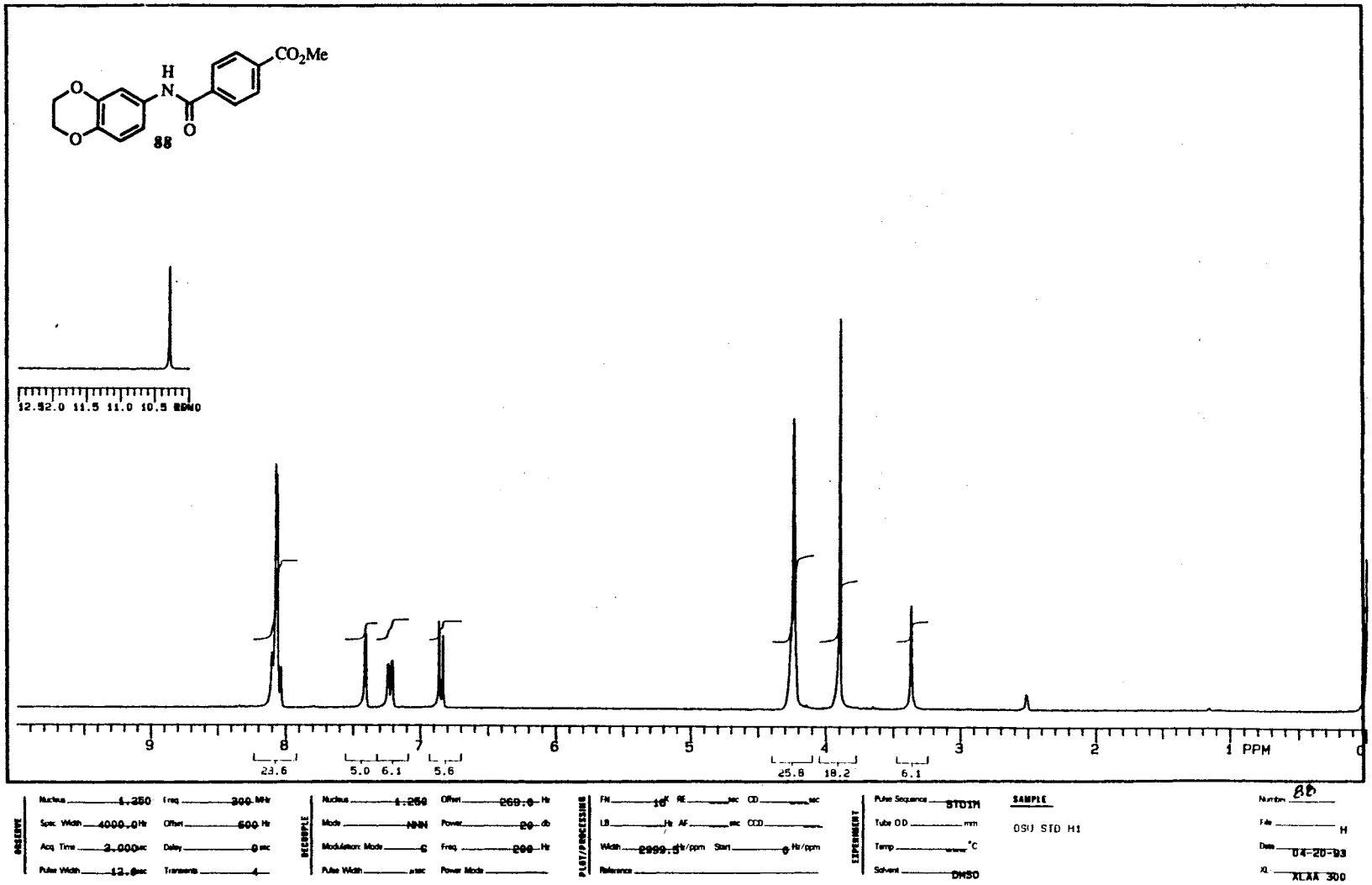
^{13}C NMR Spectrum of **87**

Plate XXXIV



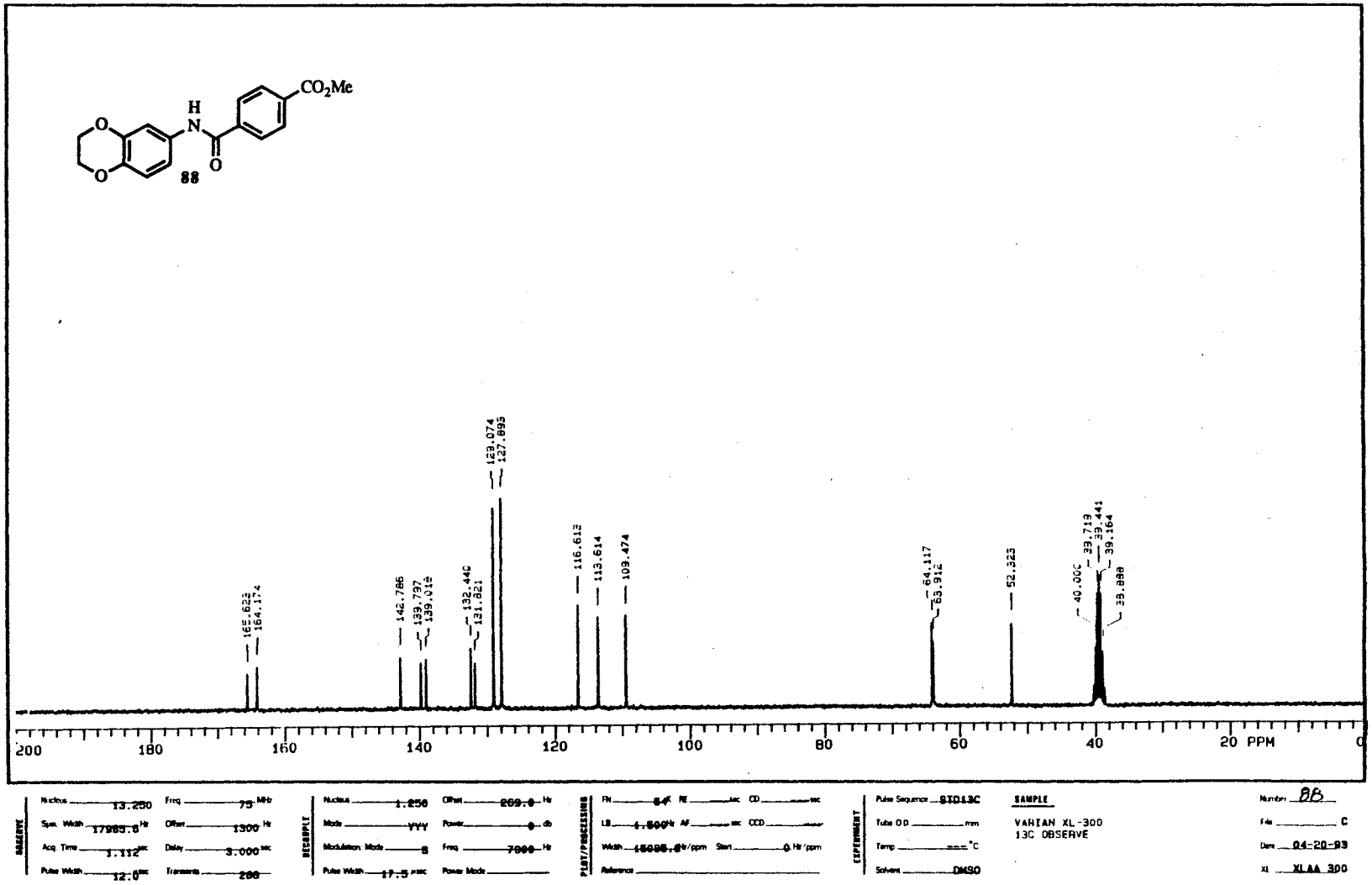
IR Spectrum of **88**

Plate XXXV



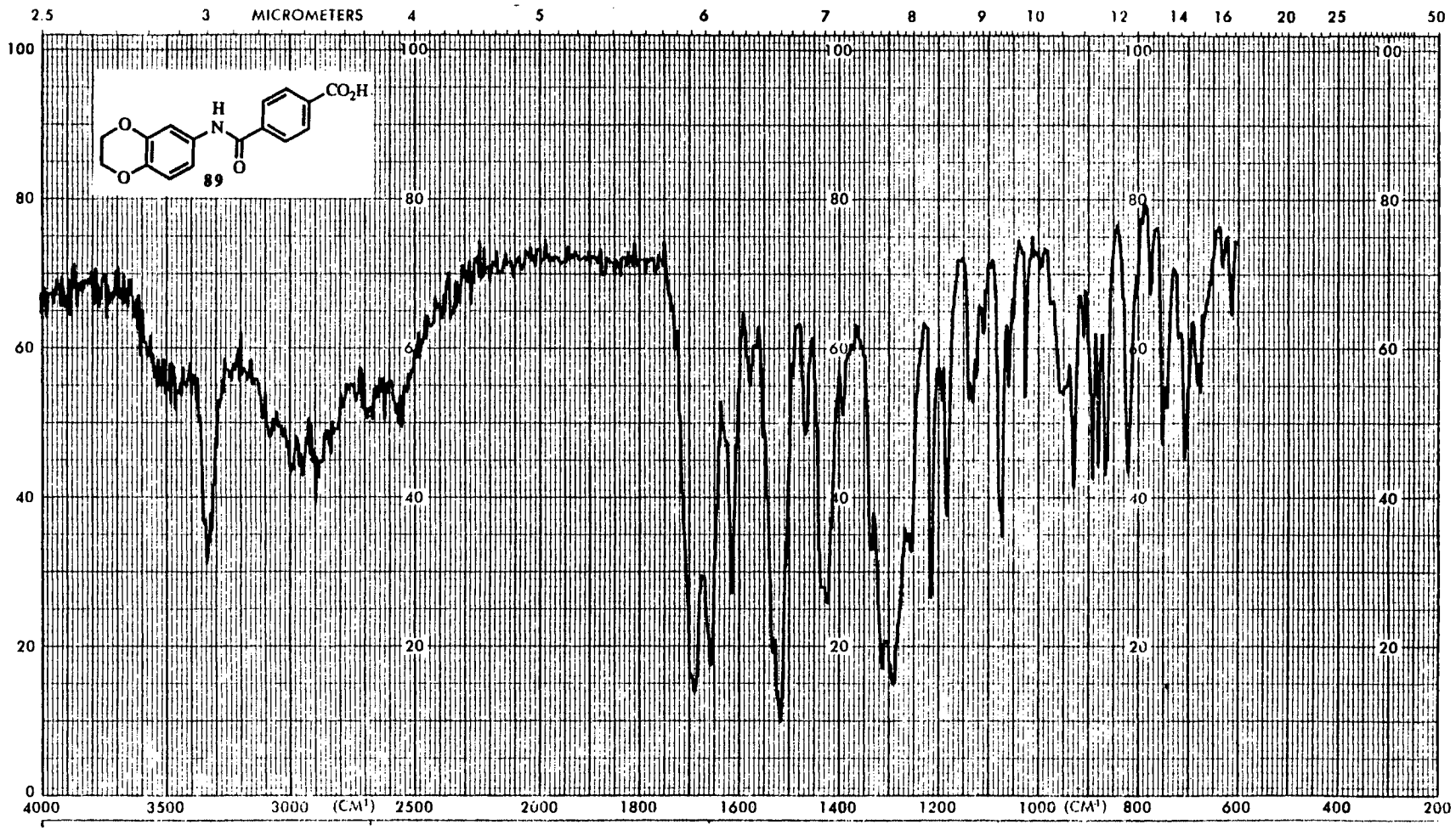
^{1}H NMR Spectrum of **88**

Plate XXXVI



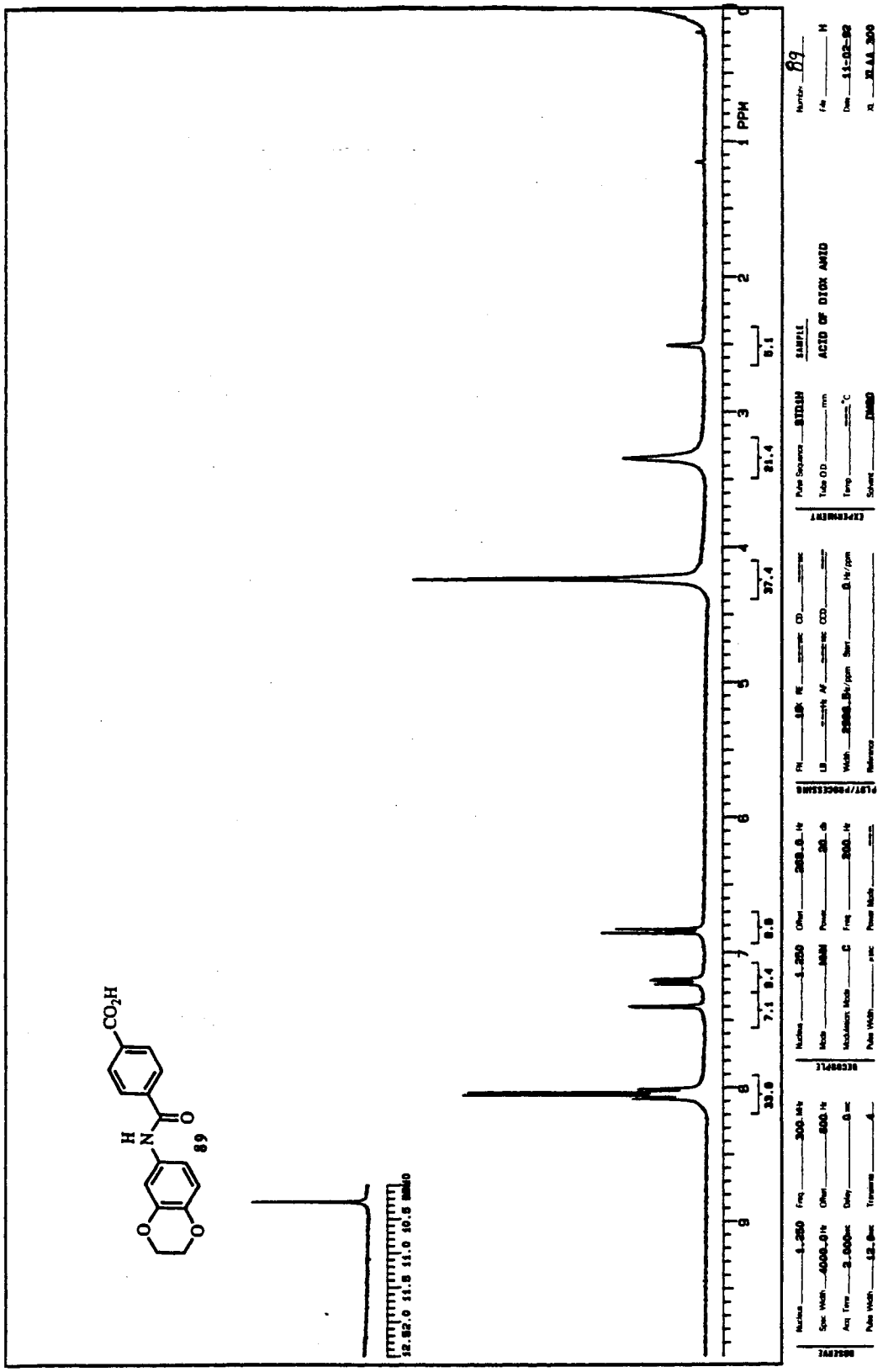
^{13}C NMR Spectrum of **88**

Plate XXXVII



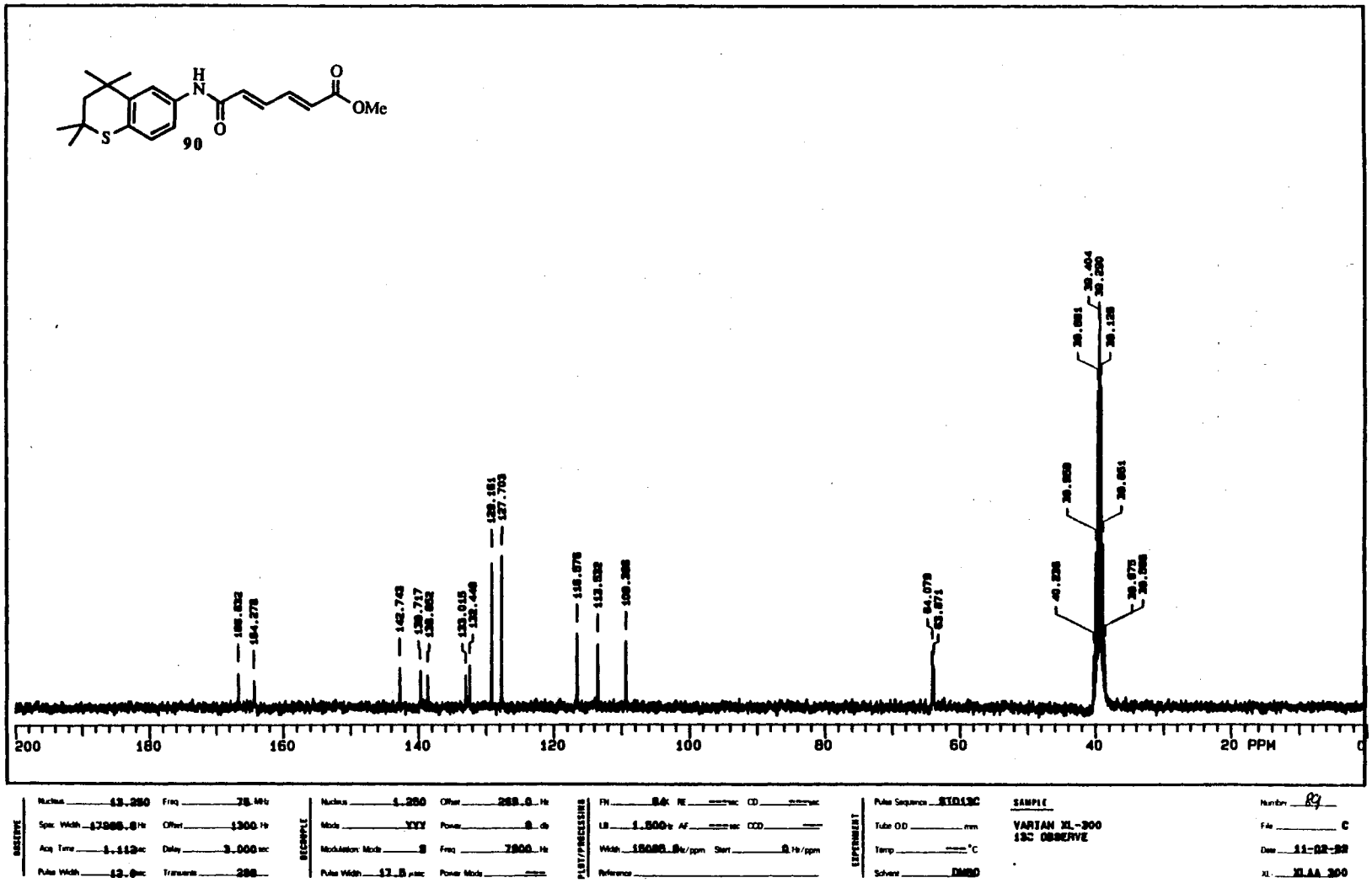
IR Spectrum of **89**

Plate XXXVIII



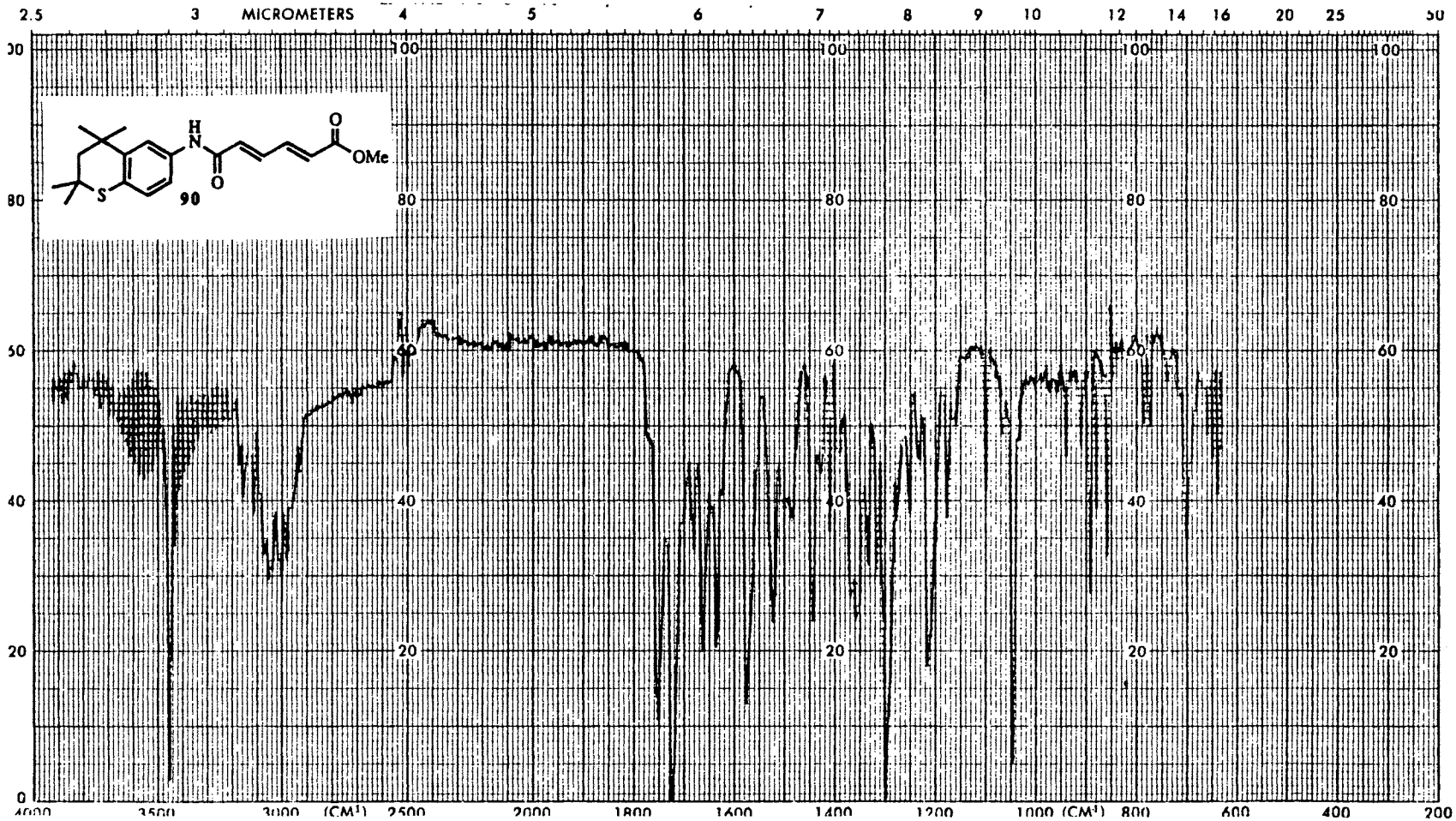
^{1}H NMR Spectrum of **89**

Plate XXXIX



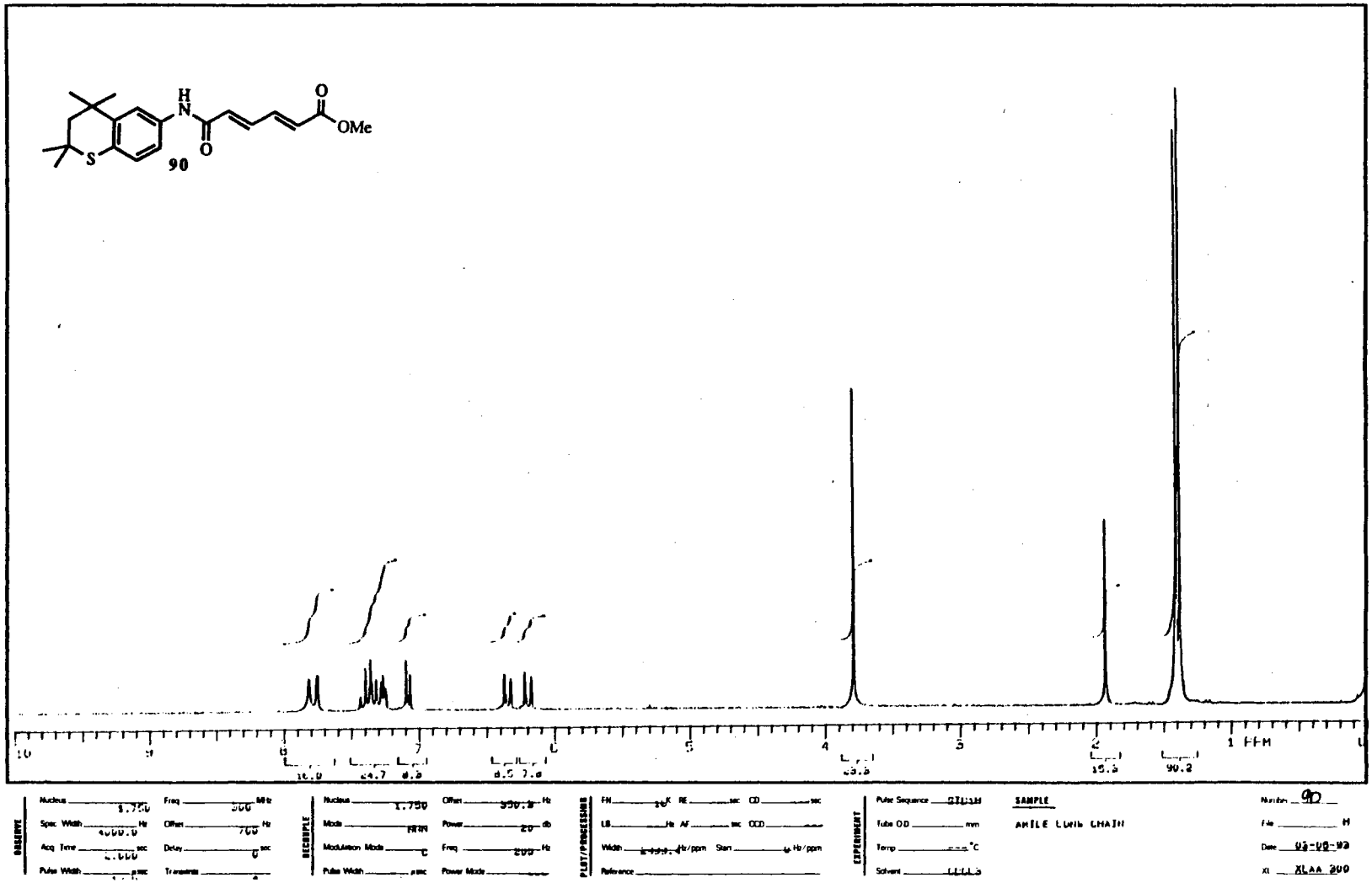
^{13}C NMR Spectrum of **89**

Plate XL



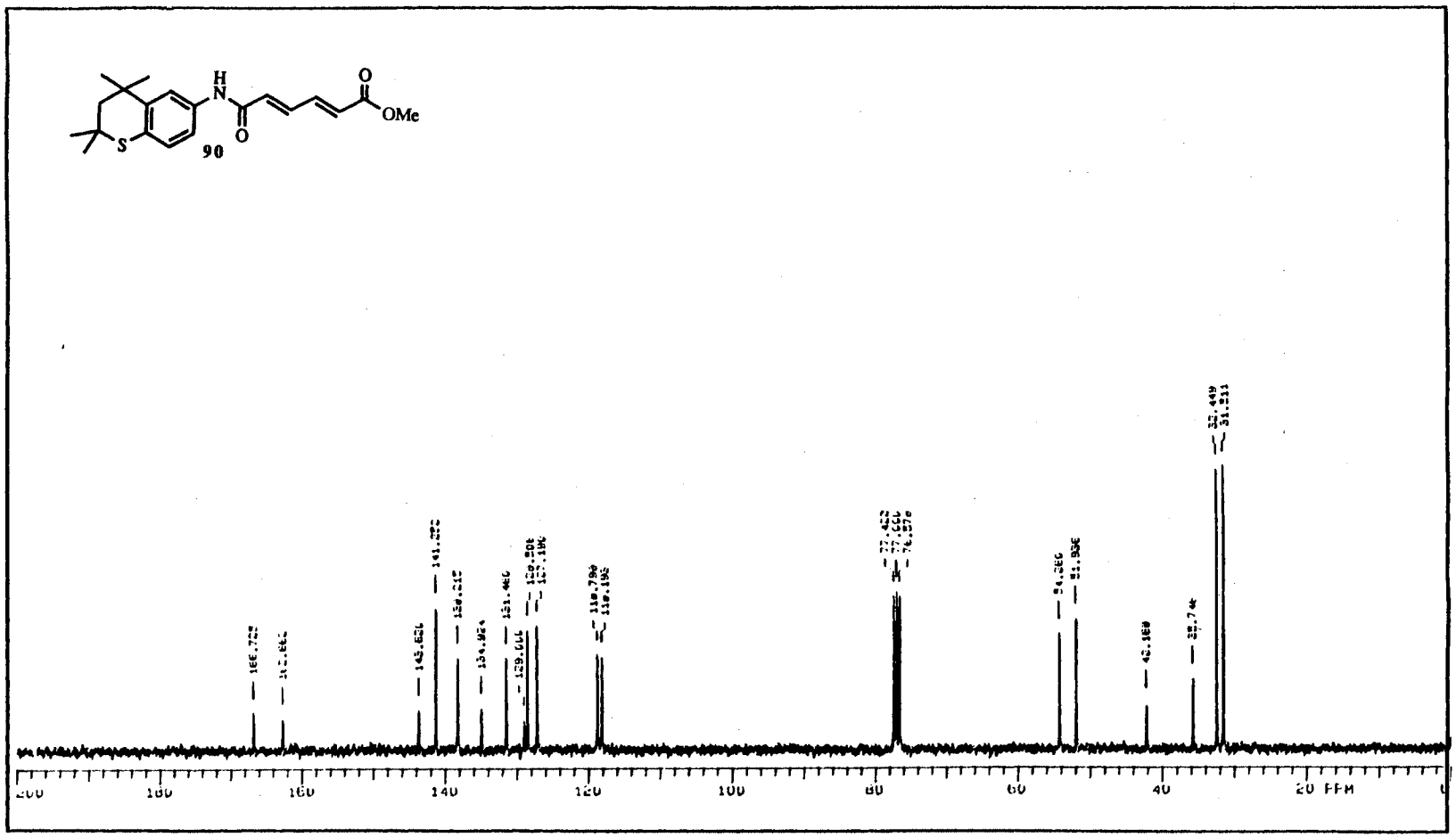
IR Spectrum of **90**

Plate XLI



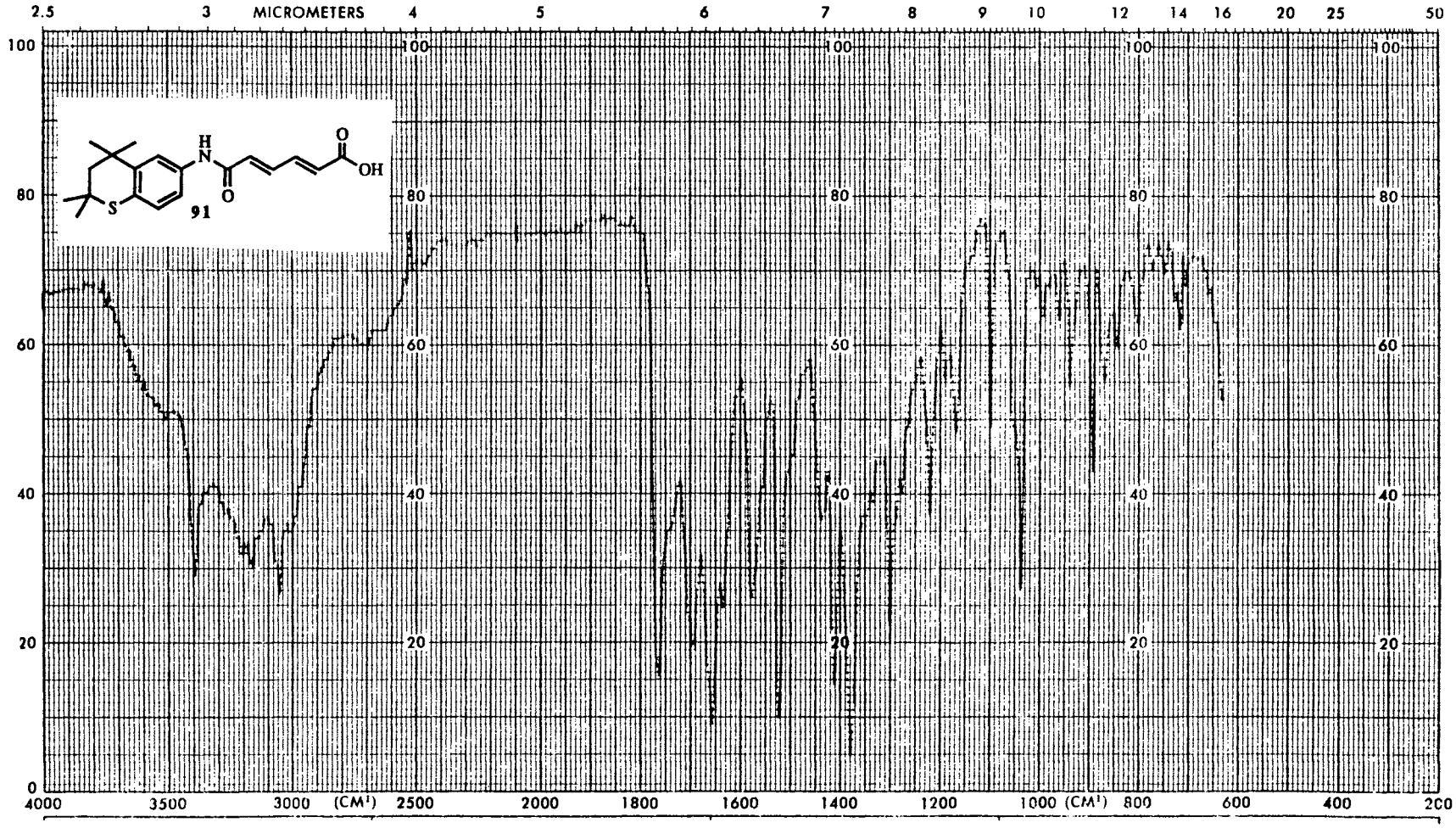
^{1}H NMR Spectrum of **90**

Plate XLII



^{13}C NMR Spectrum of **90**

Plate XLIII

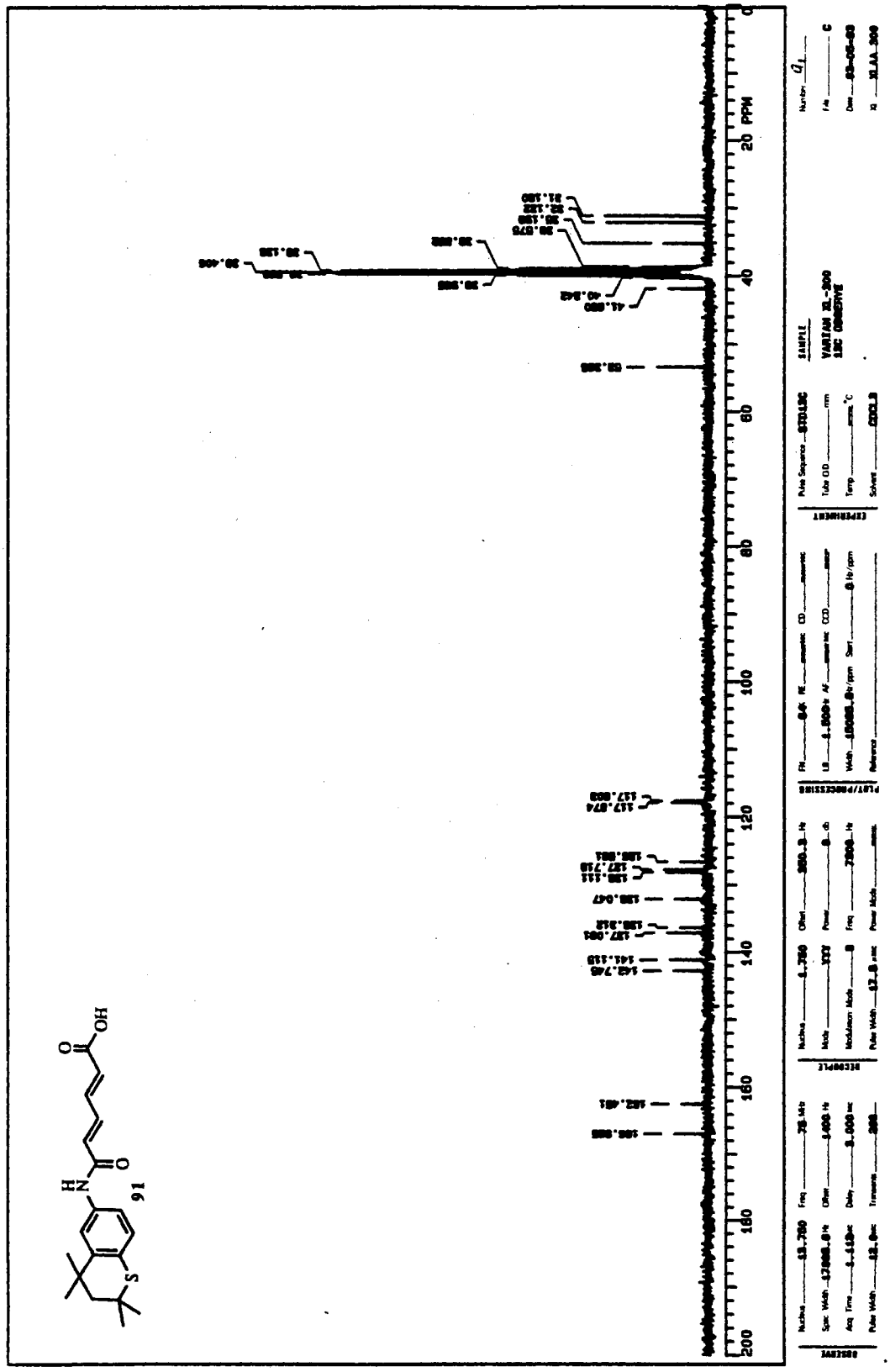


IR Spectrum of **91**

Plate XLIV

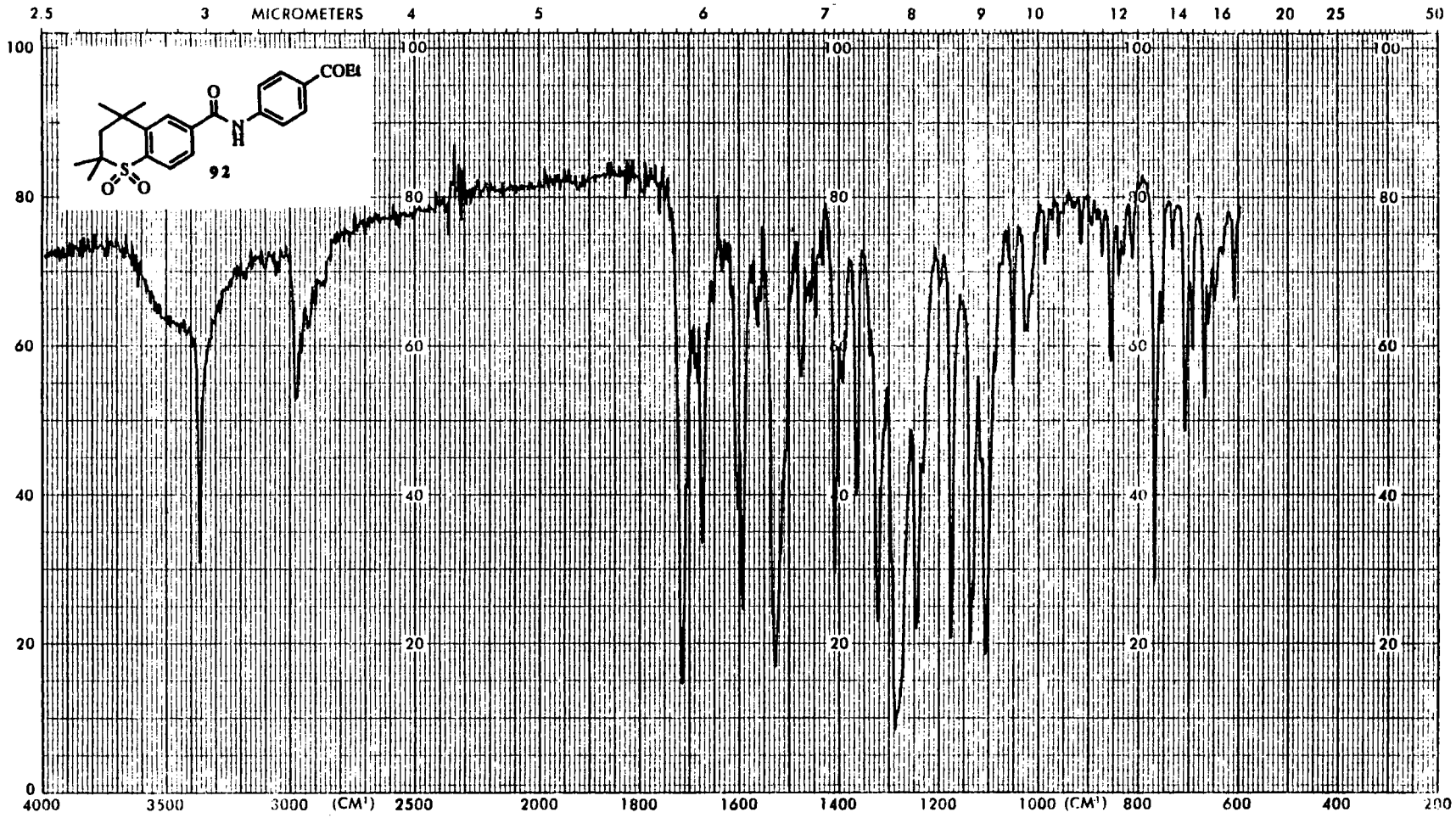
^{1}H NMR Spectrum of **91**

Plate XLV



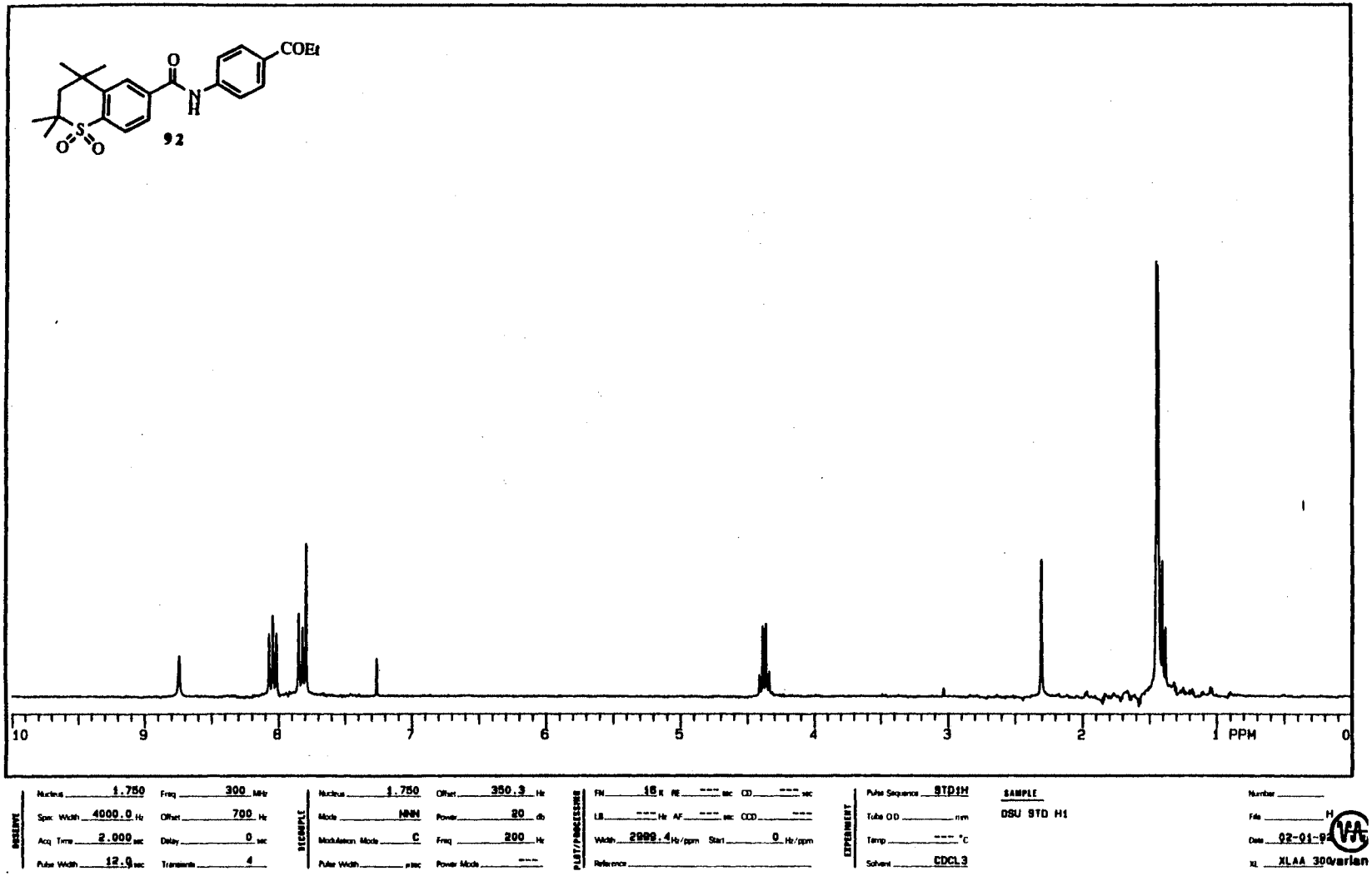
^{13}C NMR Spectrum of **91**

Plate XLVI



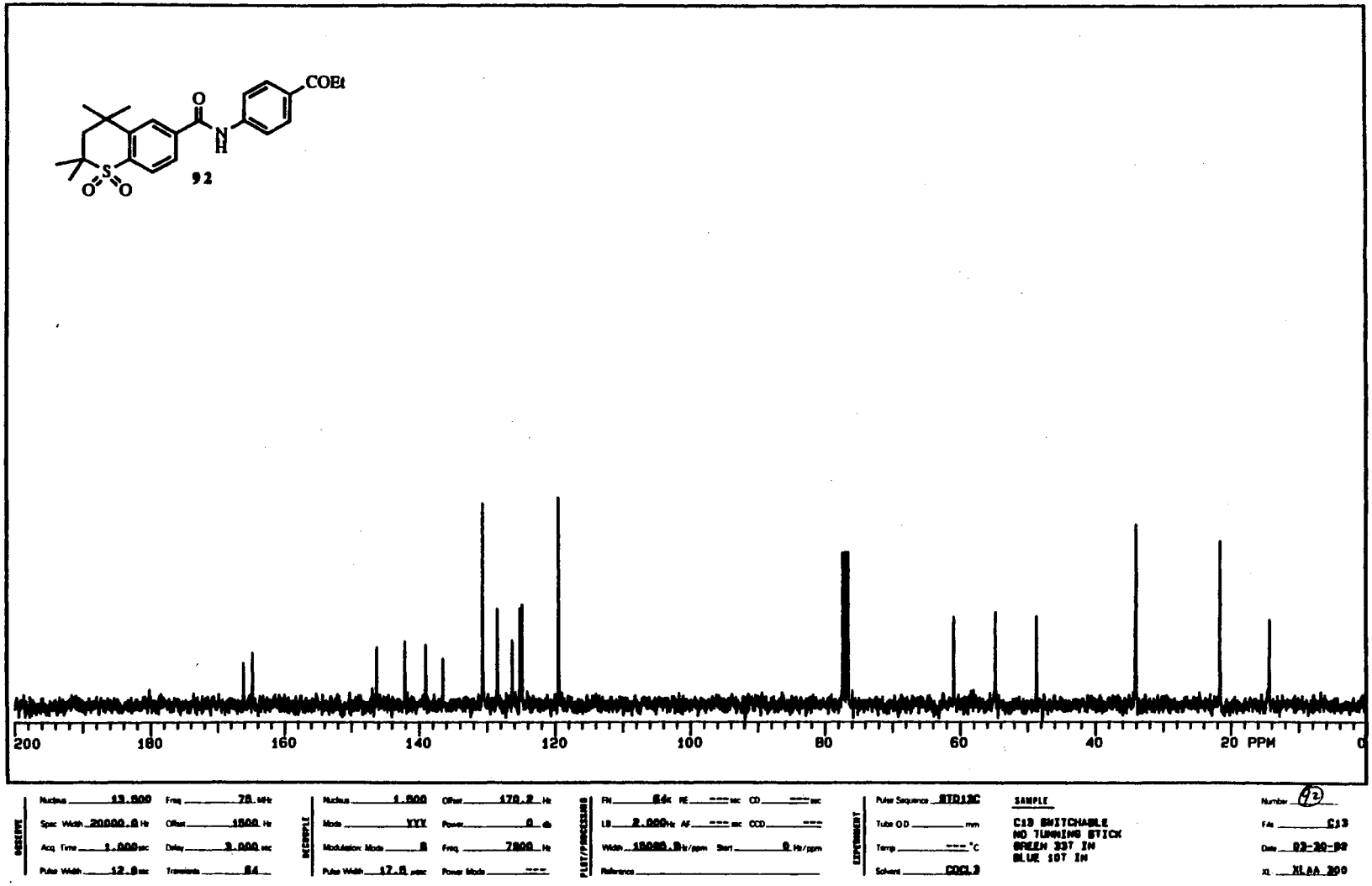
IR Spectrum of **92**

Plate XLVII



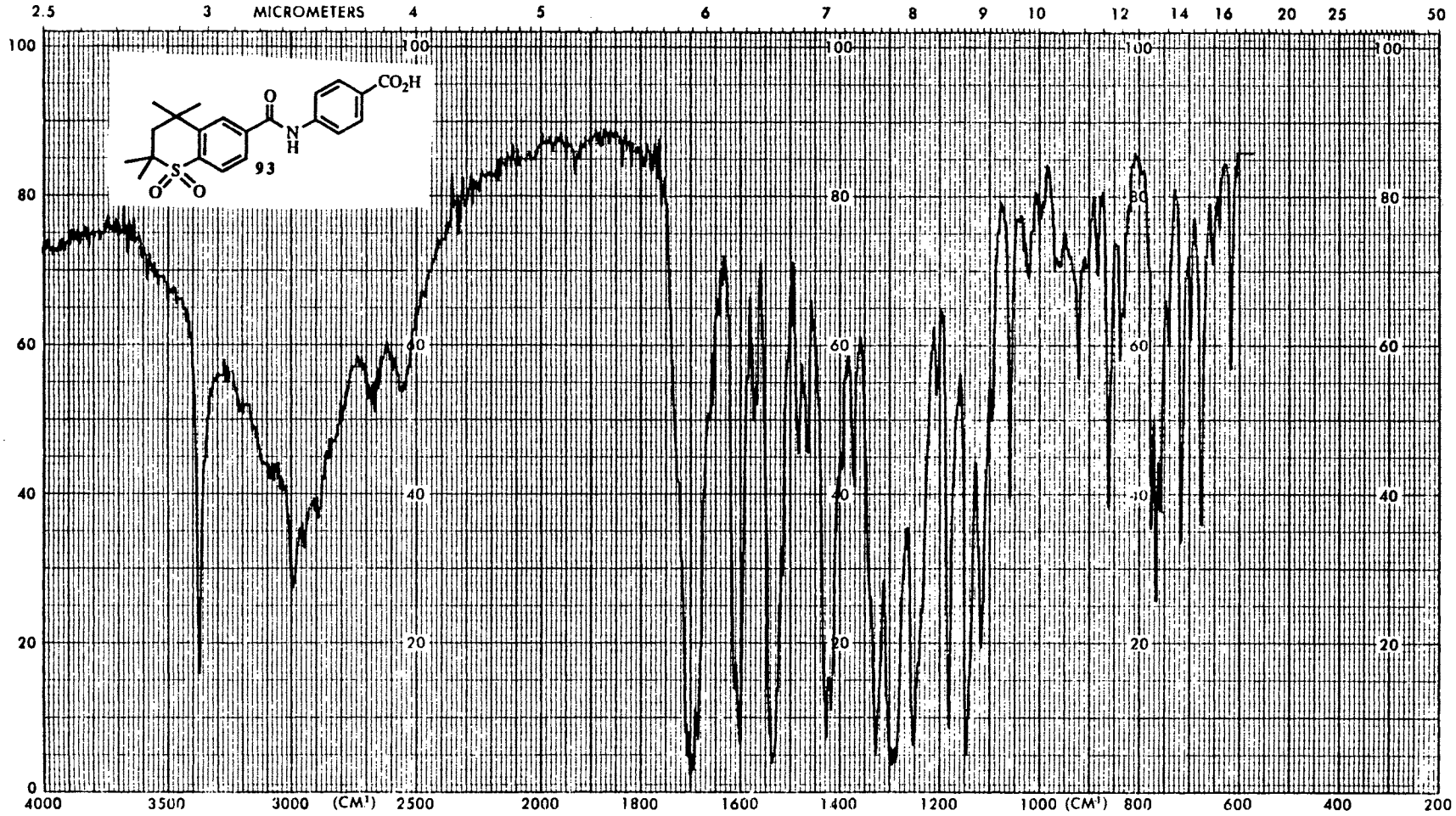
^{1}H NMR Spectrum of **92**

Plate XLVIII



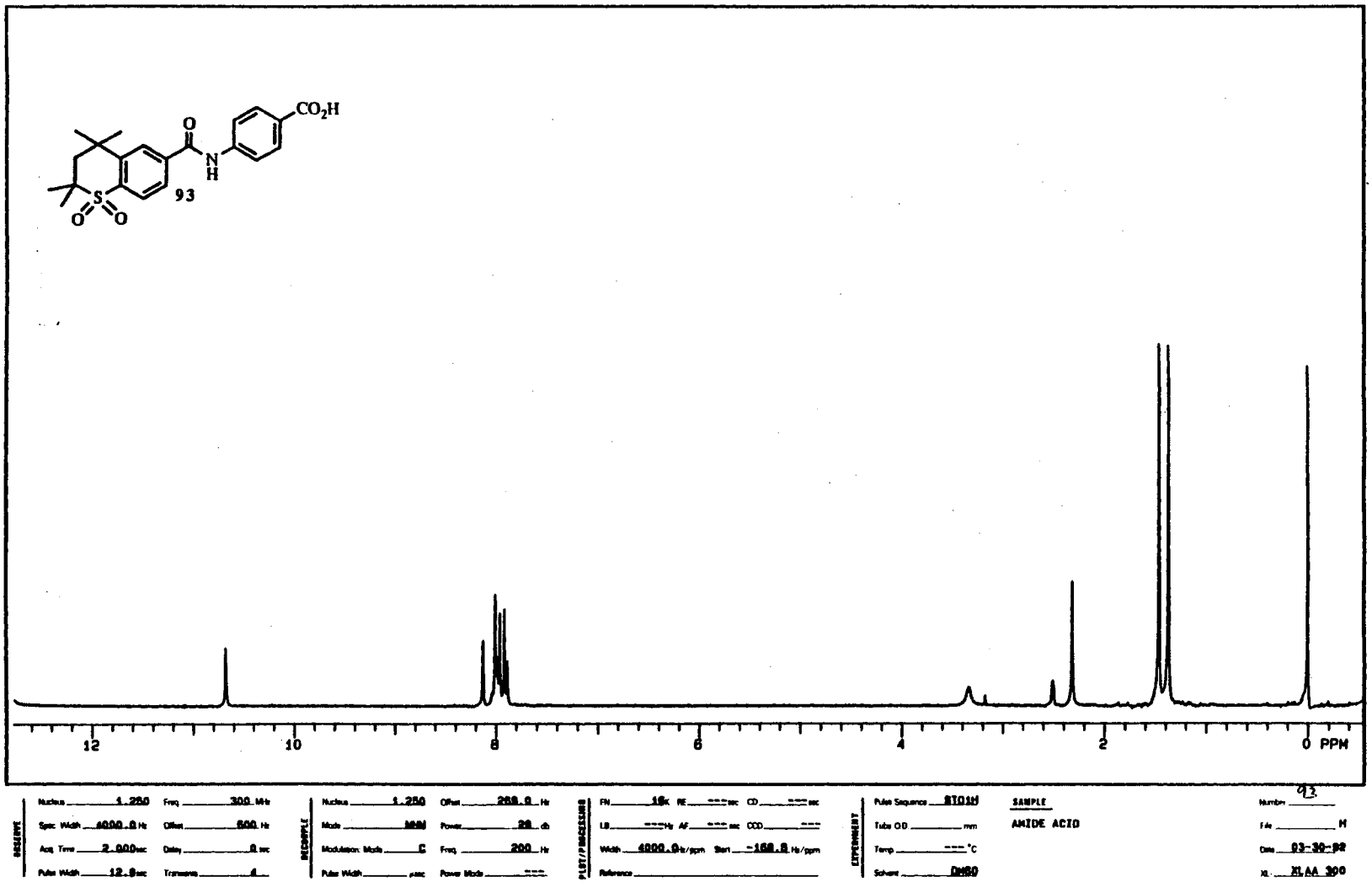
^{13}C NMR Spectrum of **92**

Plate XLIX



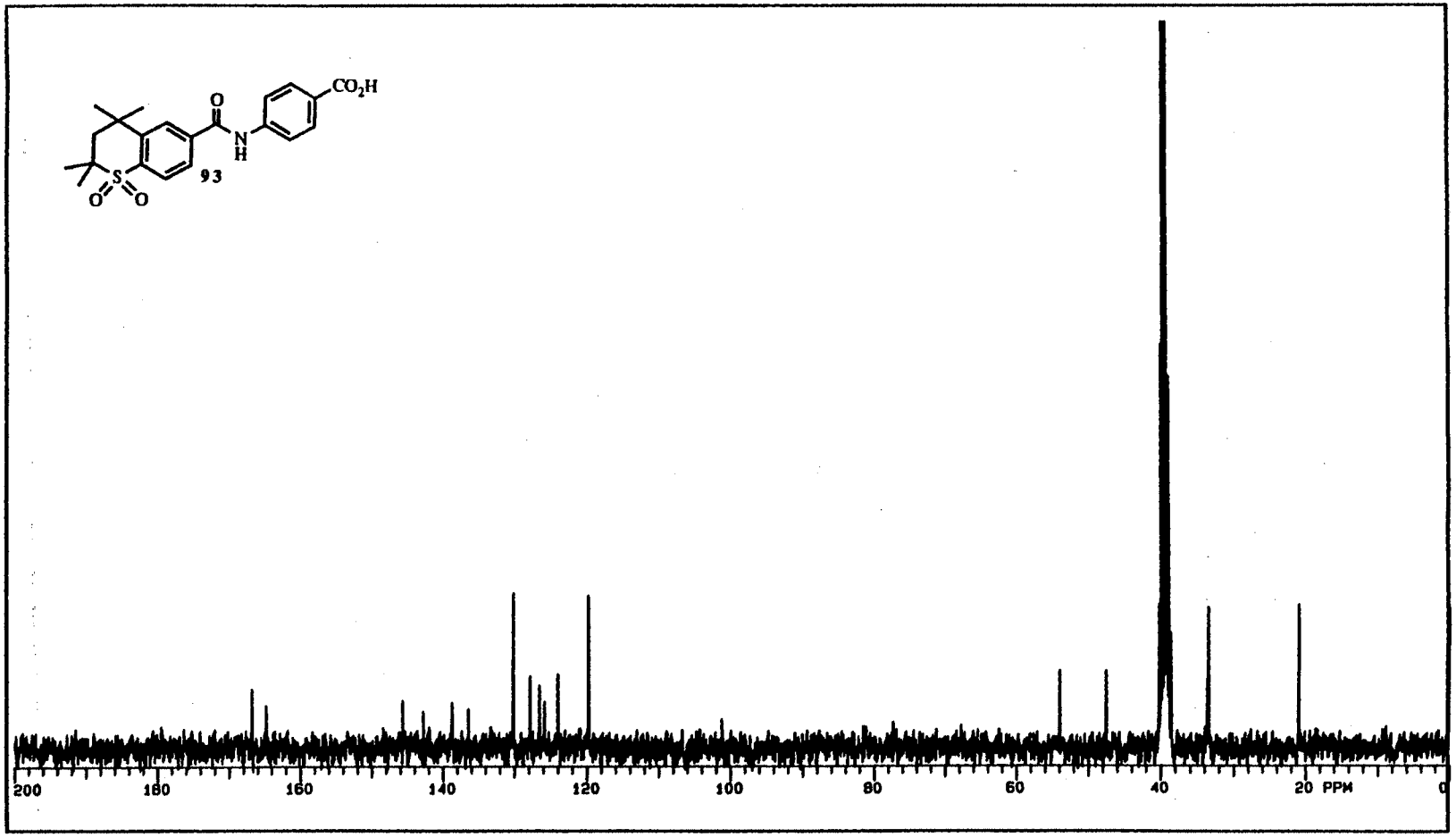
IR Spectrum of **93**

Plate L



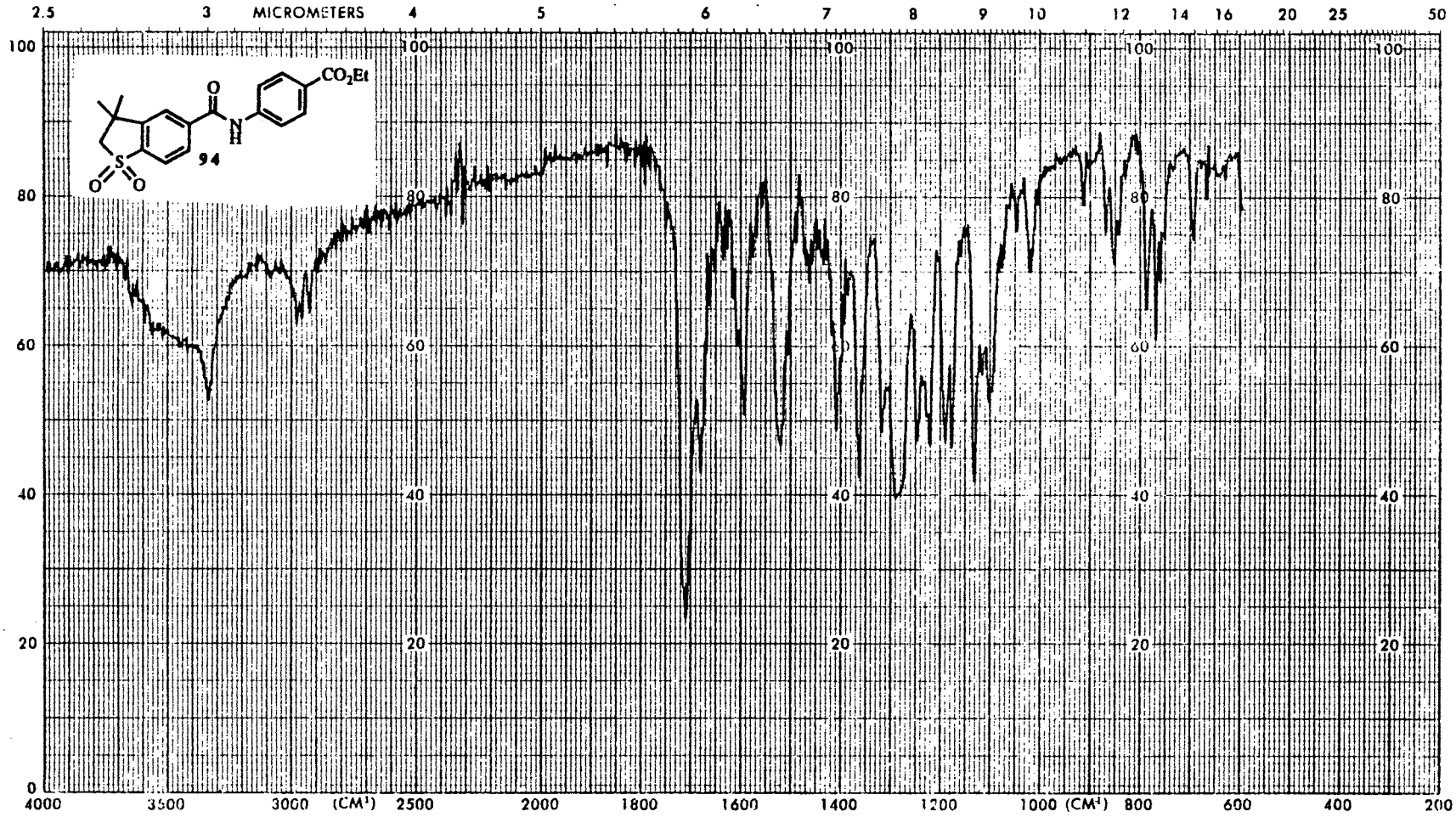
^{1}H NMR Spectrum of **93**

Plate LI



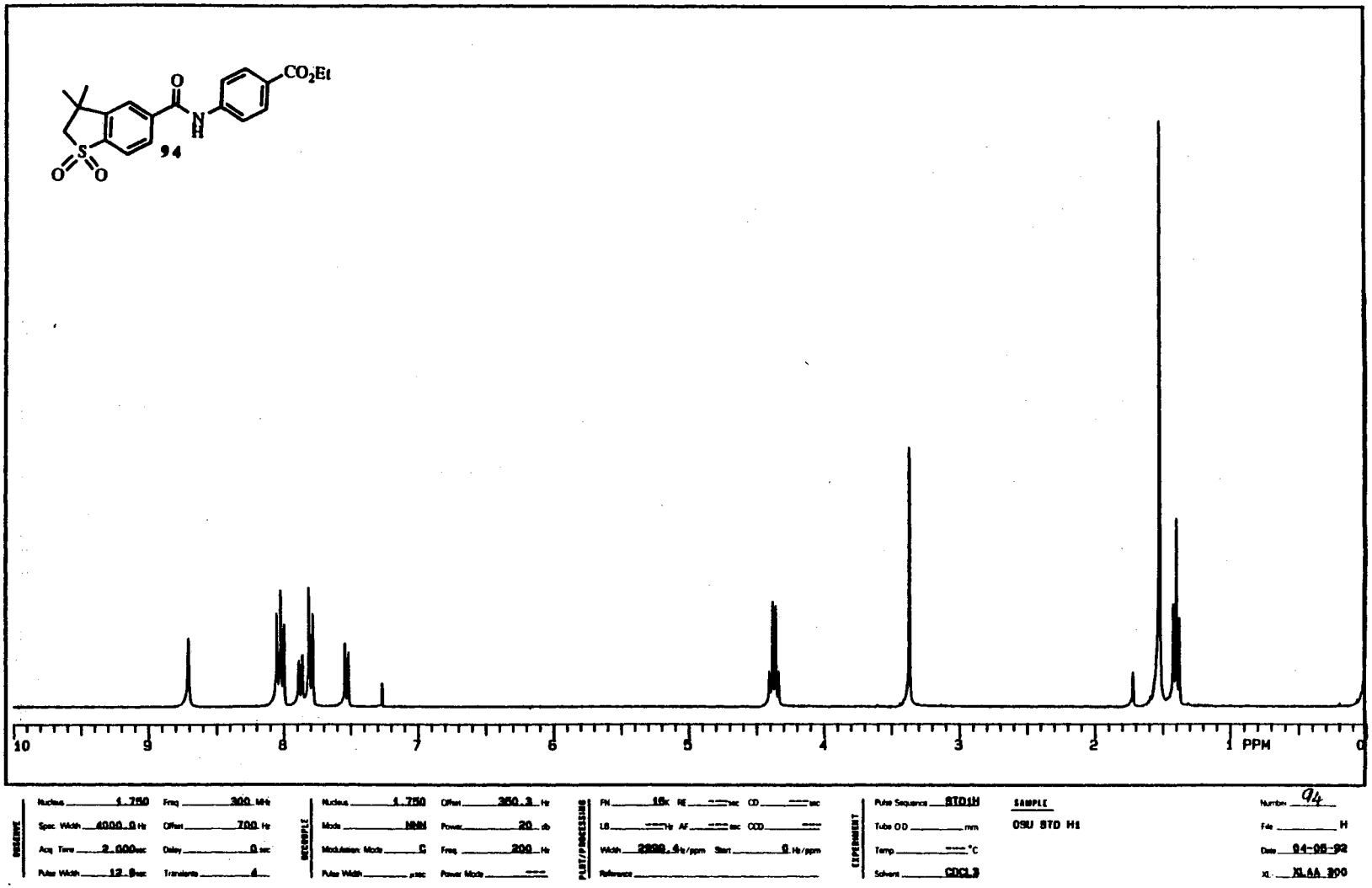
^{13}C NMR Spectrum of **93**

Plate LII



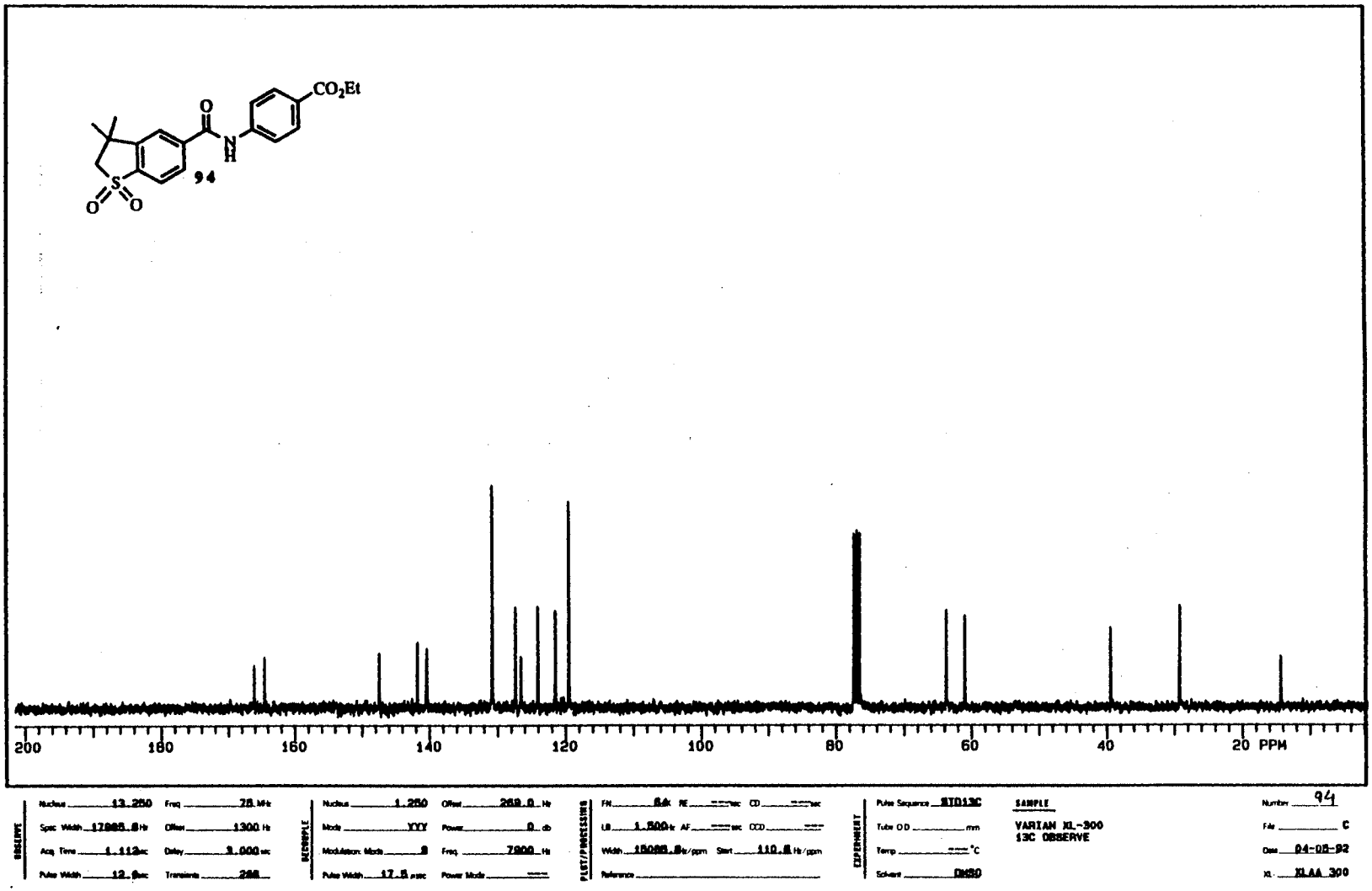
IR Spectrum of **94**

Plate LIII



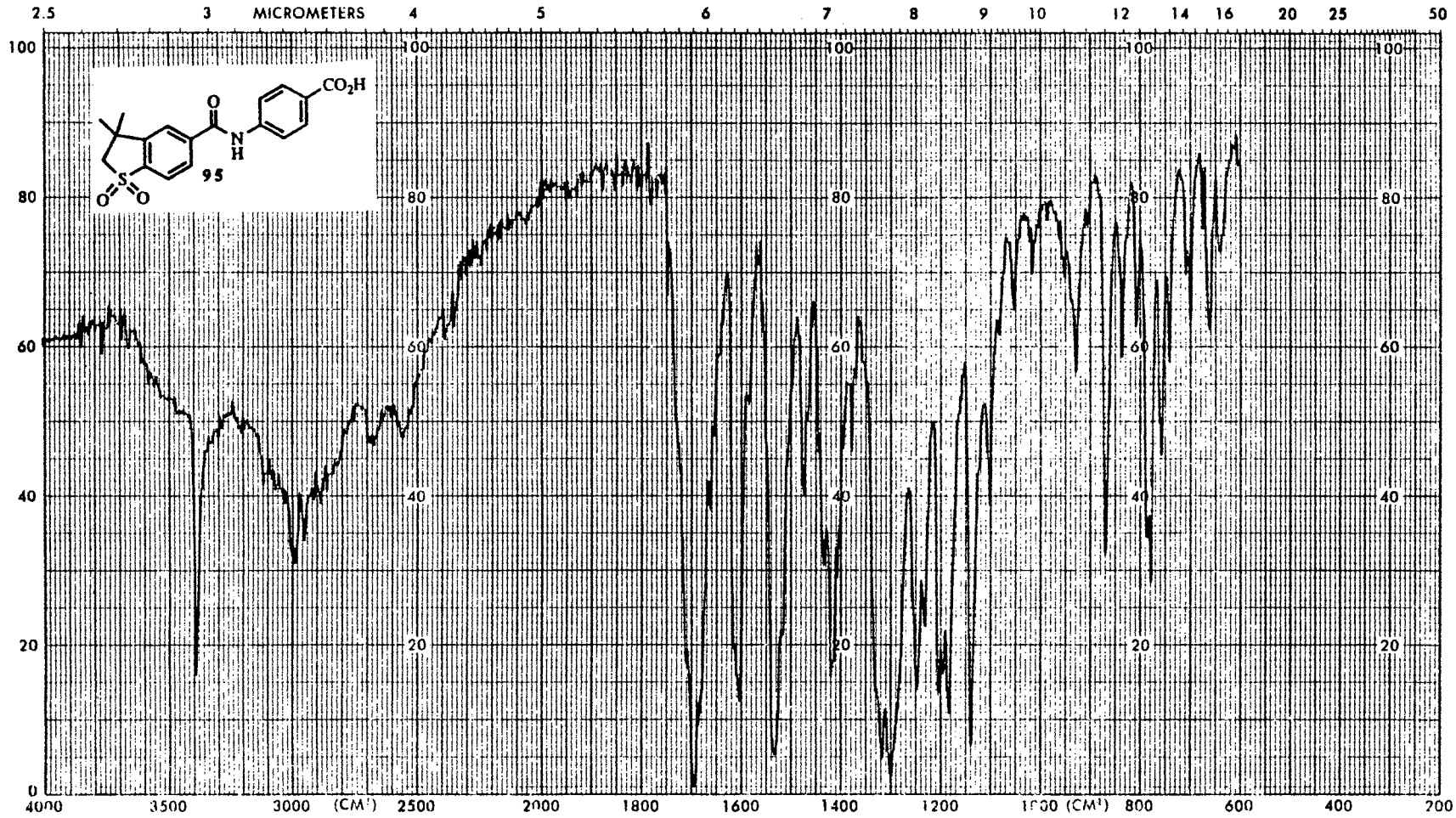
^{1}H NMR Spectrum of **94**

Plate LIV



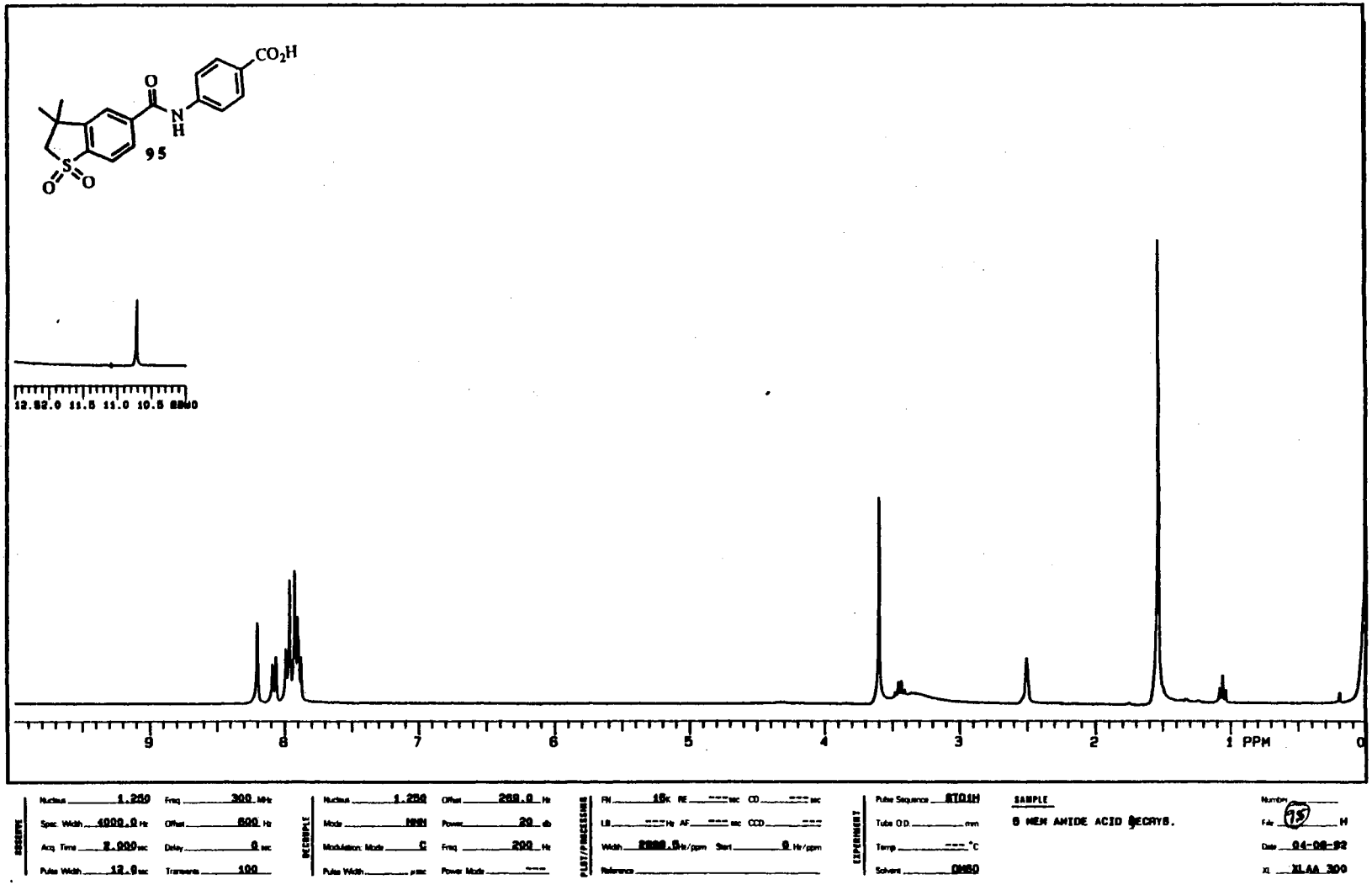
^{13}C NMR Spectrum of **94**

Plate LV



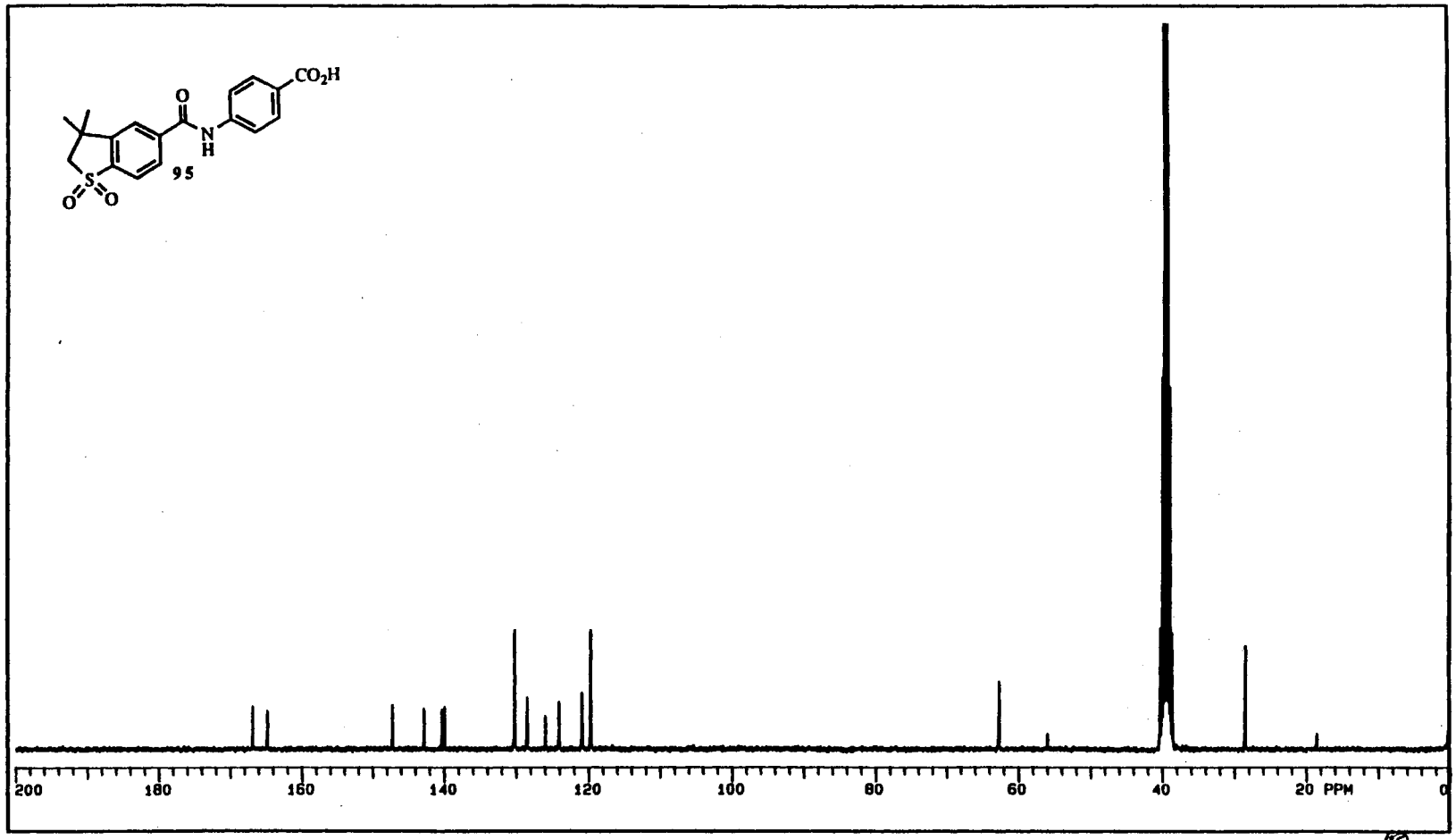
IR Spectrum of **95**

Plate LVI



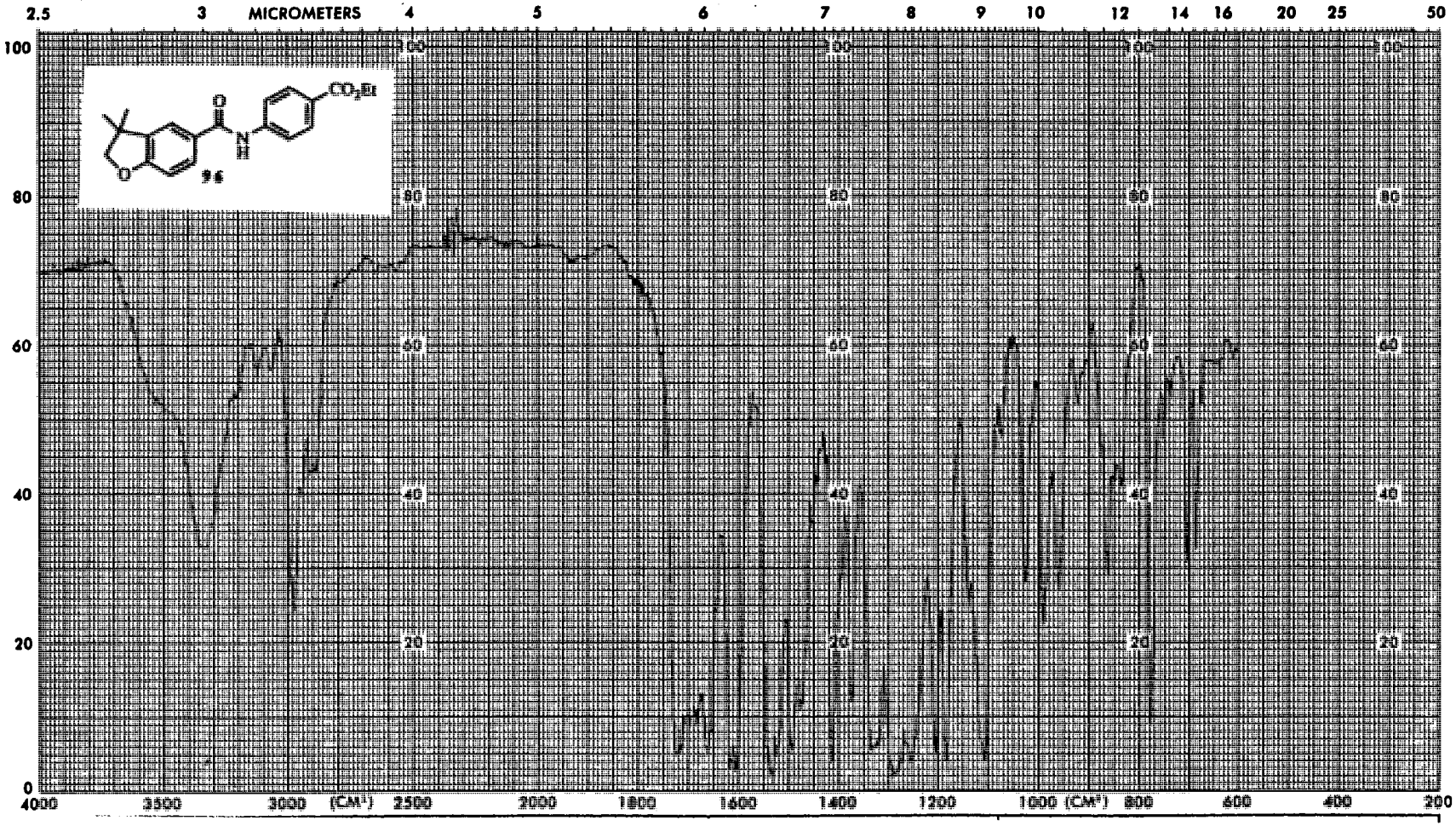
^{1}H NMR Spectrum of **95**

Plate LVII



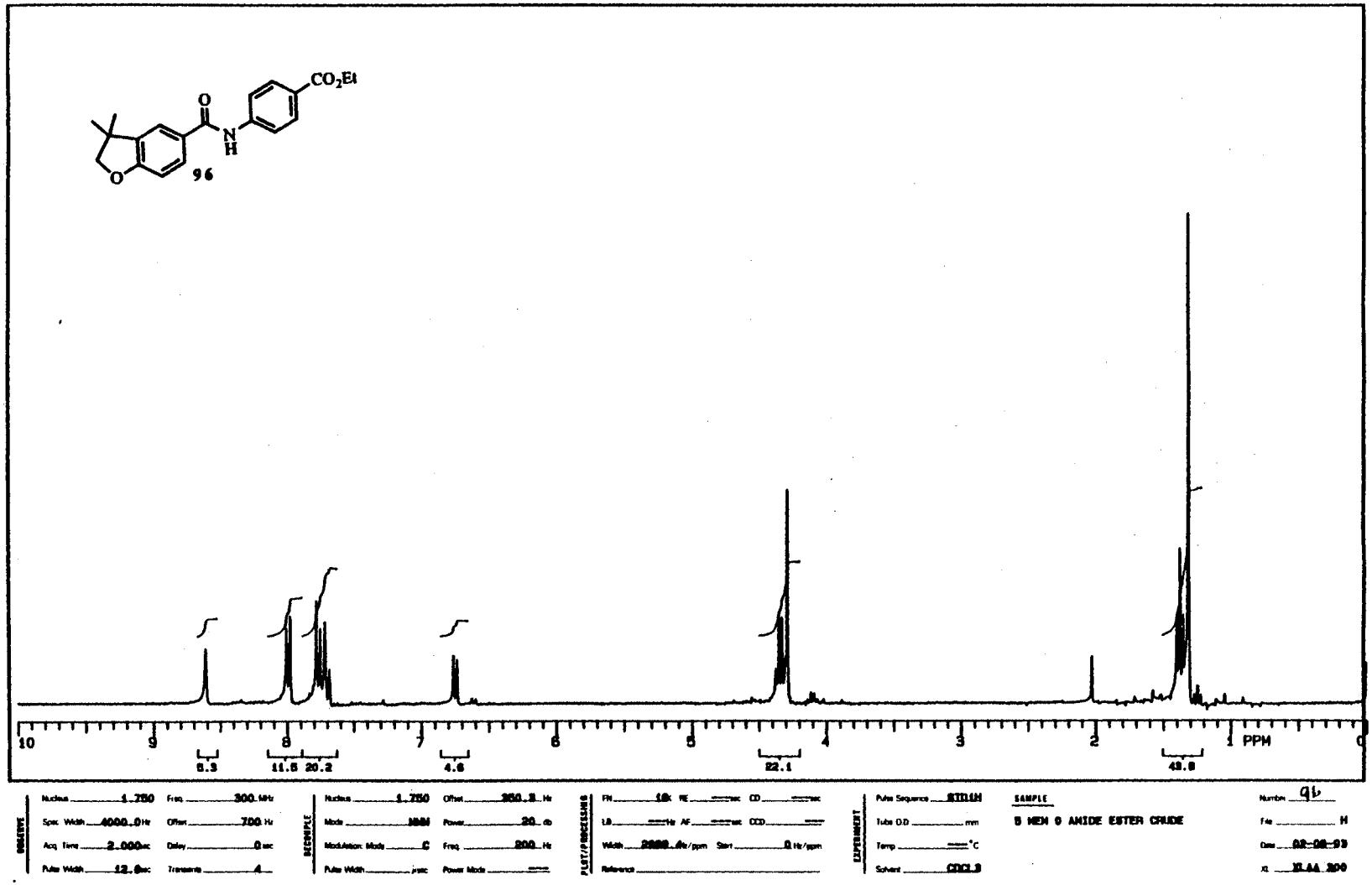
^{13}C NMR Spectrum of **95**

Plate LVIII



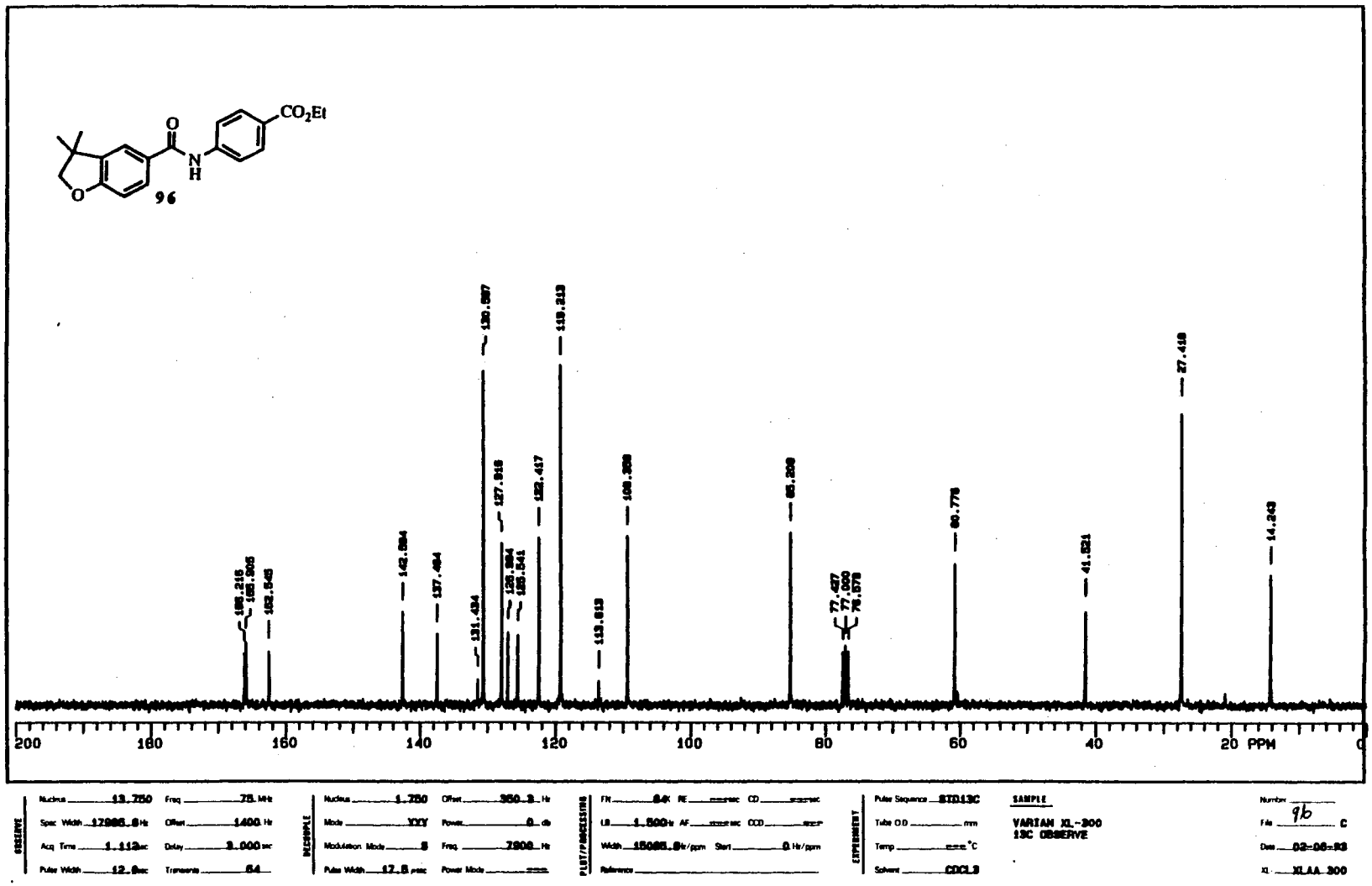
IR Spectrum of **96**

Plate LIX



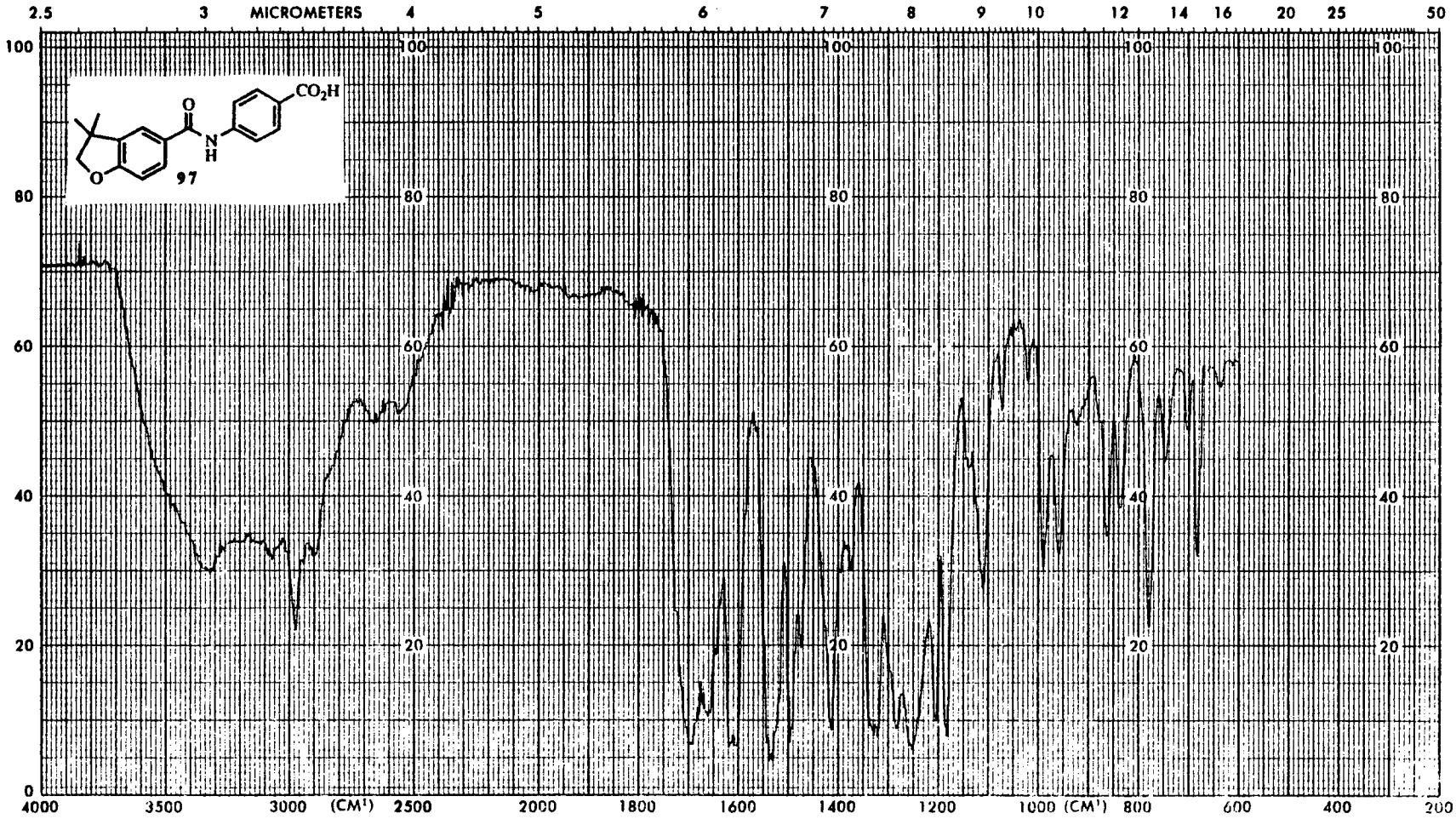
^{1}H NMR Spectrum of **96**

Plate LX



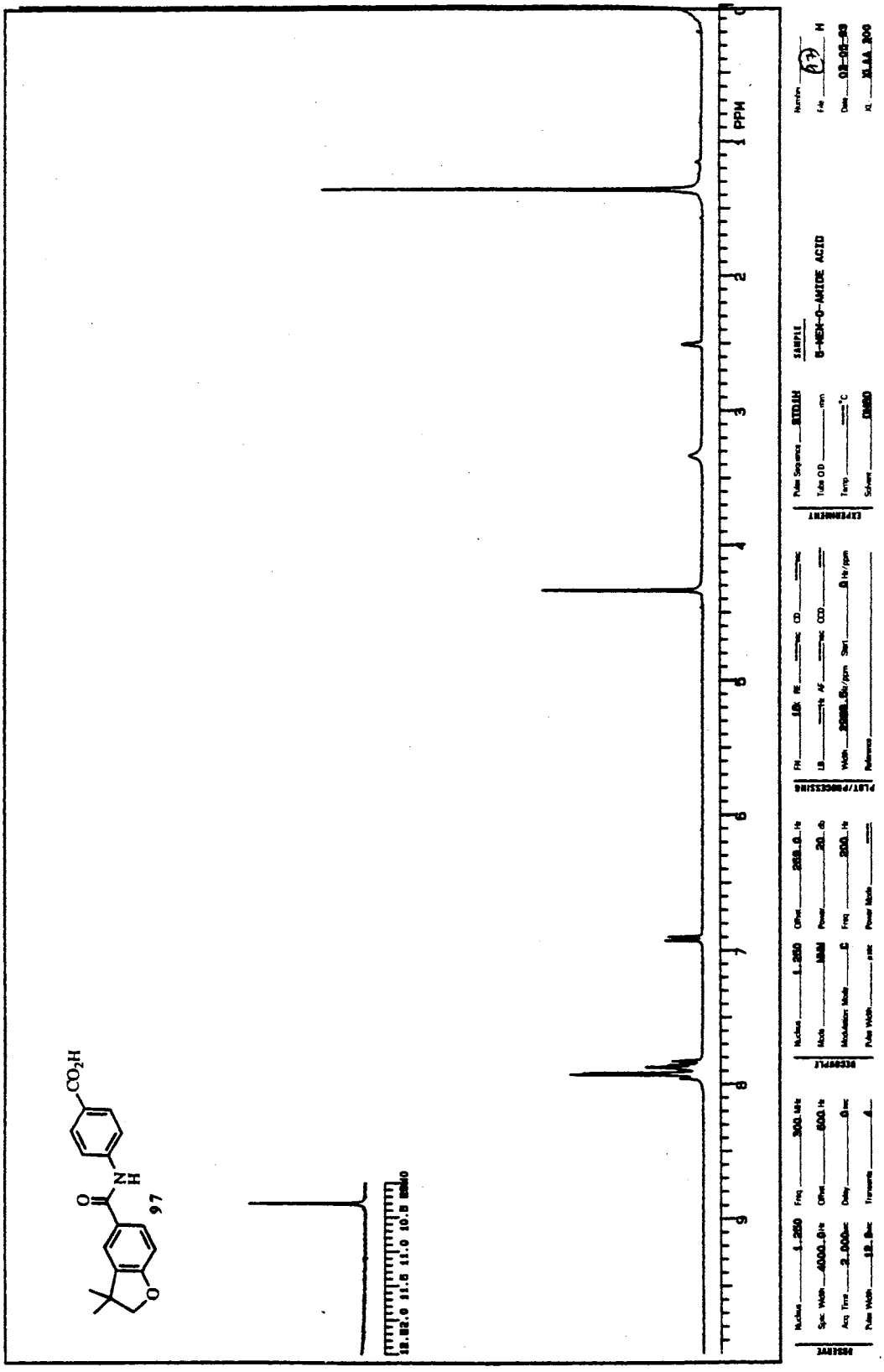
^{13}C NMR Spectrum of **96**

Plate LXI



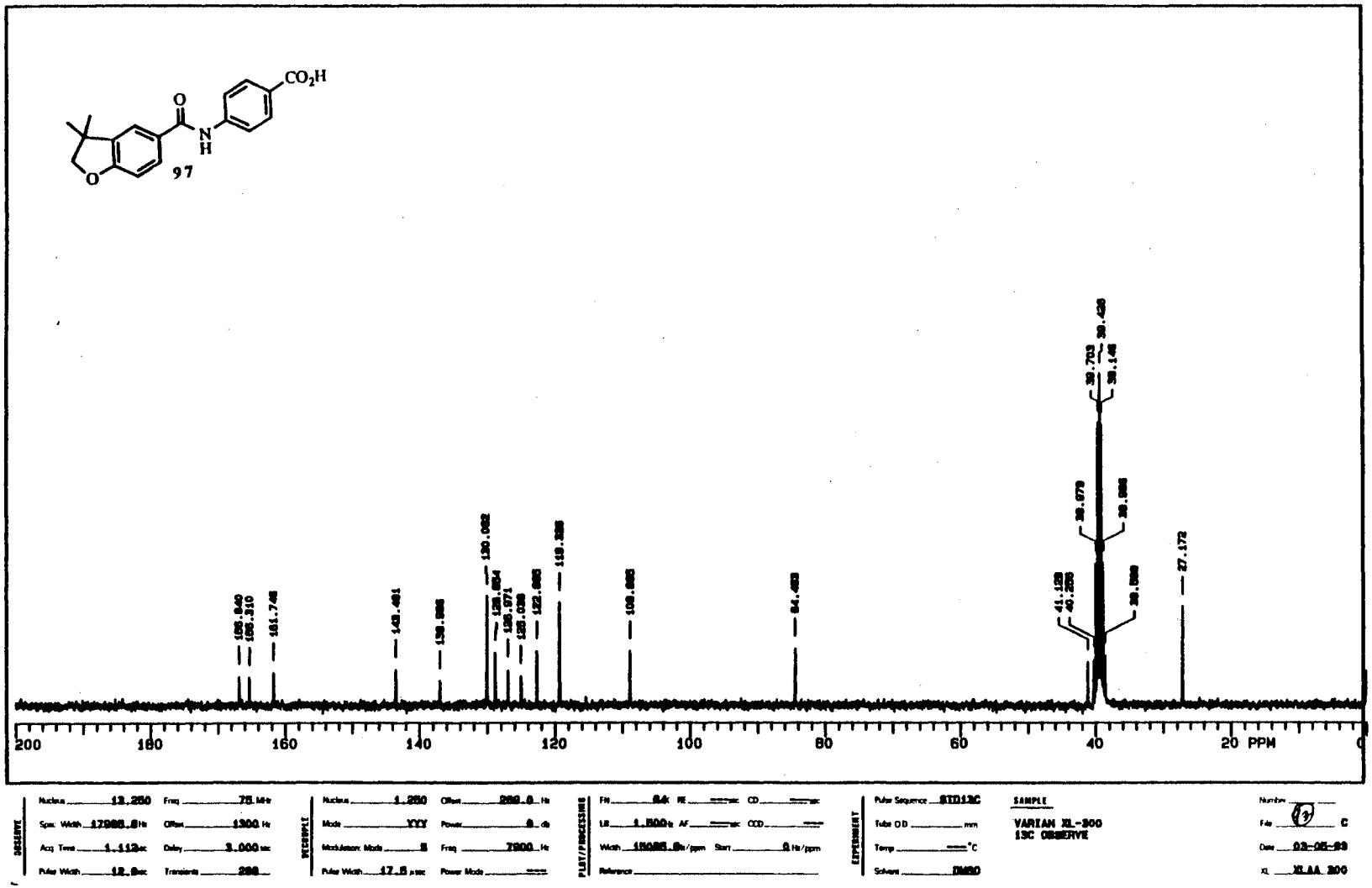
IR Spectrum of **97**

Plate LXII



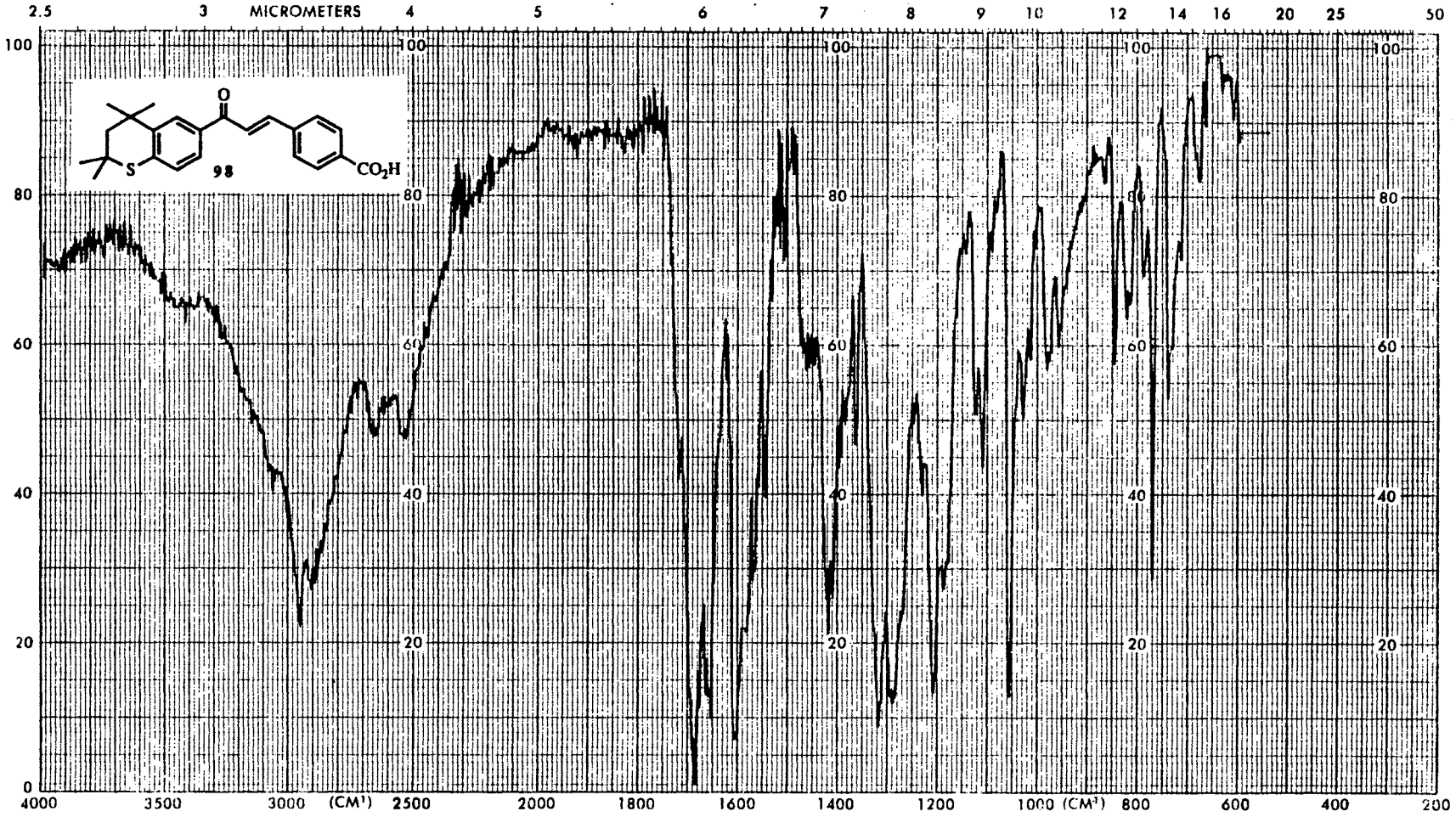
^{1}H NMR Spectrum of **97**

Plate LXIII



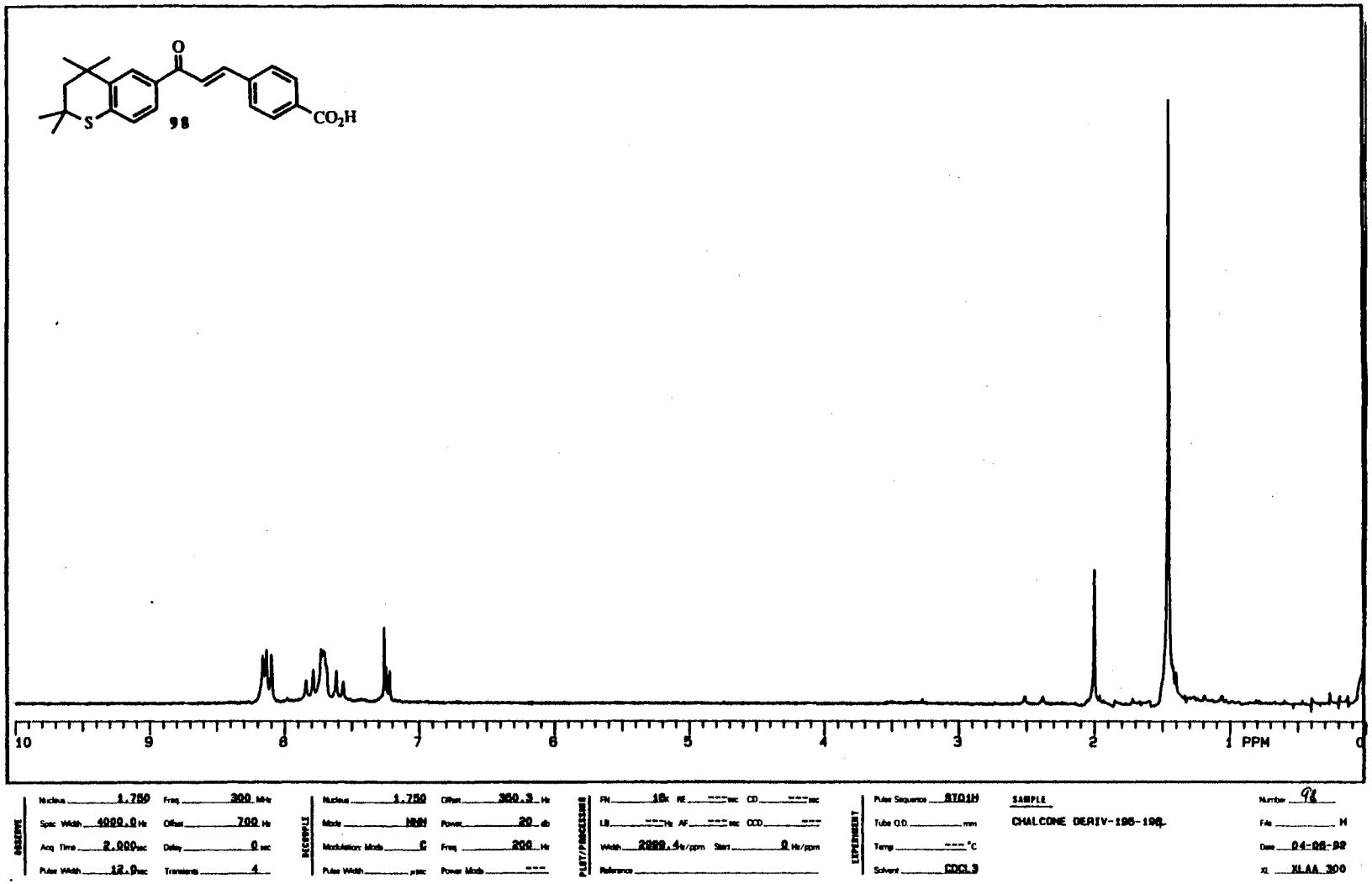
^{13}C NMR Spectrum of **97**

Plate LXIV



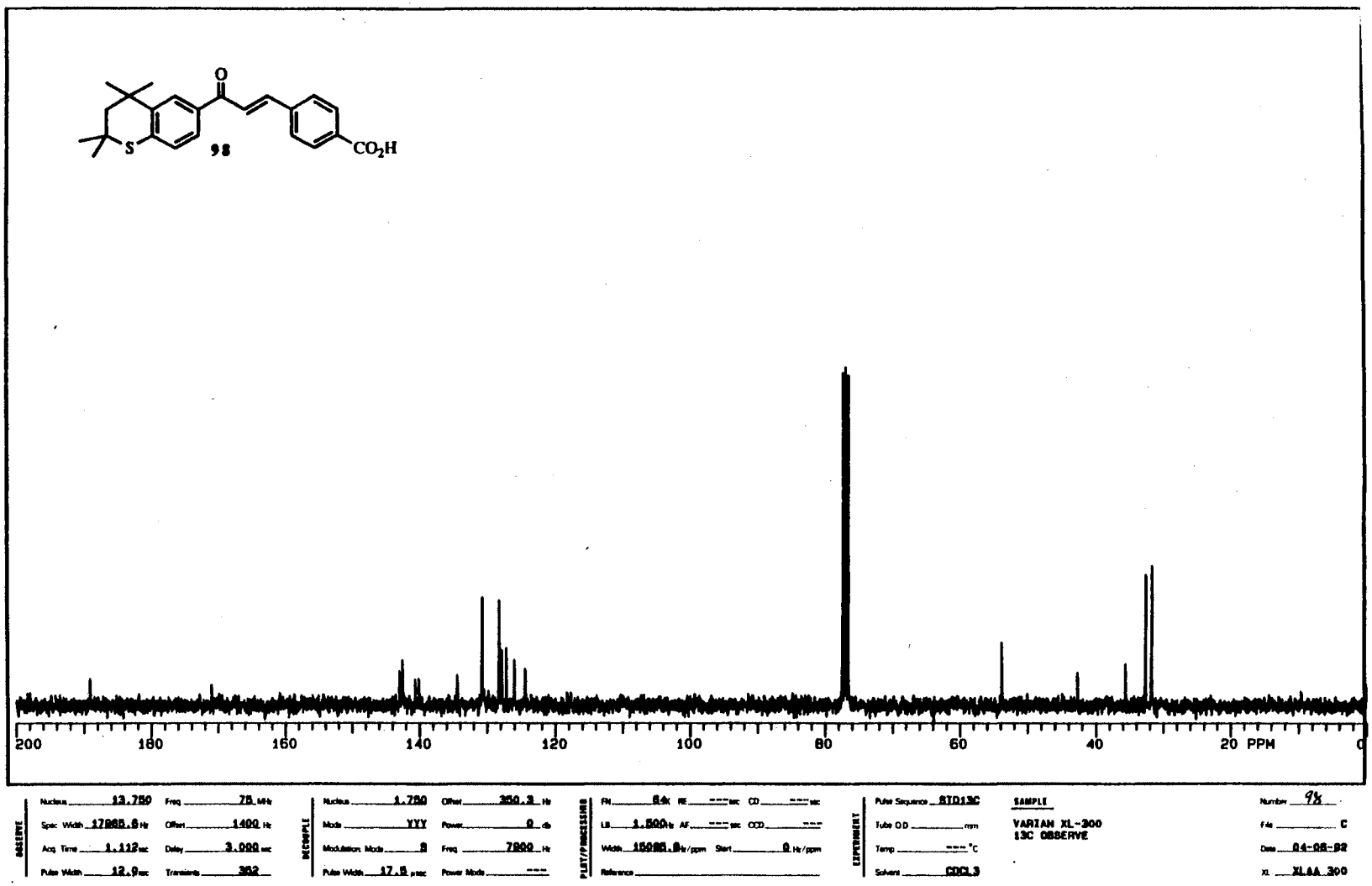
IR Spectrum of **98**

Plate LXV



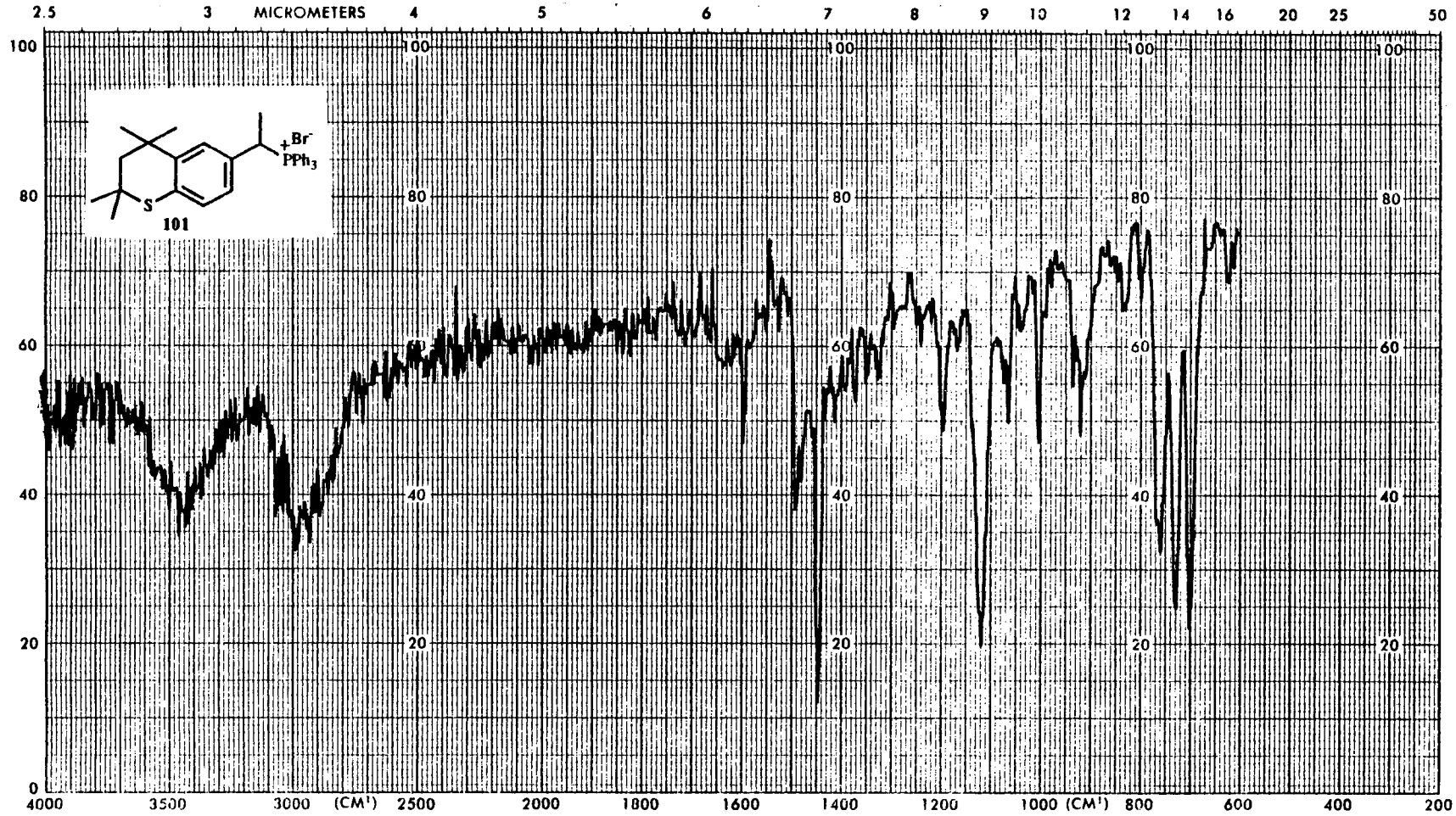
^{1}H NMR Spectrum of **98**

Plate LXVI



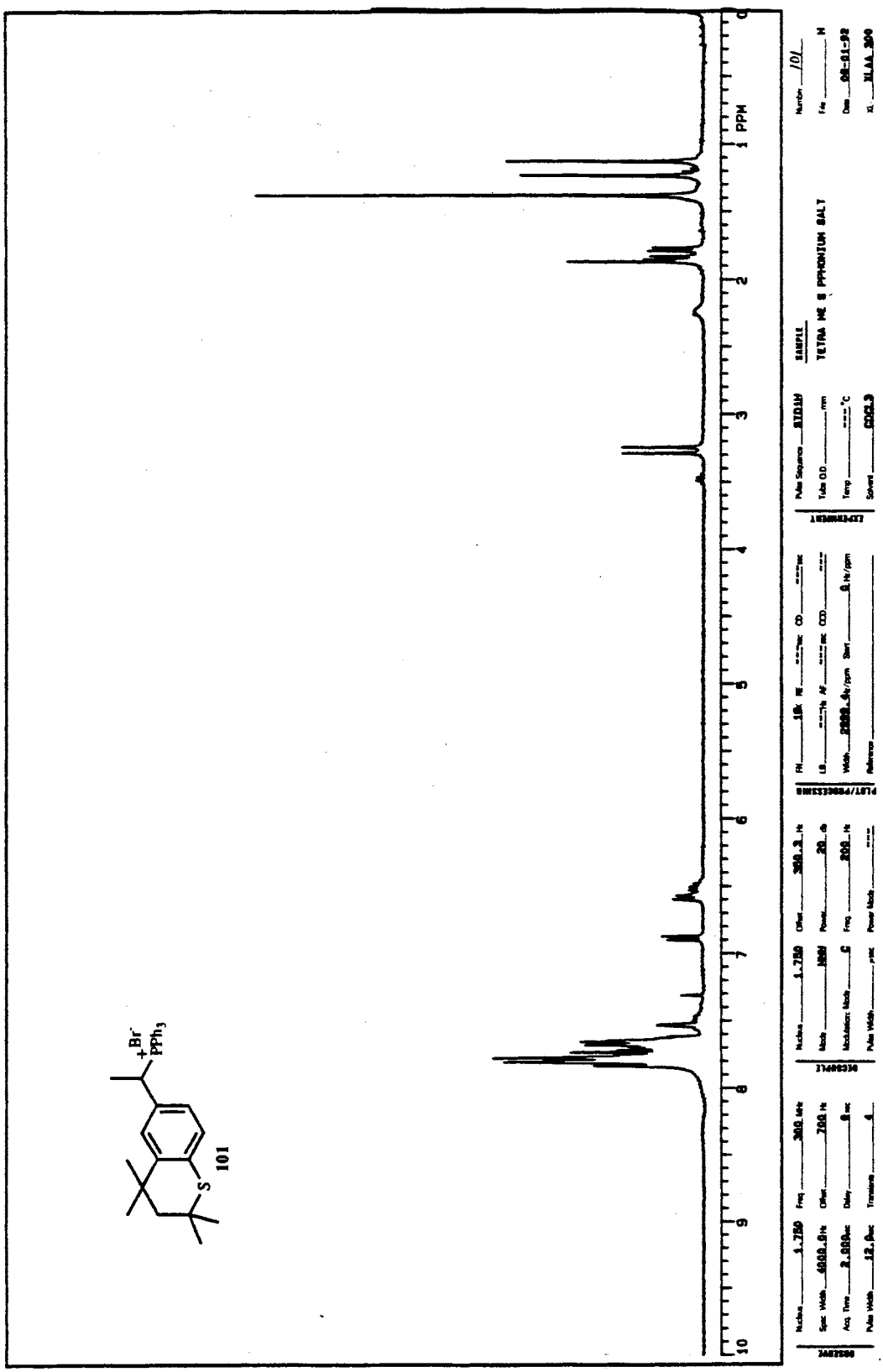
^{13}C NMR Spectrum of **98**

Plate LXVII



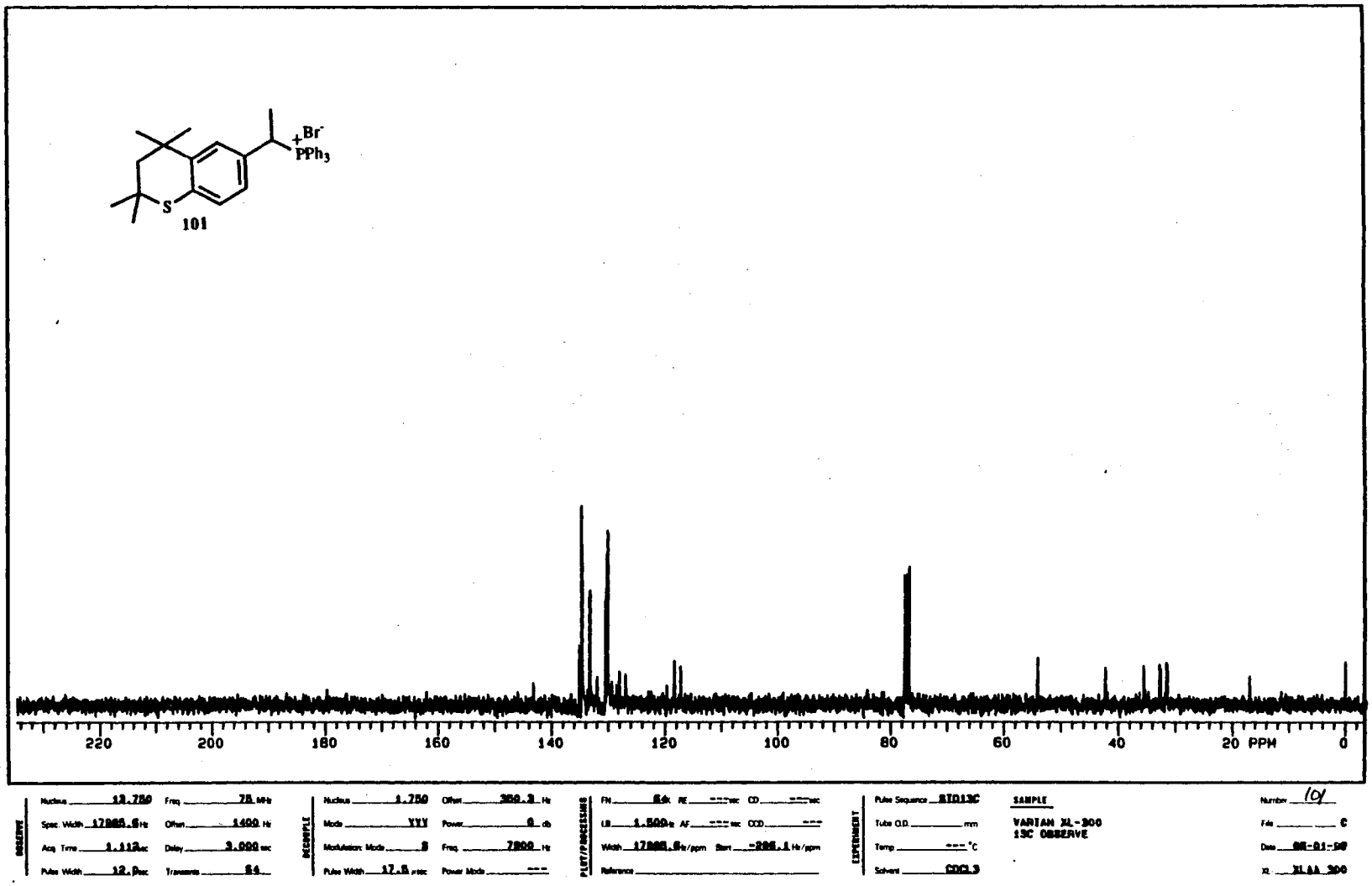
IR Spectrum of **101**

Plate LXVIII



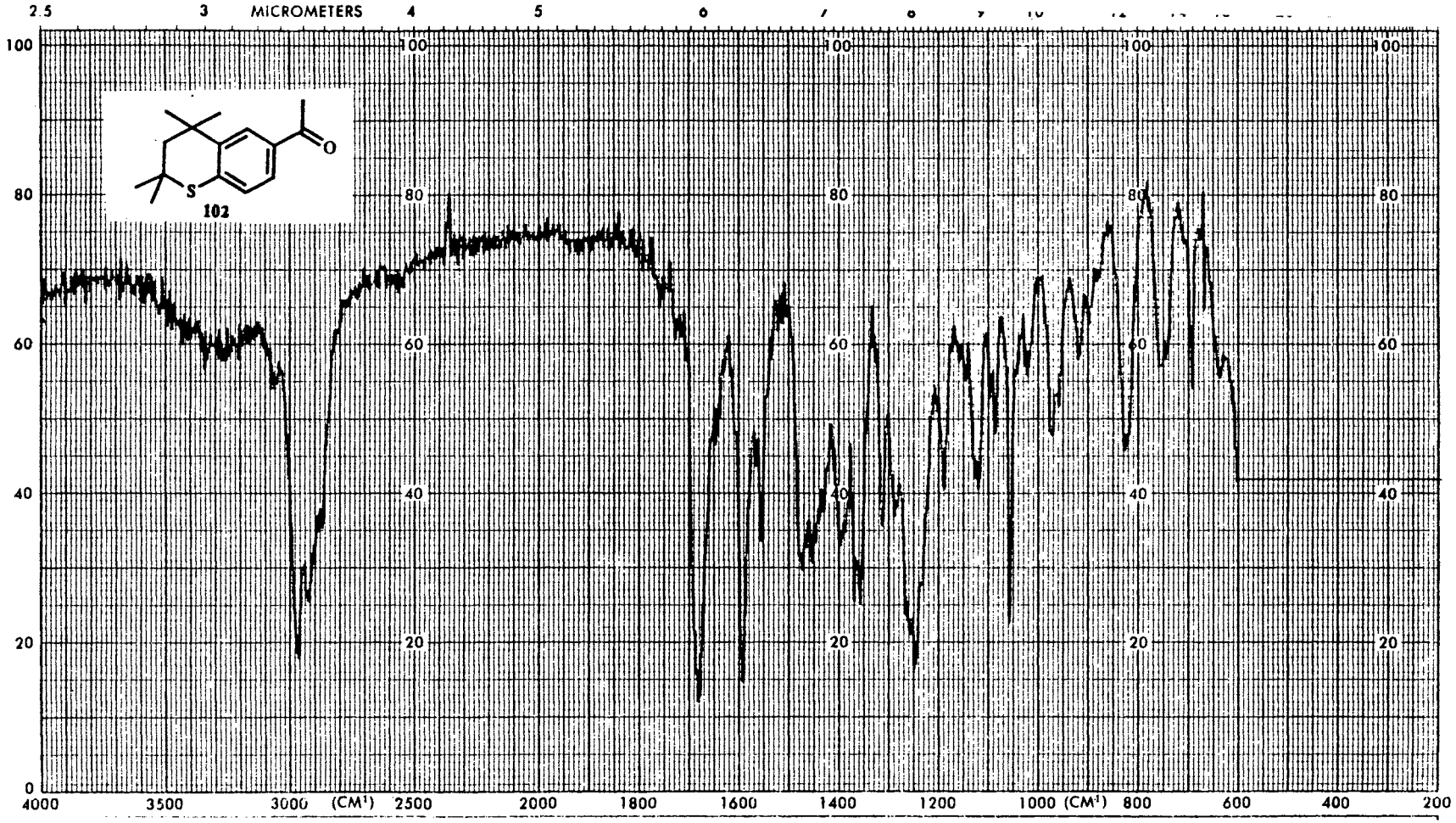
^{1}H NMR Spectrum of **101**

Plate LXIX



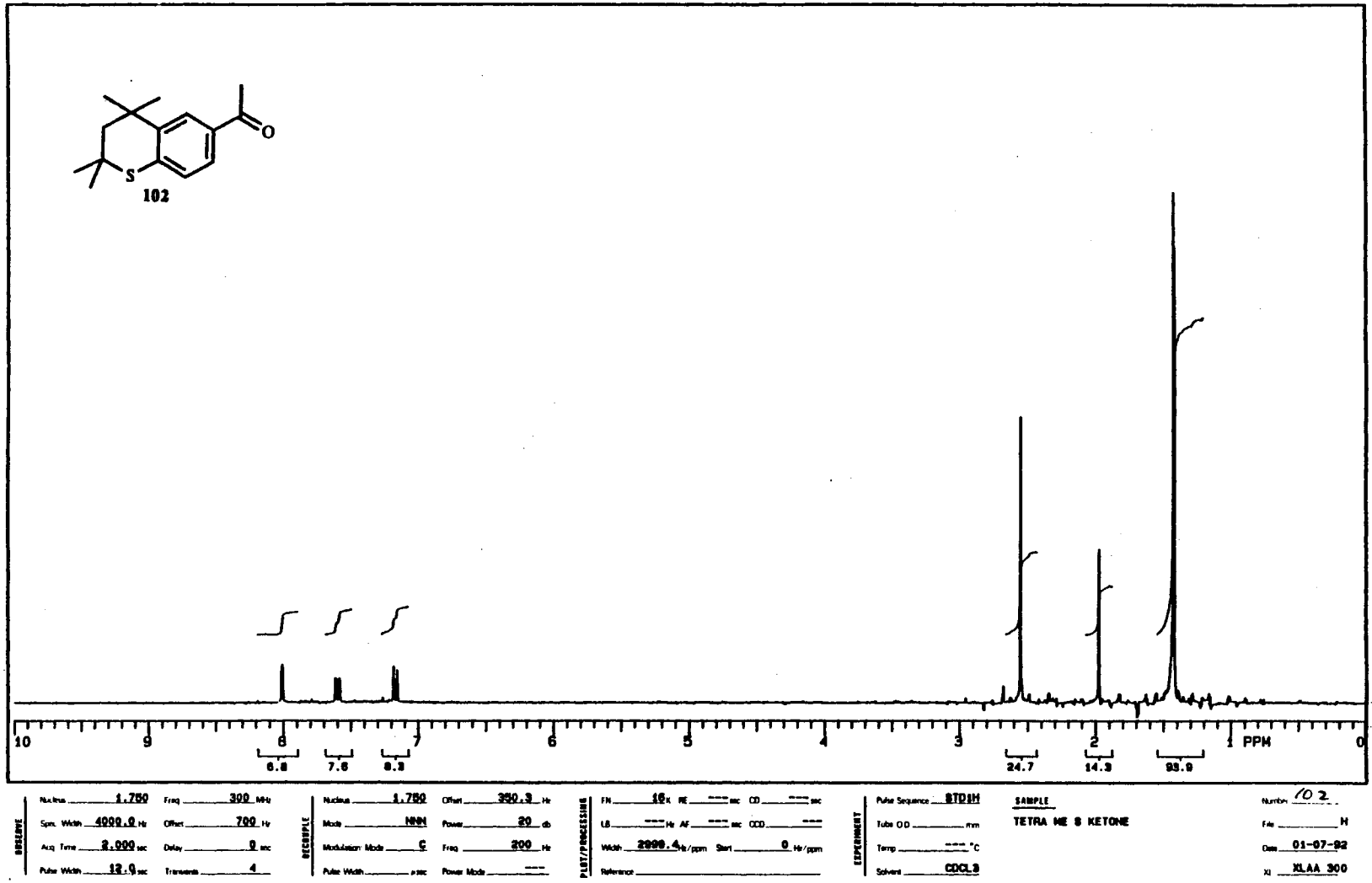
^{13}C NMR Spectrum of **101**

Plate LXX



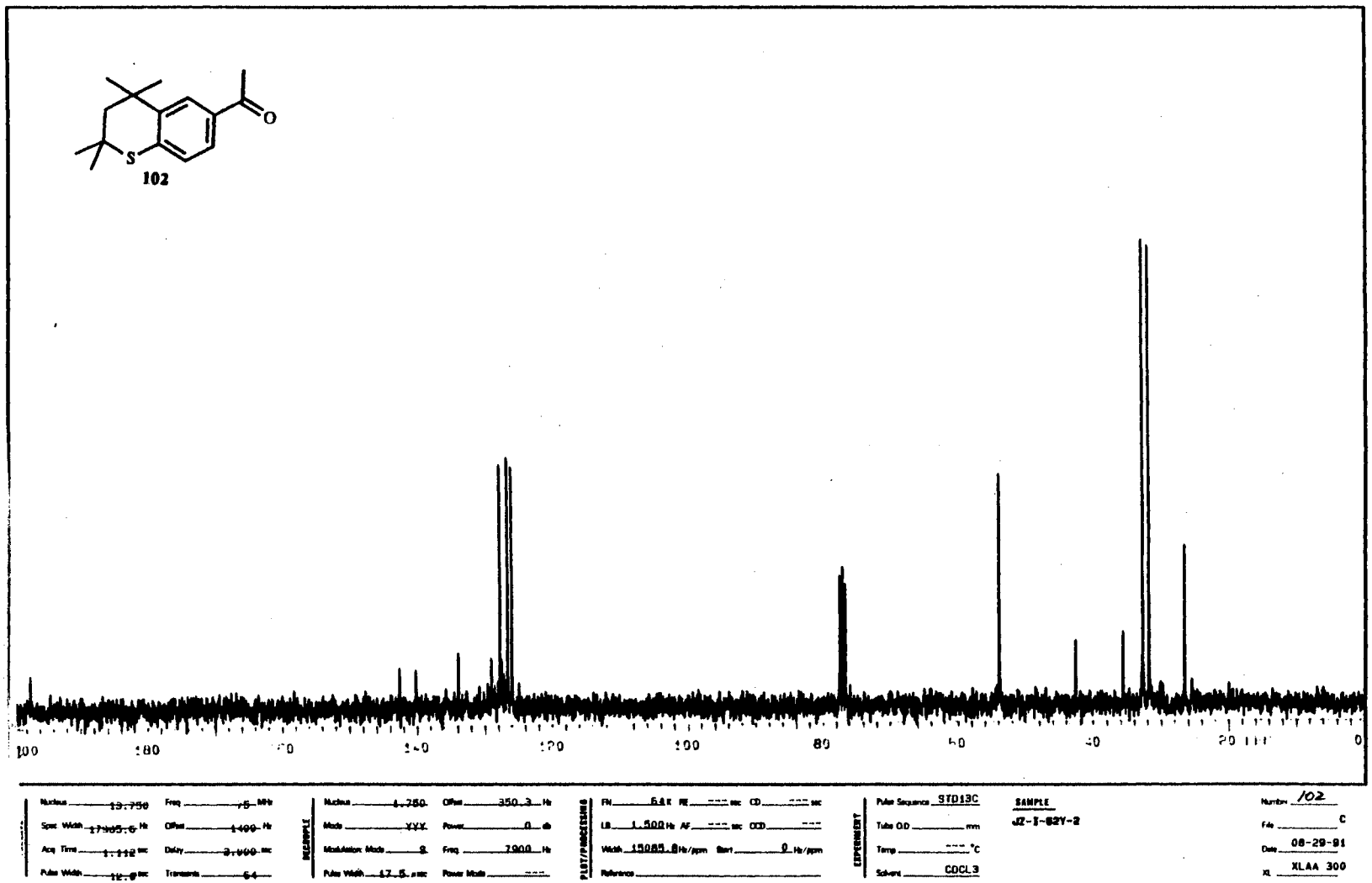
IR Spectrum of **102**

Plate LXXI



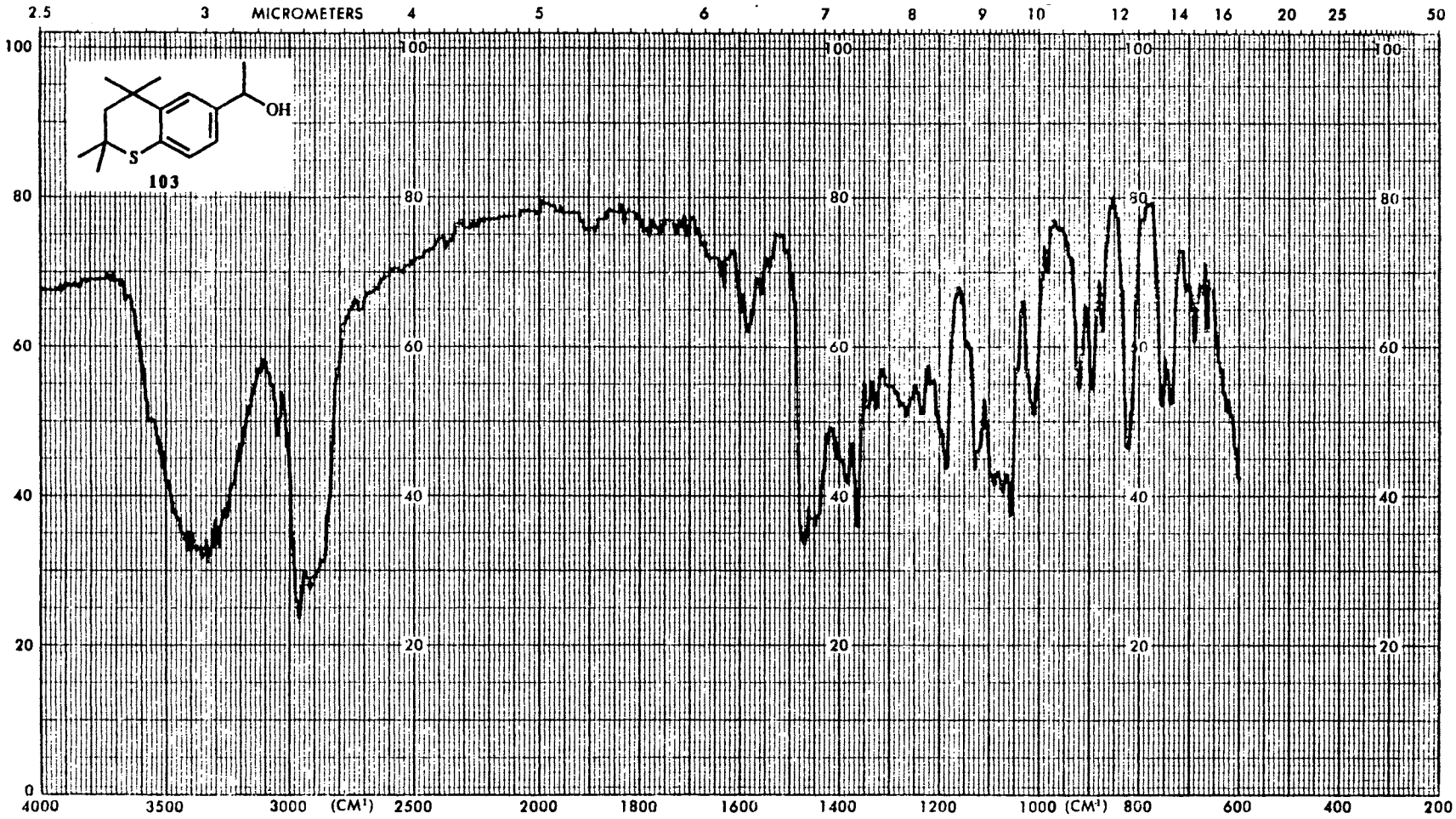
^{1}H NMR Spectrum of **102**

Plate LXXII



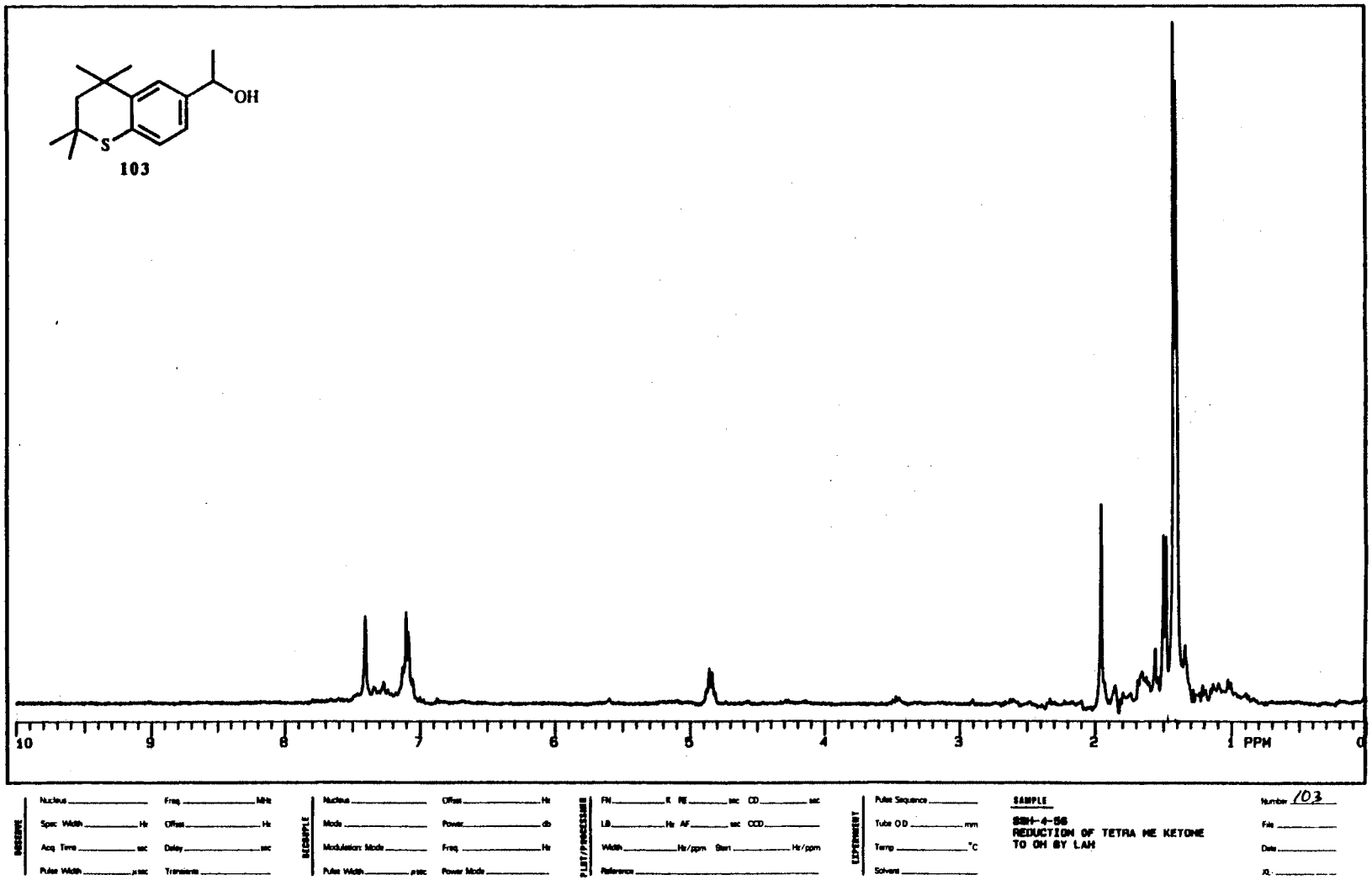
^{13}C NMR Spectrum of **102**

Plate LXXIII



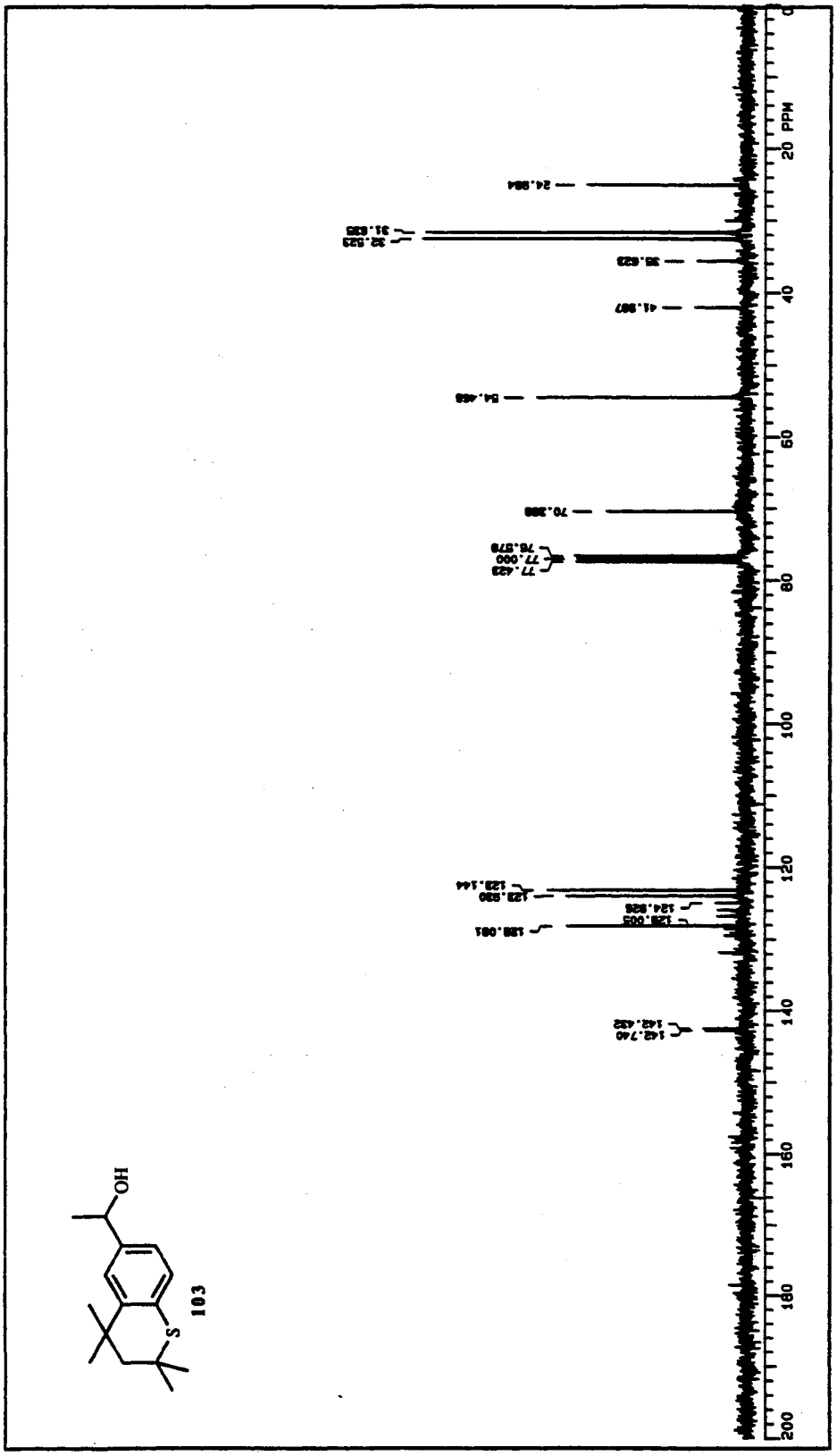
IR Spectrum of **103**

Plate LXXIV



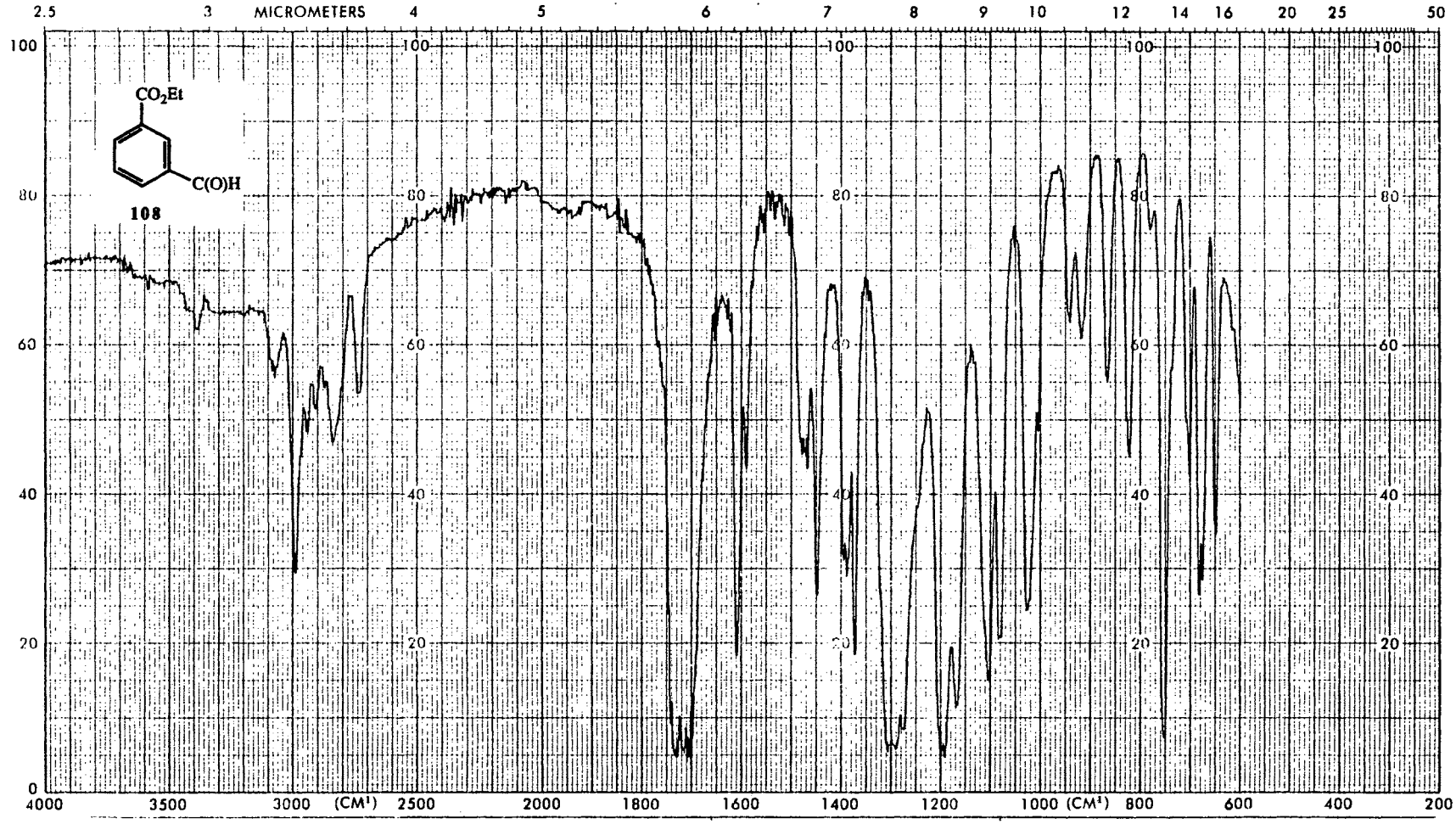
^{1}H NMR Spectrum of **103**

Plate LXXV



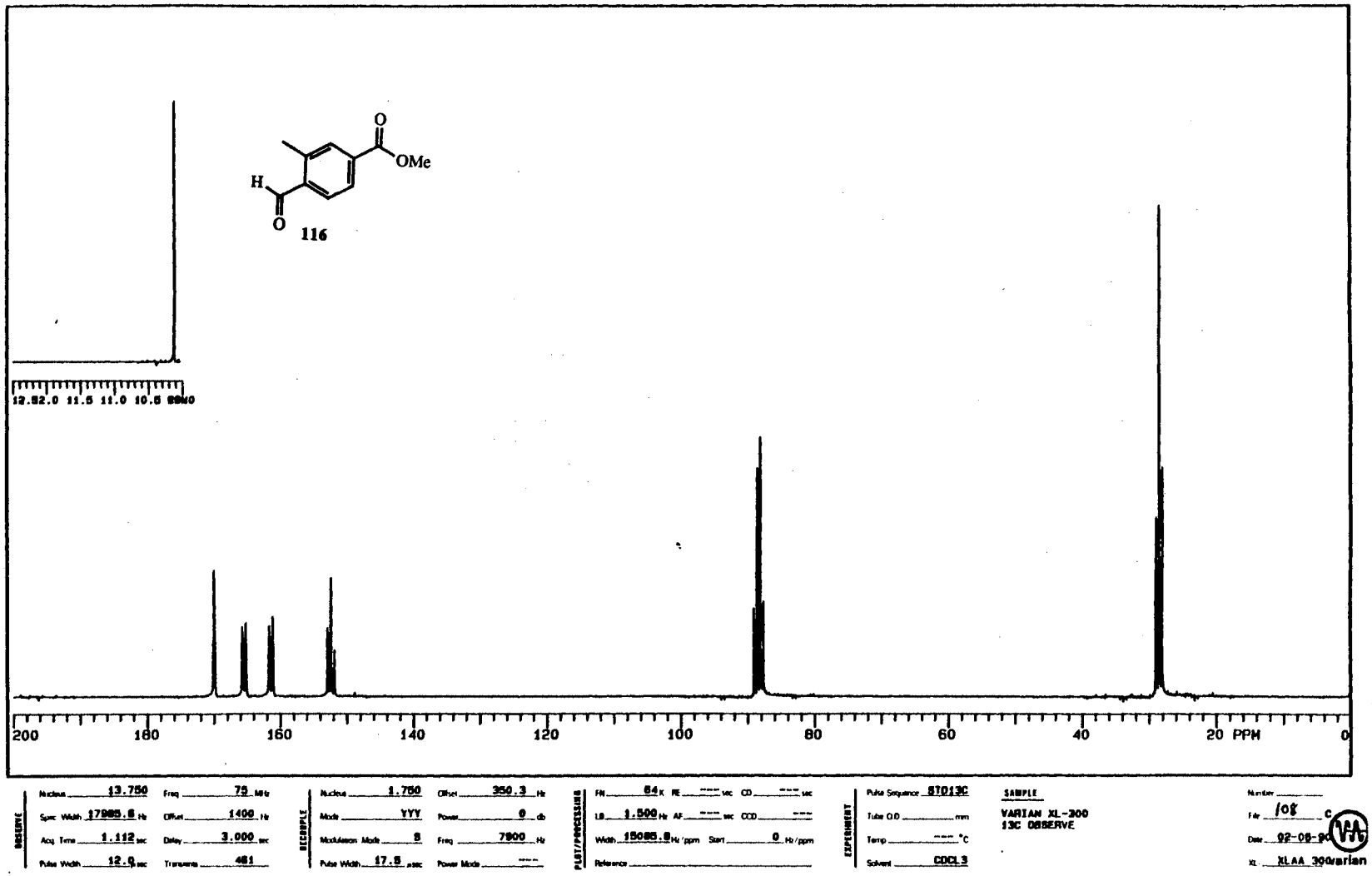
^{13}C NMR Spectrum of **103**

Plate LXXVI



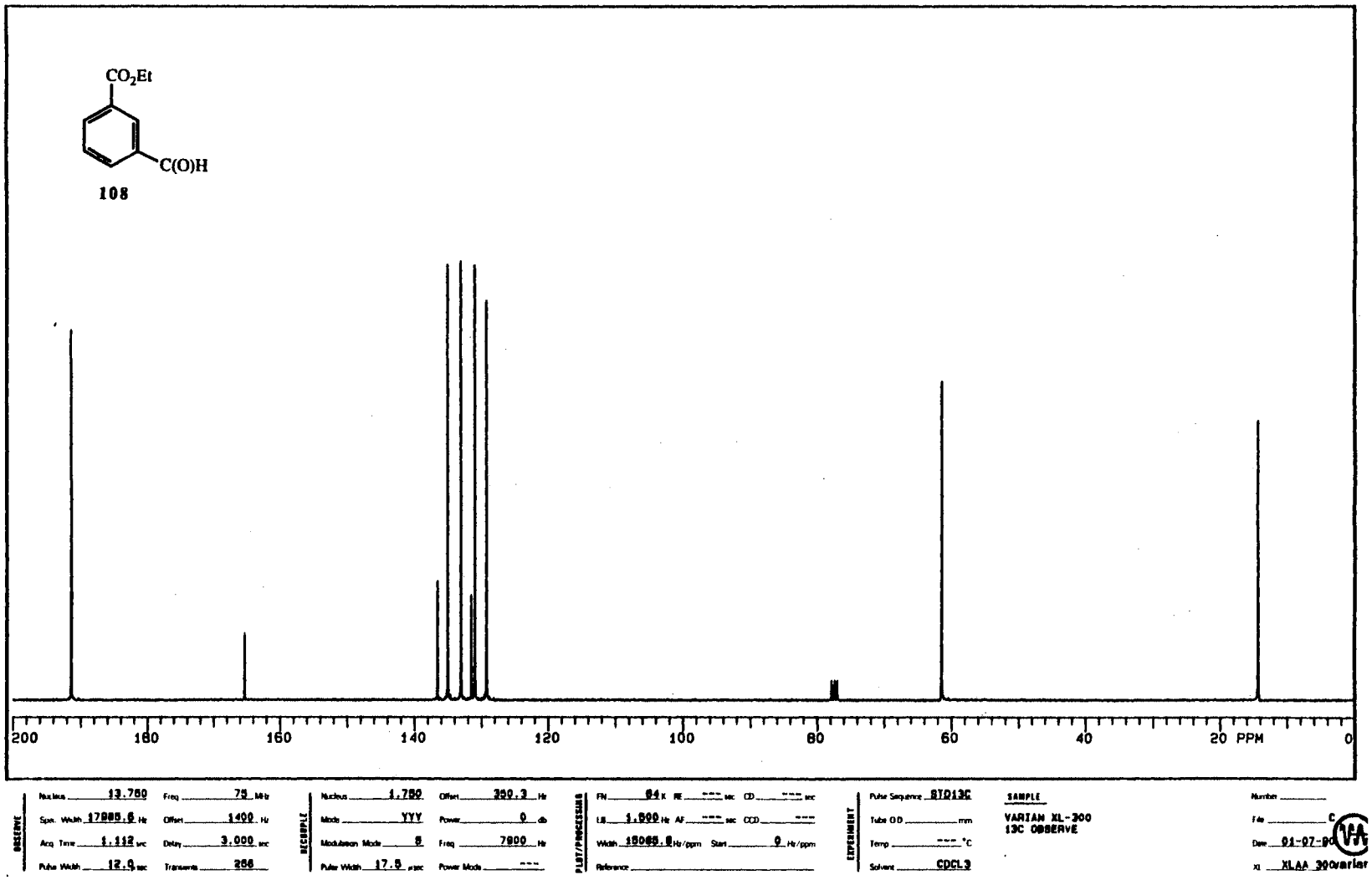
IR Spectrum of **108**

Plate LXXVII



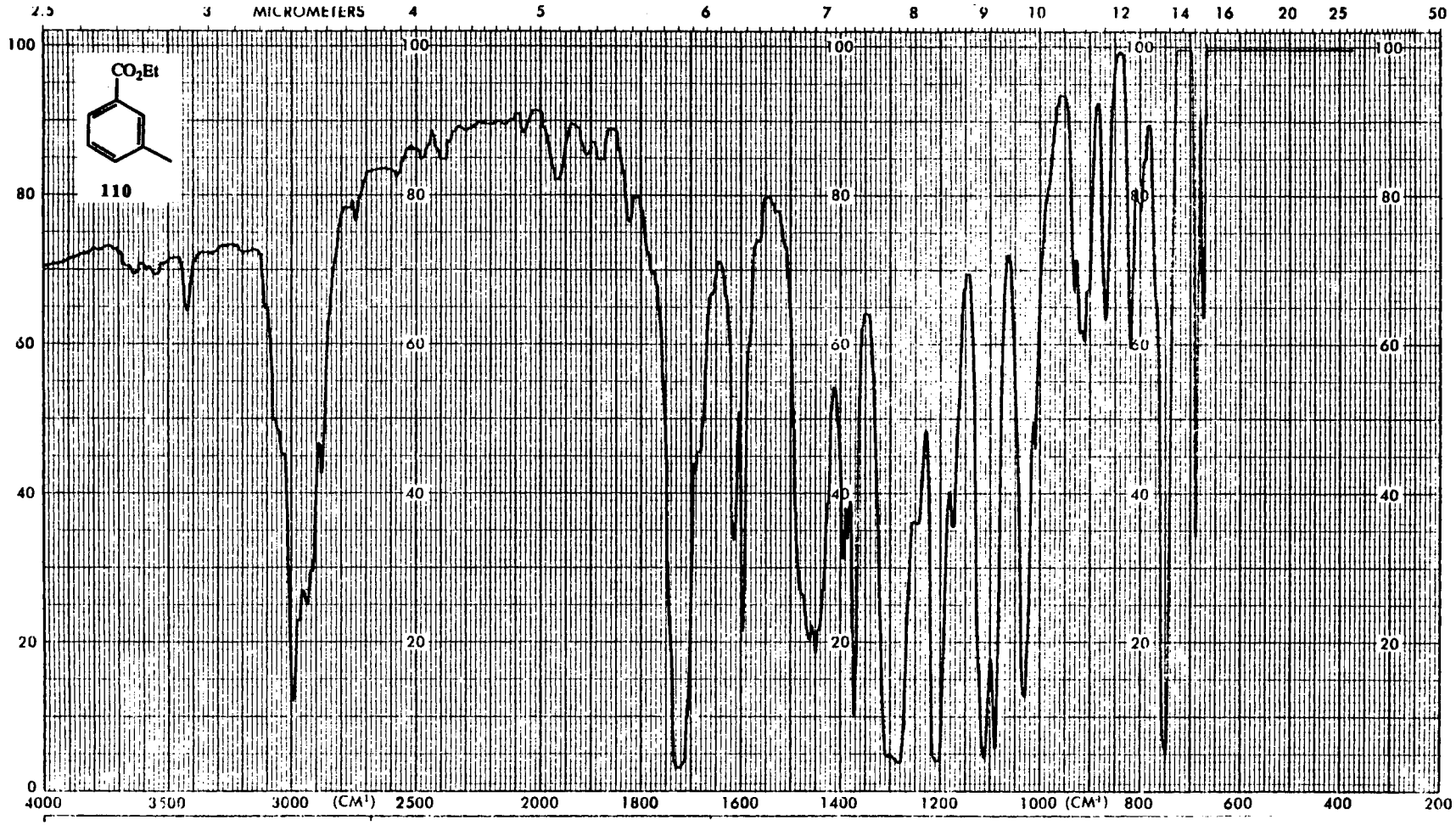
^{1}H NMR Spectrum of **108**

Plate LXXVIII



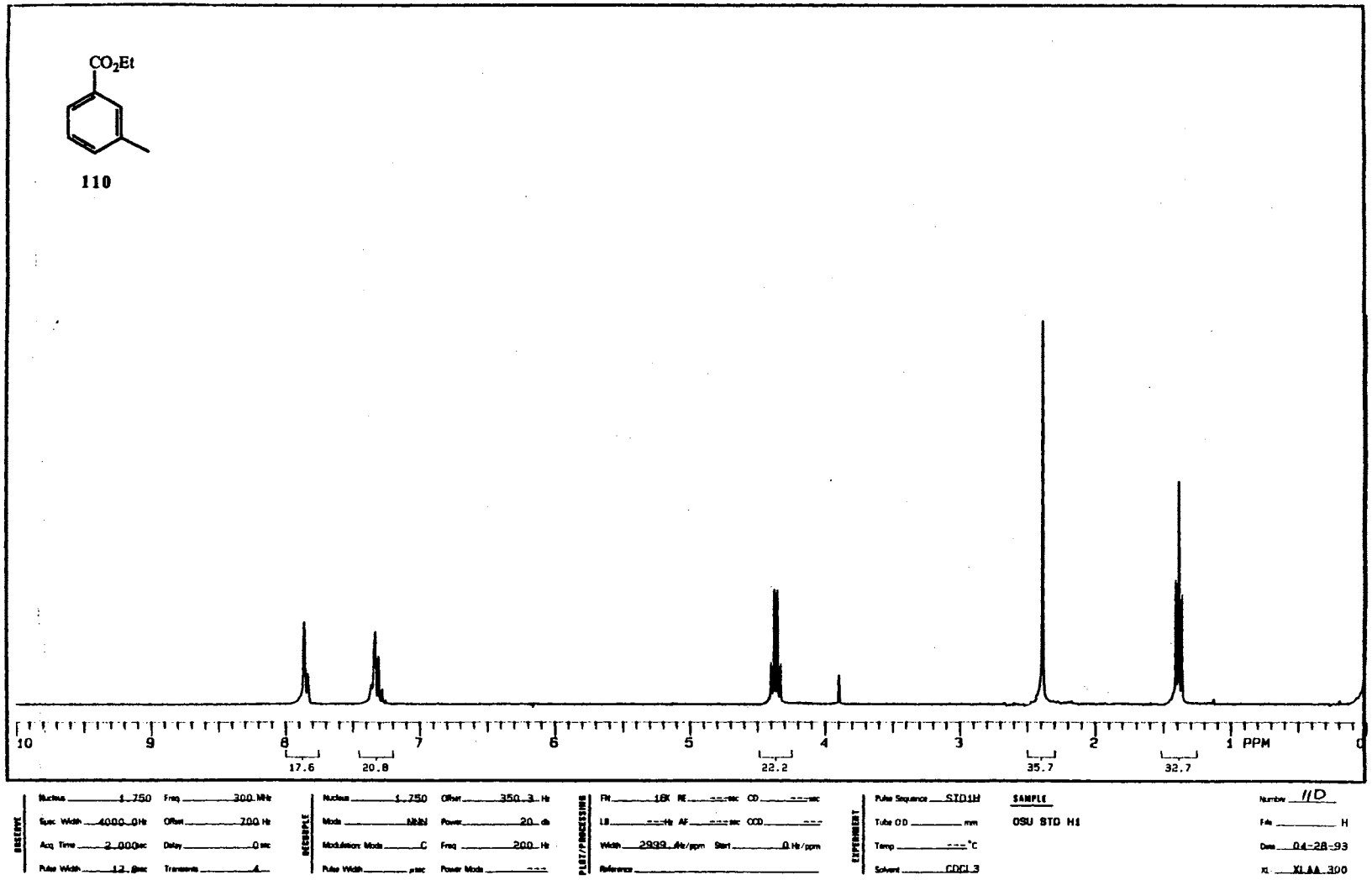
^{13}C NMR Spectrum of **108**

Plate LXXIX



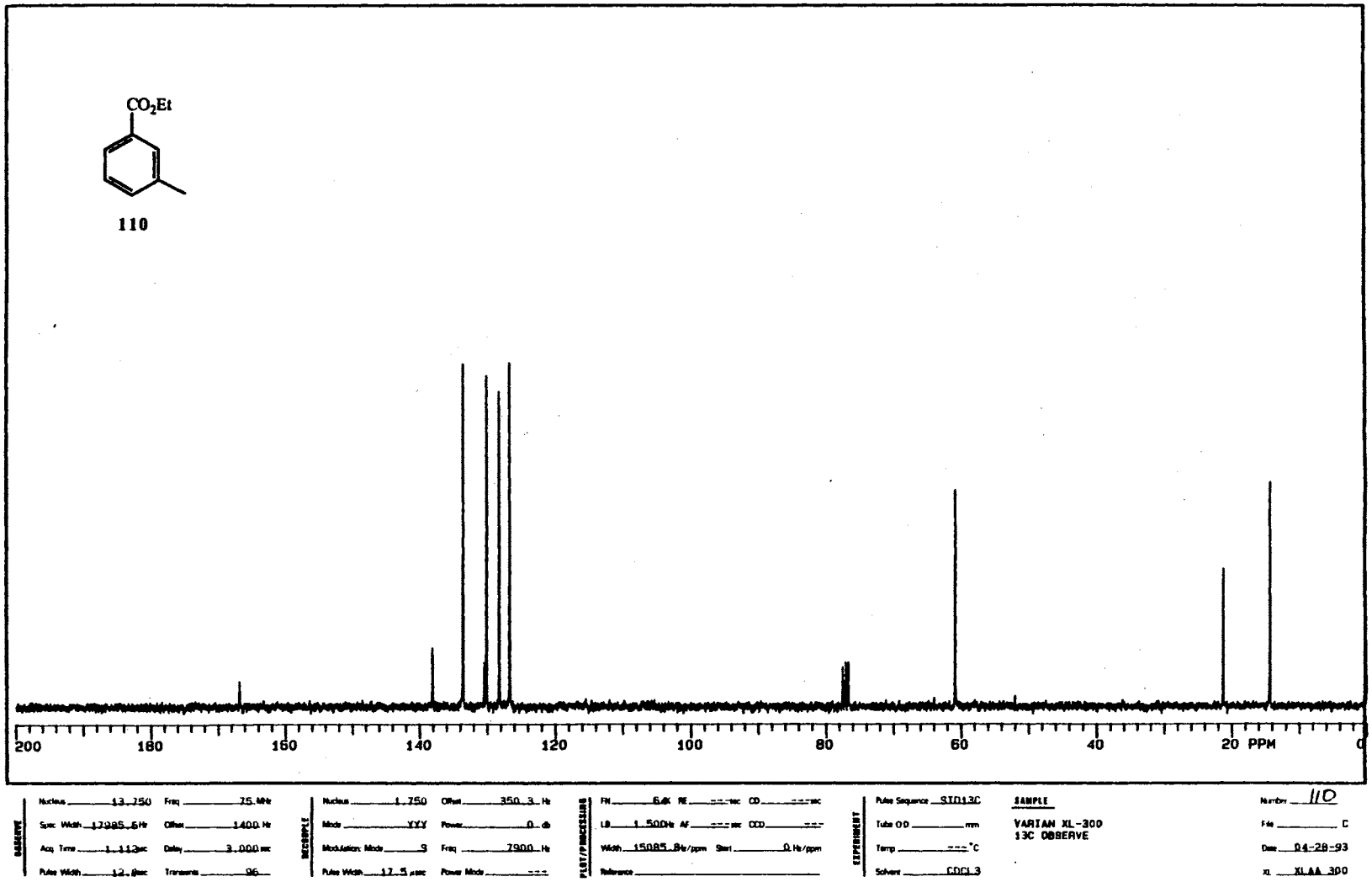
IR Spectrum of **110**

Plate LXXX



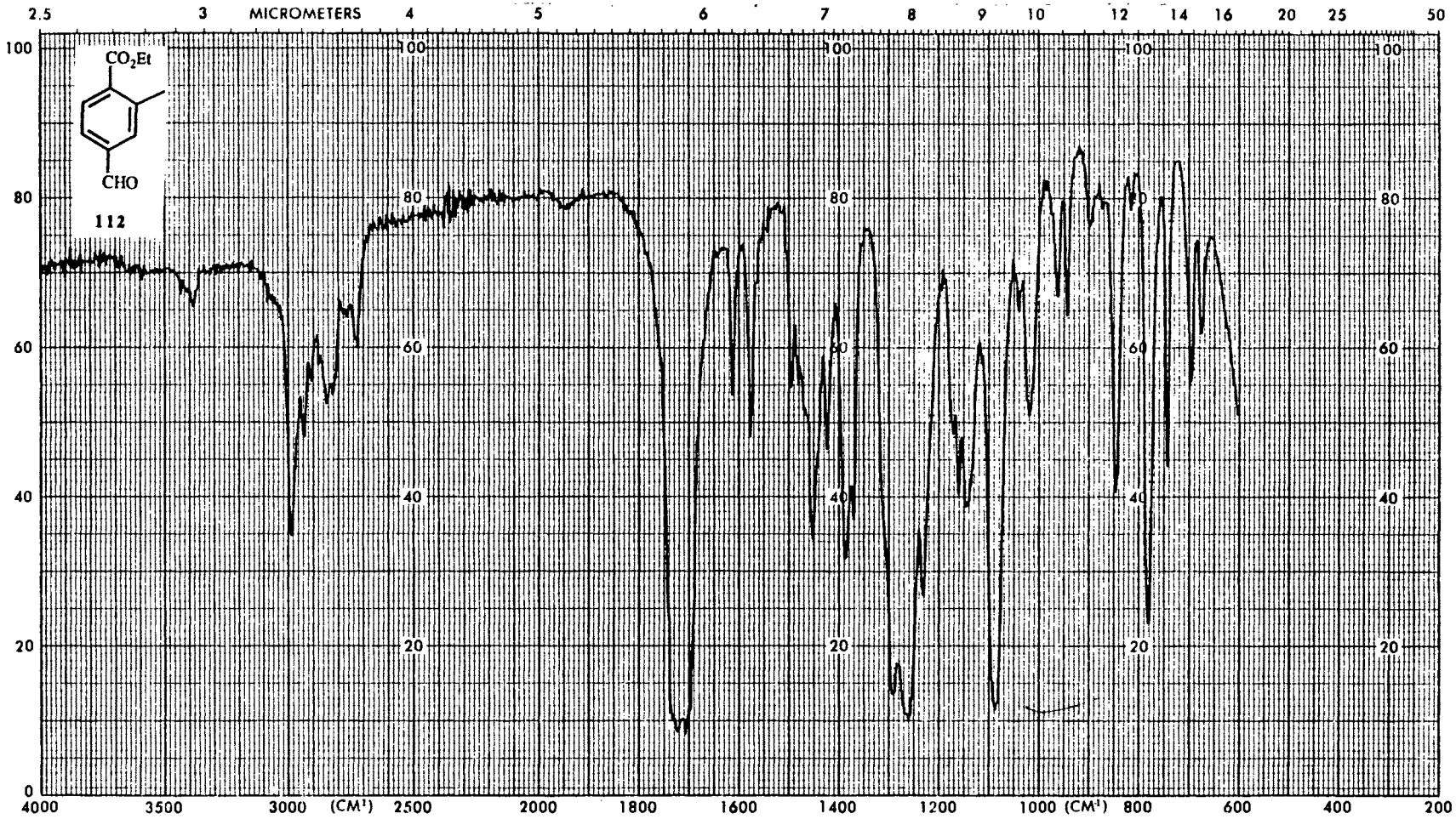
^{1}H NMR Spectrum of **110**

Plate LXXXI



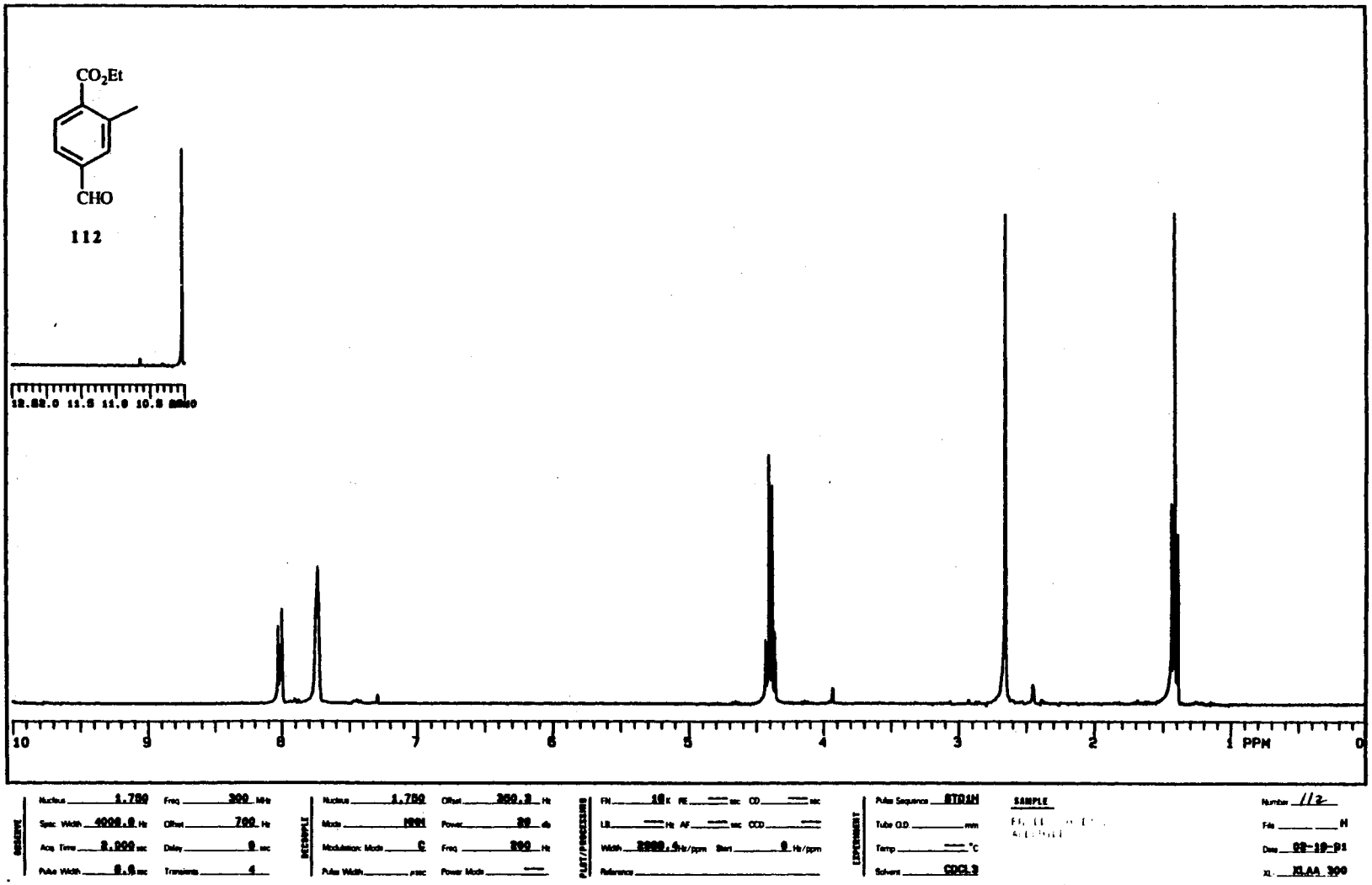
^{13}C NMR Spectrum of **110**

Plate LXXXII



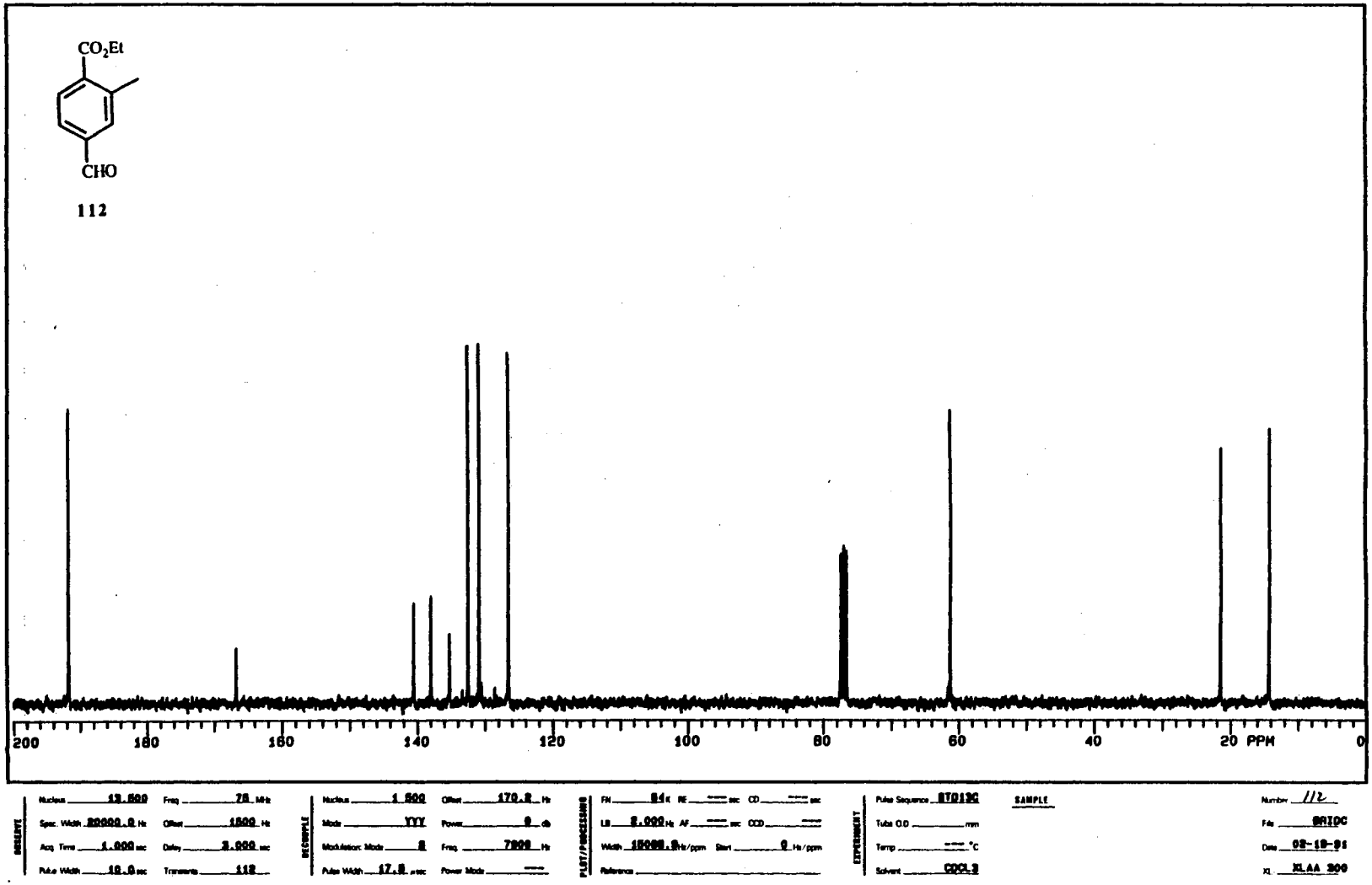
IR Spectrum of **112**

Plate LXXXIII



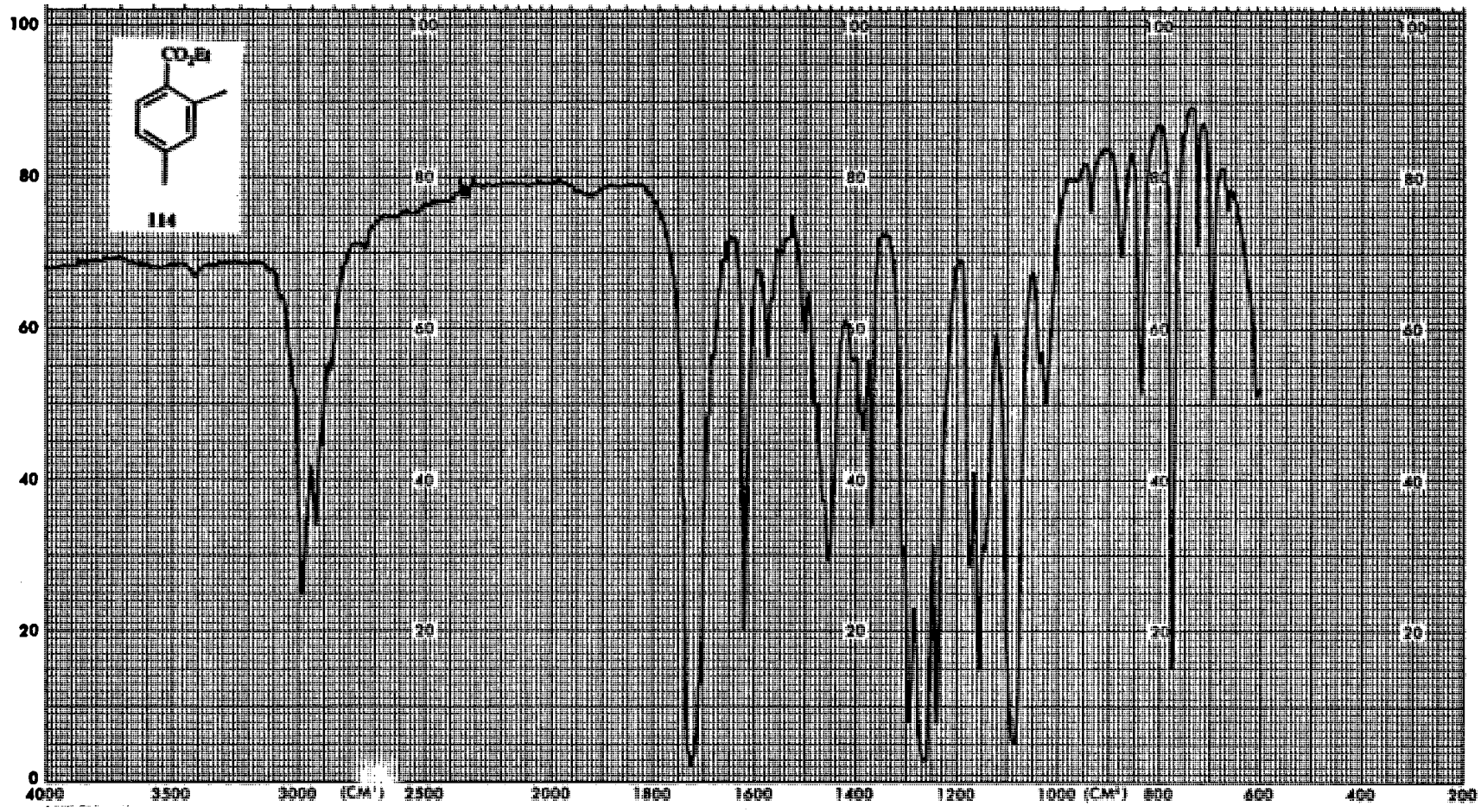
^{1}H NMR Spectrum of **112**

Plate LXXXIV



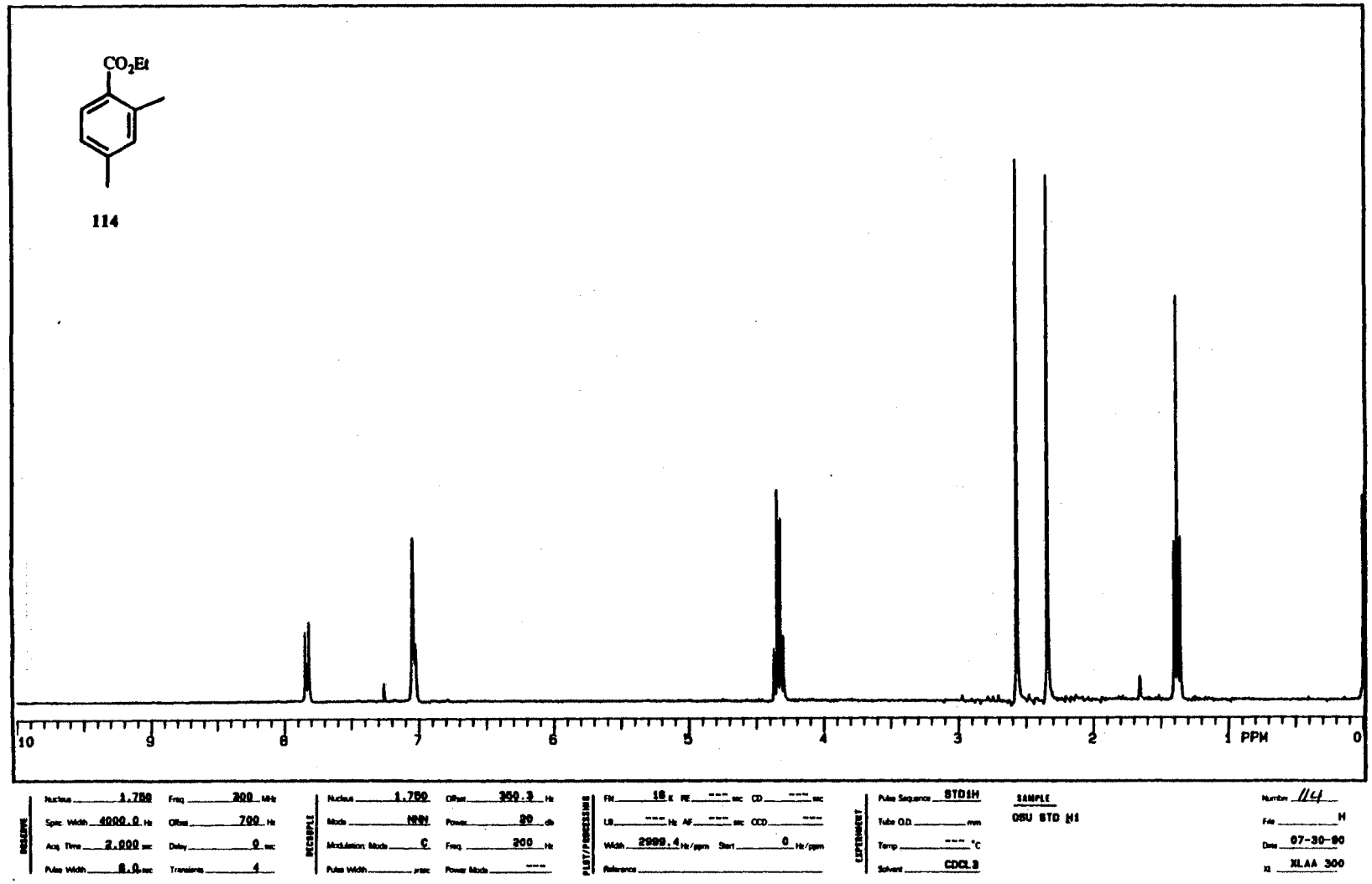
^{13}C NMR Spectrum of **112**

Plate LXXXV



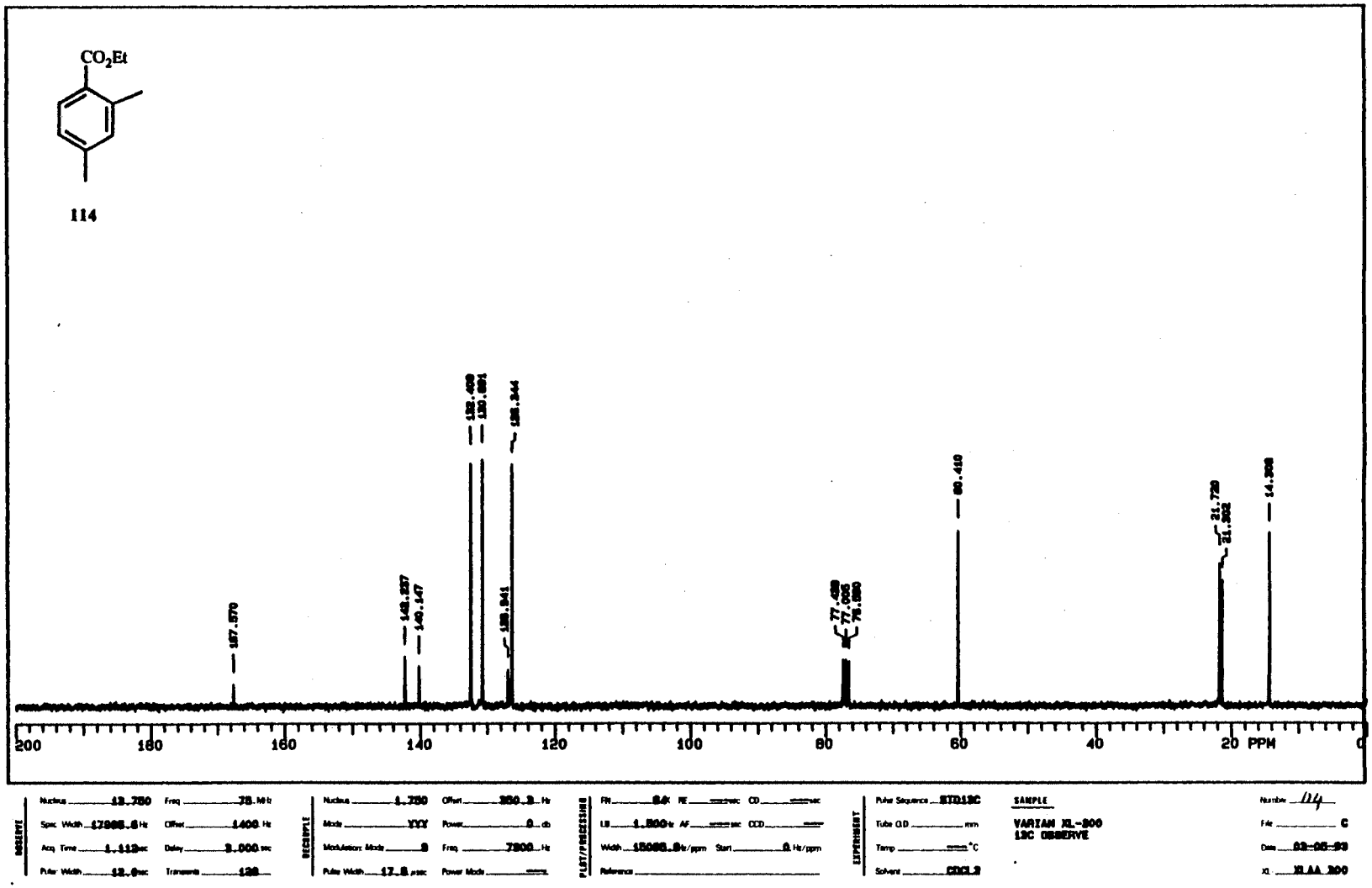
IR Spectrum of **114**

Plate LXXXVI



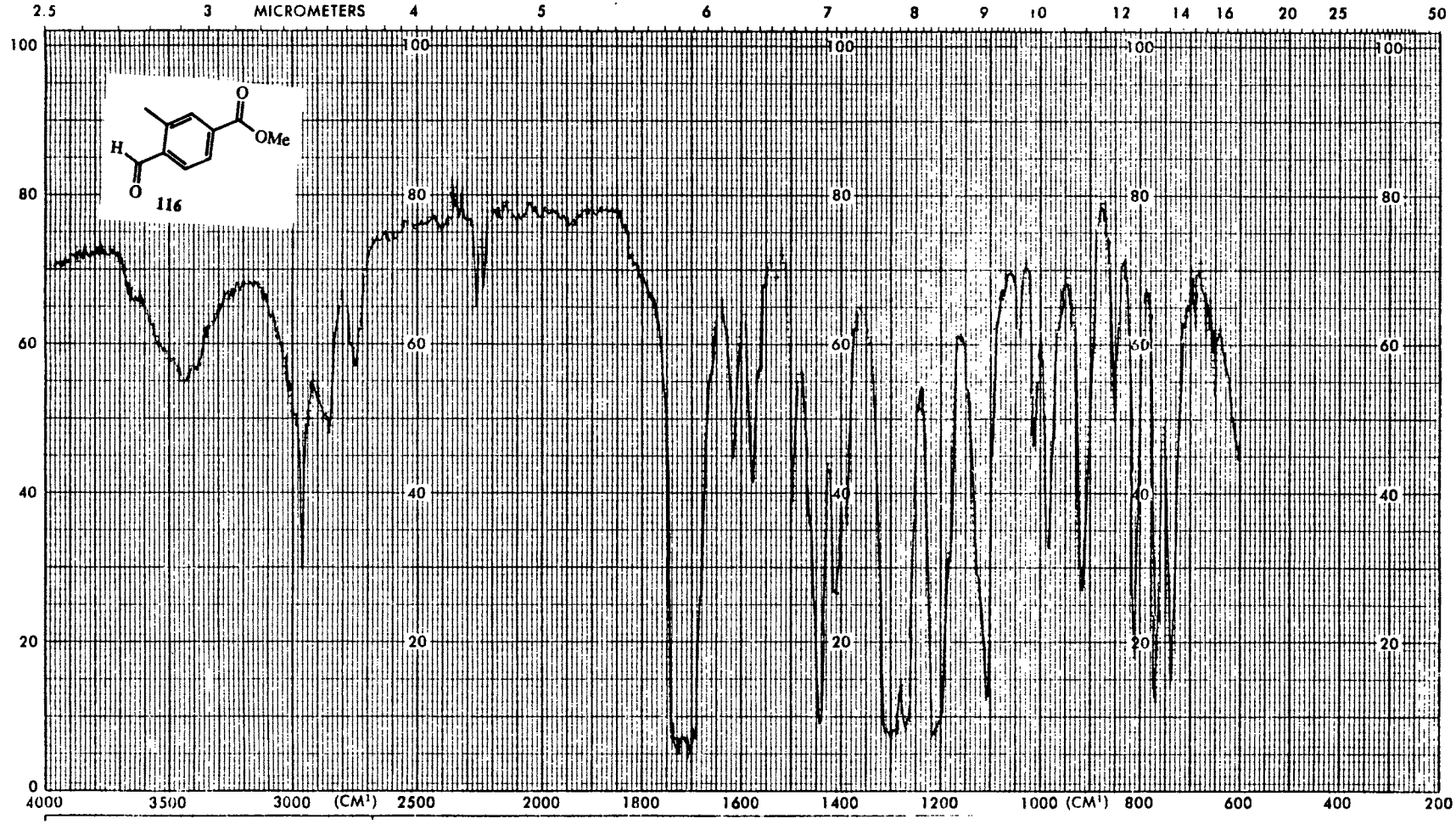
^{1}H NMR Spectrum of **114**

Plate LXXXVII



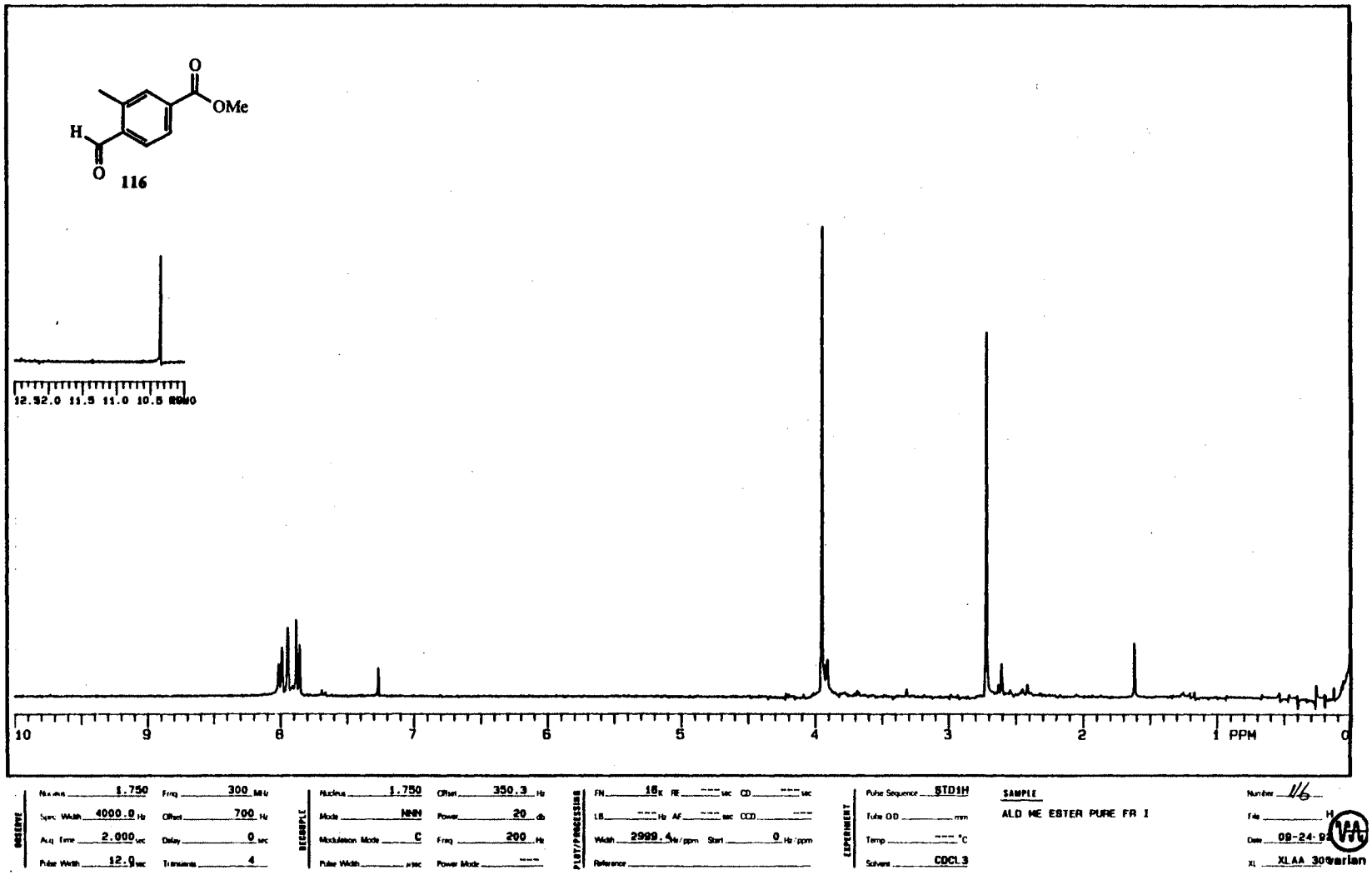
^{13}C NMR Spectrum of **114**

Plate LXXXVIII



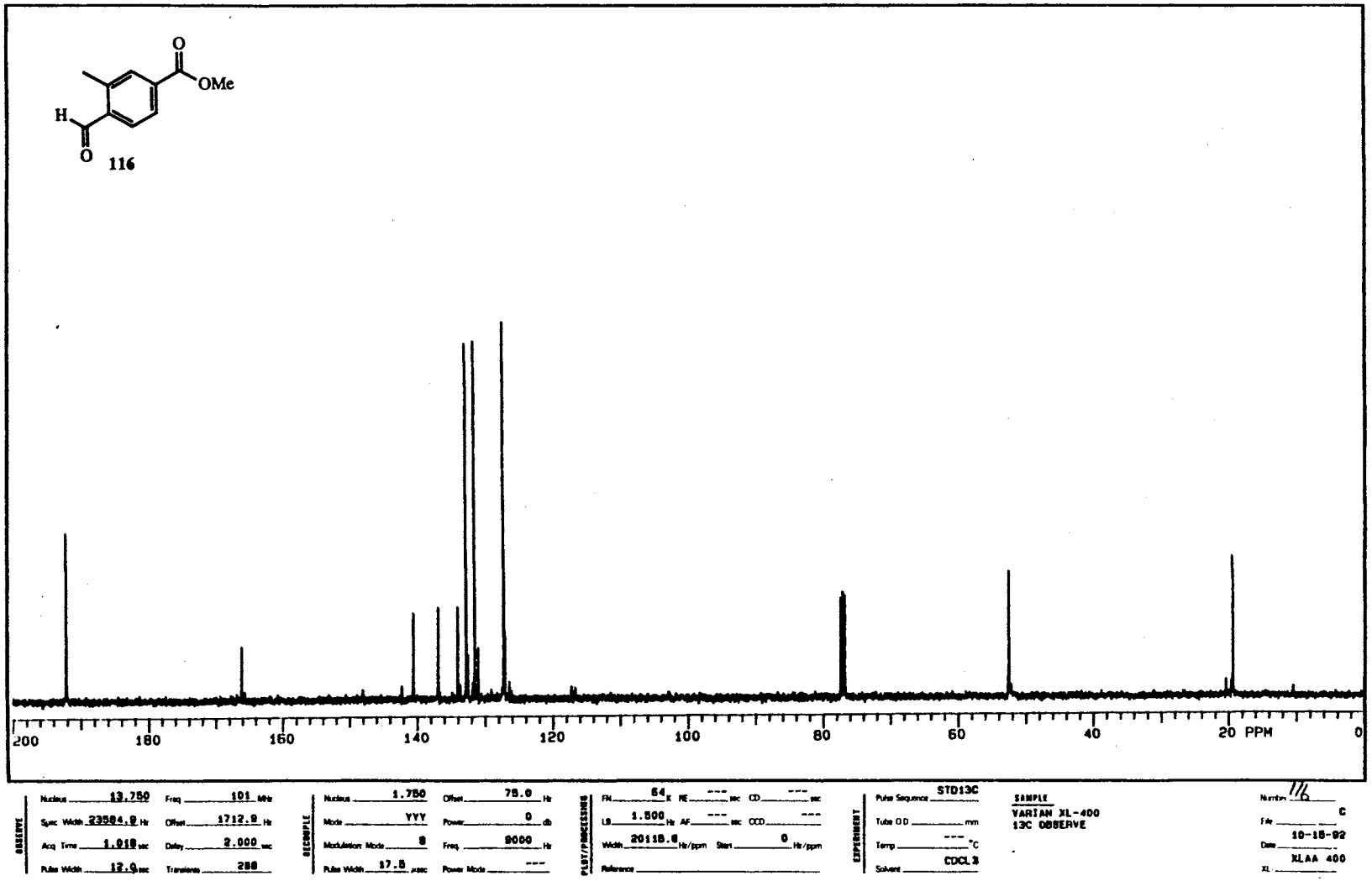
IR Spectrum of **116**

Plate LXXXIX



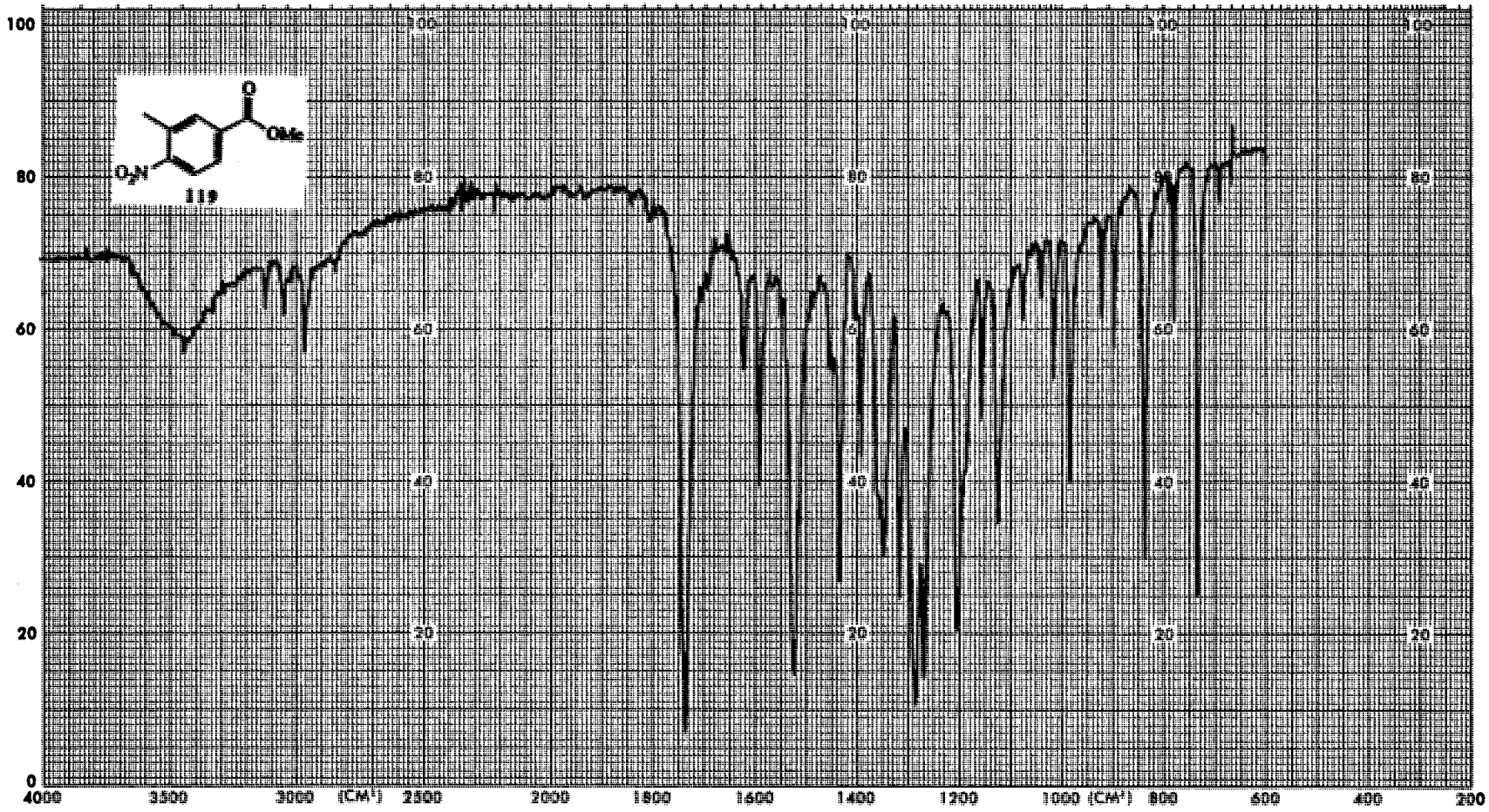
^{1}H NMR Spectrum of **116**

Plate XC



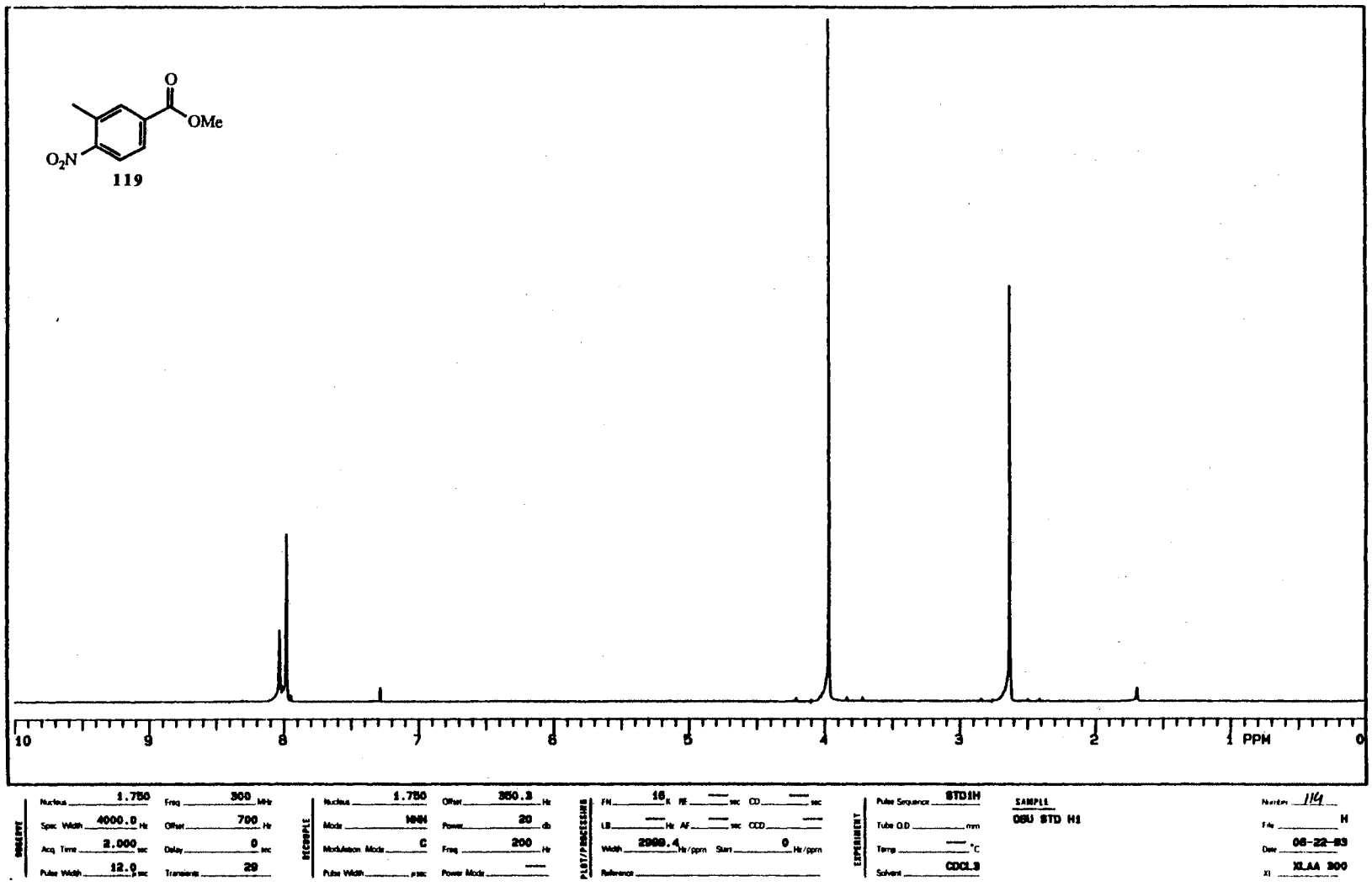
^{13}C NMR Spectrum of **116**

Plate XCI



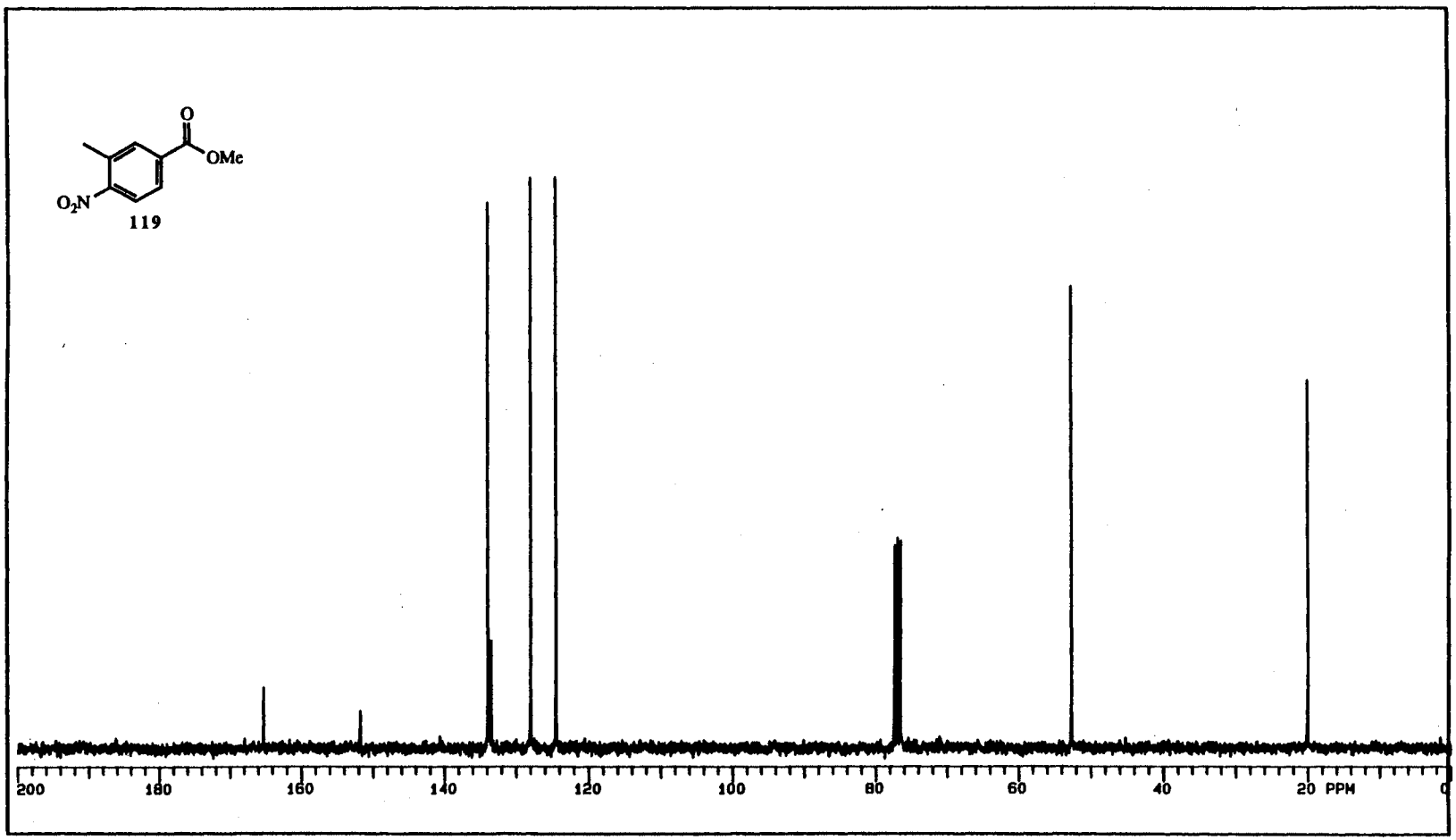
IR Spectrum of **119**

Plate XCII



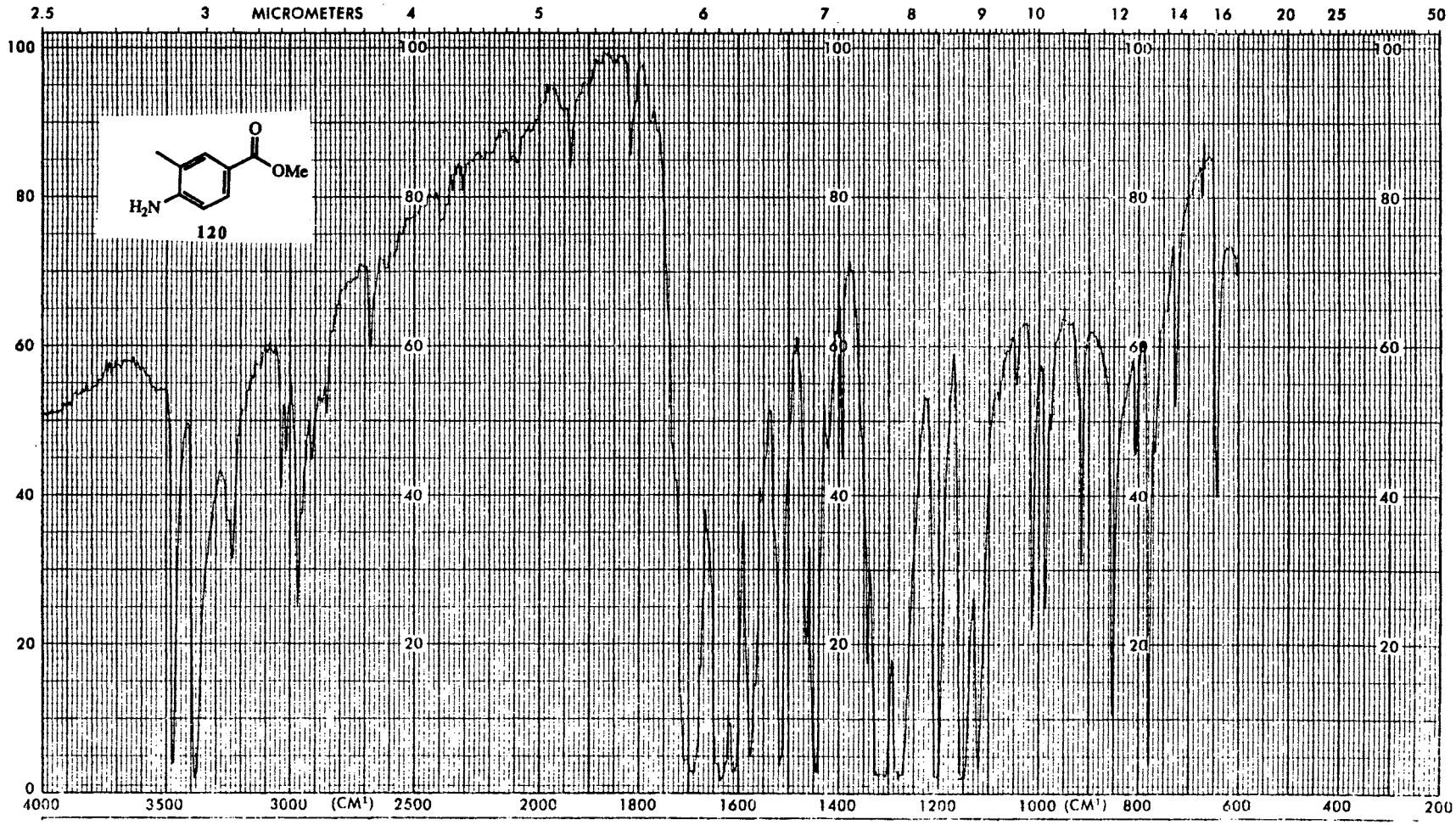
^{1}H NMR Spectrum of **119**

Plate XCIII



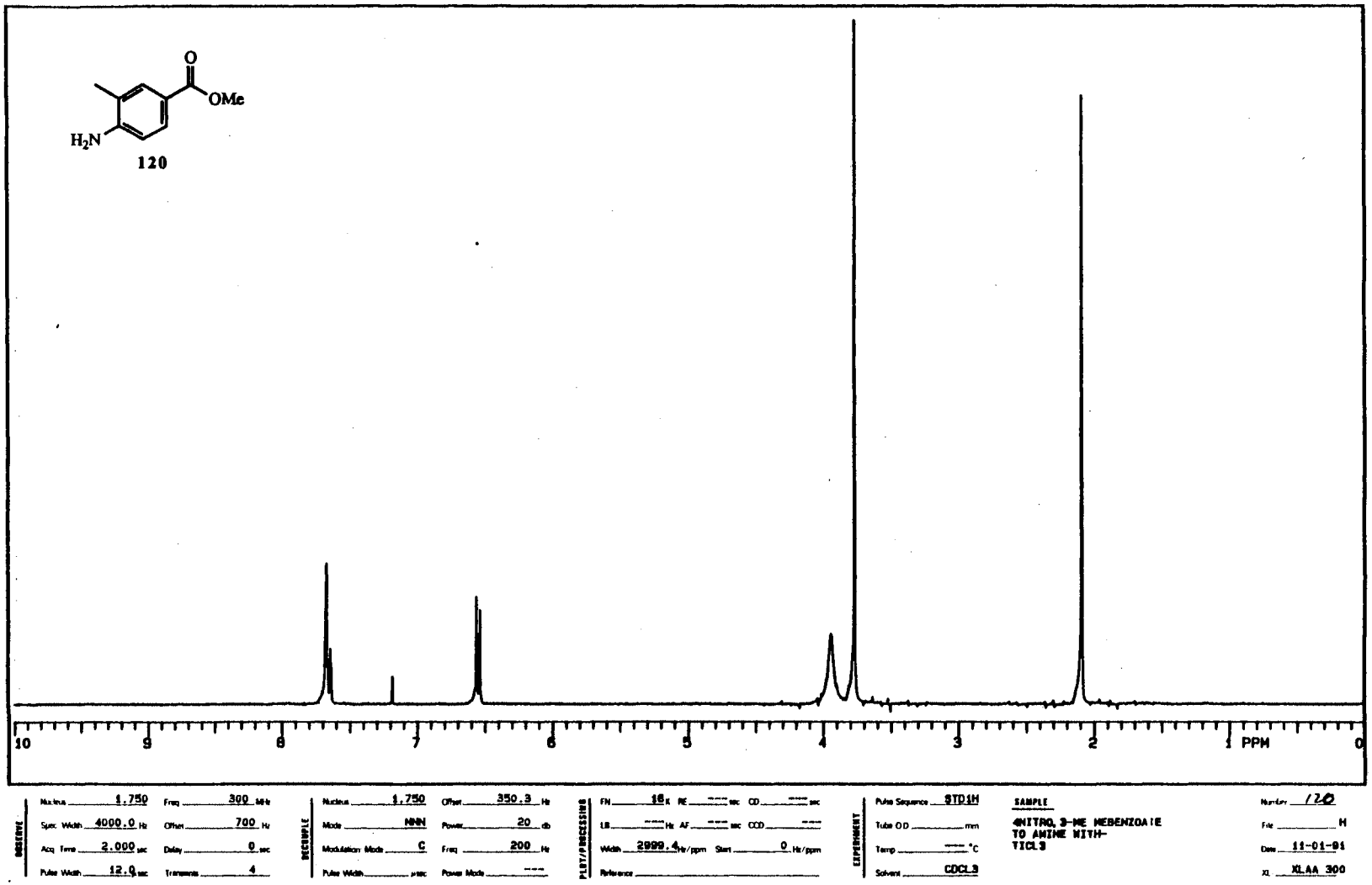
^{13}C NMR Spectrum of **119**

Plate XCIV



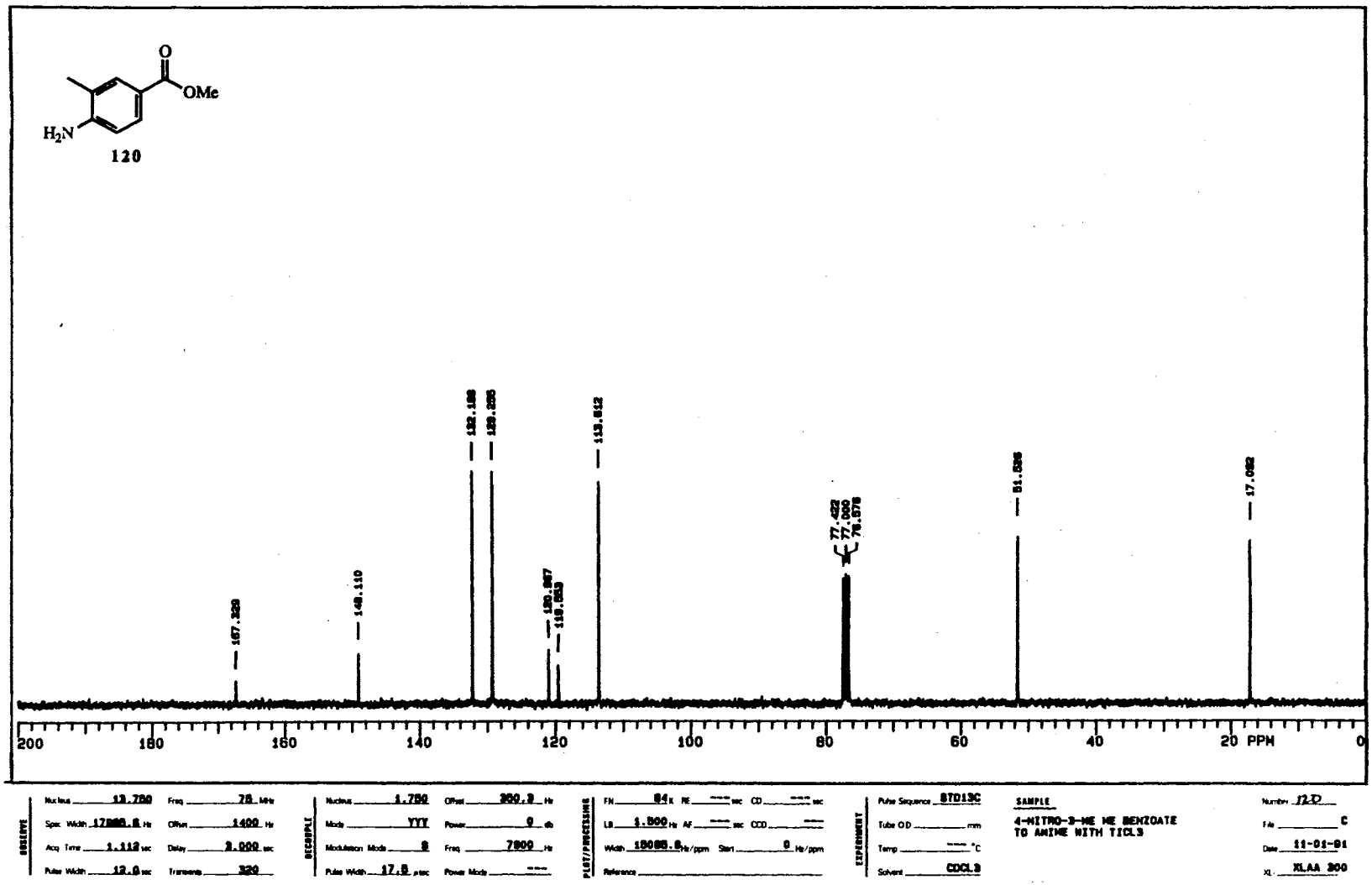
IR Spectrum of **120**

Plate XCV



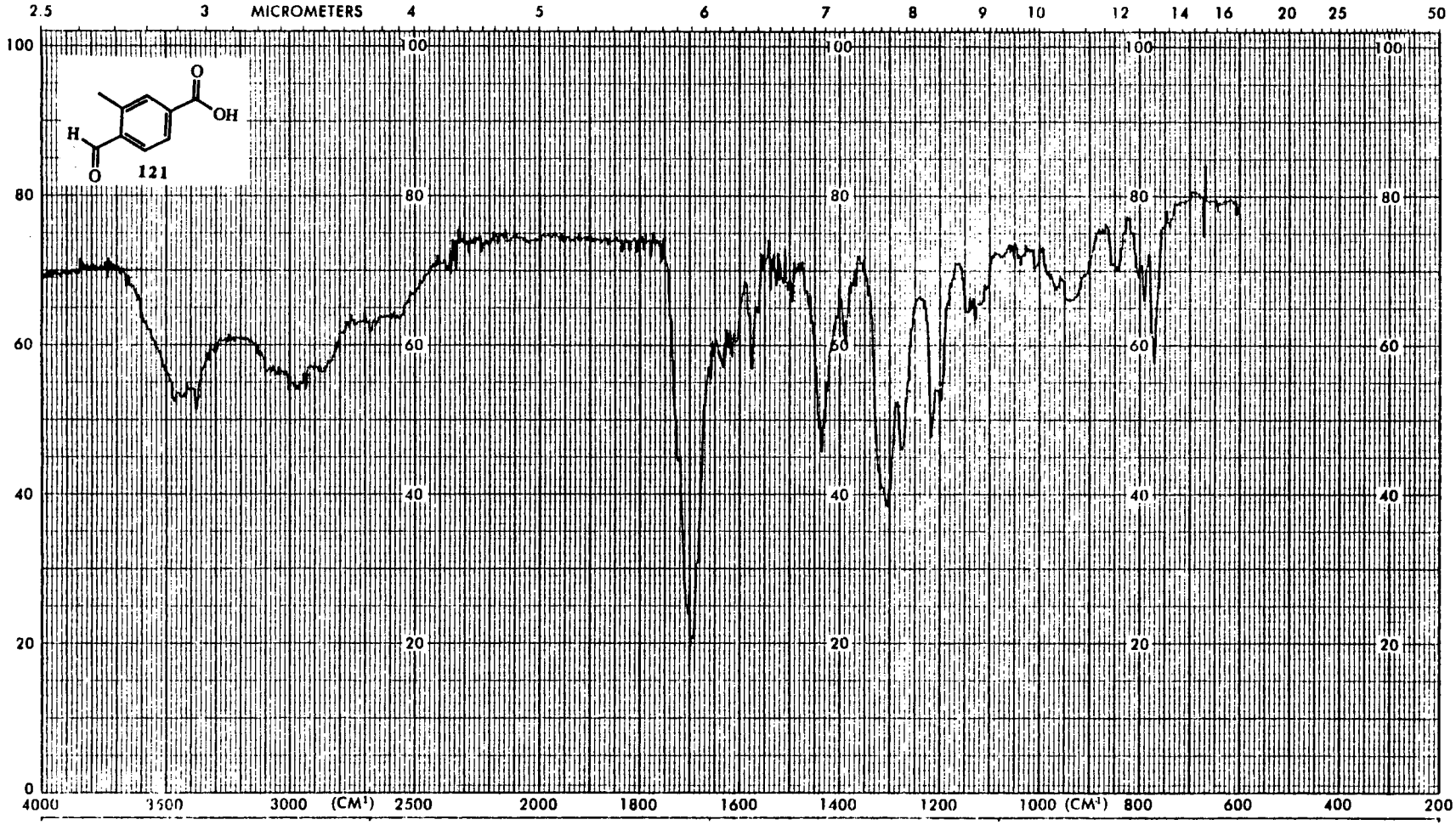
^{1}H NMR Spectrum of **120**

Plate XVI



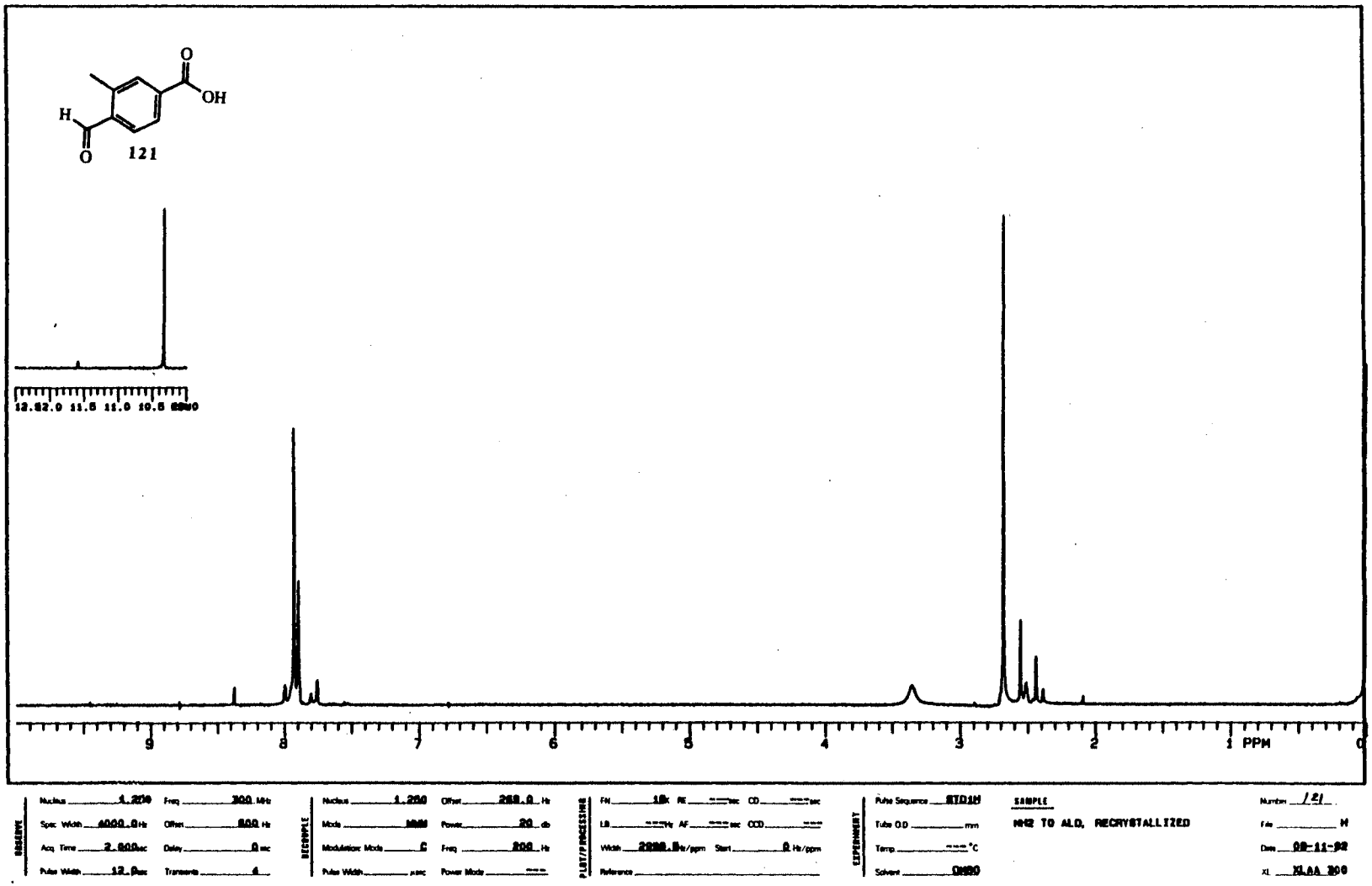
^{13}C NMR Spectrum of **120**

Plate XCVII



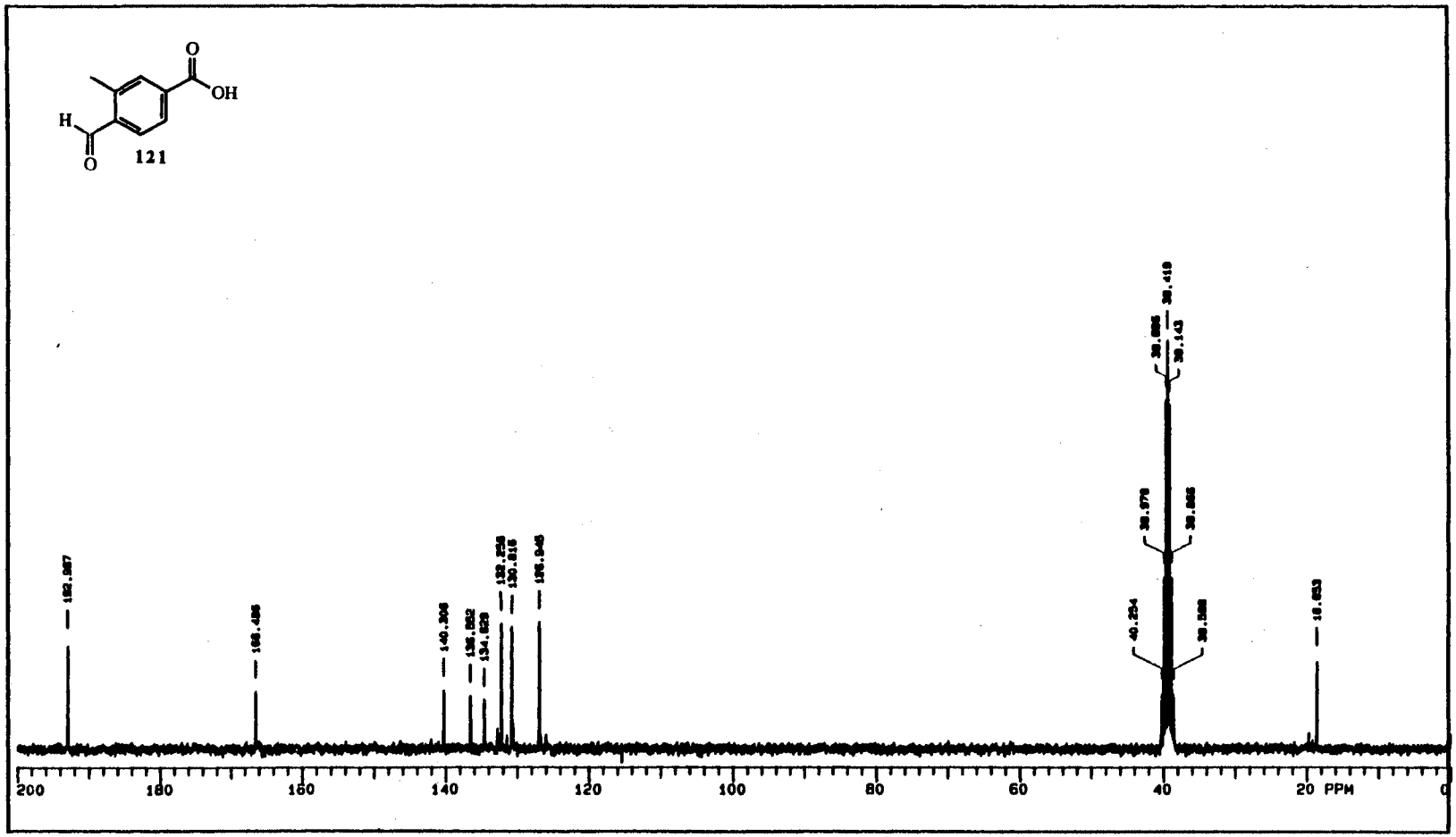
IR Spectrum of **121**

Plate XCVIII



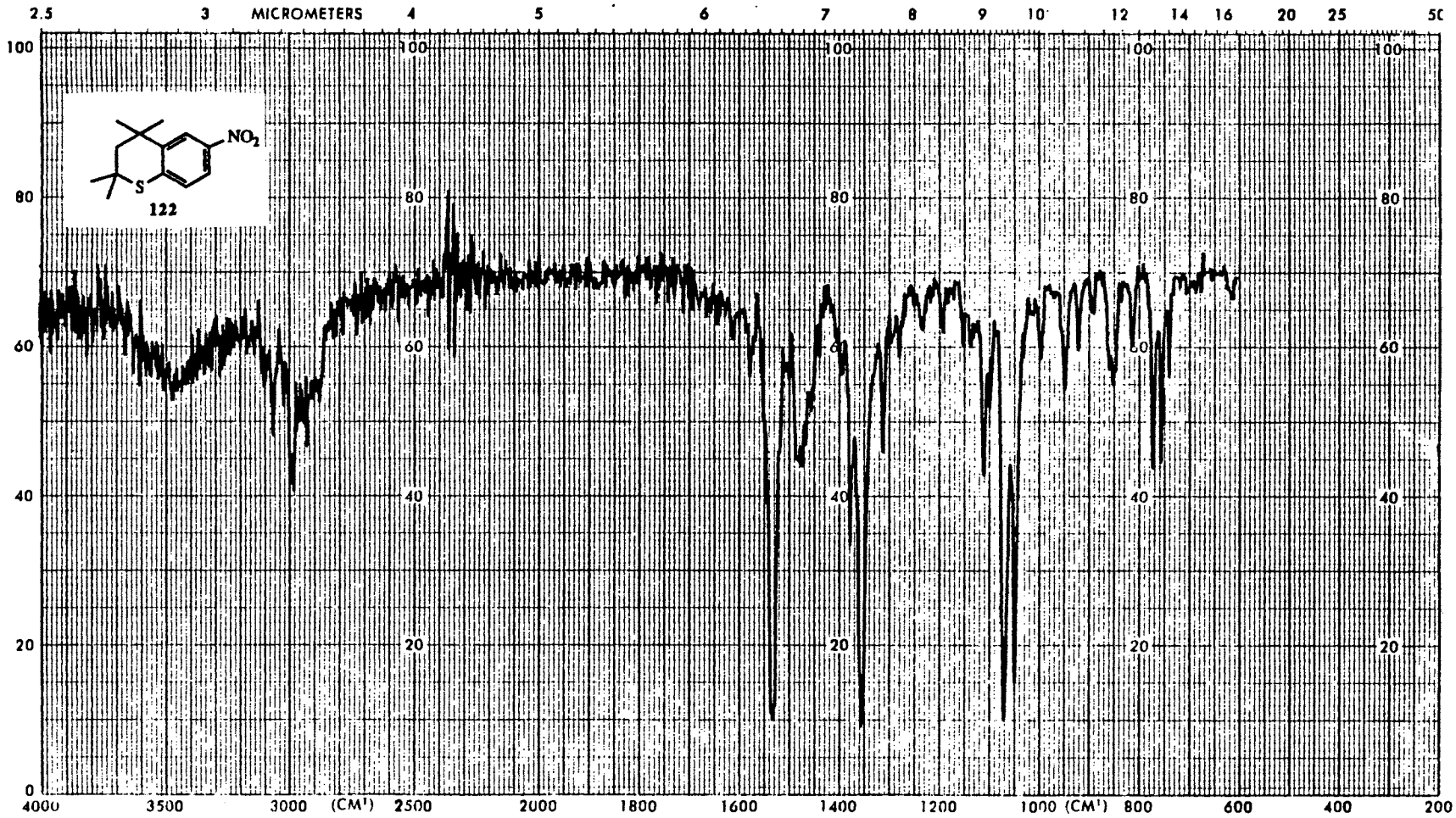
^{1}H NMR Spectrum of **121**

Plate XCIX



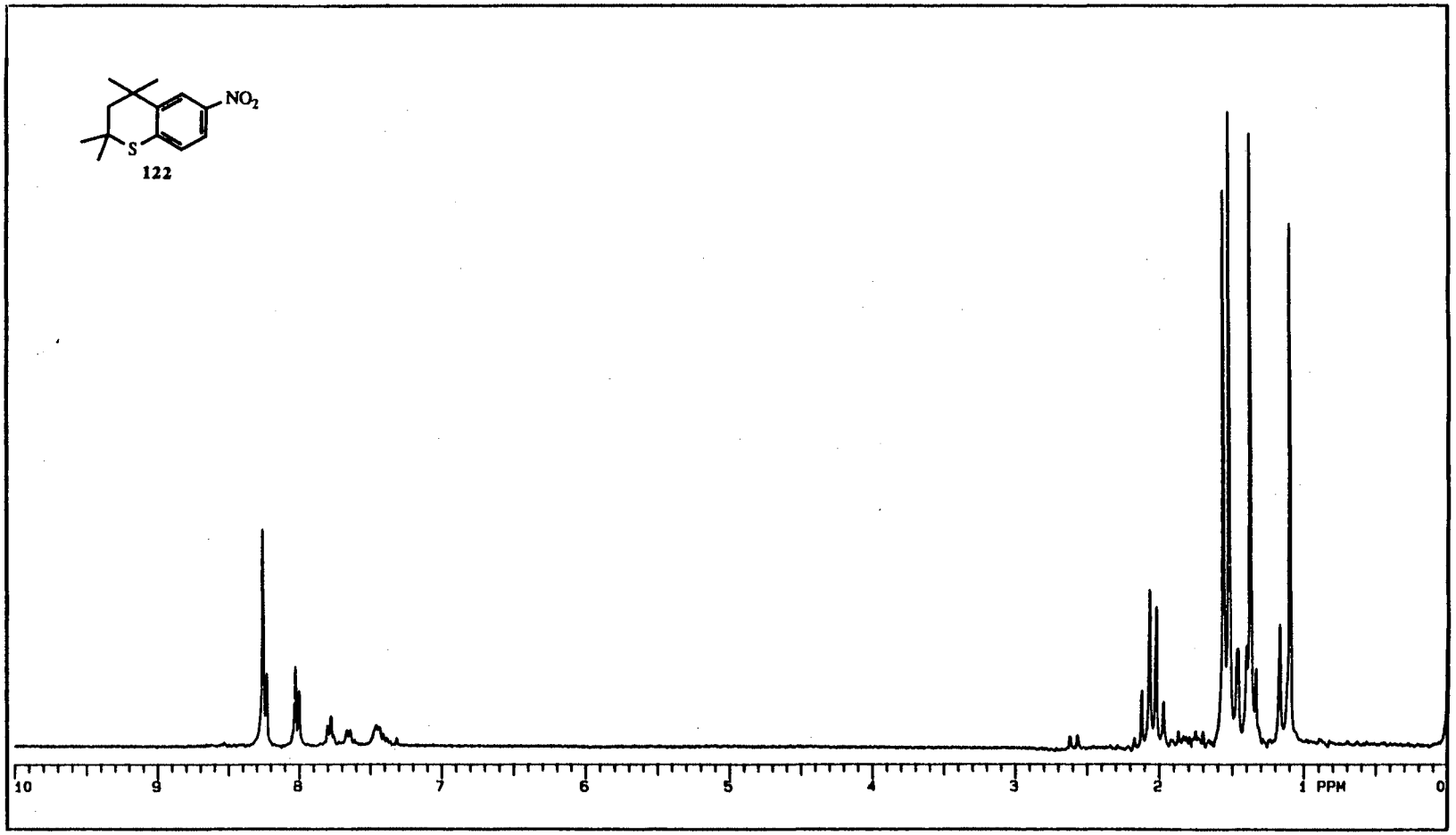
^{13}C NMR Spectrum of **121**

Plate C



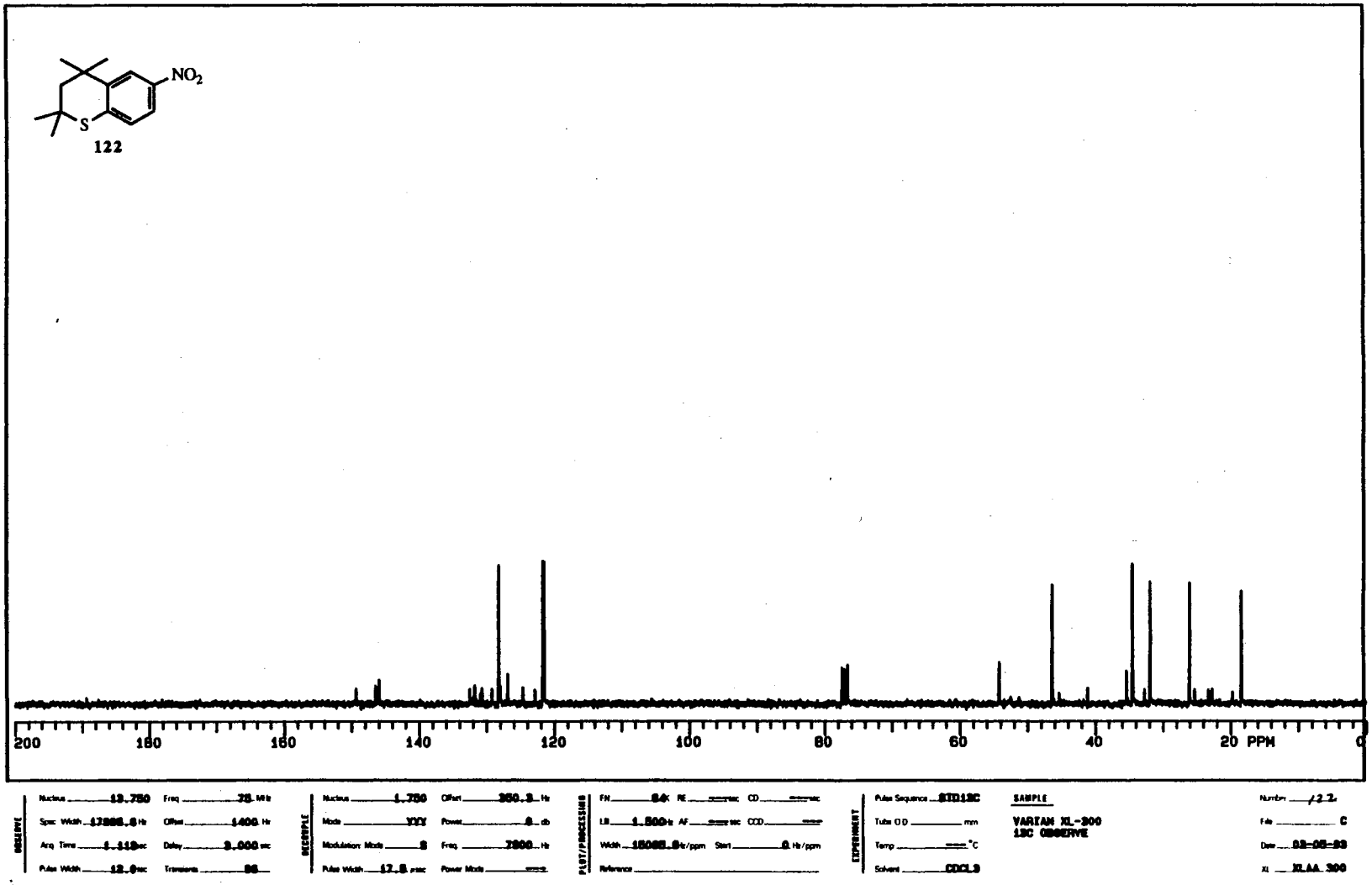
IR Spectrum of **122**

Plate CI



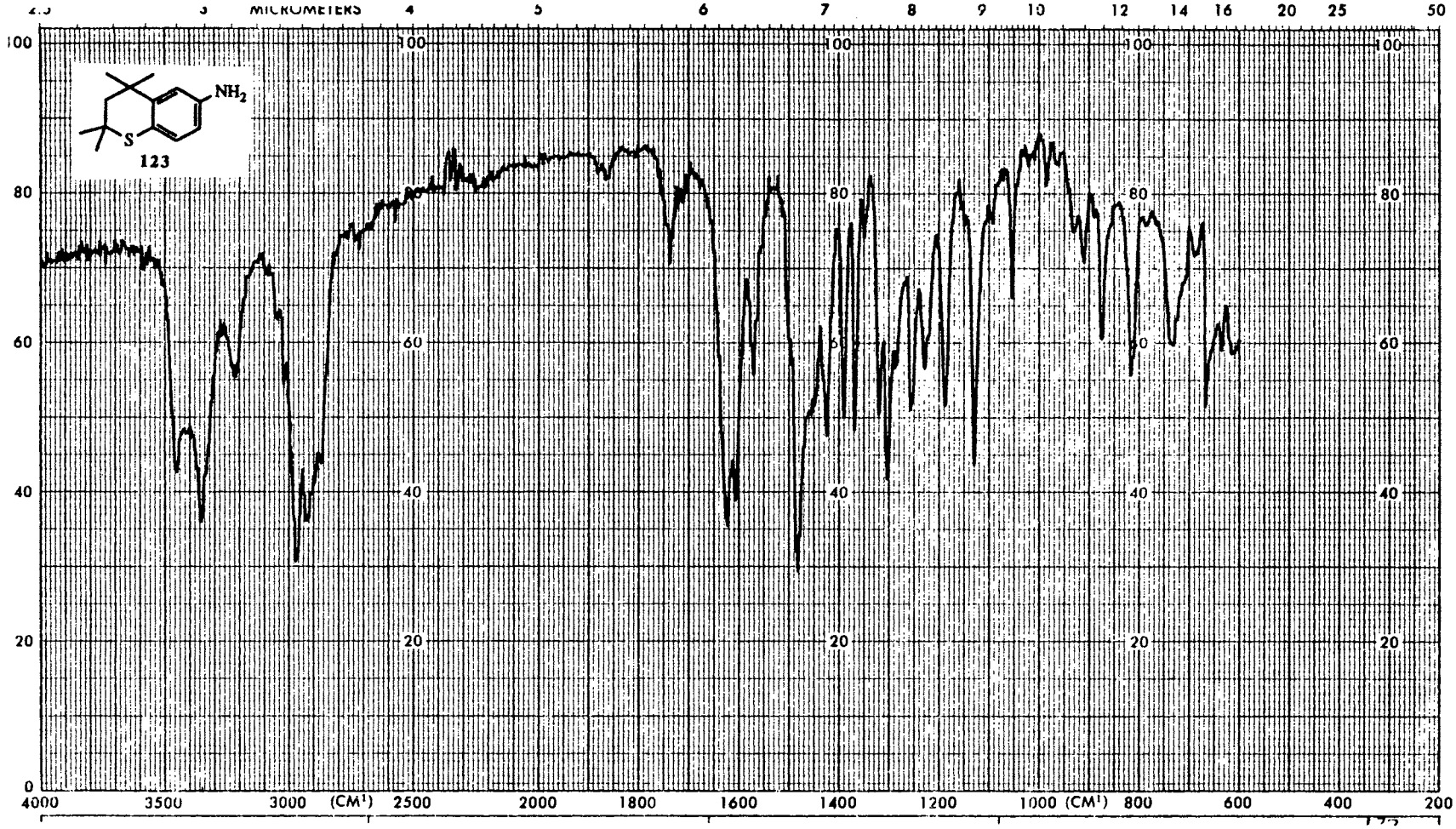
^{1}H NMR Spectrum of **122**

Plate CII



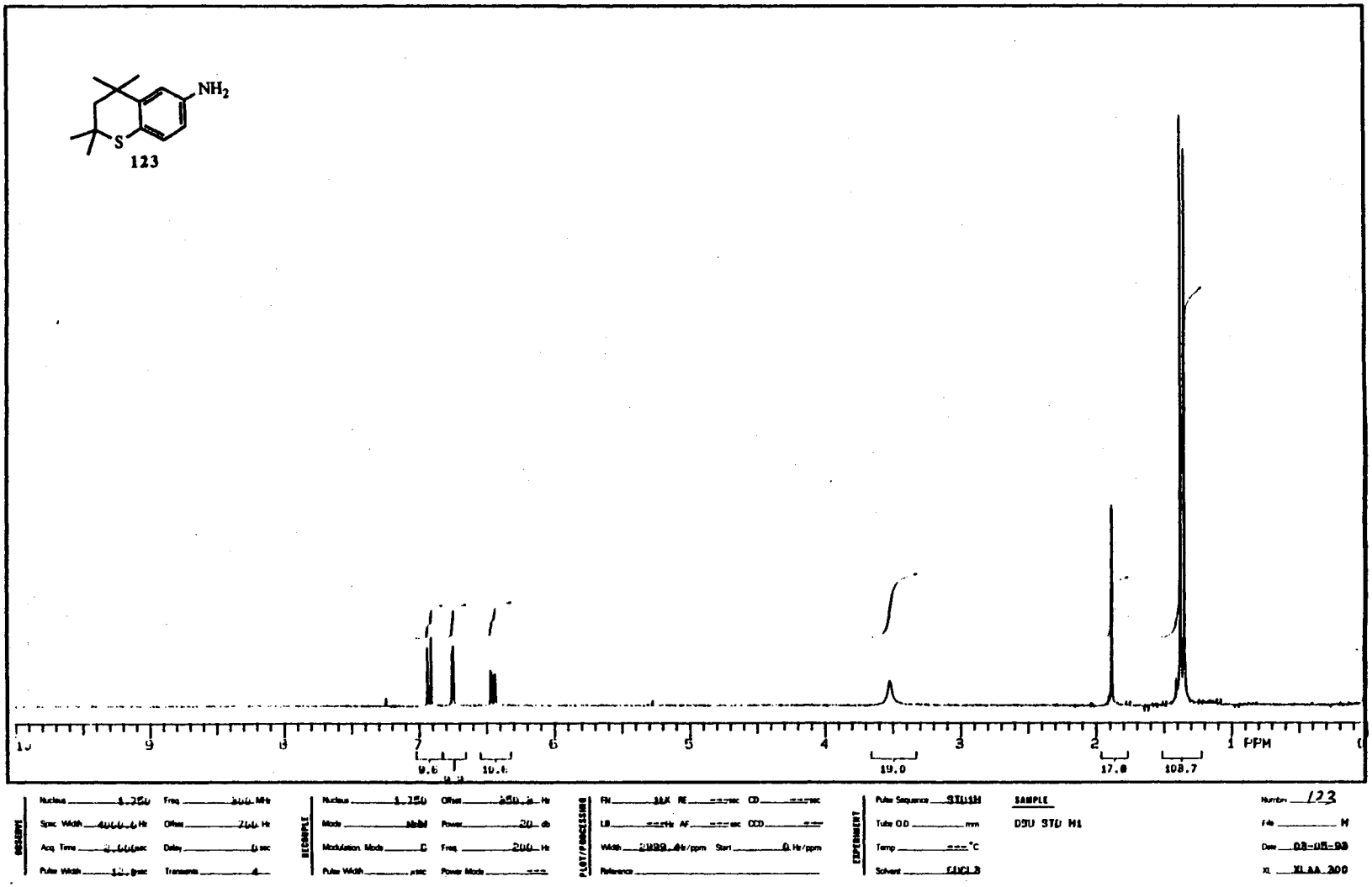
^{13}C NMR Spectrum of **122**

Plate CIII



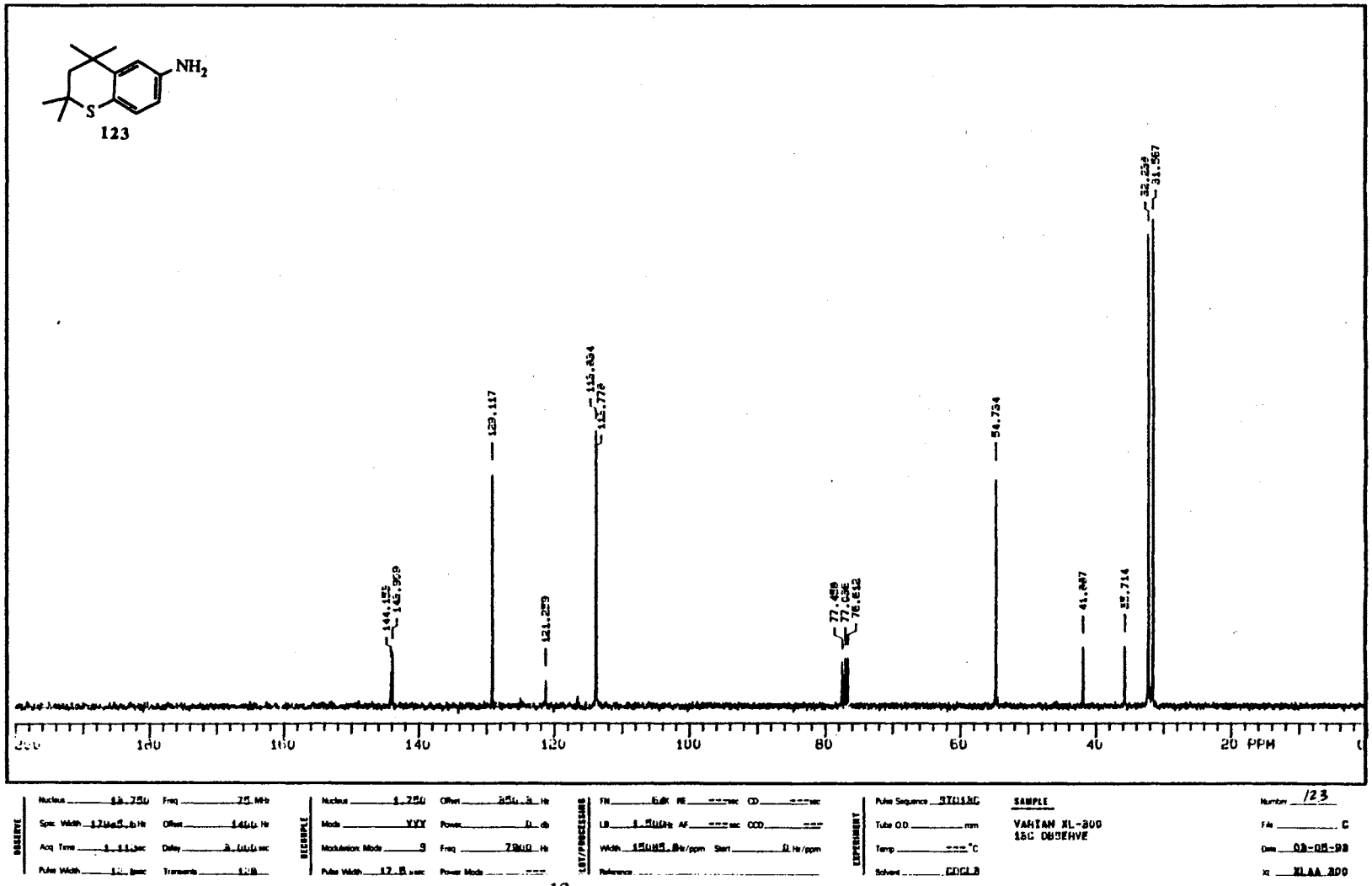
IR Spectrum of **123**

Plate CIV



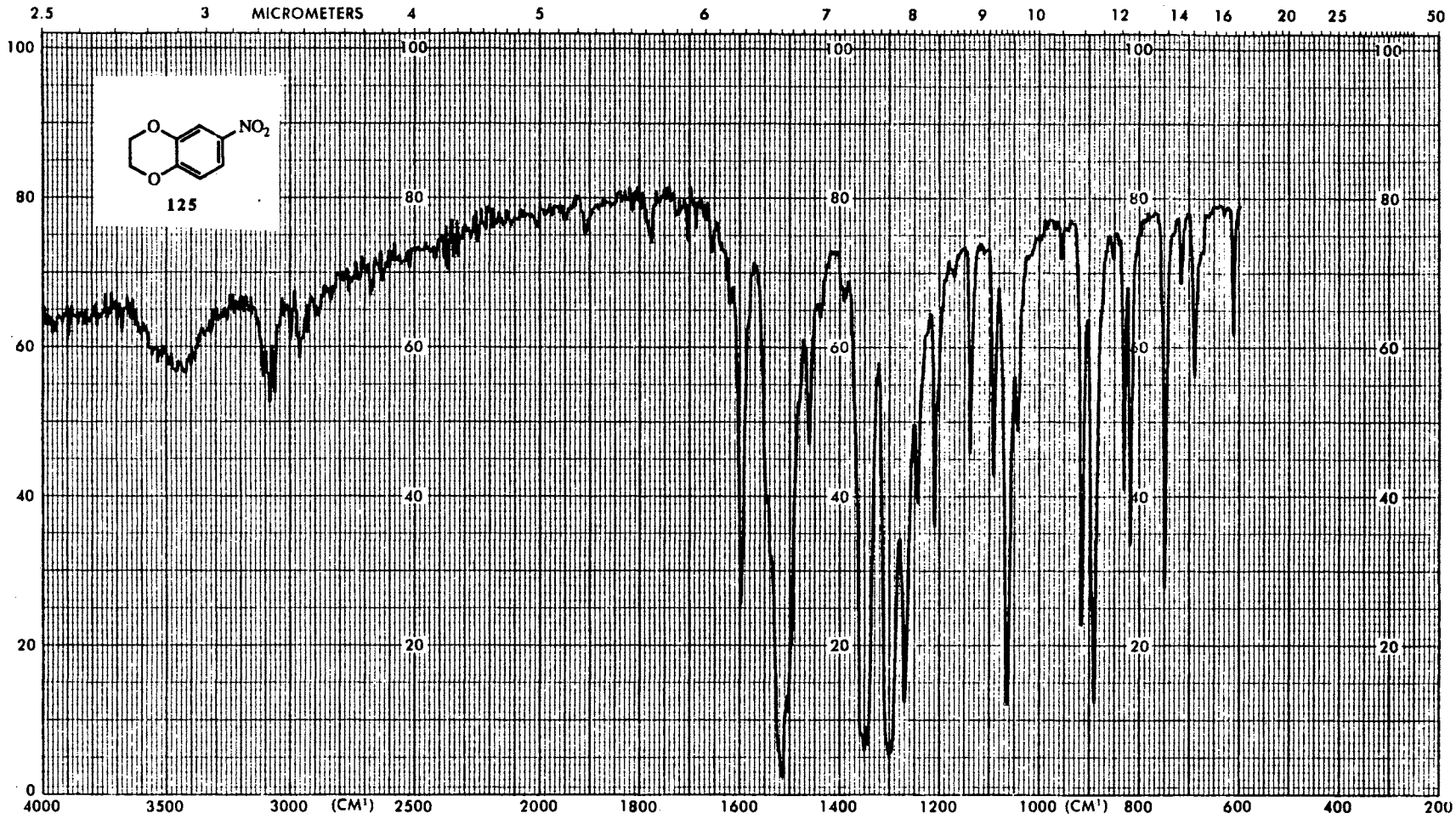
^{1}H NMR Spectrum of **123**

Plate CV



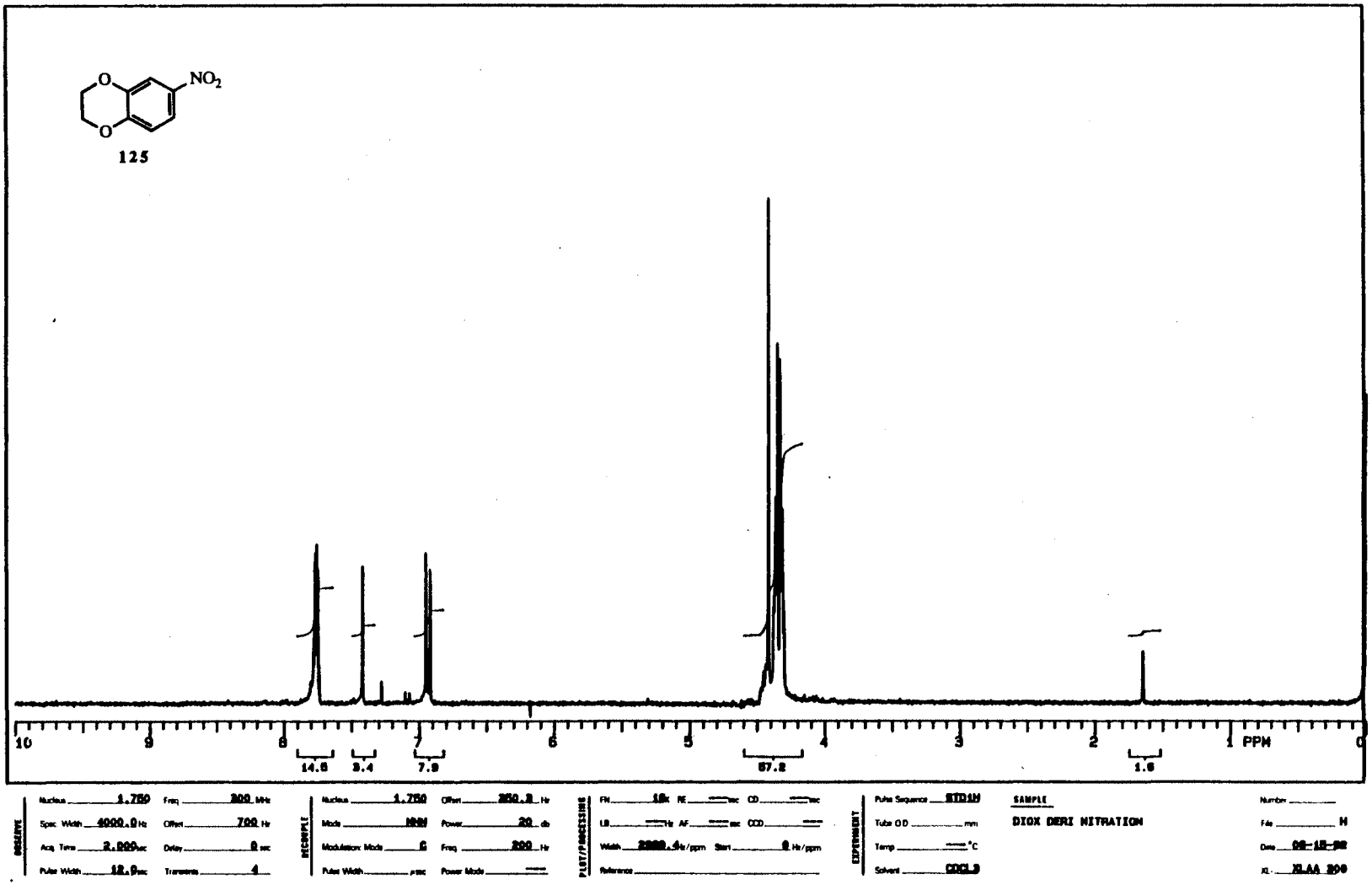
^{13}C NMR Spectrum of **123**

Plate CVI



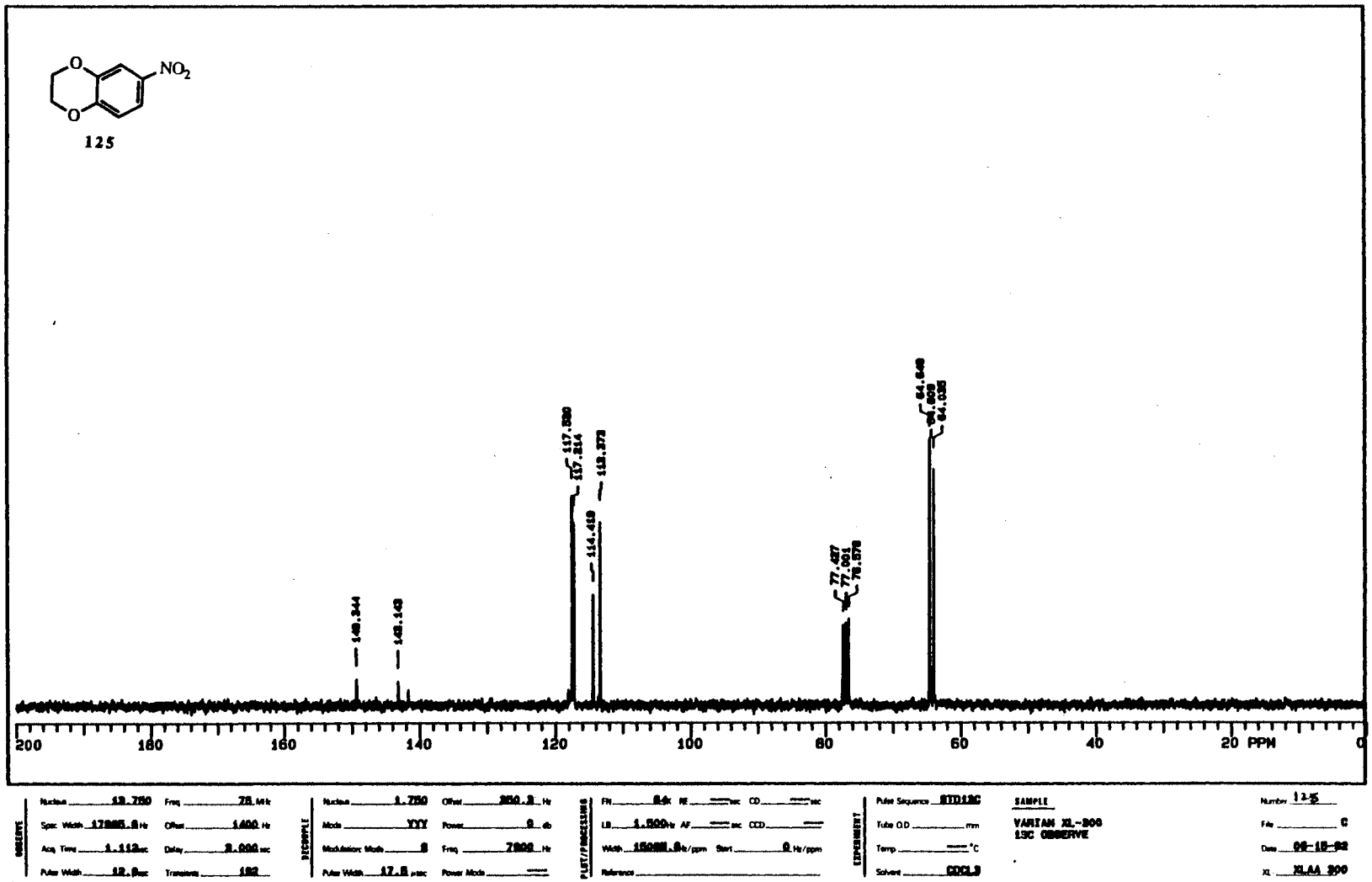
IR Spectrum of **125**

Plate CVII



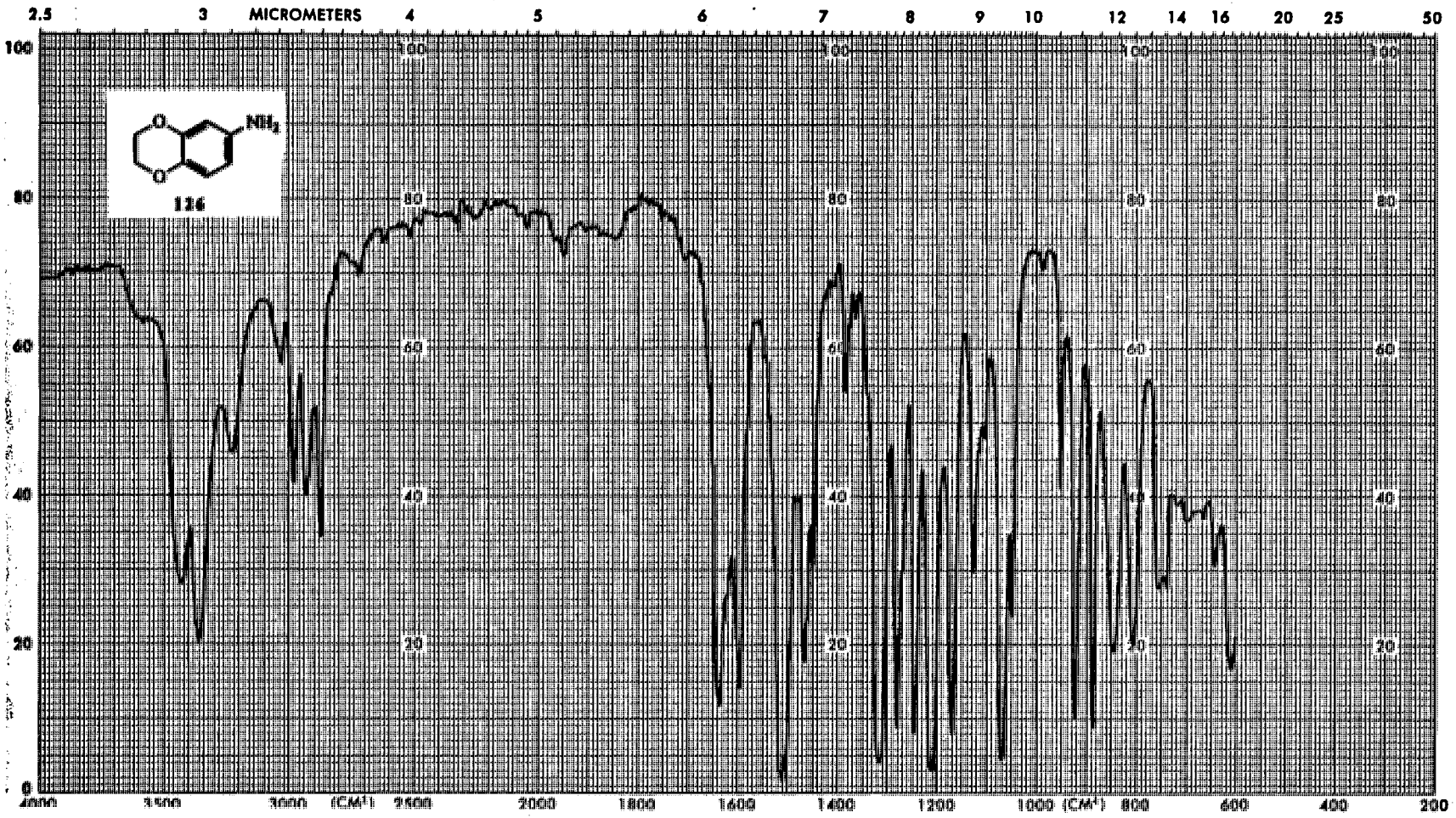
^{1}H NMR Spectrum of **125**

Plate CVIII



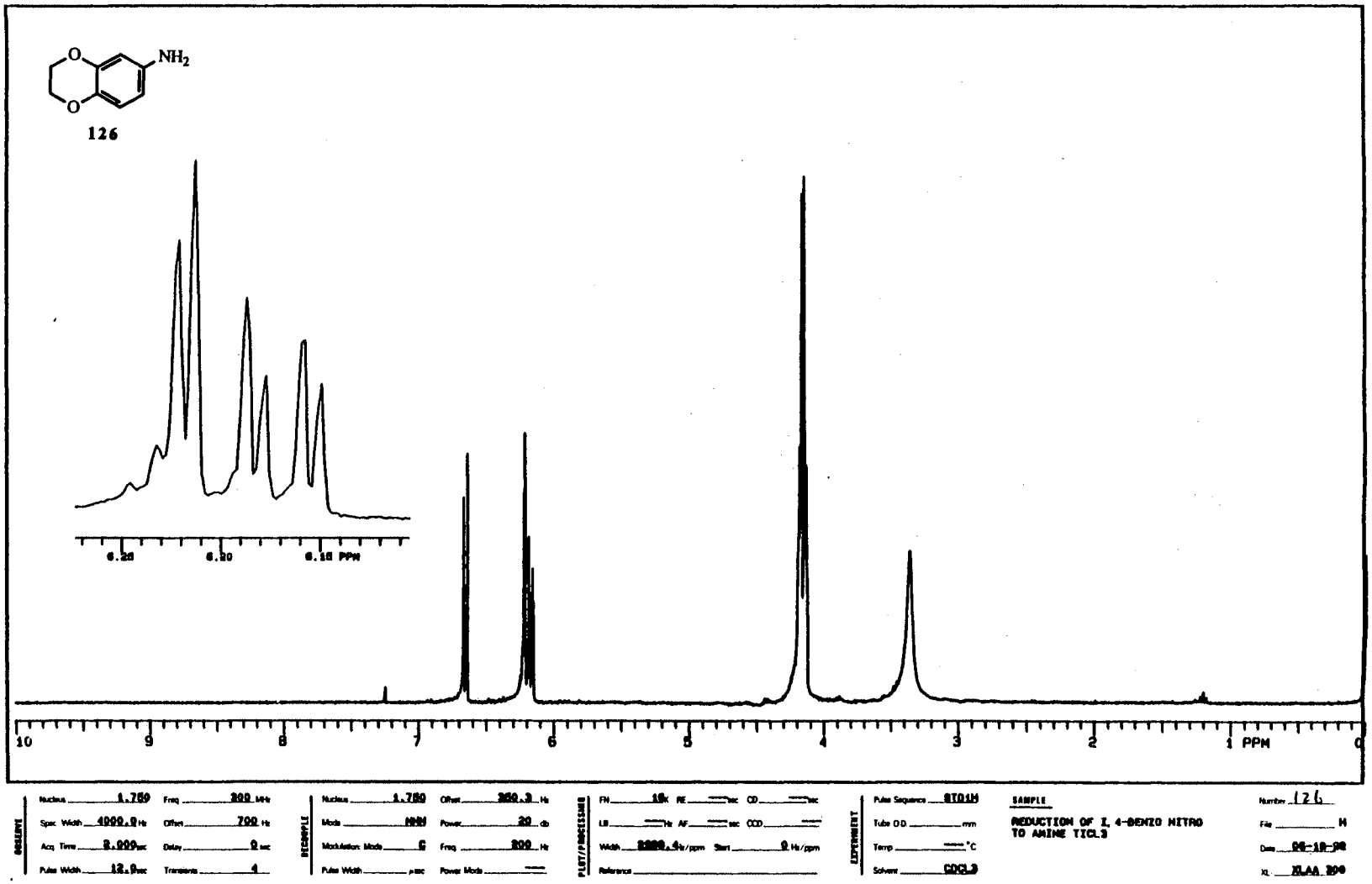
^{13}C NMR Spectrum of **125**

Plate CIX



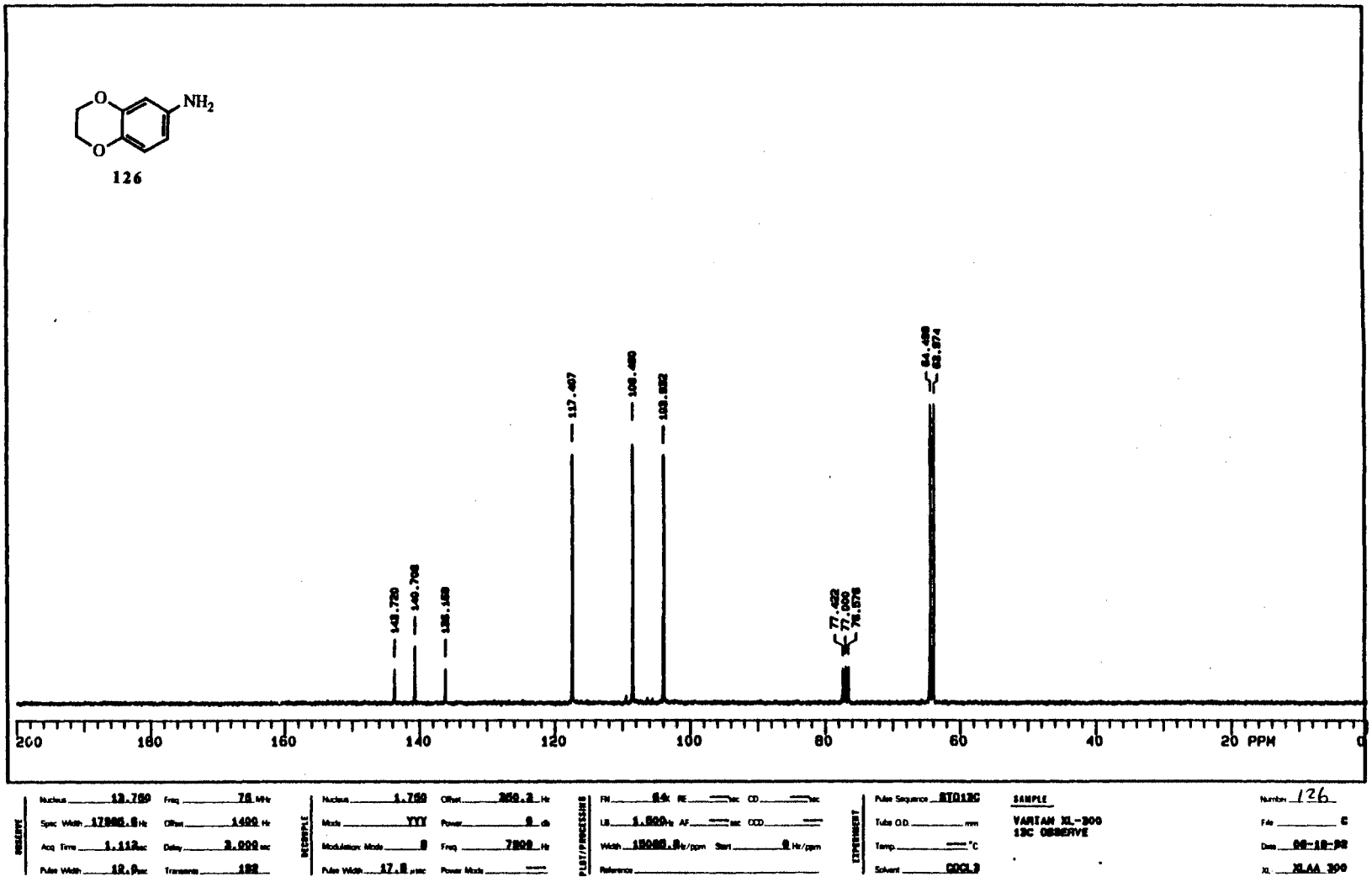
IR Spectrum of **126**

Plate CX



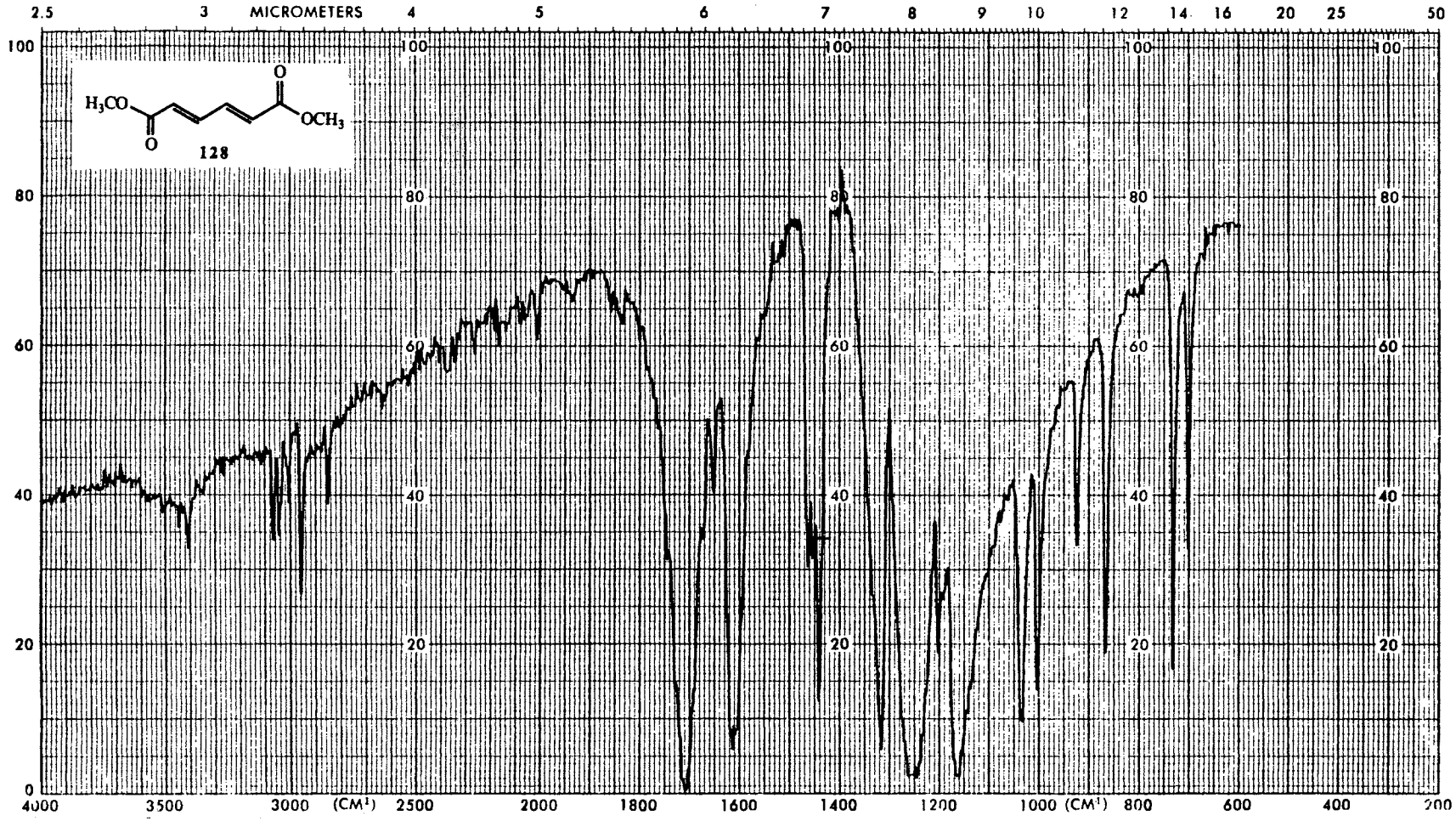
^{1}H NMR Spectrum of **126**

Plate CXI



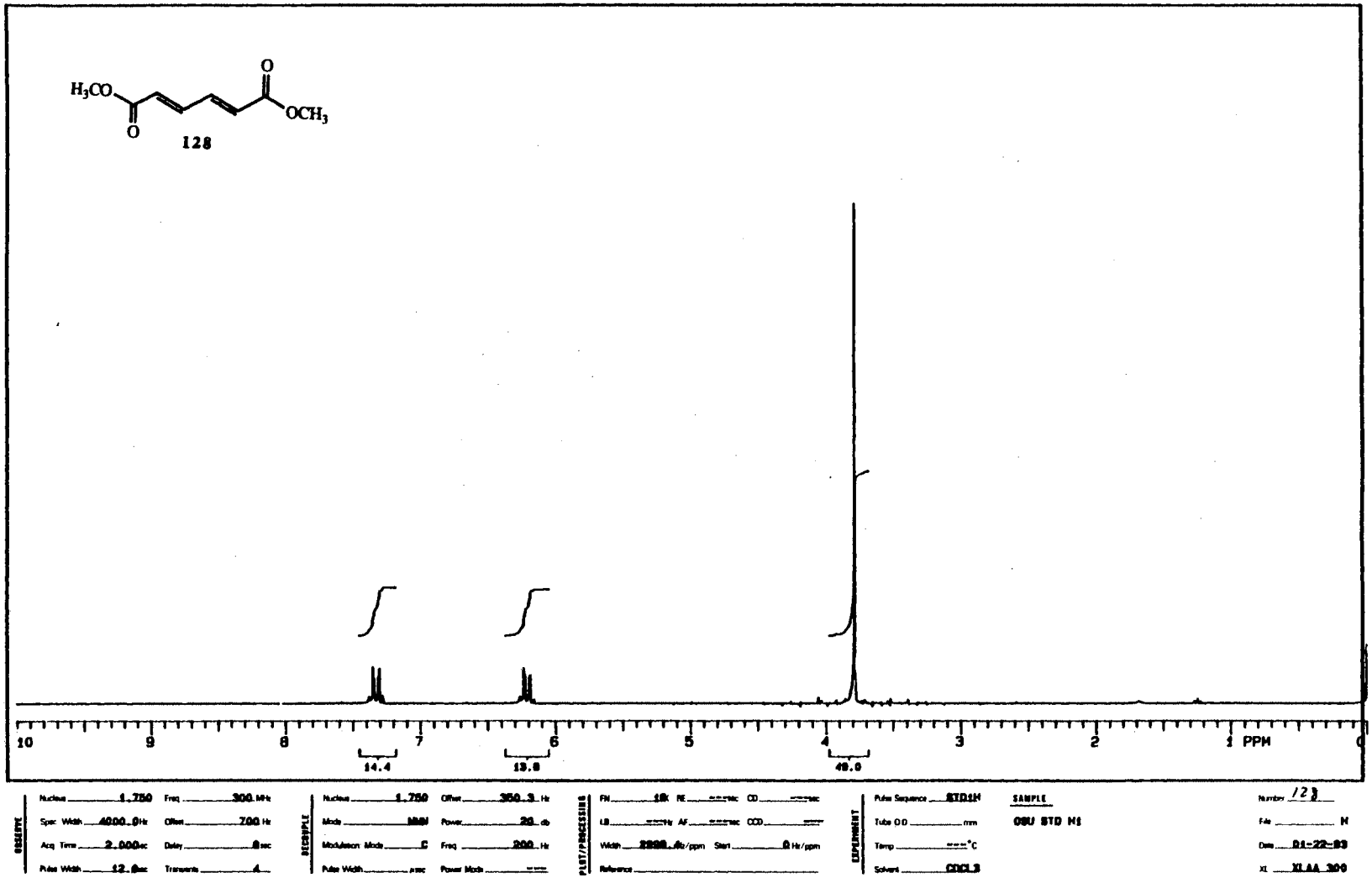
^{13}C NMR Spectrum of **126**

Plate CXII



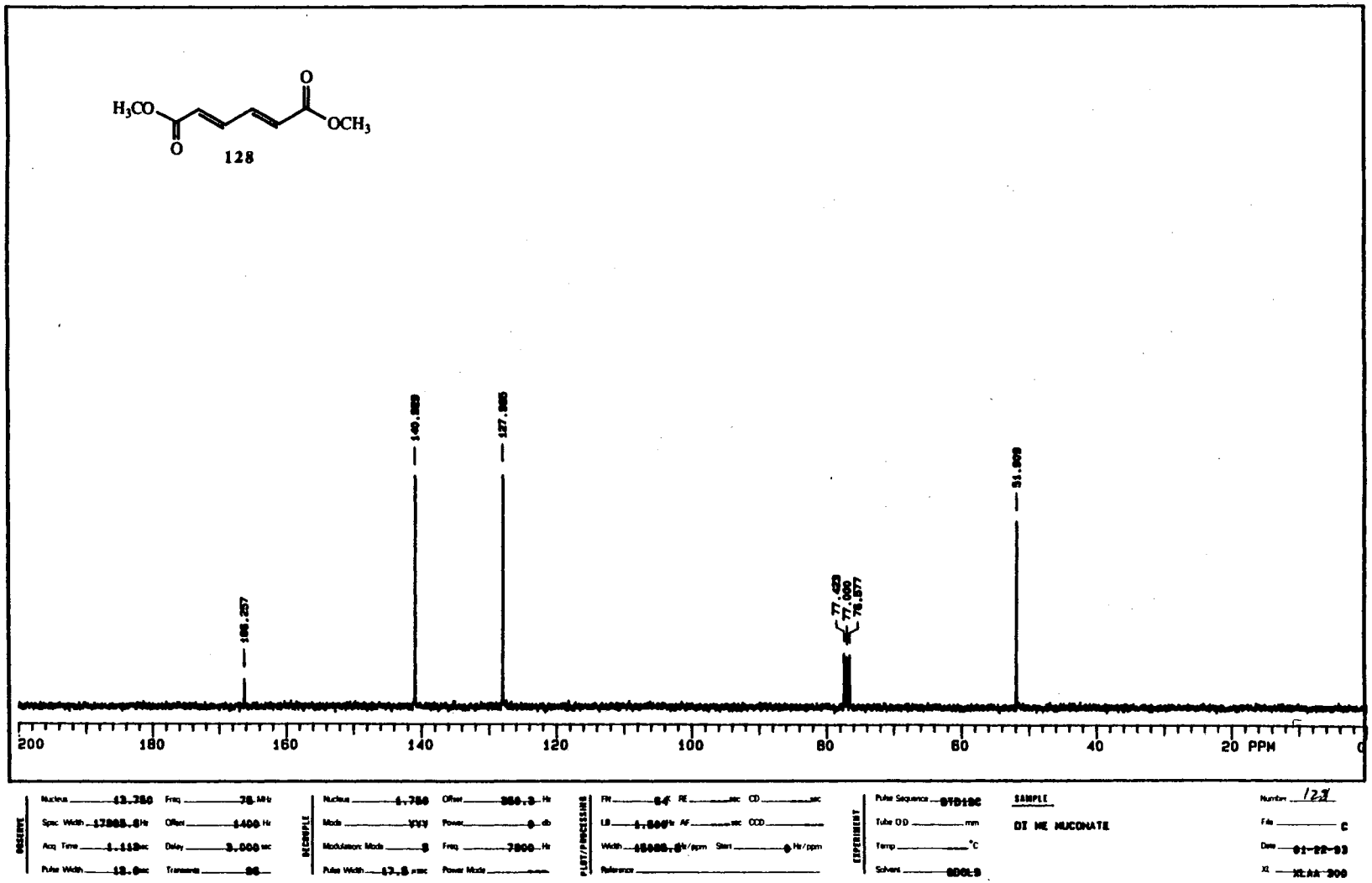
IR Spectrum of **128**

Plate CXIII



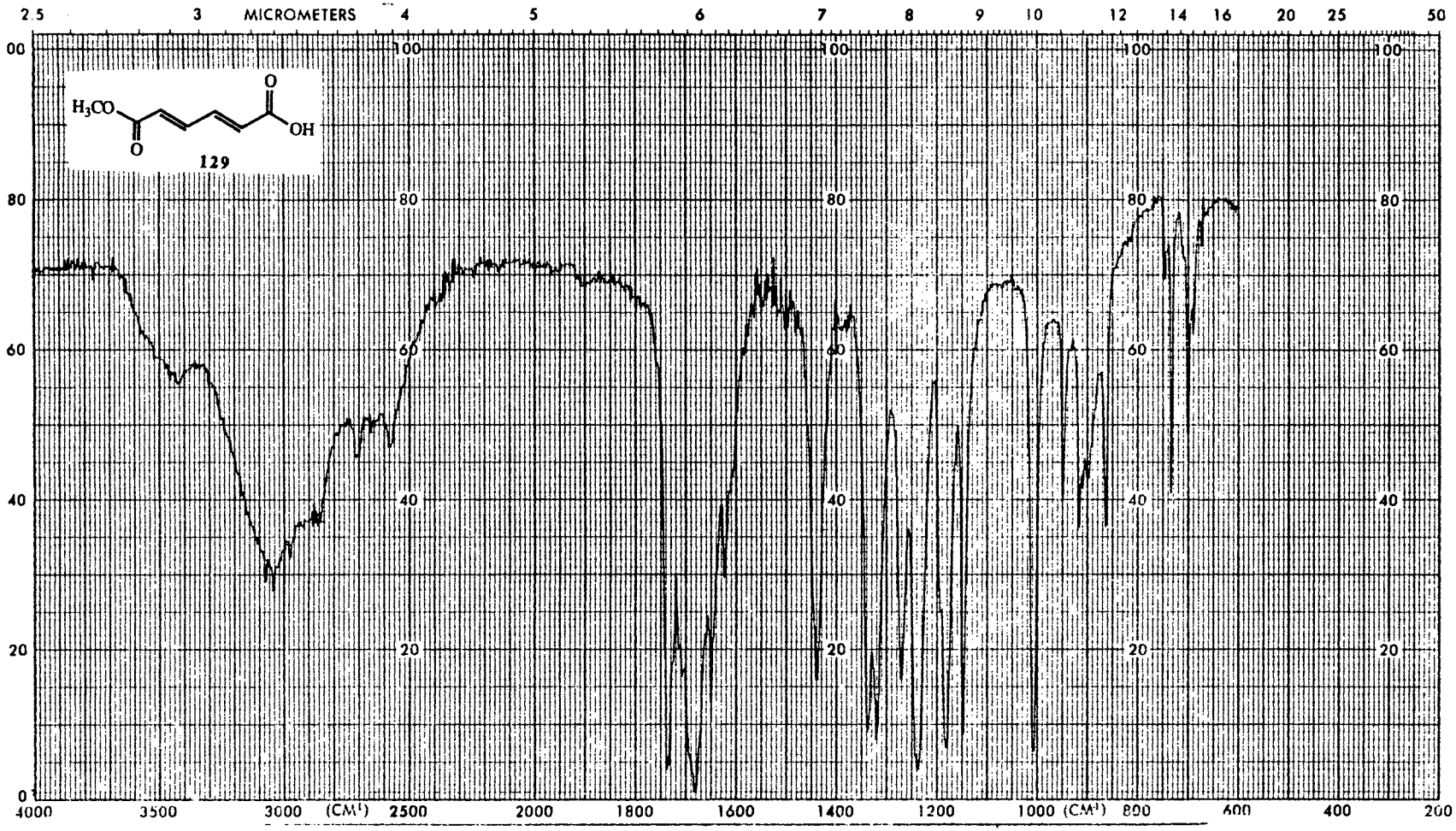
^{1}H NMR Spectrum of **128**

Plate CXIV



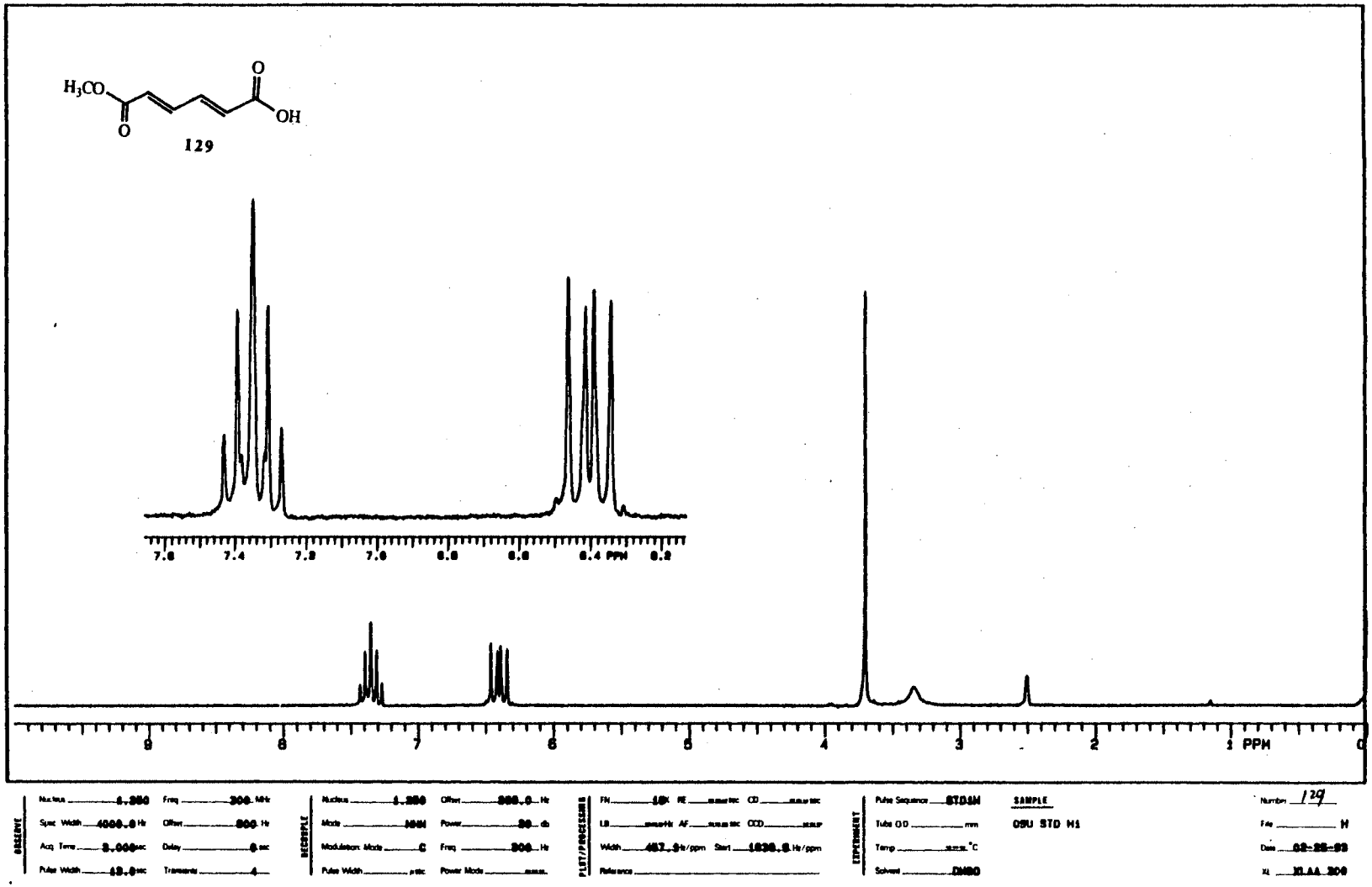
^{13}C NMR Spectrum of **128**

Plate CXV



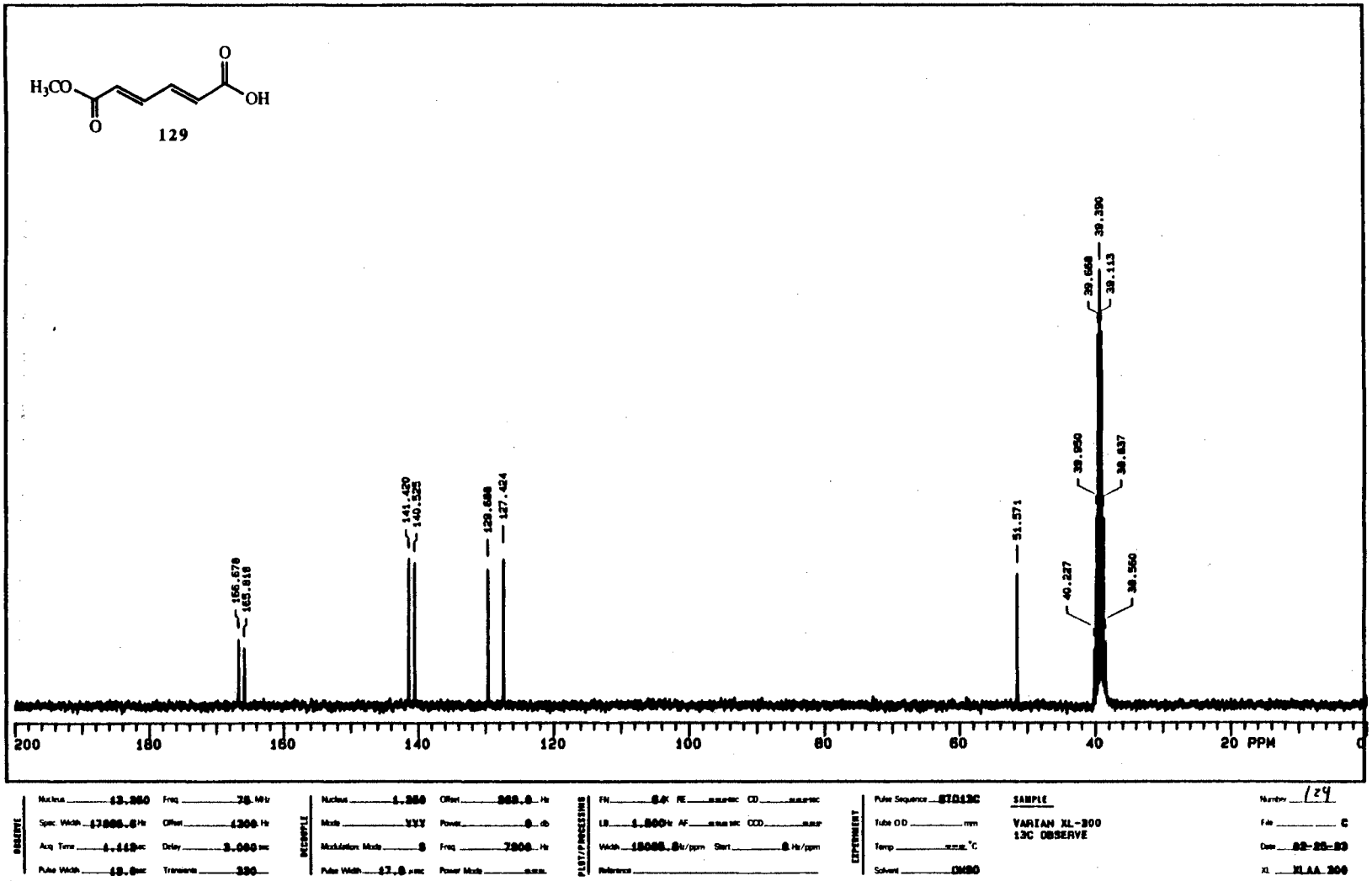
IR Spectrum of **129**

Plate CXVI



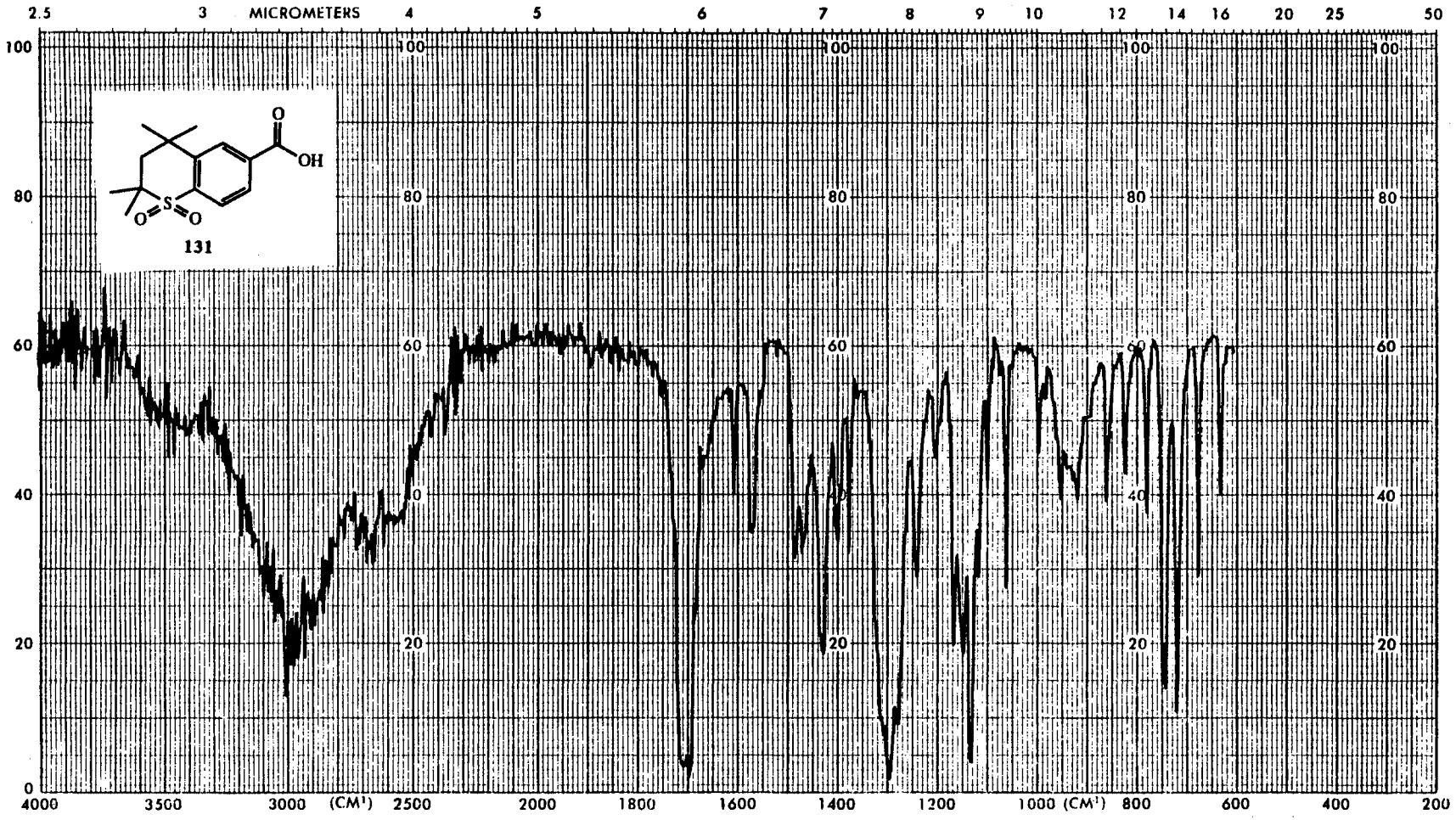
^{1}H NMR Spectrum of **129**

Plate CXVII



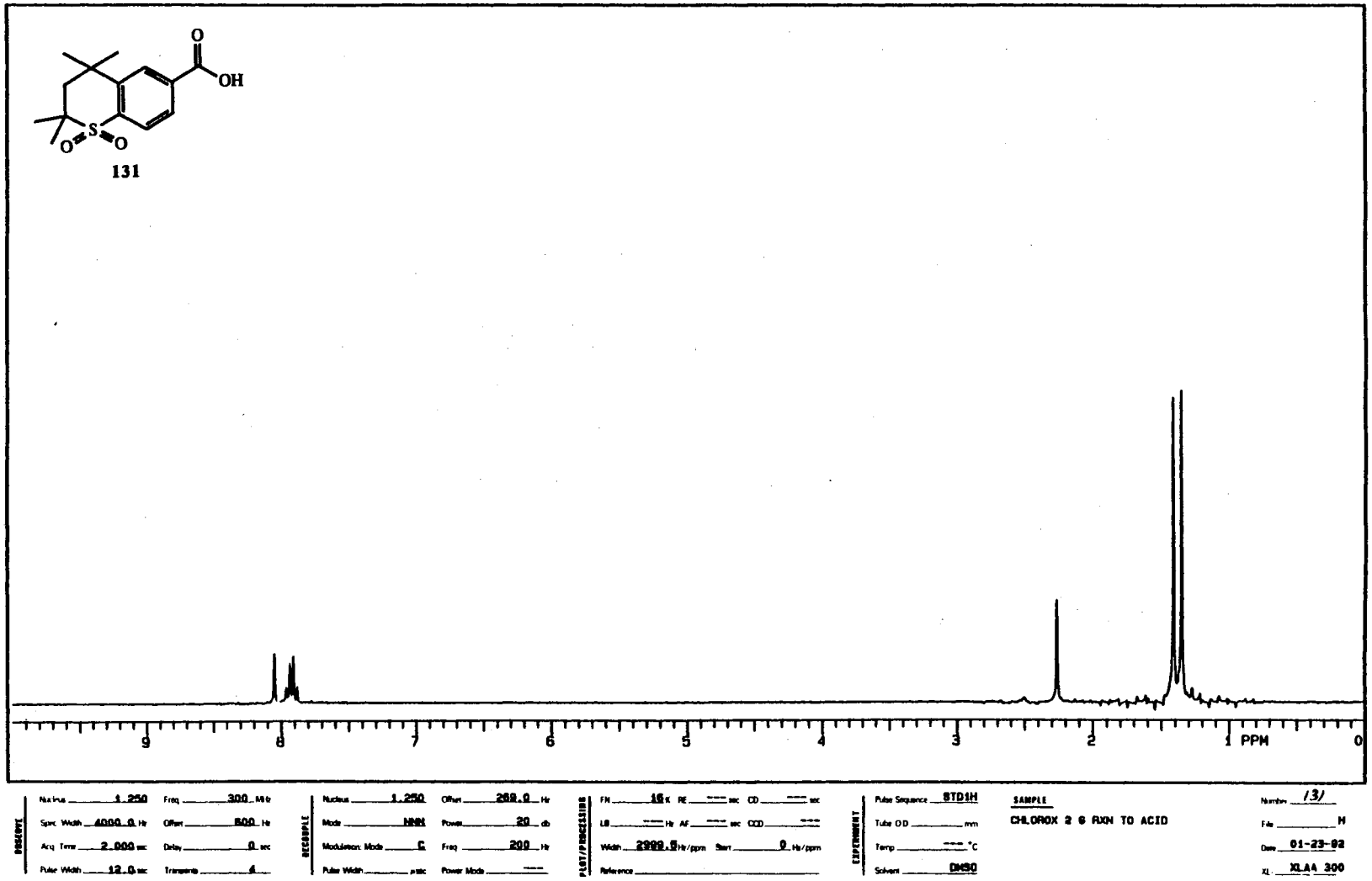
^{13}C NMR Spectrum of **129**

Plate CXVIII



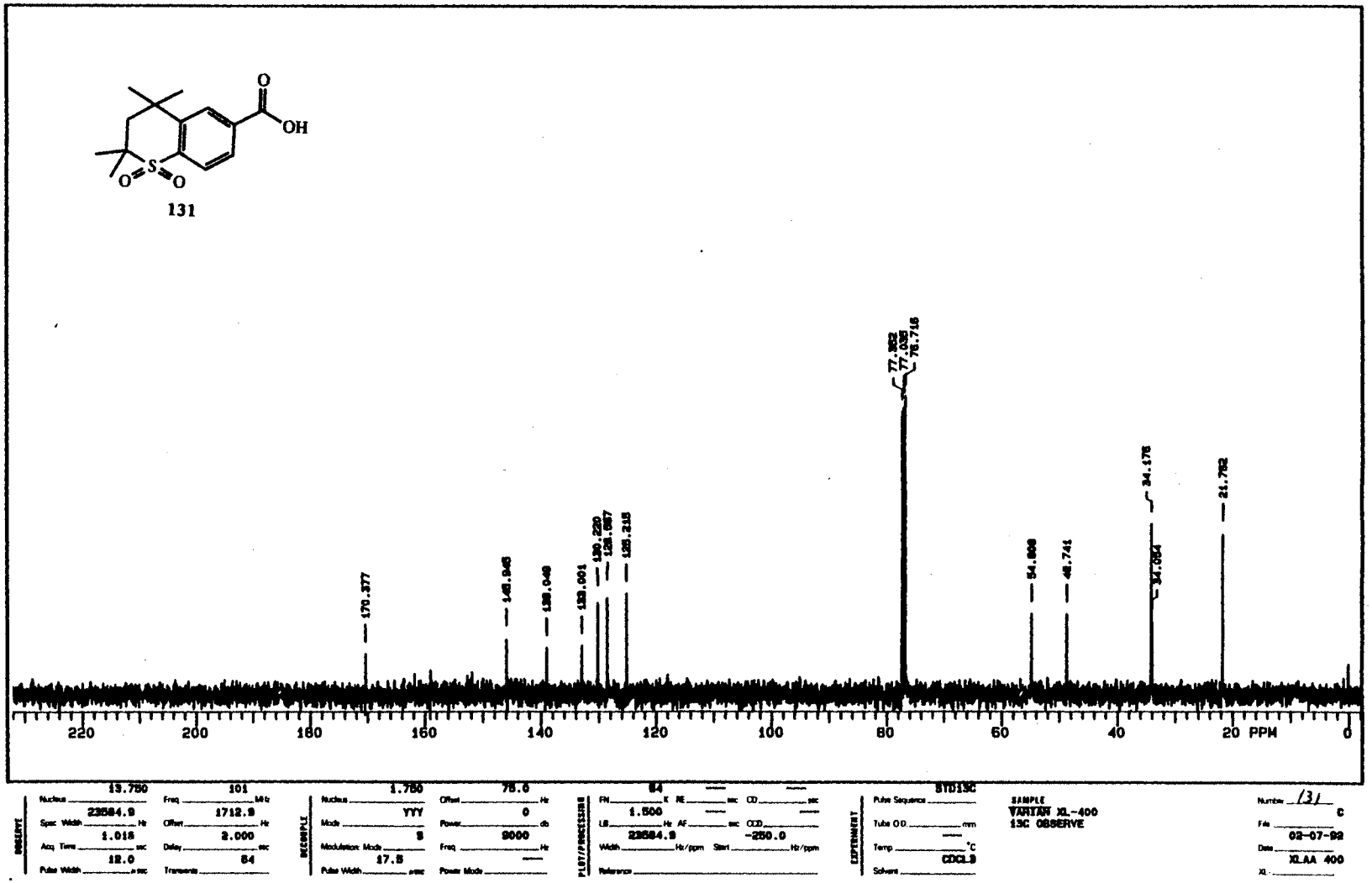
IR Spectrum of **131**

Plate CXIX



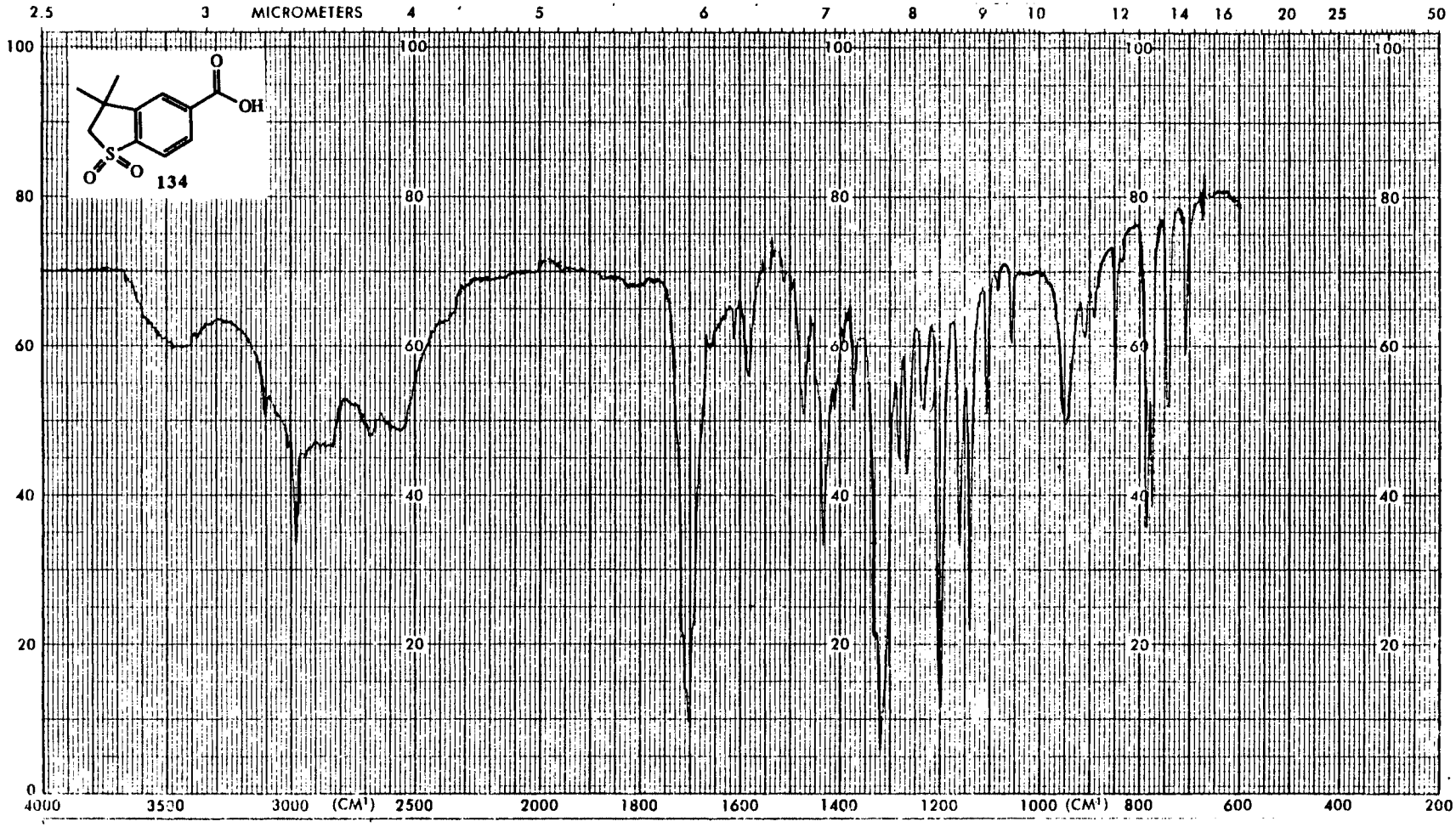
^{1}H NMR Spectrum of **131**

Plate CXX



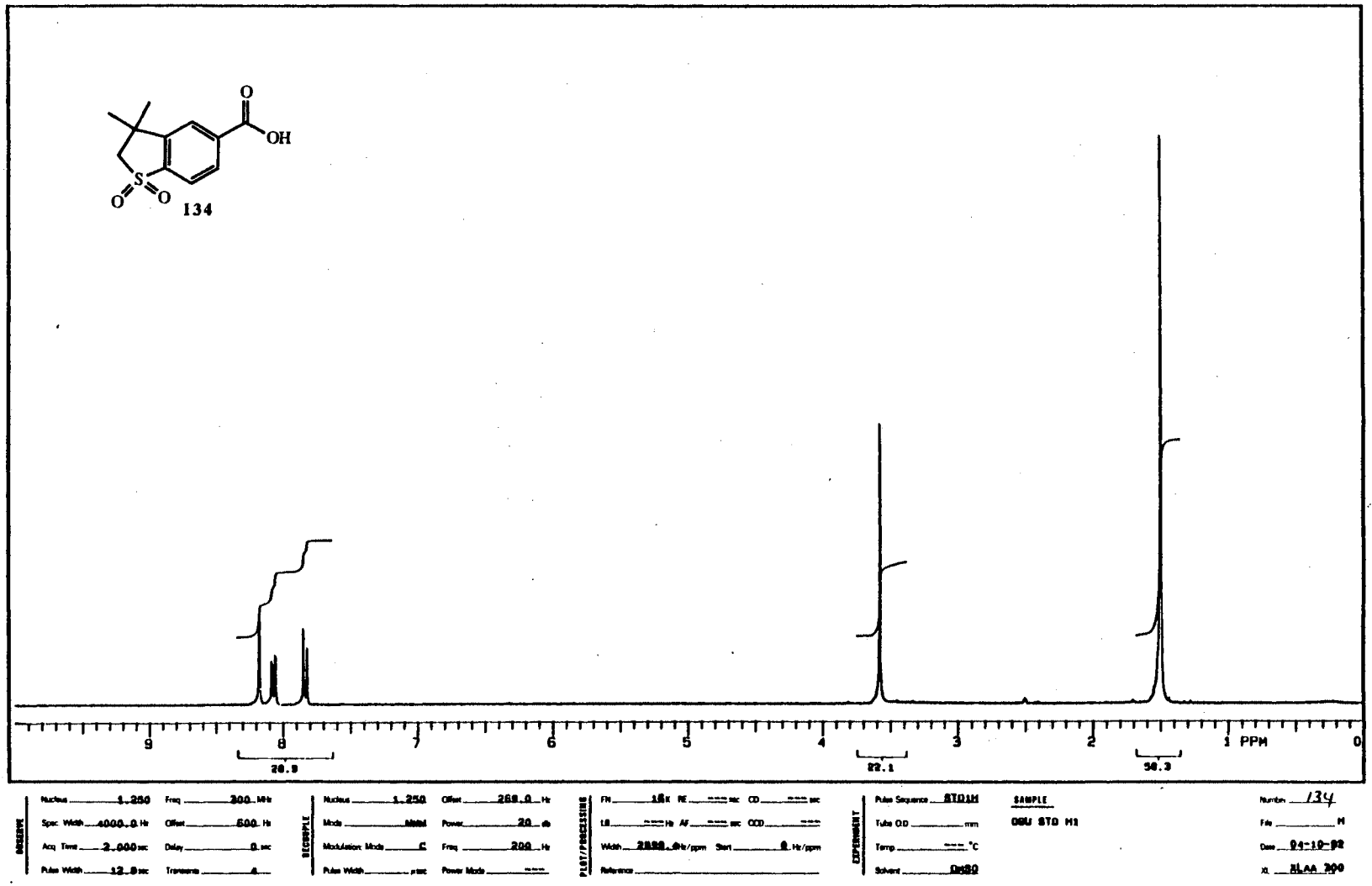
^{13}C NMR Spectrum of **131**

Plate CXXI



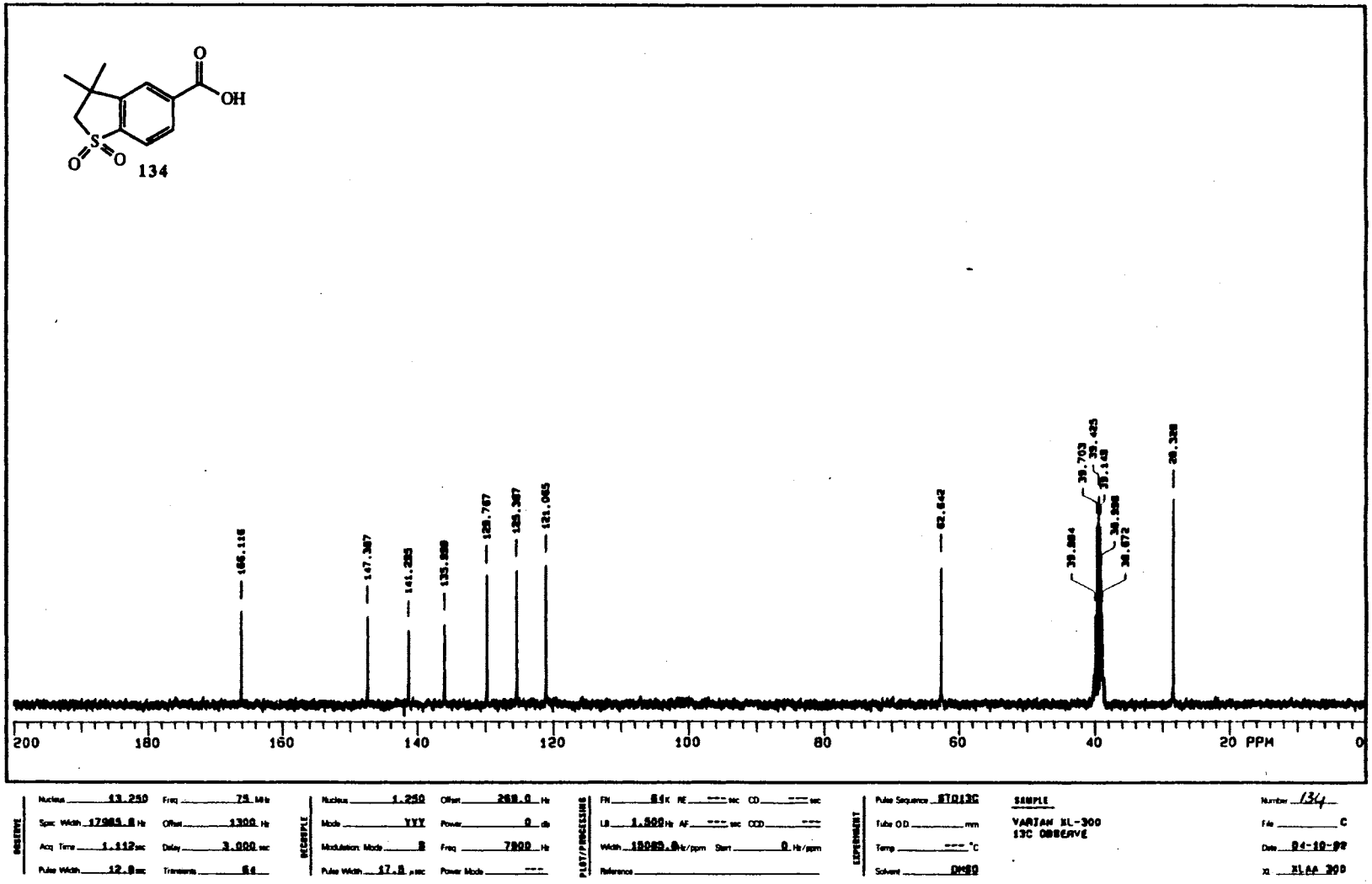
IR Spectrum of **134**

Plate CXXII



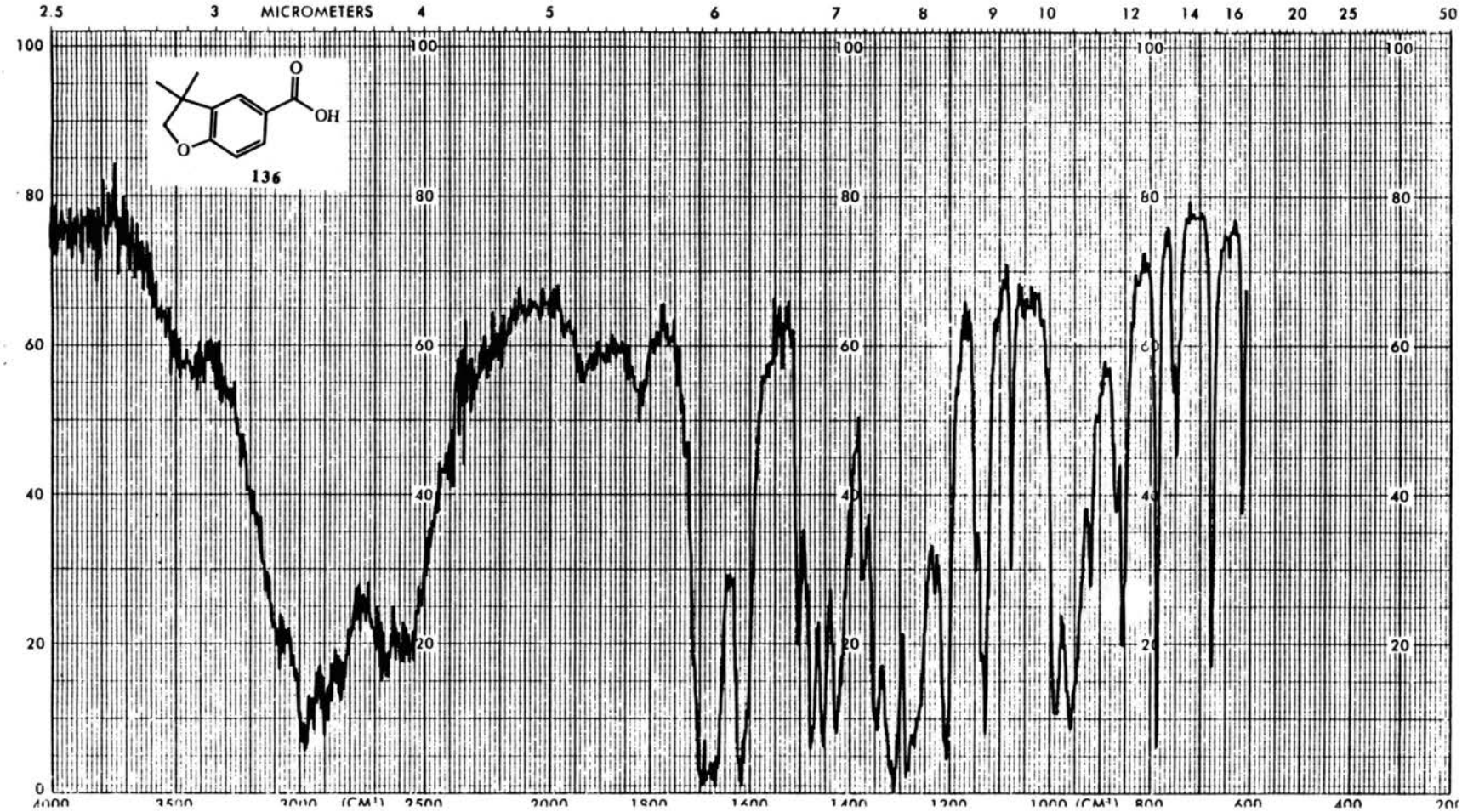
^{1}H NMR Spectrum of **134**

Plate CXXIII



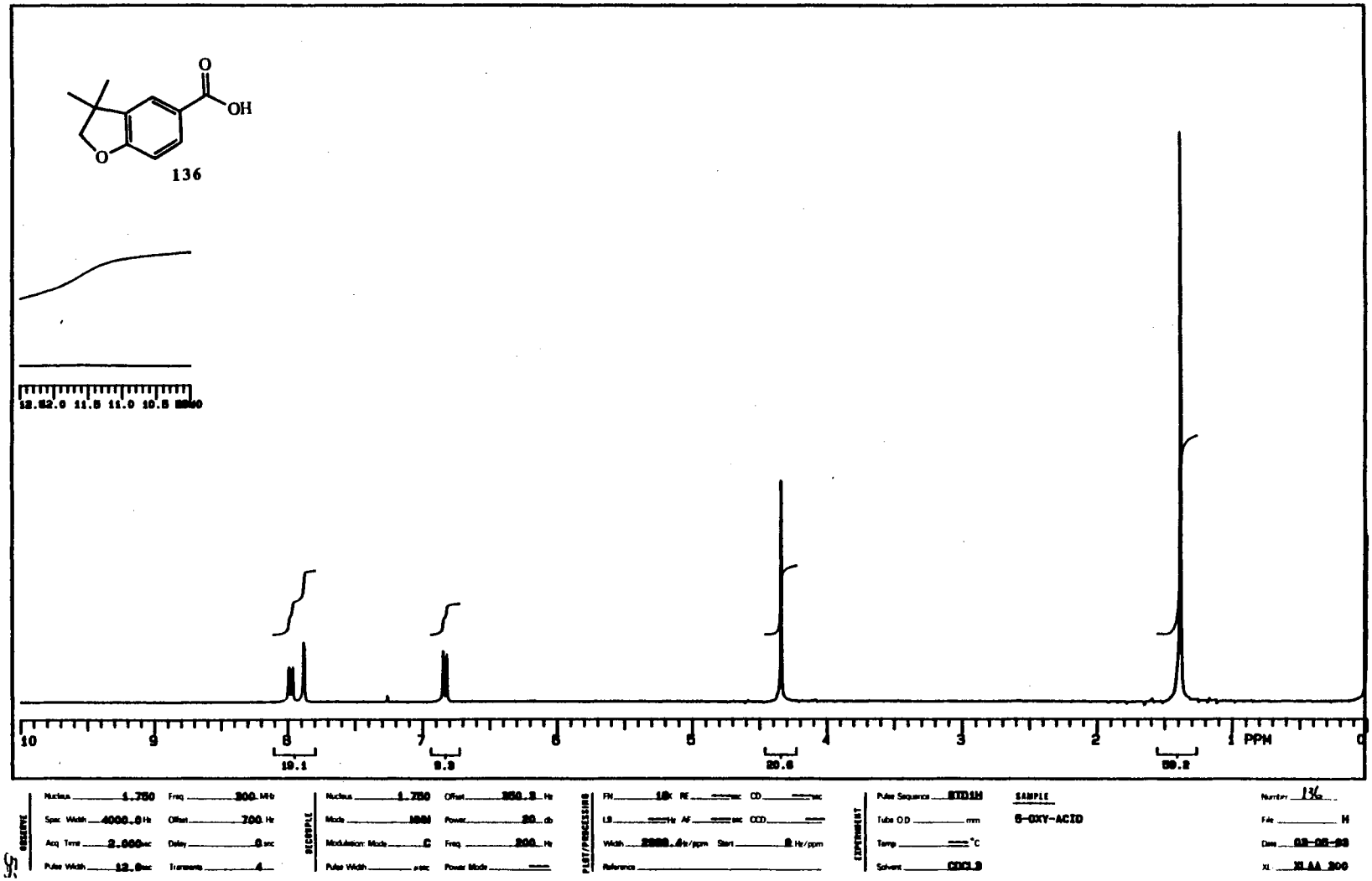
^{13}C NMR Spectrum of **134**

Plate CXXIV



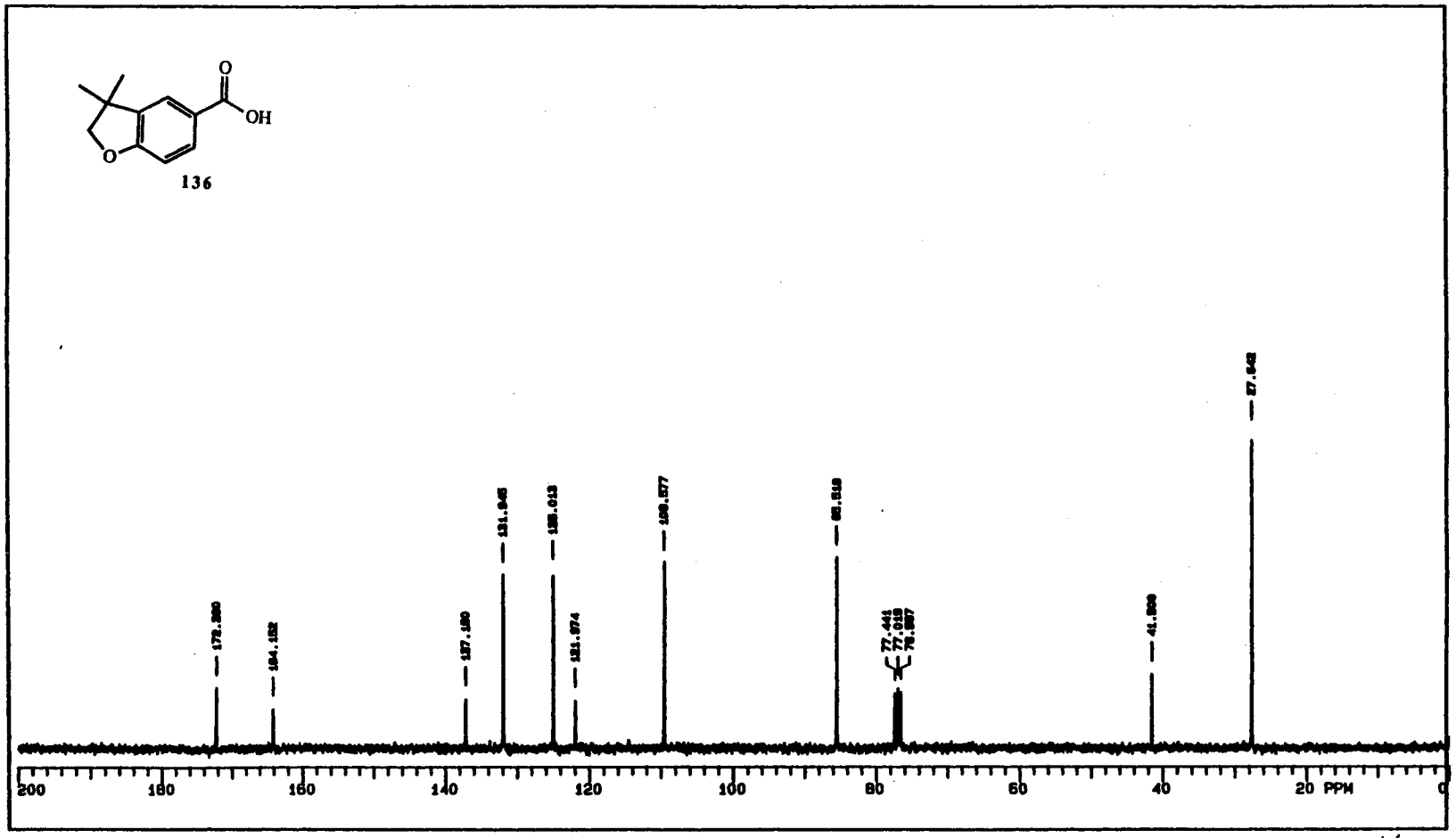
IR Spectrum of **136**

Plate CXXV



^{1}H NMR Spectrum of **136**

Plate CXXVI



^{13}C NMR Spectrum of **136**

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