HETEROAROTINOIDS: 1,4-BENZODIOXAN DERIVATIVES AS RETINOID MIMICS

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CHAPTER I

HISTORICAL

Introduction

The term "retinoids" is employed to encompass members of the vitamin A group of compounds, their derivatives, and their analogues.[92] Vitamin A refers to the all-*trans*-retinol (**1**), and some of the relatives **2-5** of vitamin A (**1**) are illustrated below.

1 [Retinol, vitamin A] **2** [Retinal]

3 [ß-Carotene]

4 [Retinoic acid] **5** [Retinyl acetate]

As far back as about 1500 B.C., Egyptian writings have described the benefits of liver as a food for the treatment of night blindness.[60] In 1909, a fat soluble extract from egg yolk was found to be essential to life.[97,98] This substance, initially called "fat soluble A",[62] was also found in animal fats and fish oils and was subsequently called vitamin A (**1**).[26] In 1931, Karrer and co-workers[29,54] isolated and purified vitamin A (**1**) from

halibut liver oil and determined its structural formula. In the next two decades, retinal (**2**)[3,105] and retinoic acid (**4**)[1,75,93,94] were synthesized and shown to be important in the visual cycle and in growth, respectively. Since then, various research groups have directed efforts at the total synthesis of vitamin A (**1**), and, among them, the contributions from Hoffmann-La Roche (1947)[47] and Badische Anilin und Sodafabrik (BASF)[78] have been particularly noteworthy. Isler and co-workers[47] at Hoffmann-La Roche effected the chemical synthesis of **1** as illustrated.

6 → $2\ N\ H_2SO_4$ → **7**

7 → (1) $ClCH_2CO_2Et$ (2) $NaOCH_3$ → **8** (CHO)

8 → (1) Mg, EtBr, Ether, Δ (2) HO, H (**9**) (3) **8** → **10** (OH, HO)

10 → H_2, Pd/C → **11** (OH, OH)

11 → (1) C_6H_6, Pyr (2) $CH_3C(O)Cl$ → **12** (OH, OAc)

12 → (1) d, l-α-Tocopherol (2) I_2 → **5**

5 → (1) $\bar{O}H/H_2O$ (2) H_3O^+ → **1**

In the first step, pseudoionone (**6**) was cyclized to give ß-ionone (**7**) using sulfuric acid.[45] The ß-C_{14} aldehyde **8** was obtained by adding ethyl chloroacetate to ß-ionone (**7**) using the Darzens condensation method. Upon treatment with *cis*-3-methyl-2-penten-4-yn-1-ol (**9**), aldehyde **8** gave diol **10**. Partial hydrogenation of **10** yielded the diol **11** which, upon monoacetylation, dehydration, and rearrangement, gave the acetate **5**. Vitamin A (**1**) was finally obtained by saponification of **5**. Pommer[78] of BASF attempted the synthesis of vitamin A (**1**) using the known Wittig reaction[110] in the penultimate step as outlined below.

1. HC≡CNa (13)
2. H_3O^+

7 → **14**

H_2

14 → **15**

$Ph_3P{\cdot}HCl$ (16)

15 → **17** ($\overset{+}{P}Ph_3\overset{-}{Cl}$)

1.Base
2. OHC–C(CH₃)=CH–CH₂–OAc (18)

17 → **5** → **1**

In this route ß-ionone (**7**) was also used as the starting material to which sodium acetylide (**13**) was added followed by hydrolysis to yield **14**, which on partial hydrogenation gave ß-vinyl ionol (**15**). The phosphonium salt **17** was obtained upon treating alcohol **15** with triphenylphosphine hydrochloride (**16**). Finally, a Wittig reaction gave retinyl acetate (**5**) which was then saponified to yield vitamin A (**1**).

Chemistry and Biology of Retinoids

Vitamin A (**1**) and its naturally occurring analogues are 20-carbon diterpenes isolated chiefly from fish liver oils, visceral parts of fish, eggs, animal kidney, lungs, eyes, and intestinal mucosa.[2] Investigative work on retinoids began with the observation that vitamin A (**1**) regulates the differentiation and proliferation of epithelial tissues.[89,109] Wolback[111] in 1925 was the first to describe cell differentiation by retinol (**1**). His study showed that changes from normal epithelium to squamous keratinization in mucous membranes arose from deficiencies of retinol (**1**). Since deficiencies of vitamin A (**1**) produce hyperkeratinization, it was hypothesized that analogues of vitamin A (**1**) might be beneficial in the treatment of dermatological disorders like psoriasis, acne and similiar maladies.[101] In 1926, Fujimaki[36] demonstrated that rats fed on a diet deficient in vitamin A (**1**) developed carcinomas of the stomach. Vitamin A (**1**) has also been found to inhibit tracheobronchial tumors in a study which used Syrian golden hamsters.[82] In this investigation, the carcinogen used was benzo[*a*]pyrene suspended in saline. Normal exposure to such a carcinogen generally results in 100% formation of respiratory tract tumors. It was found that of the 46 hamsters treated with vitamin A (**1**) only two developed tumors.[82]

A controversy arose when ß-carotene (**3**), a plant product, was also discovered to be effective in curing the symptoms of vitamin A (**1**) deficiency in rats when the former was administered in daily doses of 5 µg.[28] Later, Moore[65,66] and Capper[14] demonstrated the appearance of vitamin A (**1**) in the liver of vitamin A (**1**) deficient rats which had been given ß-carotene (**3**). The enzymatic conversion of ß-carotene (**3**) to retinol (**1**), and then to retinal (**2**), in the intestinal mucosa and liver of animals has been confirmed.[40] In 1930, Karrer and co-workers established the structure of ß-carotene (**3**).[52,53]

In 1946, retinoic acid (**4**), another derivative of vitamin A (**1**), was synthesized and many in-depth studies were completed on the chemistry and biochemistry of acid **4**.[1,93,94]

It was shown by Bollag[6-9] that acid **4** had a prophylactic effect on the generation of papillomas (induced originally by 7,12-dimethylbenz[*a*]-anthrene) by either diminishing or delaying the occurrence of these tumors as compared to a control. Additional studies led to the hypothesis that retinoids may play a role in the prevention and treatment of tissue disorders, including cancer.[42,95] Therefore, it seemed logical to conclude that within the retinoid family certain chemotherapeutic agents may exist which might reduce the number of deaths resulting from epithelium malignancies.[2]

A problem with vitamin A (**1**) or its esters is that they are stored in the liver, and thus it is not possible to raise its concentration significantly in the blood stream.[88,89] In addition, it has been shown that upon administration of high concentrations the natural retinoids become toxic leading to a condition called as "hypervitaminosis".[18] Thus, the natural retinoids have limited clinical utility and this has provided an impetus to search for modified retinoids which might possess not only enhanced activity but also low toxicity.

Mechanism of Retinoid Action

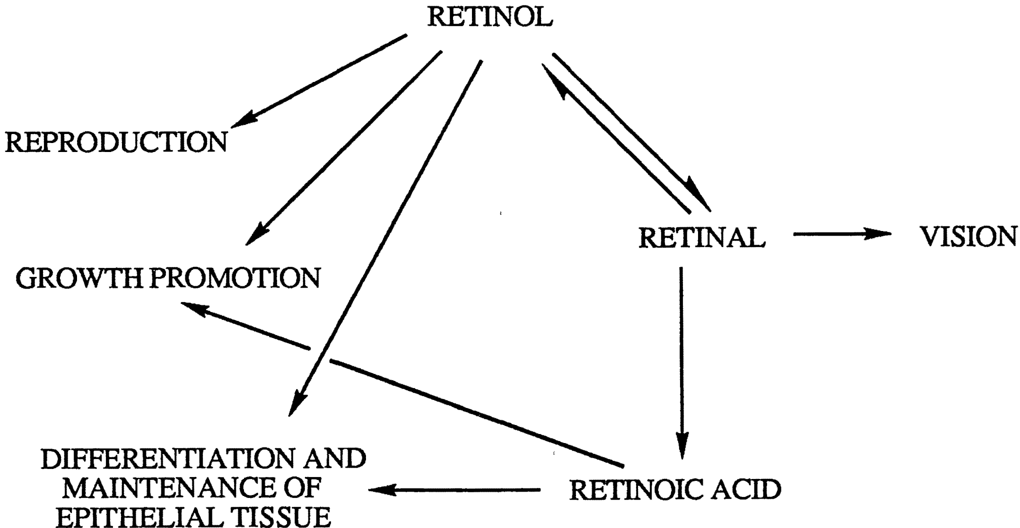
To date, there are various mechanisms that have been proposed to explain the mechanism of retinoid action.[33,39,40,43,72,81,89] It was shown by Green and co-workers that retinoids suppress the synthesis of a 67-kDa keratin (a fibrous protein) at the level of mRNA synthesis.[35] Moreover, Wang and co-workers[107] found that *trans*-retinoic acid (**4**) regulated the transcription of nucleotide sequences in DNA of F9 cells (a certain type of murine cancer cell). In 1935 Wald demonstrated the importance of "retinene", a vitamin A derivative in the visual cycle.[104,105] Later, Morton established the identity of retinene as vitamin A aldehyde [retinal (**2**)], which is the product of enzymatic cleavage of ß-carotene (**3**) via synthesis from retinol (**1**)[67,68] In other tissues, the fate of retinol (**1**) is not yet clear and is still a subject of investigation.[39]

Retinoic acid (4) was shown to be important for growth.[1] Intracellularly, retinoic acid (4) binds to cellular retinoic acid binding protein (cRABP)[16,73,83,85,86] and can be translocated within the cell as the RA-cRABP complex.[83] It is believed that cRABP may have a major role in mediating some of the biological effects of retinoic acid (4).[16,58,100] Most normal cells also appear to possess a 14, 600-dalton, cellular retinol-binding protein (cRBP) that binds specifically to retinol (1). This protein, although immunologically different from cRABP, has a similar molecular weight and a related amino acid sequence.[27] Nevertheless, the cRBP and cRABP each appear to have a single specific retinoid binding site.[15] A recent discovery made by Chambon, Evans and co-workers identified a protein receptor that contained a DNA binding domain as well as a ligand binding site.[38,77] Among the possible ligands, it was found that *trans*-retinoic acid (4) was a ligand to which the polypeptide receptor bound not only specifically but also with a high degree of affinity. A cloned full length cDNA was isolated and characterized by Evans and co-workers.[38] This cDNA (called as λhK1R) encodes a 462 amino acid polypeptide whose molecular mass is 50,772 and is labelled as hRR (human retinoic acid receptor). Experimentally, they discovered a gene sequence whose polypeptide product contained a DNA binding domain as well as a region for the specific binding of *trans*-retinoic acid (4). The retinoids may function in a more sophisticated manner then realized at present, but the work of Evans, Chambon, and co-workers provides insight as to how retinoid "mimics" might be constructed which could possibly be quite effective in controlling the replication of malignant cells.

There is evidence which suggests that the transportation of vitamin A (1) involves two plasma proteins, namely RBP (retinol binding protein) and transthyretin.[39,90] Vitamin A (1) is bound to RBP as a complex and this, in turn, forms another protein-protein complex with transthyretin.[91] It is these proteins which play an important role in the translocation of vitamin A (1) from its storage point to the tissues. Deficiency of retinol (1) blocks RBP secretion with the consequence that while the level of plasma RBP falls, the level of the

liver RBP rises.[39] It has been shown that when retinol (**1**) was injected into vitamin A deficient rats there was a rapid secretion of RBP from the liver into the plasma.[103] In the treatment of actinic and non-actinic keratosis, it was revealed that retinal (**3**) is effective in concentrations between 0.05 and 1.00 weight percent in 95% ethanol or propylene glycol.[76,112]

The interrelationship of retinol (**1**), retinal (**2**) and retinoic acid (**4**), as well as their biological roles has been reviewed by Lotan[58] and is outlined in the diagram below. In a reversible process, retinol (**1**) is oxidized *in vivo* to retinal (**3**) which is important in the



vision process. Although retinoic acid (**4**) is a major oxidative metabolite of retinol (**1**), it is rapidly excreted, unlike **4** which is stored in the liver. Retinol (**1**) is necessary for growth, differentiation, maintenance of epithelial tissue, and also for reproduction. Retinoic acid (**4**) can substitute for retinol (**1**) in vitamin A deficient animals in growth promotion, in epithelial differentiation and in maintenance. However, **4** can neither substitute completely for retinol (**1**) in maintaining the reproductive function nor replace retinal (**3**) in the visual cycle.

Arotinoids and Heteroarotinoids

Incorporation of an aromatic ring into the skeleton of a retinoid system in some cases vastly improves the therapeutic ratio relative to retinoic acid (**4**) by a factor of as much as 10.[93,94] Such compounds are called "arotinoids", and the name has been reserved for systems containing an aryl ring within the side chain or fused to the cyclohexyl ring.[94] Some of these retinoids were prepared at Hoffmann-La Roche by Bollag and co-workers[8] during the 1970's. Swiss albino mice were tested and the therapeutic ratio of test retinoids was determined from the dose (mg/kg) which caused regression of papillomas in the mice relative to that dose which produced hypervitaminosis. Many arotinoids have shown a high activity in the tracheal organ culture (TOC),[20,84] ornithine decarboxylase (ODC),[103] and human promyelocytic leukemia cell line (HL-60)[10-13] assays. However, a major disadvantage was the high toxicity of arotinoids. Thus, although these arotinoids appeared quite promising, alternate compounds had to be found in order to decrease the high toxicity. Several research groups have prepared modified retinoids with goals to produce both high activity and low toxicity.[20,21,79,96,108] Efforts have been expended to change the cyclohexyl skeleton, leaving the rest of the molecule intact as shown below.

Polyene chain

Nonpolar end

Polar end

1 [Retinol, vitamin A]

It has been found that replacing C(4) with heteroatoms, such as O, NR, or S, produced "heteroarotinoids" with useful properties. These new compounds have been investigated separately by the research groups of Berlin[79,96,108] and Dawson.[20-27,64] Heteroarotinoids

are a class of retinoids which possess at least one aryl ring and a heteroatom, usually in the partially saturated ring as shown. One can see that there is a resemblance in **19** to the basic

4 **19**

structure in *trans*-retinoic acid (**4**) as illustrated by the broken lines in the drawing. A few papers have appeared on the chemistry and structure-activity (SA) relationships[24,49-51,64,79,96,108] of some heteroarotinoids, but the data are relatively sparse to date. However, preliminary results from two studies[24,37,55] on the toxicity of certain heteroarotinoids have indicated the presence of the heteroatom resulted in reduced toxicity compared to *trans*-retinoic acid (**4**) which has exhibited powerful activity in several assays but is highly toxic.[24] The heteroarotinoids **20-51** currently in the literature or available in our laboratory are illustrated.

20 X = O, R = CO_2Me
21 X = S, R = CO_2Me
22 X = O, R = CO_2H
23 X = S, R = CO_2H

24 X = O, R = CO_2Me
25 X = S, R = CO_2Me

26 X = O, R = CO_2Me

27 X = S, R = CO_2Me

28

29 X = O, R = CO_2H

30 X = S, R = CO_2H

31 R = CO_2Et

32 R = CO_2H

33

34 Z = CO_2H

35 Z = CO_2CH_3

36 Z = CN

37 Z = CHO

38 Z = CH_2OH

39 Z = CH_2NH_2

40 Z = $C(O)CH_3$

R, Ar

41 Ar = [furan-2-yl] CH_2OAc

42 Ar = 4-$C_6H_4CO_2CH_3$

R = CH_2OH

R, Z

43 Z = CO_2CH_3, R = CH_3

44 Z = CN, R = CH_3

45 Z = CO_2CH_3, R = CH_2OH

46 Z = CO_2H, R = CH_2OH

CO_2H, H, N, O

47

CO_2H, O, N, H

48

CH_3, N, O, CH_2OH

49

O, N, CH_3, CH_2OH

50

51

52

A commonly held belief is that the carboxylic acid forms of retinoids are the active forms *in vivo* because of their ability to bind to cRABP, and therefore many tests have been reported for retinoids containing the carboxylic acid moiety as shown in Table I.[7] The arotinoids prepared by Dawson and co-workers[20-25] also showed good activity as illustrated in Table II. Among these, the naphthalene derivative **52** showed good activity in the ornithine decarboxylase (ODC) assay and also an activity better than retinoic acid in the tracheal organ culture (TOC) assay.[24] Later, it was found that **52** is extremely toxic.[24,37,55] The heteroarotinoids **20-23** have been assessed both for their ODC activity and their ability to induce differentiation in HL-60 cells.[37] Results of the ODC assay correlate well with the ability of the test substance to inhibit tumor formation in mice and for the general procedure see the section on Assays of Retinoids. It can be inferred from Table III[37] that the heteroarotinoids **21** and **23**, which contain a sulfur atom, showed very high activity as compared to the control **4**. The oxygen containing heteroarotinoids **20** and **22** showed less activity than **4**. The increase in activity upon replacing oxygen with sulfur has also been noted by Berlin and co-workers.[96]

In order to provide a background for the discussion of our results, it is necessary to describe a working hypothesis which was to be tested via several bioassays of the expected products. If binding to a transport molecule is important in the biological function of *trans*-retinoic acid (**4**), it is implied that any retinoid mimic must possess certain structural features for effective binding. Such parameters have never been probed from the viewpoint of a heteroarotinoid mimic. Although it is conceivable that the nonpolar, hydrocarbon ring

TABLE I

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

Arotinoid	Induction of differentiation HL-60 assay[b] ED_{50}	Complete inhibition of scale formation, M
CO_2H 4[c]	1×10^{-7} (1×10^{-8})[d]	10^{-5}
CO_2H 53	8×10^{-8}	10^{-7}
CO_2H 54	8×10^{-9}	10^{-8}
CO_2H 55	e	10^{-7}
CO_2H 56	7×10^{-8}	10^{-8}

[a]Reference 7. [b]See Assays of retinoids. [c]For comparison only, not an arotinoid. [d]Reference 12. [e]Not reported.

TABLE II

ACTIVITY OF SELECTED AROTINOIDS IN THE TOC AND ODC ASSAYS[a]

Arotinoid	TOC Assay ED_{50}, M (mg/kg/day)	ODC dose, nmol	ODC % inhibition[c] of control
4[b]	1×10^{-11}	1.7	88
52	3×10^{-12}	17	80
		1.7	56
56	1×10^{-12}	17	91
		1.7	89
57	6×10^{-10}	17	69
		1.7	33

[a]Reference 13

[b]For comparison only, not an arotinoid

[c]
$$\% \text{ Inhibition} = \frac{[100 \times \text{ODC activity (control)} - \text{ODC activity (retinoid)}]}{\text{ODC activity (control)}}$$

TABLE III

ODC ACTIVITY OF HETEROAROTINOIDS **20-23**[a]

20 X = O, R = CO_2Me
21 X = S, R = CO_2Me
22 X = O, R = CO_2H
23 X = S, R = CO_2H

Test system	Retinoid dose, nmol	ODC Activity	Percent Inhibition[b]
Acetone + TPA	0	5.3 ± 0.7[c]	0 (control)
4 + TPA	34	1.0 ± 0.1[c]	81
20 + TPA	34	1.5	72
Acetone + TPA	0	1.02[d]	0 (control)
4 + TPA	34	0.13[d]	87
21 + TPA	34	0.062[d]	94
22 + TPA	34	0.283[d]	72
23 + TPA	34	0.09[d]	91

[a]Reference 37

[b]Percent Inhibition = $\dfrac{\text{ODC activity (control) - ODC activity (retinoid + TPA)}}{\text{ODC activity (control)}}$

[c]nmol CO_2/60 min/mg protein

[d]nmol CO_2/30 min/mg protein

system and polar carboxyl group at opposite ends of the molecule in *trans*-retinoic acid (**4**) are critical for binding, one might envision that the replacement of certain atoms in the nonpolar section might be tolerated which in turn would also produce a mimic with greater aqueous solubility. Thus it was decided that a somewhat more polar group would be inserted into the nonpolar end of some heteroarotinoids.

The presence of the geminal dimethyl groups in the partially saturated ring system of most natural retinoids is well documented.[94] The size of two methyl groups might be assessed on the basis of van der Waals radii and expected C-C bond lengths. As a substitute, a group with nonbonding pairs of electrons might be inserted for the $C(CH_3)_2$ group. The choice of such a substitute group is limited since it would be anticipated that a steric factor might arise with a large group or additional interactive forces might prevent or alter binding in an unacceptable manner if, for example, hydrogen bonding were involved with an O-H, N-H, or S-H group. If one compares structural characteristics of an oxygen atom (with its two sets of nonbonding electron pairs) to the $C(CH_3)_3$ group, the former will occupy less space [van der Waals radii for O (140 pm, 1 pm = 10^{-2} Å) and CH_3 (200 pm)[5]]. The smaller radius for oxygen would be expected since the lone pairs of electrons are drawn closer to the nucleus.[5] In an ideal situation, that is, when a tetrahedral carbon is surrounded by four identical groups or atoms, the bond angles will be 109° 27'.[5] In the case of propane (**58**), the C-C-C bond angle (112°) is larger than the tetrahedral angle owing to the "Thorpe-Ingold" effect.[5] The latter arises when groups attempt to maximize nonbonding contact. This, in turn, causes a compression of the angle between the remaining two groups, especially if the groups are C-H. In case of the ether **59**[5], the bond angle between the two methyl groups is 111° which suggests that the angle between the orbitals of the two sets of nonbonding electron pairs is compressed. Thus orbitals holding nonbonding pairs are commonly more compressed than orbitals holding bonding electron pairs such as in C-C bonds or in C-H bonds. An interesting point of comparison concerning the C-O-C angle is found in **60** from X-ray diffraction data on a crystal.[4] The

internal angle in question was determined to be 114.0°(4)[4] which indicates considerable expansion probably to minimize internal strain resulting from both attached or nearby groups as well as from angular deformation in the ring. Thus, the two orbitals on the oxygen atom in **60** must be compressed and are possibly shielded to some degree by the adjacent methylene hydrogens and the peri hydrogen as illustrated above. Since **60** is a close model system to compounds in our work, analogous conclusions regarding structural characteristics may be reasonable but must be taken with caution since no X-ray data are available on our systems.

Assays of Retinoids

There are numerous methods developed to test for antitumor activity in a retinoid.[94] Among these, two methods shall be discussed briefly, namely: (1) *in vivo* methods[102,103] [ornithine decarboxylase (ODC) and tracheal organ culture (TOC) assays], and (2) *in vitro* methods[12,13][the human promyelocytic leukemia cell line (HL-60) assay]. Regarding the *in vivo* method, Verma and co-workers[102,103] have shown that the ability of a test substance (in our case, a retinoid) to inhibit the biosynthesis of the enzyme ornithine decarboxylase (ODC) can be measured and reflects the extent to which the test substance is capable of inhibiting skin tumor production. A potent promoter of cancerous activity is 12-*O*-tetradecanoylphorbol-13-acetate (TPA), and the ability of a retinoid to inhibit ODC synthesis can be regarded as a measure of its ability to inhibit skin tumor promotion.[102,103]

In this method, the backs of mice are shaven 3-4 days prior to treatment with TPA. An hour prior to treatment with TPA (8-17 mmol in acetone), mice are pretreated with a test retinoid (usually 17 or 34 mmol in acetone). After 4-5 hours, the mice are sacrificed and the epidermis is separated, homogenized, and centrifuged. The ODC activity is then determined from the soluble extracts by measurement of the release of $^{14}CO_2$ from ^{14}C-labelled ornithine. The percent inhibition of ODC is determined as follows:

$$\% \text{ Inhibition} = \frac{[100 \times \text{ODC activity (control)} - \text{ODC activity (retinoid)}]}{\text{ODC activity (control)}}$$

The experiment is run simultaneously with mice treated similarly but with a standard, for example all-*trans*-retinoic acid (**4**), which shows high activity in this assay.[24,94,96,108]

Regarding an *in vitro* assay, one such method is called the HL-60 assay which is used to assess the potential of the test substance to induce differentiation in cells derived from a patient with acute promyelocytic leukemia.[12,13] The HL-60 cells do *not* produce superoxide anions upon stimulation by agents like TPA, whereas differentiated HL-60 cells do so. The presence of such anions can be detected by their ability to reduce the water soluble yellow dye nitroblue tetrazolium (NBT)[3] to the water insoluble blue-black formazan[13] by phagocytizing neutrophils,[87] a reaction mediated by superoxide ions, (O_2^-) obtained from the reduction of oxygen during phagocytosis.[17] The percentage of differentiated cells (obtained by counting the number of cells containing this dark precipitate under a light microscope) is a direct indication of the ability of a test substance (retinoid) to induce differentiation and is also one convenient way to determine ED_{50} values.[7,13,99]

It should be noted that the positive or negative results from any one assay do not eliminate or establish the potential carcinostatic activity of a test retinoid *in vivo* in humans.[102] For a nearly complete biological profile, several tests are necessary.[103]

Retinoids in Clinical Use

There have been numerous studies relating retinoids to epidermal disorders and cancer.[93,94] However, in the U.S. only two retinoids are currently used for the dermatologic conditions and none are in use for the treatment of cancer.[109] Accutane®, the trade name for 13-*cis*-retinoic acid, or isoretinoin (**61**), is the only retinoid approved for oral use.[109] Acid **61** has been adopted for the systemic treatment of severe cystic and conglobate acne where the usual remedies have proven ineffective.[109] In fact, 13-*cis*-retinoic acid (**61**) proved sufficiently promising to reach clinical trials in the treatment of cancer of skin and bladder.[109] Tretinoin [all-*trans*-retinoic acid (**4**)] has been effective in treating acne topically[109] and was found to be more selective than vitamin A (**1**) in preventing established epithelial tumors from developing in mice.[109] Tigason® (**62,**

4 [Tretinoin]

5 [Retinyl acetate]

61 [Accutane or Isoretinoin]

62 [Etretinate or Tigason]

Etretinate), a synthetic retinoid, has attracted considerable attention in Europe for the treatment of a large number of previously highly resistant skin disorders.[18] Ester **62** has been introduced clinically (orally) for treating complicated psoriasis and icthyotic conditions.[109] Etretinate (**62**) acts by normalizing the keratinization process and its effect is largely suppressive rather than curative. Retinyl acetate (**5**), given orally, has been shown to abolish carcinogen-induced mammary cancer in mice.[109]

Toxicology of Retinoids

Natural vitamin A (**1**) is too unselective and highly toxic in the doses required for a positive therapeutic response and causes gastrointestinal disorders, fatigue, cracking of lips, anaemia, liver dysfunction, visual disturbances, pain in limbs and intracranial hypertension.[56,101,109] In general, the toxic effects of retinol (**1**), *trans*-retinoic acid (**4**) and 13-*cis*-retinoic acid (**61**) have been well documented.[56,101] The toxic side effects of retinol (**1**), *inter alia* include chelitis, severe headaches, conjuctival inflammation, nausea, vomiting, bulging fontanelles in infants, dryness and scaling, and tenderness of bones.[101] Side effects from the topical treatment of all-*trans*-retinoic acid (**4**, Tretinoin) include skin irritation (redness and scaling) and reversible hypopigmentation.[56] Side effects from the oral treatment of 13-*cis*-retinoic acid (**61**, Accutane) include abdominal pain, conjunctivitis, chelitis, xerosis, and excessive thirst.[101] Tigason (**62**, Etretinate) has side effects which include fissured lips, dryness of mouth and nasal mucosa, and alopecia. Two additional problems are: (1) increasing reports of abnormalities in liver function in patients who received this drug and (2) marked teratogenic properties due to long half-life of the drug after chronic therapy.[56]

CHAPTER II

RESULTS AND DISCUSSION

Over the past few years, the research groups of Berlin[79,96,108] and Dawson[20-25] have synthesized several heteroarotinoids with a stilbene (1,2-diarylethene) skeleton which show good activity in selected assays. As discussed earlier (see the section on Chemistry and Biology of Retinoids), the impetus for the search of new heteroarotinoids arose from the desire to reduce the inherent toxicity associated with most retinoids. The X-ray crystallography[30] has shown that certain stilbene arotinoids resemble *trans*-retinoic acid (**4**) structurally and also bind well with cellular retinoic acid binding proteins (cRABP).[64] The commercial availability of 1,4-benzodioxan-6-yl methyl ketone (**63**) allowed an extension of our original work to yield several relatives which could be potential metabolites of the basic systems namely, acid **4** and ester **5**.

Several assays[24,57,96,108] have revealed that the arotinoids and heteroarotinoids are biologically quite active. Deluca and co-workers[69] have demonstrated that certain active metabolites obtained from the metabolism of *trans*-retinoic acid (**4**) contained polar groups. Along the same lines, we felt that incorporating a heteroatom into the cyclohexyl skeleton and attaching a polar group in the side chain would not only increase the polarity of the molecule but also enhance its ability for hydrogen bonding. The objectives of our work were to obtain heteroarotinoids with high hydrophilicity and, hopefully, low toxicity.

In our laboratory, we have been able to develop methods which have produced a family of heteroarotinoids **64-69** as illustrated in Figure 1. In addition to alcohol **68,** we reasoned that the incorporation of a glycerol derivative might enhance transport *in vivo*

CO_2R 10 5 9 12 3 2 11 7 8

64 R = Me

65 R = H

10 15 5 12 14 CO_2R 17 3 9 11 13 16 2 7 8

66 R = Et

67 R = H

OH

68

CO_2Me

69

Figure 1. Structures of New Heteroarotinoids **64-69**

and improve formulation of such a material for administration purposes. We have been able to obtain ester **69** but only in a low yield to date although we are working to optimize the yield. Acid **65** is currently at the National Cancer Institute for screening in a new evaluation program with a large variety of assays involved. We have been informed that the tests will be made available sometime in 1990.

Syntheses of New Heteroarotinoids

The six new heteroarotinoids **64-69** can be categorized into two groups, namely (1) **64**, **65**, **68** and **69** which have incorporated an aryl moiety that confers a locked cisoid conformation, and (2) **66** and **67** which possess a triene side chain similar to the natural retinoids. The fixed trans geometry in the first group of compounds **64**, **65** and **69** is believed to be responsible for biological activity in similar systems.[16] Certain heteroarotinoids containing a triene side chain have also shown good activity. In one HL-60 assay,[96] heteroarotinoids with the octatrienoic carboxyl side chain have shown better activity than the stilbene like heteroarotinoids.

Initiation of the synthesis of the heteroarotinoid **64** began with the reduction of commercial ketone **63** to the alcohol **70** using $LiAlH_4$. In early attempts to make **70**, excess $LiAlH_4$ was destroyed by treatment with ethyl acetate. However, even after drying **70** overnight in the Abderhalden at room temperaure, the presence of ethyl acetate was visible in the ^1H NMR spectrum. Therefore, we elected to quench the excess $LiAlH_4$ very cautiously (dropwise) by adding ordinary tap water to a flask previously cooled with ice and water. It was observed that employing such a method produced a clean ^1H spectrum of **70**. Alcohol **70** was obtained in a yield of more than 98%. It was converted to the phosphonium salt **71** by treatment with triphenylphosphine hydrobromide ($Ph_3P{\cdot}HBr$) in methanol as shown in Figure 2.

63

$LiAlH_4$

THF

70

$Ph_3P\ HBr$

MeOH

71

$\overset{+}{P}Ph_3$, $\overset{-}{Br}$

1. *n*- Buli/hexanes, -78°C

2. p-OHC-C_6H_4-CO_2Me

(72)

CO_2CH_3

64

1. NaOH/H_2O/EtOH

2. Conc. HCl

CO_2H

65

Figure 2. Synthesis of **64** and **65**

Isolation of salt **71** posed problems in that a foam obtained upon evaporation of the solvent had to be crushed with a spatula, and this proved difficult on many occasions. The problem was circumvented by evaporating the solvent at 75°C, over a half hour period during which time formation of the foam was maximized. The phosphonium salt obtained

in this manner could be used directly without any further purification. Ester **64** was generated by forming the Wittig reagent from **71** with a slight excess of *n*-BuLi and then adding an equimolar amount of methyl 4-formylbenzoate (**72**) at -78°C. The temperature was allowed to rise to room temperature over 48 h. Ester (*E*)-**64**, was isolated as white flakes (mp 91.5-92.5°C) in a yield of 25%. Saponification of (*E*)-**64**, using NaOH in absolute alcohol:tap water (1:3), yielded acid (*E*)-**65** which had a high melting point (mp 171-172°C) and appeared as shiny white crystals.

Syntheses of triene heteroarotinoids **66** and **67** began by treating starting ketone **63** with vinylmagnesium bromide to yield the alcohol **73**. The reaction was initiated with a tiny amount of methyl iodide, and **73** was obtained in a quantitative yield. Alcohol **73** was converted to phosphonium salt **74** using $Ph_3P \cdot HBr$ in methanol. Salt **74** was utilized without purification to form a Wittig reaction using *n*-BuLi in hexanes. Treatment of the Wittig reagent with ethyl-3-methyl-4-oxocrotonate (**75**) gave ester **66** (28% yield) as fine yellow crystals (mp 86-87°C). Saponification of **66**, using KOH in absolute alcohol:tap water (2:1), gave the carboxylic acid **67** as yellow crystals (35%)with a melting point of 181-182°C. The scheme for the preparation of the triene heteroarotinoids **66** and **67** is outlined in Figure 3.

Oxidation of allylic carbons to give allylic alcohols has been well documented.[19] To prepare the heteroarotinoid **68**, ester **64** was treated with SeO_2 in 95% ethanol at reflux over 24 h. During the course of the reaction, elemental selenium precipitated and was filtered out during workup. A yellow oil was obtained, which upon being subjected to separation by chromatography using the Chromatotron [silica gel (7:3, hexanes:EtoAc)],

Figure 3. Synthesis of **66** and **67**

gave the allyl alcohol **68** (20%). A scheme for the method is shown in Figure 4. The mechanism believed to be operating in the formation of allylic alcohols using SeO_2 has been reported.[110]

The glycerol derivative **69** was prepared from the acid **65** using dicyclohexylcarbodiimide (DCC),[70] 4-dimethylaminopyridine (DMAP), and 2,2-dimethyl-1,3-dioxolane-4-methanol (**76**, Solketal) as illustrated in Figure 5.

Figure 4. Preparation of Heteroarotinoid **68**

Figure 5. Preparation of Heteroarotinoid **69**

A massive excess of the reagents compared to acid **65** was required since stoichiometric amounts resulted only in the recovery of the starting material. The yield of **69** is low and we are currently trying alternate conditions to optimize the same.

Spectral Data for Heteroarotinoids **64-69**

In general, the C=O group in the IR spectra for **64-69** were observed in the range of 1690 to 1720 cm^{-1} depending upon conjugation. In the case of acids **65** and **67**, a broad peak from 2400 to 3400 cm^{-1} was diagnostic for the carboxyl group. In the proton NMR spectrum, the aromatic protons appeared in the range of ∂ 6.8-7.8. The protons H(2) and H(3) were visible as one sharp singlet around ∂ 4.27 for **64-69** while C(2) and C(3) appeared at 64 ppm in the ^{13}C NMR. The vinyl proton H(11) in **64**, **65** and **69** was consistently visible as a broad singlet around ∂ 6.8. In the case of alcohol **73**, the J value for the cis coupling between H(11) and H(12a) was found to be 10.6 Hz while that for the ttrans coupling between H(11) and H(12b) was 17.2 Hz. These values agree well with the observed normal cis and trans J values.[75] A comparison of the UV spectra of the diaromatic compounds **64** and **65** versus the triene compounds **66** and **67** reveals that the λ_{max} in the former is around 312 nm (*trans*-stilbene has a λmax of 295 nm[89]) whereas that of the latter is around 347 nm. This is expected due to increased conjugation in the triene compounds.[75]

For testing the activity of our compounds, ester **64** and acid **65** were sent to the National Cancer Institute (NCI) more than 1.5 years ago. The NCI personnel have indicated that the testing will be done in 1990. We have also sent the above samples to the Cancer Center at the University of Wisconsin for testing by Dr. A. K. Verma, and we are awaiting the results. We are hopeful that the blockage of oxidative sites and increased solubility would result in enhanced biological activity as compared to their hydrocarbon

counterparts. Moreover, Dr. Verma will determine if the compounds complex with RABP. He would also screen compounds **64** and **65** in certain tumor lines which he has in house.

Conclusions

In conclusion, the objectives of the work have been met to some degree with the development of the syntheses of the target 1,4-benxodioxans. Both systems have been obtained, namely that with a phenyl ring as part of the side group and that with a polyene chain as the attached side group. In addition, we have been able to regiospecifically oxidize the methyl group on the double bond to a hydroxymethyl function. This compound is a potential metabolite of the parent ester **64** and acid **65**. We have also prepared, although in low yield, a glycerol derivative of the acid **65**. The glycerol ester **69** is definitely more hydrophilic than either ester **64** or acid **65**, and thus the former may be more easily formulated for improved biological assay. These novel systems have an oxygen atom (with lone pairs) at the 4-position rather than the geminal dimethyl group normally associated with retinoids related to *trans*-retinoic acid (**4**). The presence of the two orbitals containing two pairs of electrons will offer an opportunity to probe the capability of the RABP to bind such a system for the generation of a complex that is transported to the cells. Unfortunately, no biological data has arrived as yet from NCI or Dr. Verma to allow us to assess our reasoning for improved design of other heteroarotinoids. Future research in the area should take such biological data into account before arriving at a strategy for constructing new members of this family of heterocycles.

Suggestions for Future Work

The glycerol derivative **69** was synthesized as a prodrug on the assumption that cleavage of the ketal would likely occur *in vivo*. However, to date, we have obtained **69** only in low yields. One can attempt to increase the yield of **69** by employing alternate

approaches such as by using a Ph_3P/CCl_4 system,[80] or by employing the acid chloride method in the presence of a powerful amine. Once a suitable esterification method has been found, one can envision an array of such glycerol derivatives of the known heteroarotinoids, if activity is at a useful level in appropriate assays.

Our present work has focused on the synthesis of 1,4 benzodioxan derivatives, which contain incorporated within them oxygen atoms at the 1- and 4-positions. There is evidence which suggests that toxicity reduction is associated with compounds containing sulfur as the heteroatom.[24] Therefore, it would be interesting to synthesize and determine the biological activity of similar compounds which contain sulfur as the heteroatom(s) instead of the oxygen(s).

CHAPTER III

EXPERIMENTAL

All reactions were carried out in an N_2 atmosphere using a magnetic stirrer unless otherwise stated. Solvents were concentrated using a rotary evaporator. NMR data was obtained using a Varian XL-300 NMR spectrometer. The NMR spectra were recorded at 300 MHz and 75.43 MHz for ^1H and ^{13}C, respectively. NMR data were recorded in δ or ppm values downfield from TMS using $DCCl_3$. IR spectra were taken on a Perkin-Elmer 681 IR spectrophotometer. All IR spectra were recorded as films or KBr pellets (for solids). UV spectra were taken on the Varian UV Visible Spectrophotometer Model DMS 200 equipped with an Epson LX-800 printer. Melting Points were determined using a Thomas Hoover melting point apparatus and are uncorrected. Mass spectra were taken on a VG Analytical Instrument Model ZAB-2SE.

1-(2,3-Dihydro-1,4-benzodioxan-6-yl)-ethanol (**70**)

A 300-mL, three-necked, round-bottomed flask equipped with a magnetic stirrer, addition funnel and N_2 inlet (positive pressure from an oil bubbler) was flushed with N_2 for 5 min. Into this flask was placed $LiAlH_4$ (1.264 g, 33.3 mmol) and dry THF (10 mL). A little "fizzing" was observed, and the resulting suspension was stirred under N_2 (2 min). A solution of the ketone (**63**, 2.0 g, 11.24 mmol) dissolved in dry THF (20 mL) was added dropwise via the addition funnel over a period of 10 min. This mixture was boiled for 24 h during which time dry ether (2 x 10 mL) was added after 8 h and after 16 h to

maintain the volume. After the flask had attained room temperature (about 1 h), it was cooled with ice (30 min). Very cautious addition of 5 mL of tap water followed from an addition funnel over 20 min to destroy excess $LiAlH_4$. A white precipitate formed. While still cool, the solution was treated with 5% HCl (80 mL) over 10 min with stirring. The precipitate fragmented and two layers formed with the aqueous layer containing a suspension. After being stirred for 5 min, the contents of the flask were transferred to a separatory funnel, and the aqueous layer was extracted (ether, 5 x 15 mL). The combined ether extracts were washed with 5% Na_2CO_3 (4 x 25 mL) and finally with saturated NaCl (4 x 25 mL). The ether extracts were dried (Na_2SO_4, overnight) and evaporated (rotovap) to yield a colorless oil. Removal of traces of solvent (Abderhalden, RT, 0.025 mm, 2 h) gave the alcohol **70** as a colorless oil (2.009 g, 98.68%). The material was used without further purification: IR (neat) 3700-3100 (O-H) cm^{-1}; ^1H NMR ($DCCl_3$) ∂ 1.45 [d, 3 H, H(10)], 4.25 [s, 4 H, H(2,3)], 4.79 [q, 1 H, H(9)], 6.87 [m, 3 H, Ar-H(5,7,8)]; ^{13}C NMR ($DCCl_3$) ppm 24.96 [C(10)], 64.35 [C(2,3)], 69.91 [C(9)], 117.19 [C(8)], 118.46 [C(7)], 139.29 [C(6)], 142.82 [C(8a)], 149.39 [C(4a)]. Anal. calcd. for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.84; H, 6.66.

[1-(2,3-Dihydro 1,4-benzodioxan-6-yl)ethyl]triphenyl-
phosphonium Bromide (**71**)

In a single-necked, 300-mL, round-bottomed flask equipped with a magnetic stirrer and N_2 inlet (positive pressure from an oil bubbler) was placed $Ph_3P{\cdot}HBr$ (5.33 g, 15.54 mmol) in dry CH_3OH (25 mL). To this suspension, was added, dropwise, via an addition funnel, a solution of the alcohol (**70**, 2.813 g, 15.54 mmol) in dry CH_3OH (20 mL) over a peroid of 10 min. Additional dry CH_3OH (60 mL) was added to form a homogenous solution, and the resulting clear colorless solution was stirred at room temperature under N_2 (24 h). Upon rotary evaporation, a colorless foam (which solidified) was obtained.

The foam was partially crushed with a spatula, and dry ether (30 mL) was added. The suspension was stirred at room temperature under N_2 (3 h), filtered (aspirator suction), and washed (dry ether, 25 mL). Removal of traces of solvent (Abderhalden, RT, 0.025 mm, 10 h) gave **71** as an off-white powder (7.72 g, 98.30%). The material was used without further purification: mp 153-158°C(dec); IR (KBr) 1110 (C-O) cm^{-1}. ^1H NMR ($DCCl_3$) ∂ 1.74 [dd, 3 H, H(10)], 4.24 [s, 4 H, H(2,3)], 6.25 [m, 1 H, H(9)], 6.41-6.78 [m, 3 H, Ar-H(5,7,8)], 7.46-7.85 [m, 15 H, $P(C_6H_5)_3$].

Methyl (*E*)-4-[2-(2,3-Dihydro-1,4-benzodioxan-6-yl)-
1-propenyl]benzoate (**64**)

A three-necked, 300-mL, round-bottomed flask equipped with a magnetic stirrer, addition funnel, rubber septum, and N_2 inlet (positive pressure from an oil bubbler) was flushed with N_2 for 5 min. In this flask was placed **71** (4.83 g, 9.57 mmol) in dry ether (10 mL). To the stirred suspension was added dropwise *n*-BuLi (2 mL, 0.906 M, 1.81 mmol) in hexanes over 2 min at room temperature. The appearance of the solution changed from colorless to light brown to dark brown at the end of the addition period. The solution of the anion was stirred at room temperature (30 min). This Wittig reagent was then cooled in a dry ice-acetone bath to -78°C for 1 min, and a solution of methyl 4-formylbenzoate (**72**, 1.57 g, 9.57 mmol) in dry ether (10 mL) was added via the addition funnel over 3 min. After being stirred for an additional 5 min in the cold bath, the mixture was allowed to stir at room temperature for 48 h. The color changed from brown to yellow when temperature of the mixture reached room temperature and persisted throughout the period. The contents of the flask were filtered (aspirator suction) and washed (dry ether, 100 mL). Evaporation (rotovap) of the filtrate and removal of traces of solvent (Abderhalden, RT, 0.025 mm, 30 min) yielded a yellow oil which solidified at room temperature. Recrystallization (minimum absolute EtOH) and seeding with a pure crystal gave ester **64**

(0.747 g, 25.19%): mp 91.5-92.5°C; IR (KBr) 1710 (C=O) cm^{-1}; ^1H NMR ($DCCl_3$) ∂ 2.24 [d, J = 1.4 Hz, 3 H, H(10)], 3.92 [s, 3 H, H(1)], 4.27 [s, 4 H, H(2,3)], 6.78 [br s, 1 H, H(11)], 6.85-8.04 [Ar-H]; ^{13}C NMR ($DCCl_3$) ppm 17.6 [C(10)], 52.1 [C(19)], 64.4 [C(2,3)], 114.9 [C(5,8)], 117.1 [C(11)], 119.2 [C(7)], 125.6 [C(13,17)], 127.8 [C(14,16)], 129.0 [C(15)] 129.4 [C(6)], 137.0 [C(9)], 138.7 [C(12)], 143.2 [C(4a,8a)], 166.9 [C(18),(C=O)]; mass spectral data for $C_{19}H_{18}O_4$: m/e (M^+) 310.1205; Found: 310.1206. Anal. calcd. for $C_{19}H_{18}O_4$: C, 73.53; H, 5.84. Found: C, 73.75; H, 5.82.

(*E*)-4-[2-(2,3-Dihydro-1,4-benzodioxan-6-yl)-
1-propenyl]benzoic Acid (**65**)

In a single-necked, 25-mL, round-bottomed flask equipped with a magnetic stirrer, spiral condenser, and N_2 inlet (positive pressure from an oil bubbler) was placed the ester (**64**, 0.2 g, 0.644 mmol). To this solid was added NaOH pellets (0.129 g, 3.23 mmol), absolute alcohol (3 mL), and tap water (9 mL). The suspension became clear on warming and was boiled for 5 h. After cooling to room temperature (about 30 min), concentrated HCl (12 N) was added dropwise with stirring until the solution became acidic to litmus. The white precipitate formed was filtered, and the filtrate was checked for any further precipitation (conc. HCl was added until precipitation ceased). The precipitate was washed with tap water (30 mL) and air dried for 30 min. Recrystallization (95% EtOH) and drying over toluene (Abderhalden, RT, 0.025 mm, 10 h) yielded **65** as shiny, white, fluffy crystals (0.148 g, 77.6%): mp 171-172°C; IR (KBr) 3400-2400 (CO_2H) cm^{-1}; ^1H NMR ($DCCl_3$) ∂ 2.26 [d, J = 1.35 Hz, 3 H, H(10)], 4.29 [s, 2 H, H(2,3)], 6.80 [br s, 1 H, H(11)], 6.89-8.12 [(Ar-H)]; ^{13}C NMR ($DCCl_3$) ppm 17.6 [C(10)], 52.1 [C(19)], 64.4 [C(2,3)], 114.9 [C(5,8)], 117.1 [C(11)], 119.2 [C(7)], 125.6 [C(13,17)], 127.8 [C(14,16)], 129.1 [C(15)], 130.1 [C(6)], 170.9 [C(18)]; mass spectral data for $C_{18}H_{16}O_4$:

m/e (M^+) 296.1048; Found: 296.1048. Anal. calcd. for $C_{18}H_{16}O_4$: C, 72.96; H, 5.44. Found: C, 73.07; H, 5.34.

2-(2,3-Dihydro-1,4-benzodioxan-6-yl)-3-buten-2-ol (**73**)

A 300-mL, three-necked, round-bottomed flask equippped with a magnetic stir bar, dry-ice condenser, and N_2 inlet (positive pressure from an oil bubbler) was flushed with N_2 for 5 min. In the flask was placed Mg turnings (1.52 g, 62.53 mmol) and dry THF (10 mL). Vinyl bromide (10 g, 93.45 mmol), collected separately in a 50 mL, single-necked, round-bottomed flask without solvent and at 0°C, was added dropwise over 5 min to the original flask via an addition funnel. To initiate the reaction, CH_3I (0.5 mL) was added and the solution began to bubble immediately. After 5 min, an additional quantity of vinyl bromide (10 g, 93.45 mmol) in 25 mL of dry THF was added dropwise over 15 min. Stirring the mixture for 30 min at room temperature ensured that nearly all of the Mg had dissolved while the color of the solution changed from colorless to light brown. Then the ketone (**63**, 4.0 g, 22.45 mmol), dissolved in dry THF (25 mL), was added dropwise through the addition funnel over 15 min. The dry-ice condenser was replaced by a spiral water condenser, and the solution was boiled for 2 h. After being stirred at room temperaure for 10 h, the mixture was cooled in a water bath, and a solution of saturated NH_4Cl (20 mL) was added dropwise initially (about 5 min) and later in 1-mL portions over 10 min. Two layers formed and the contents of the flask were transferred to a separatory funnel. Extraction of the aqueous layer with ether (4 x 50 mL) followed. The combined ether extracts were washed with saturated NaCl (2 x 500 mL) and dried (Na_2SO_4, 2 h). Evaporation (rotovap) and removal of traces of the solvent (Abderhalden, RT, 0.025 mm, 1 h) gave crude **73** as a yellow oil (4.78 g, 103%). This oil was used without further purification. IR (neat) 3700-3100 (O-H) cm^{-1}; ^1H NMR ($DCCl_3$) ∂ 1.60 [s, 3 H, H(10)], 4.23 [s, 4 H, H(2,3)], 5.11 [dd, J_{cis} = 10.6 Hz, J_{gem} = 1.08 Hz, 1 H, H(12b)], 5.27

[dd, J_{trans} = 17.2 Hz, J_{gem} = 1.08 Hz, J_{gem} = 1.08 Hz, 1 H, H(12a)], 6.12 [dd, J_{trans} = 17.3 Hz, J_{cis} = 10.52 Hz, H(11)], 6.8-6.99 [m, Ar-H, 3 H, H(5,7,8)]; ^{13}C NMR ($DCCl_3$) ppm 29.15 [C(10)], 64.33 [C(2,3)], 74.25 [C(9)], 112.03 [C(5)], 114.41 [C(12)], 116.86 [C(8)], 118.26 [C(7)], 139.96 [C(11)], 142.41 [C(6)], 143.02 [C(8a)], 144.78 [C(4a)]; mass spectral data for $C_{12}H_{14}O_3$: m/e (M^+) 206.0942. Found: 206.0938.

[3-(2,3-Dihydro-1,4-benzodioxan-6-yl)-2-buten-1-yl]triphenylphosphonium Bromide (**74**)

In a 100-mL, two-necked, round-bottomed flask equipped with an addition funnel, magnetic stir bar, and an N_2 inlet (positive pressure from an oil bubbler) was placed $Ph_3P \cdot HBr$ (2.615 g, 4.92 mmol) in dry methanol (20 mL). To this stirred suspension was added dropwise, via the addition funnel, a solution of the alcohol (**73**, 1.015 g, 4.92 mmol) in dry CH_3OH (30 mL) over 10 min at room temperature. The suspension became a solution and immediately acquired a peach coloration. It was stirred at room temperature under N_2 for 10 h, concentrated to about 5 mL (rotovap), and transferred to a 600 mL beaker. Dry ether (200 mL) was added slowly with hand stirring using a glass rod to ensure complete precipitation. Filtration (aspirator suction) and washing with dry ether (20 mL) gave a white powder which dissolved in dry CH_3OH (15 mL). Ether (100 mL) was added with constant hand stirring. The beaker was covered with parafilm and stored in the freezer overnight. Filtration (aspirator suction) and drying (Abderhalden, RT, 0.025 mm, 10 h) gave **74** as fine white crystals (2.22 g, 84.92%). The salt was used without further purification; mp 234-235°C. IR (KBr) 1110 (C-O) cm^{-1}; ^{1}H NMR ($DCCl_3$) ∂ 1.58 [d, 3 H, H(10)], 4.22 [s, 4 H, H(2,3)], 4.84 [dd, J_{HP} = 15.1 Hz, J_{HH} = 8.1 Hz, H(12)], 5.57 [m, 1 H, H(11)], 6.68 [m, 3 H, H(5,7,8)], 7.65-7.93 [m, 15 H, $P(C_6H_5)_3$]; ^{13}C NMR ppm (tentative assignments)[37] 16.82 [C(10)], 25.3 [C(12)], 64.3 [C(2,3)], 109.8 [C(11)],

118.1 [d, $^{1}J_{CP}$ = 85.2 Hz, *orthogonal*-C's of $(C_6H_5)_3$], 130.2 [*meta*-C's of $P(C_6H_5)_3$] 134.9 [*ortho*-C's of $P(C_6H_5)_3$], 135.5 [*para*-C's of $P(C_6H_5)_3$], 145.6 [C(9)]. Anal. calcd. for $C_{30}H_{28}O_2PBr{\cdot}H_2O$: C, 65.58; H, 5.50. Found: C, 65.68; H, 5.45.

Ethyl (2*E*,4*E*,6*E*)-7-(2,3-Dihydro-1,4-benzodioxan-6-yl)-3-methyl-2,4,6-octatrienoate (**66**)

A three-necked, 100-mL, round-bottomed flask equipped with power stirrer, addition funnel, rubber septum, and an N_2 inlet (positive pressure from an oil bubbler) was flushed with N_2 for 5 min. In this system was placed the phosphonium salt (**74**, 4 g, 7.53 mmol) in dry ether (40 mL). To the stirred suspension was added dropwise via the septum, *n*-BuLi in hexanes (14 mL, 0.906 M, 21.7 mmol) over 5 min. The resulting dark red solution was stirred at room temperature for 30 min, and then a solution of ethyl 3-methyl-4-oxocrotonate (**75**, 1.3 mL, 9.15 mmol) in dry ether (10 mL) was added via the addition funnel. The color of the mixture became dark yellow, and it was allowed to stir at room temperature for 10 h. Hexanes (100 mL) were added and filtration (suction) followed by evaporation, gave a yellow oil. Crystals began forming in the oil at room temperature and the mixture was allowed to stand overnight. Recrystallization of the resulting solid mass of crystals (minimum absolute EtOH) gave **66** as fine yellow crystals (0.683 g, 28.85%): mp 86-87°C; IR (KBr) 1699 (C=O) cm^{-1}; ^{1}H NMR ($DCCl_3$) ∂ 1.3 [t, J = 7.1 Hz, 3 H, H(19)], 2.20 [s, 3 H, H(10)], 2.37 [d, J = 0.98 Hz, 3 H, H(19)], 4.17 [q, J = 7.1 Hz, 2 H, H(18)], 4.27 [s, 4 H, H(2,3)], 5.78 [br s, 1 H, H(16)] , 6.33 [d, J = 15.1 Hz, 1 H, H(13)], 6.51 [d, J = 11.2 Hz, H(11)], 6.87-7.04 [m, 3 H, H(5,7,8)]; ^{13}C NMR ($DCCl_3$) ppm 13.79 [C(19)], 14.34 [C(15)], 16.23 [C(10)], 59.64 [C(18)], 64.40 [C(2,3)], 114.67 [C((5)], 117.05 [C(8)], 118.68 [C(7)], 118.92 [C(16)], 125.54 [C(9)], 131.12 C[(12)], 135.52 [C(13)], 167.15 [C(17),(C=O)]; quarternary C [135.98, 139.16, 143.20, 143.35,

152.64]; mass spectral data for $C_{19}H_{22}O_4$: m/e (M^+) 314.1510. Found: 314.1518. Anal. calcd.for $C_{19}H_{22}O_4$: C, 72.59; H, 7.053. Found: C, 72.62; H, 7.11.

(2*E*,4*E*,6*E*)-7-(2,3-Dihydro-1,4-benzodioxan-6-yl)-3-methyl-2,4,6-octatrienoic Acid (**67**)

In a 10-mL, single-necked, round-bottomed flask equipped with a magnetic stir bar, and N_2 inlet was placed the ester (**66**, 0.150 g, 0.48 mmol). To this system was added absolute EtOH (2 mL) and aqueous KOH (1 mL, 35%). The mixture was boiled for 1 h and cooled to room temperaure. To the flask were added tap water (5 mL), EtOAc (50 mL), and AcOH:H_2O (1:1, 1 mL) in that order. Two layers were obtained, and the aqueous layer was extracted (EtOAc, 10 mL). The combined organic layers were dried (Na_2SO_4, overnight), filtered (aspirator suction), and dried (Abderhalden, RT, 0.025 mm, 10 h) to give **67** as yellow crystals (0.49 g, 35.88%): mp 181-182°C. IR (KBr) 3300-2400 (CO_2H) cm^{-1}; ^1H NMR ($DCCl_3$) ∂ 2.21 [s, 3 H, H(10)], 2.39 [s, 3 H, H(15)], 4.27 [s, 4 H, H(2, 3)], 5.83 [br s, 1 H, H(16)], 6.38 [d, J = 15 Hz, 1 H, H(13)], 6.53 [d, J = 11.2 Hz, 1 H, H(11)], 6.83-7.09 [m, 3 H, Ar-H(5,7,8)]; ^{13}C NMR ppm 14.07 [C(15)], 16.3 [C(10)], 64.4 [C(2,3)], 114.7 [C(5)], 117.1 [C(8)], 119.01 [C(16)], 125.54 [C(9)], 135.2 [C(13)], 172.08 [C(17),C=O]; mass spectral data for $C_{17}H_{18}O_4$ m/e (M^+) 286.1205; Found: 286.1203. Anal. calcd. for $C_{17}H_{18}O_4$: C, 71.31; H, 6.34. Found: C, 71.29; H, 6.31.

Methyl (*E*)-4-[2-(2,3-Dihydro-1,4-benzodioxan-6-yl)-3-hydroxy-2-propen-1-yl]benzoate (**68**)

In a 25-ml, single-necked, round-bottomed flask equipped with a magnetic stir bar, spiral water condenser, and N_2 inlet from the top of the condenser (positive pressure from

an oil bubbler) was placed the ester (**64**, 0.1 g, 0.322 mmol). To the flask was added SeO_2 (0.0357 g, 0.107 mmol) and 15 mL of 95% EtOH. The suspension became homogeneous on warming and was boiled for 20 h. After allowing to cool at RT for 2 h, the precipitated Se metal was filtered off via a thick pad of glasswool. Ether (10 mL) was used to rinse the original flask, and this rinse was also filtered (glasswool). The organic solution was concentrated to about 1 mL, filtered (glasswool), washed with ether (5 mL), and concentrated a total of three times to remove the precipitated selenium metal. Finally the concentrated filtrate was subjected to chromatography using the Chromatotron [silica gel (7:3 hexanes:EtOAc)]. The major product was isolated and dried (Abderhalden, <0.025 mm, RT overnight) to yield **68** as a thick oil (0.0202 g, 19.22%) (*E*)-**68**:(*Z*)-**68** ~10:1: IR (neat) 3550-3150 (O-H), 1715 (C=O) cm^{-1}; ^{1}H NMR ($DCCl_3$) ∂ 1.71 [s, O-H], 3.86 [s, 4 H, H(2,3)], 4.26 [s, 3 H, H(19)], 4.43 [d, 2 H, H(10)], 6.62 [d, 1 H, H(11)], 6.65-7.82 [Ar-H]; ^{13}C NMR ppm 50.89 [C(19)], 64.3 [C(2,3)], 68.2 [C(10)], 117.2 [C(11)], 121.8 [C(7)], 124.9 [C(13)], 128.0 [C(14)], 128.6 [C(15)], 141.4 [C (12)], 143.3 [C(4a,8a)], 166.9 [C(18),(C=O)]; mass spectral data for $C_{19}H_{18}O_5$: m/e (M^+) 326.1164; Found: 326.1161. Anal. calcd. for $C_{19}H_{18}O_5 \cdot 2/3H_2O$: C, 67.45; H, 5.76. Found: C, 67.83; H, 5.66.

2,2-Dimethyl-1,3-dioxolyl (*E*)-4-[2-(2,3-Dihydro-1,4-benzodioxan-6-yl)-1-propenyl]benzoate (**69**)[70]

In a 15-mL, two-necked, cone-shaped flask equipped with a magnetic stirrer was placed the acid (**65**, 0.050 g, 0.168 mmol). To the flask was added CH_2Cl_2 (3 mL), which had been dried overnight (molecular sieves 4A). Then dimethylformamide (DMF, 15 drops) was added to dissolve the suspension. To the clear solution was added Solketal (**76**, 0.1 mL, 0.84 mmol) and 4-dimethylaminopyridine (DMAP, 0.041 g, 0.336 mmol). The solution became pale brown, and it was cooled to $0^{\circ}C$ with ice and water.

Dicyclohexylcarbodiimide (DCC, 1 g, 4.85 mmol) was added at 0°C and the flask was allowed to warm up to RT for 0.5 h. After stirring at RT for an additional 4 h, the contents of the flask were filtered (aspirator suction), and transferred to a separatory funnel. The filtrate was washed sucessively with 0.5 N HCl (2 x 25 mL) and saturated $NaHCO_3$ (2 x 25 mL). The CH_2Cl_2 extracts were dried (Na_2SO_4, overnight) and evaporated (rotovap) to yield a yellow oil with a suspended white precipitate. The mixture was filtered through glasswool and washed with 10 mL CH_2Cl_2 to remove the precipitate. The filtrate was concentrated (rotovap) and separated by thin layer chromatography [Chromatotron, 1 mm plate (silica gel), 8:2 hexanes:EtOAc] to yield **69** as a yellow oil (0.003 g, 4.2%). IR (neat) 1720 (C=O) cm^{-1}; ^1H NMR ($DCCl_3$) ∂ 1.4 [s, 3 H, H(26)], 1.47 [s, 3 H, H(27)], 2.24 [d, 3 H, H(10)], 4.29 [s, 4 H, H(2,3)], 6.78 [br s, 1 H, H(11)], 7-8.05 [Ar-H]; ^{13}C NMR ppm 17.6 [C(10)], 25.4 [C(26)], 26.8 [C(27)], 64.4 [C(2,3)], 73.7 [C(23)], 114.9 [C(5,8)], 117.1 [C(11)], 119.2 [C(7)], 125.6 [C(13,17)], 127.3 [C(14,16)], 129.0 [C(15)], 137.0 [C(9)], 143.2 [C(4a,8a)], 166.2 [C(18),(C=O)]; mass spectral data for $C_{24}H_{26}O_6$: m/e (M^+) 410.1729; Found: 410.1732. Anal. calcd. for $C_{24}H_{26}O_6$: C, 70.23; H, 6.38. Found: C, 70.41, H, 6.61.

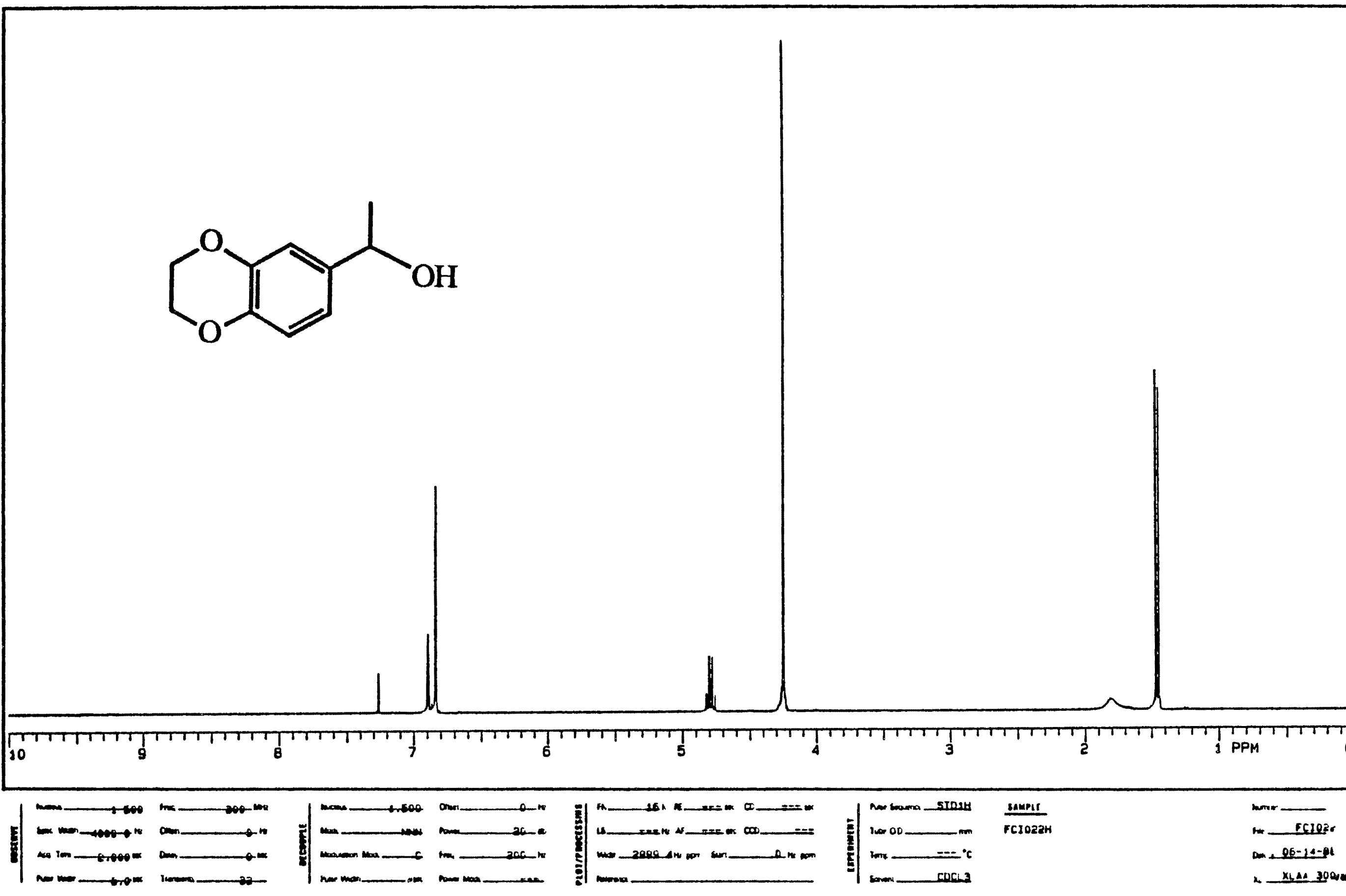
Other Methods Attempted for Preparation of **69**

In one experiment, the ratio of **65**:pyridine:$SOCl_2$:Solketal (**76**) was 1:1.2:1.1:1 and the reaction mixture was stirred at room temperature under N_2 for 12 h. Upon completion of the reaction and workup, starting material was obtained. In another experiment, the ratio of **65**:BF_3.etherate:Solketal (**76**) was 1:10:100. The mixture was heated to reflux for 4 h and, after workup, only starting material was recovered.

PLATE I

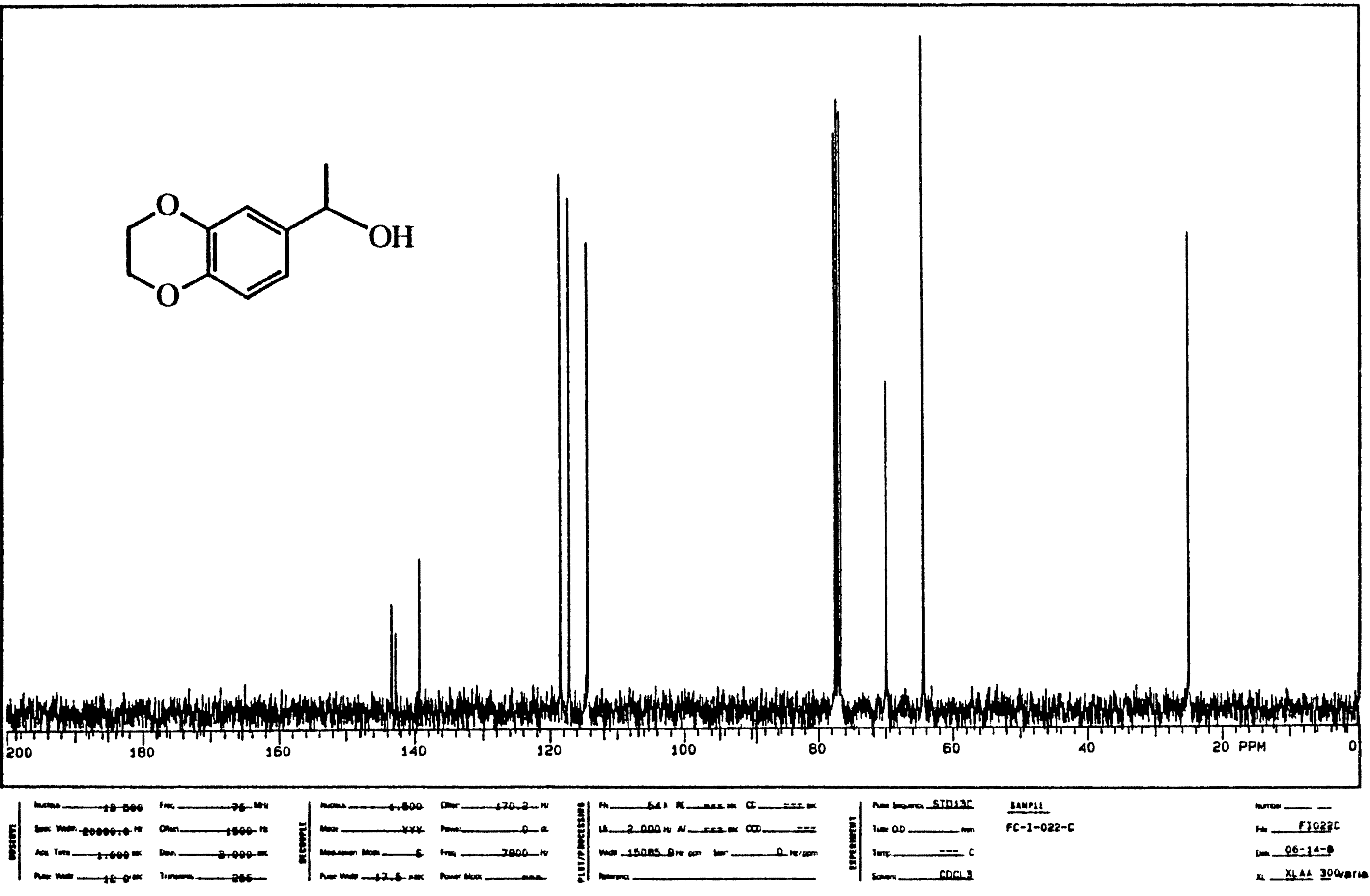
IR Spectrum of 70

PLATE II



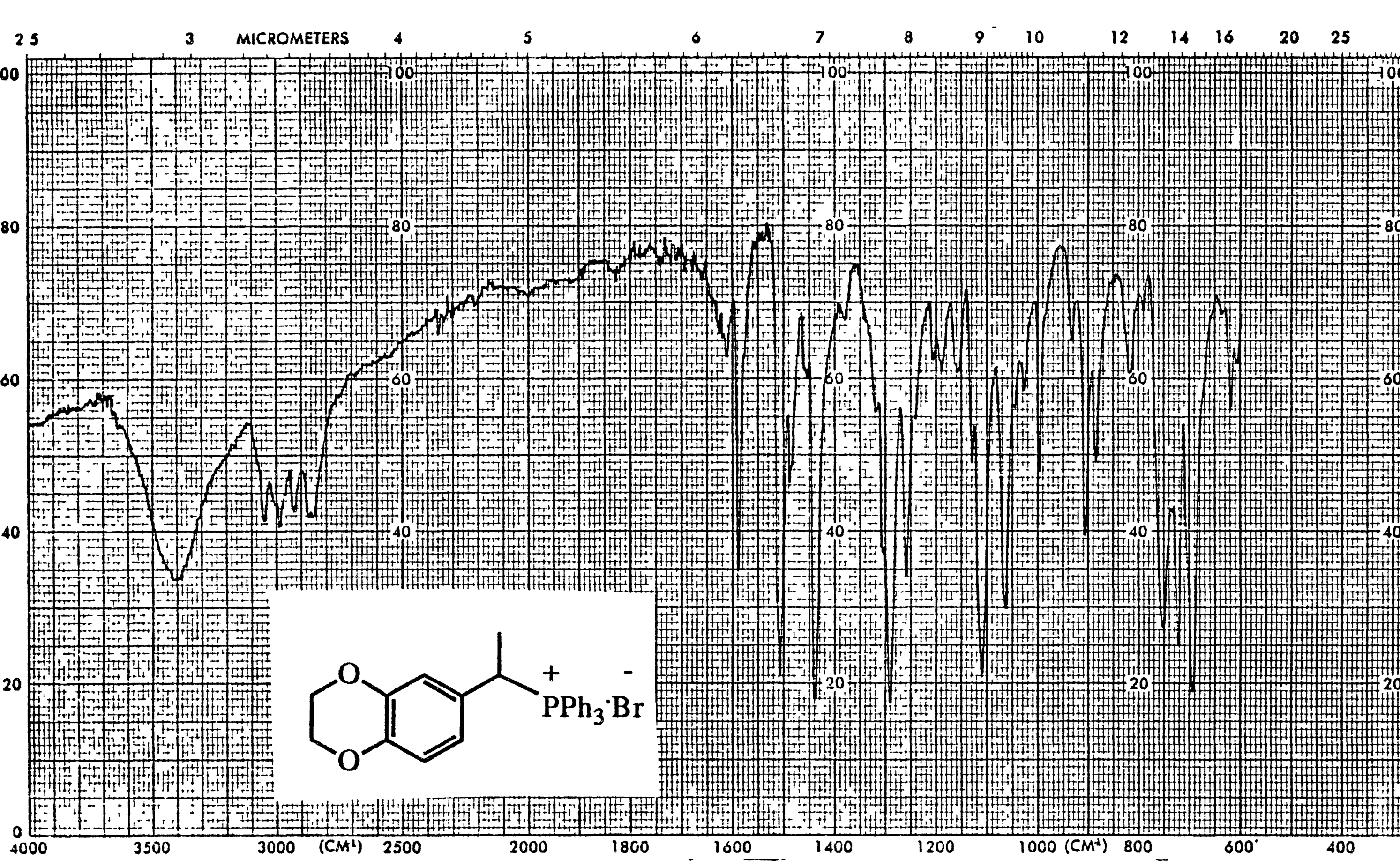
^{1}H Spectrum of **70**

PLATE III



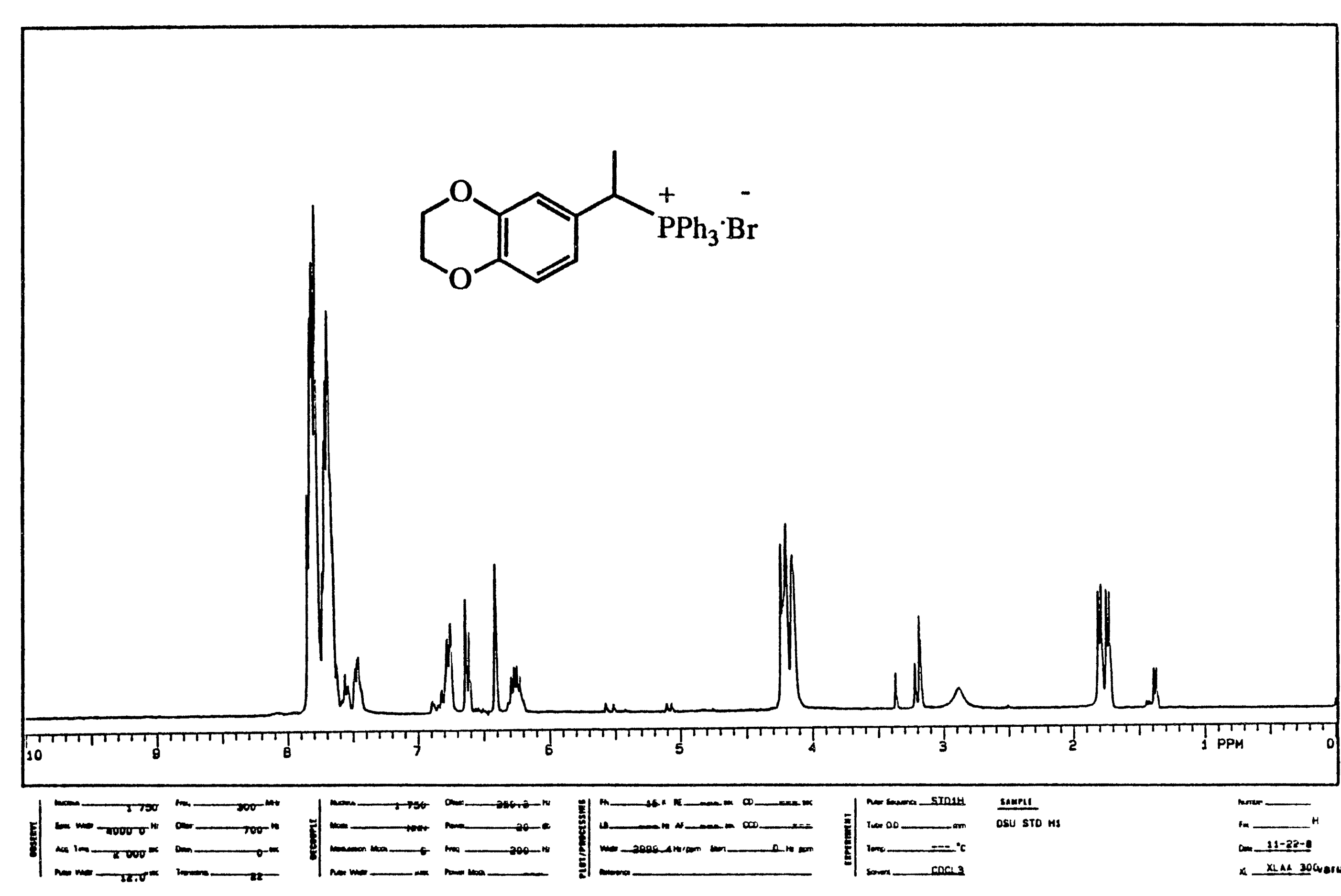
^{13}C Spectrum of **70**

PLATE IV



IR Spectrum of **71**

PLATE V

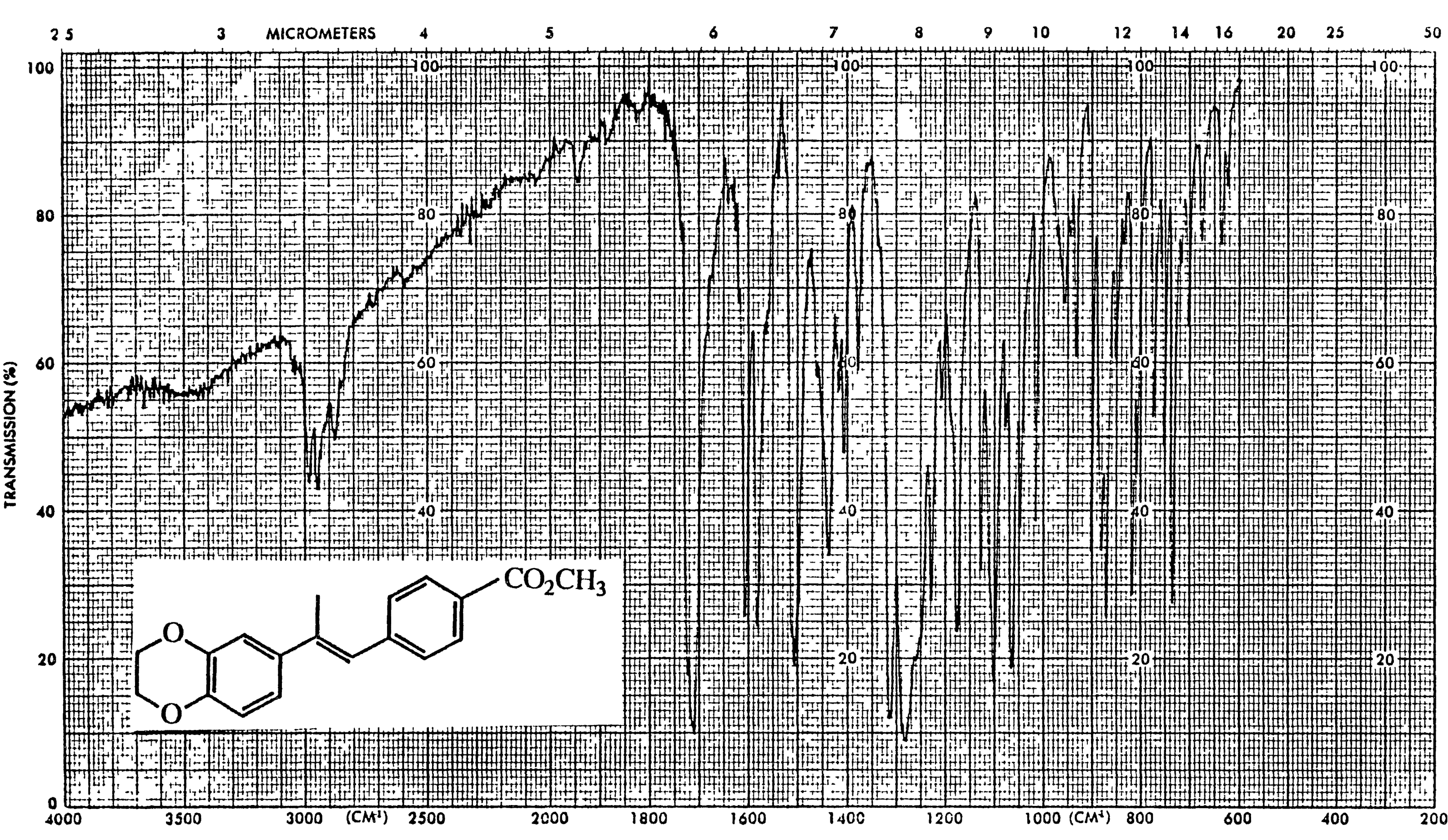


^{1}H Spectrum of **71**

PLATE VI

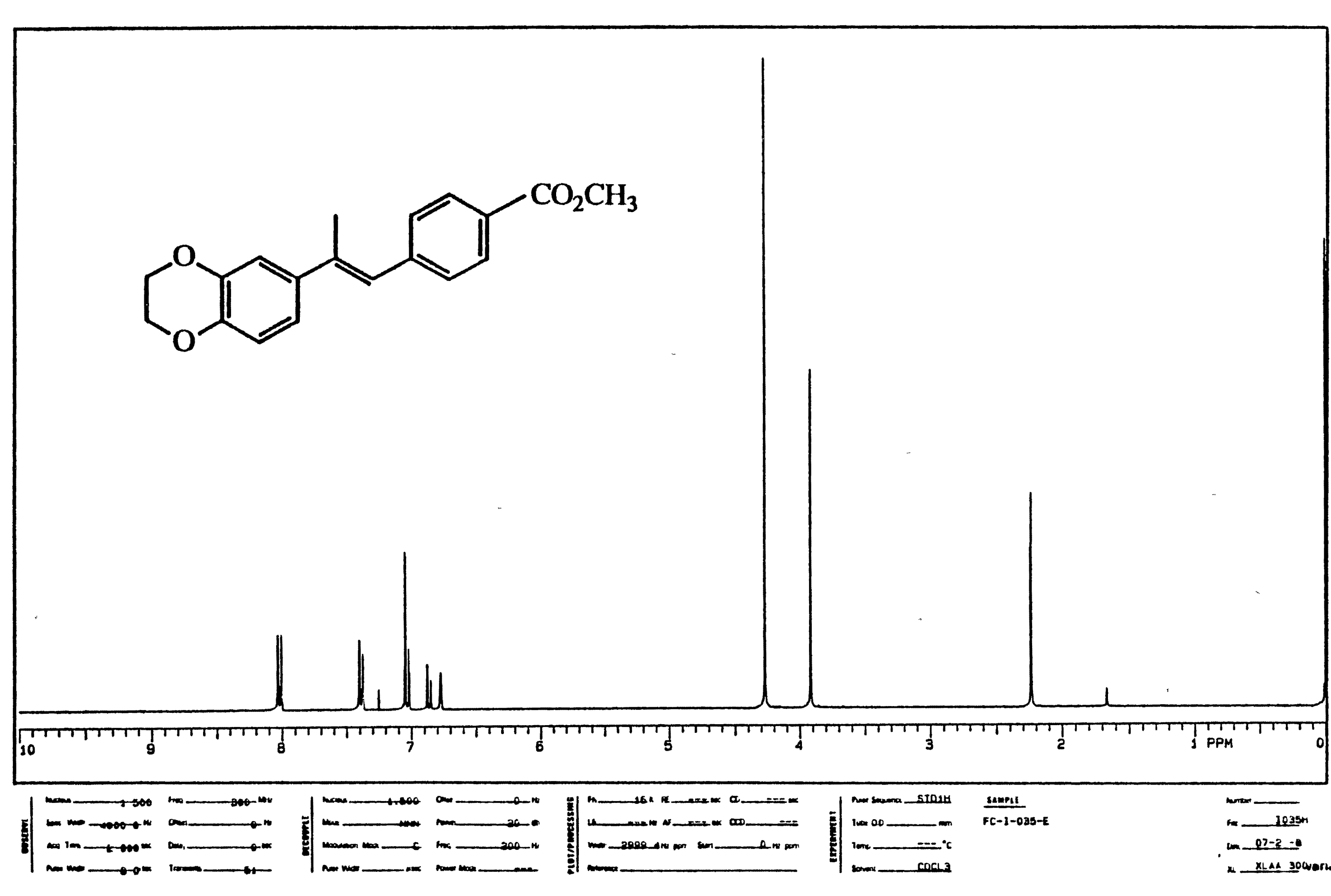
^{13}C Spectrum of **71**

PLATE VII



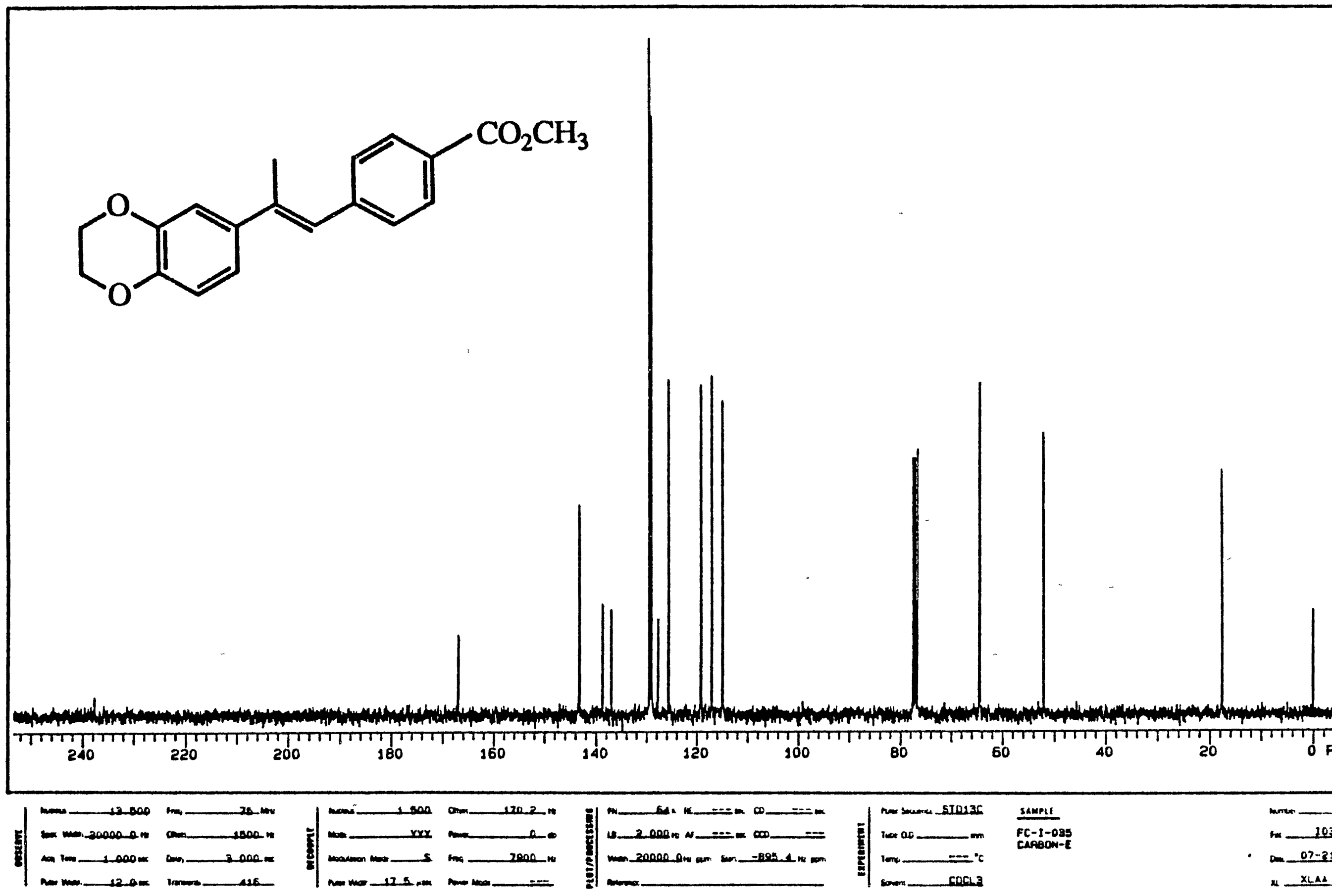
IR Spectrum of **64**

PLATE VIII



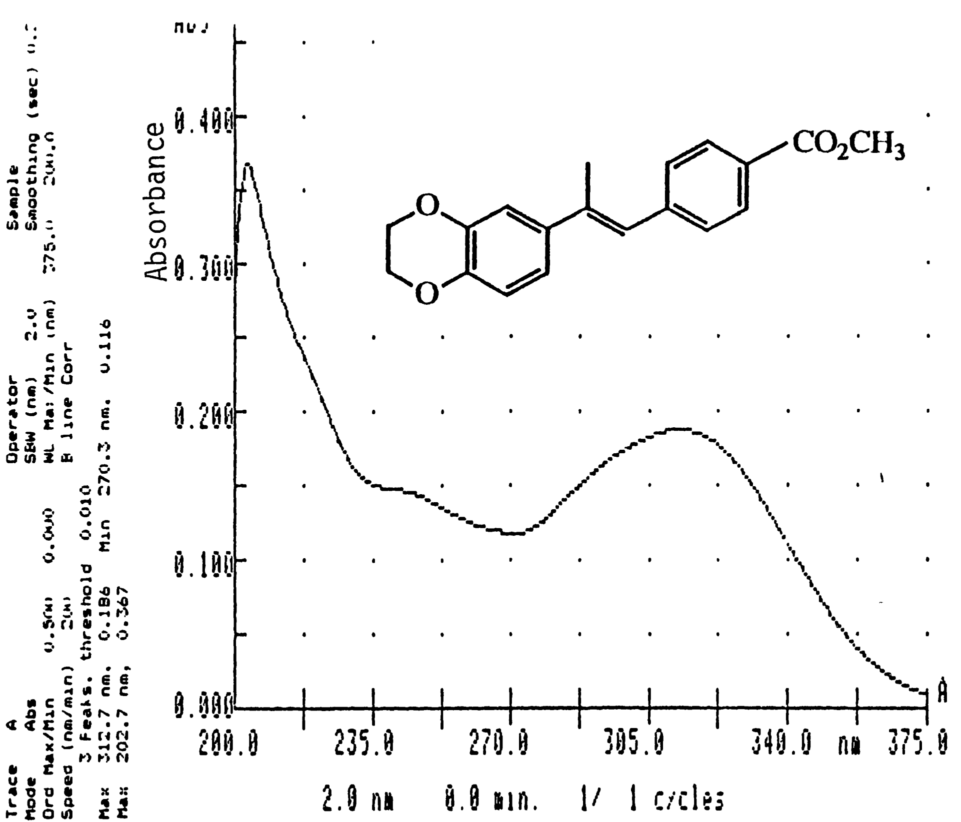
^1H Spectrum of **64**

PLATE IX



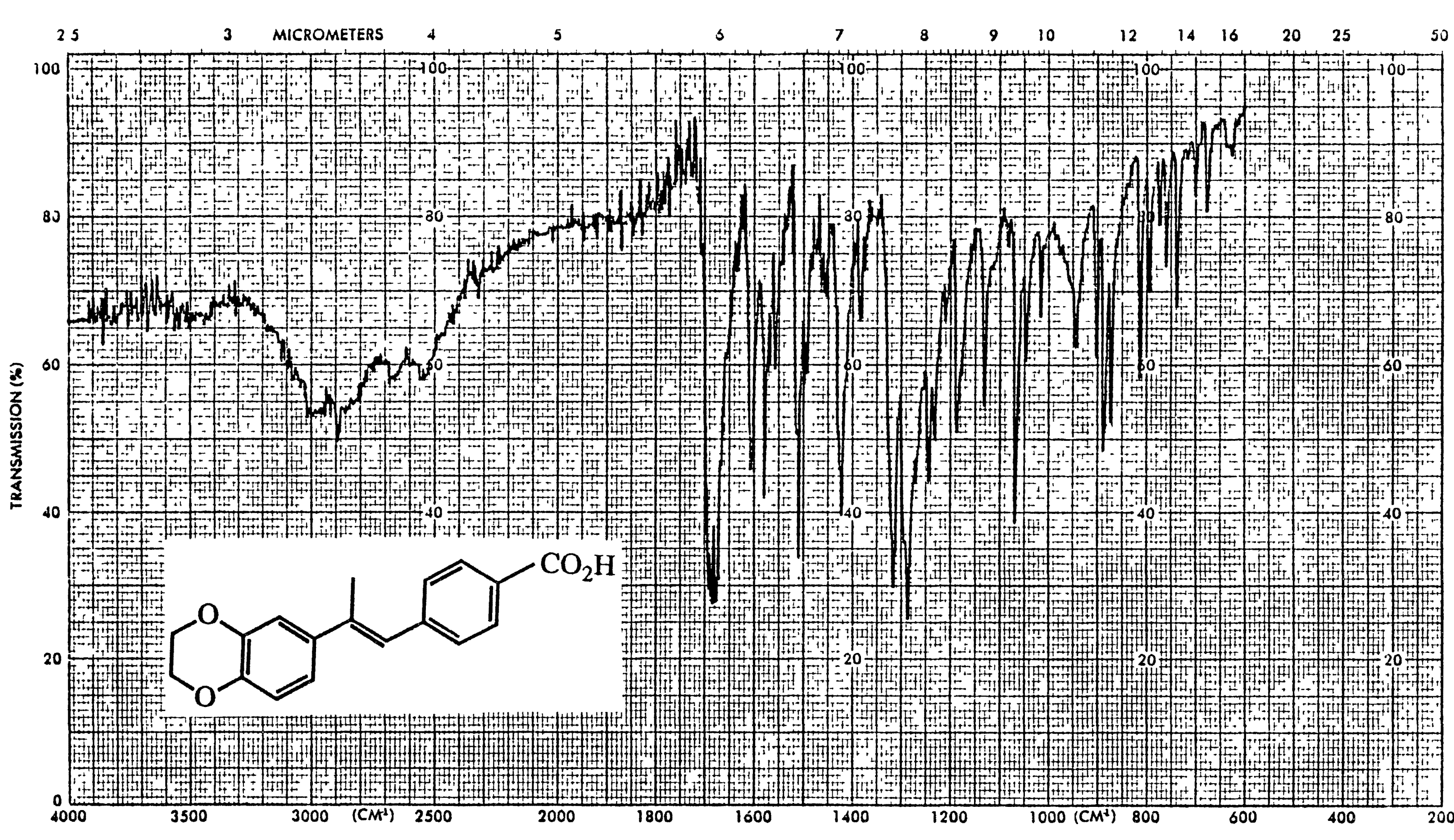
^{13}C Spectrum of **64**

PLATE X



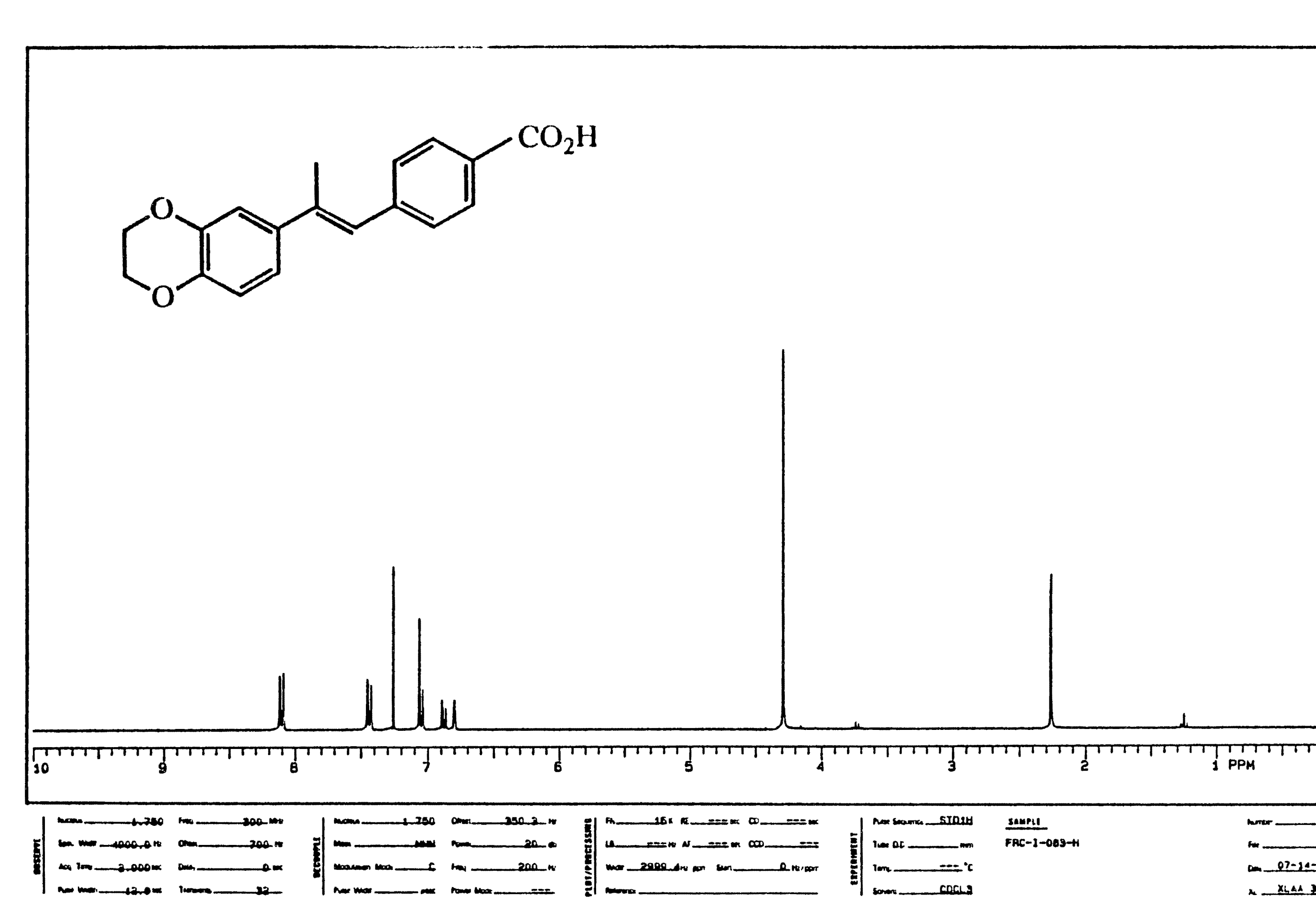
UV Spectrum of **64**

PLATE XI



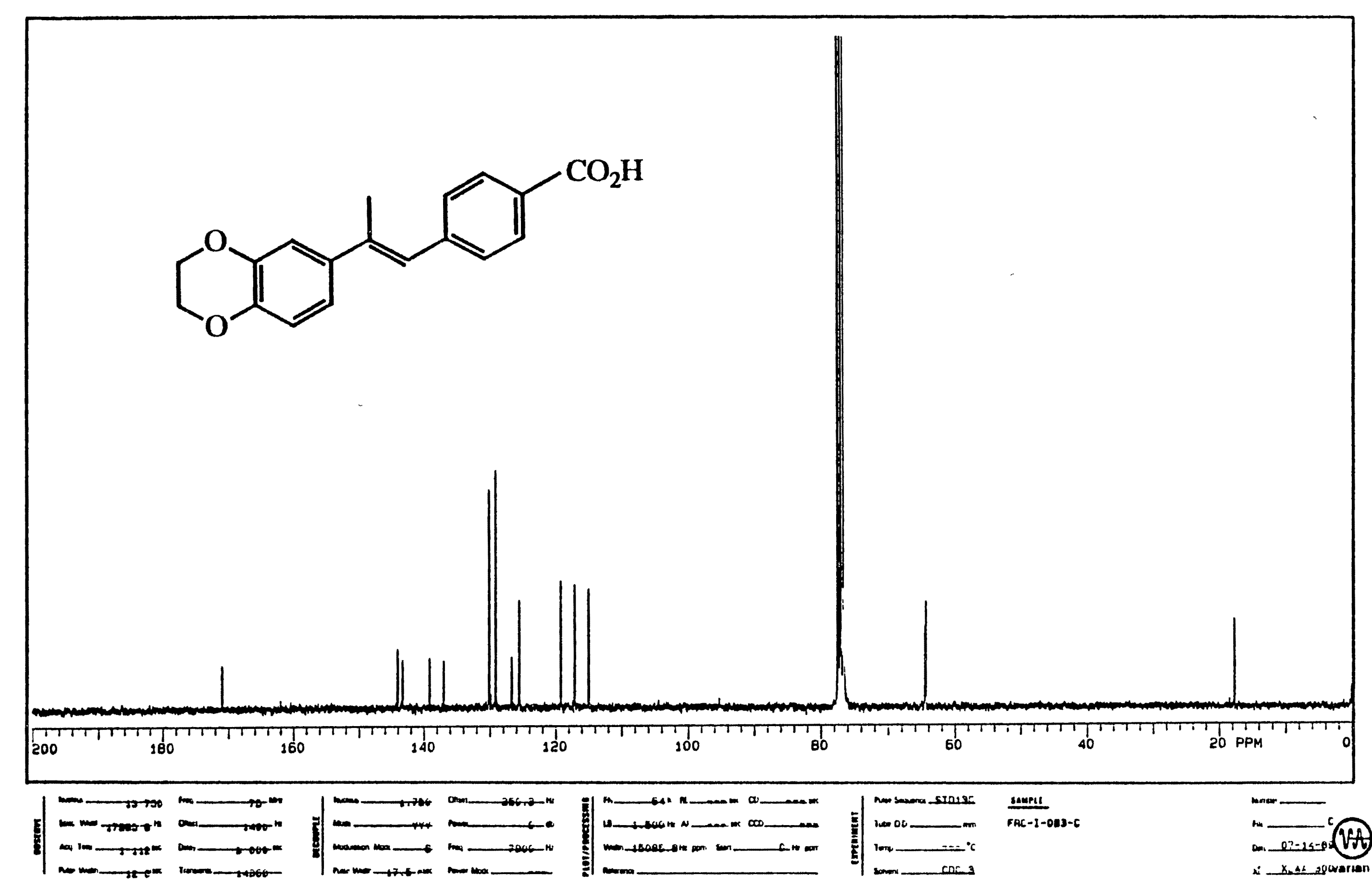
IR Spectrum of **65**

PLATE XII



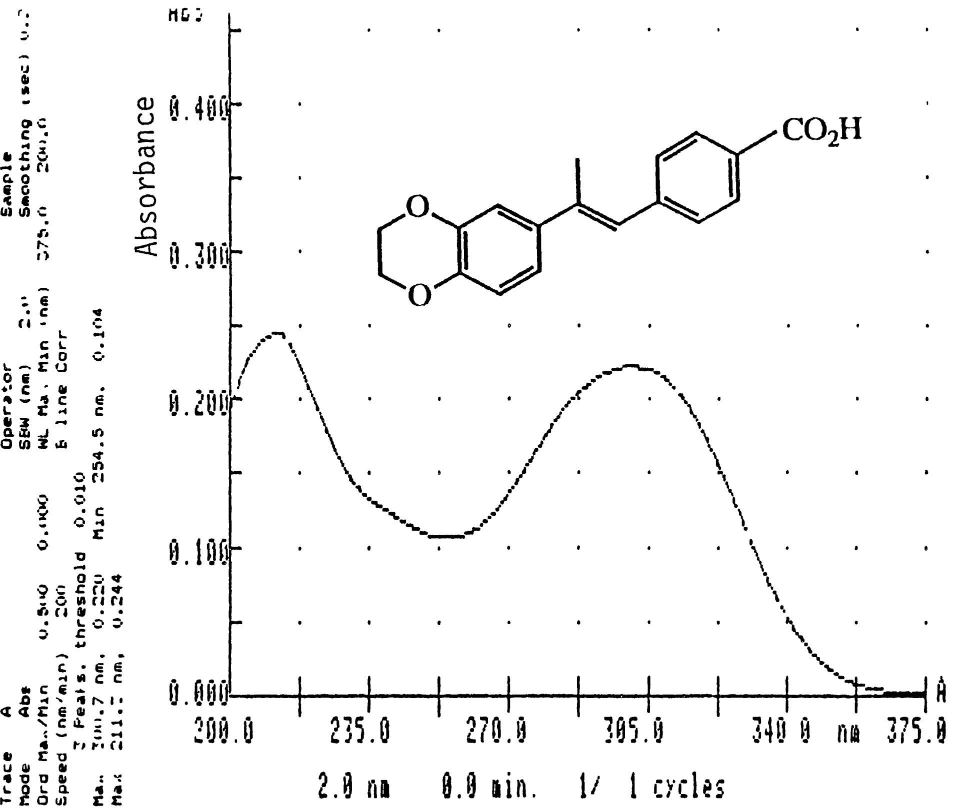
^{1}H Spectrum of **65**

PLATE XIII



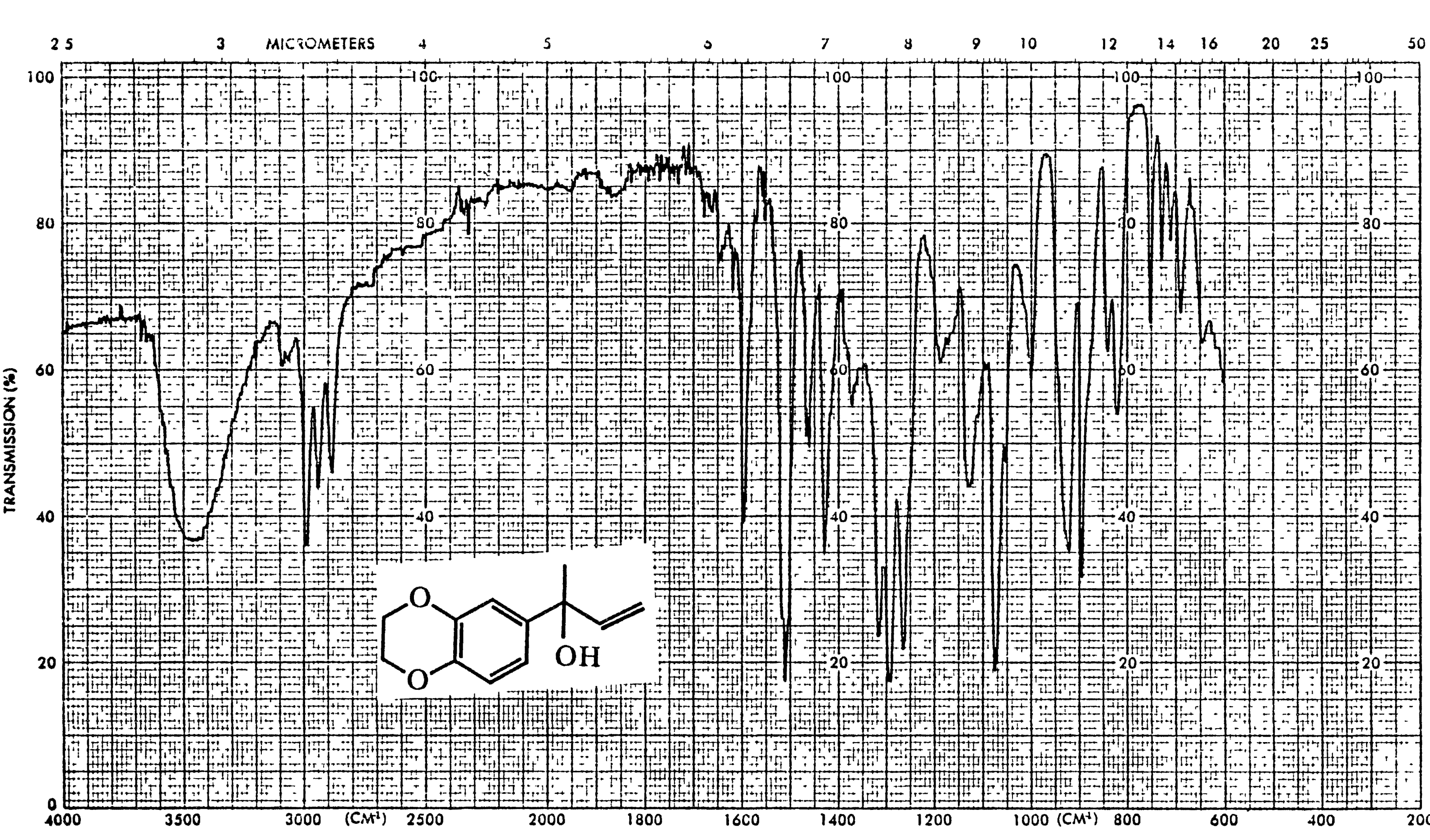
^{13}C Spectrum of **65**

PLATE XIV



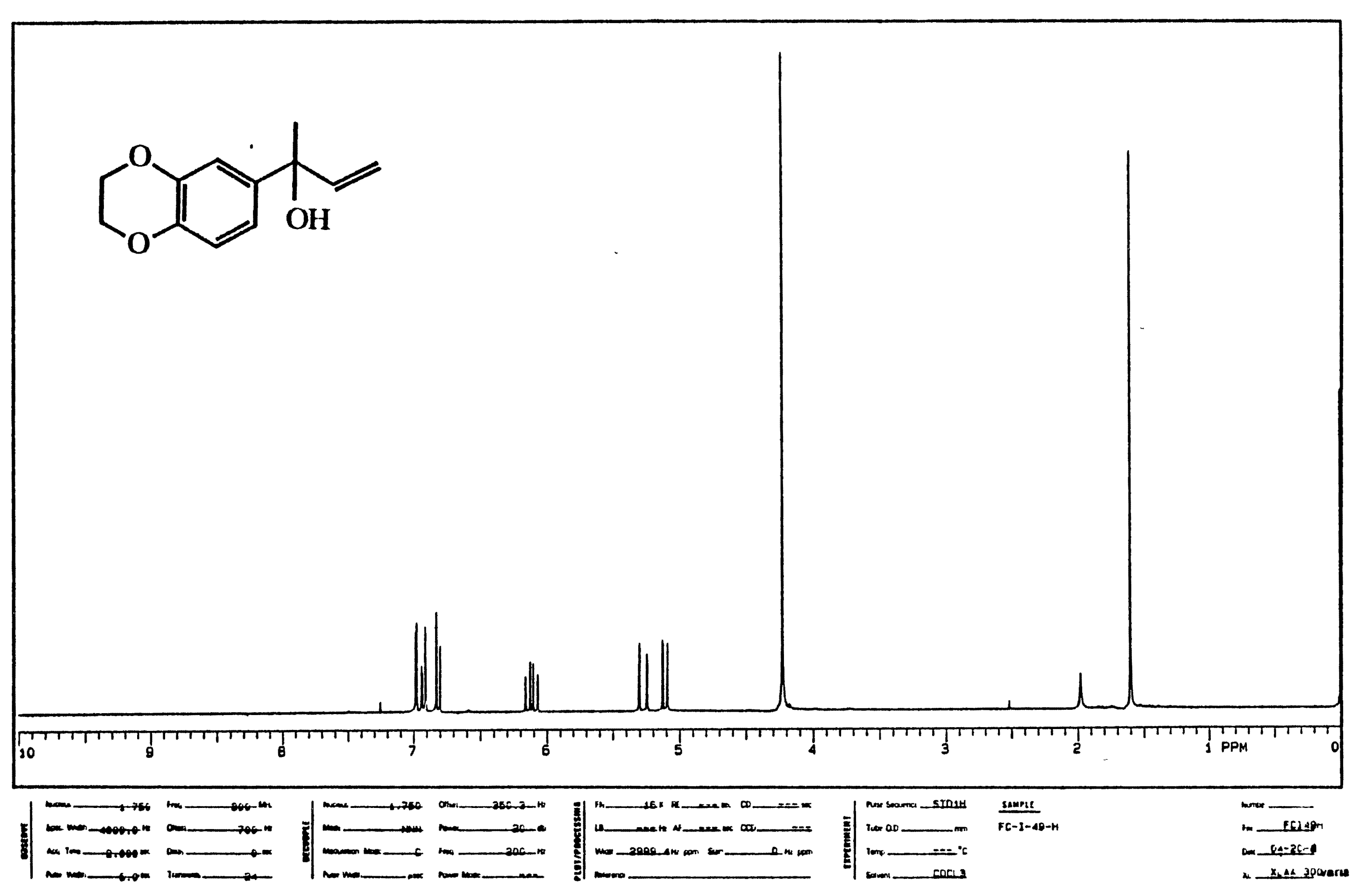
UV Spectrum of **65**

PLATE XV



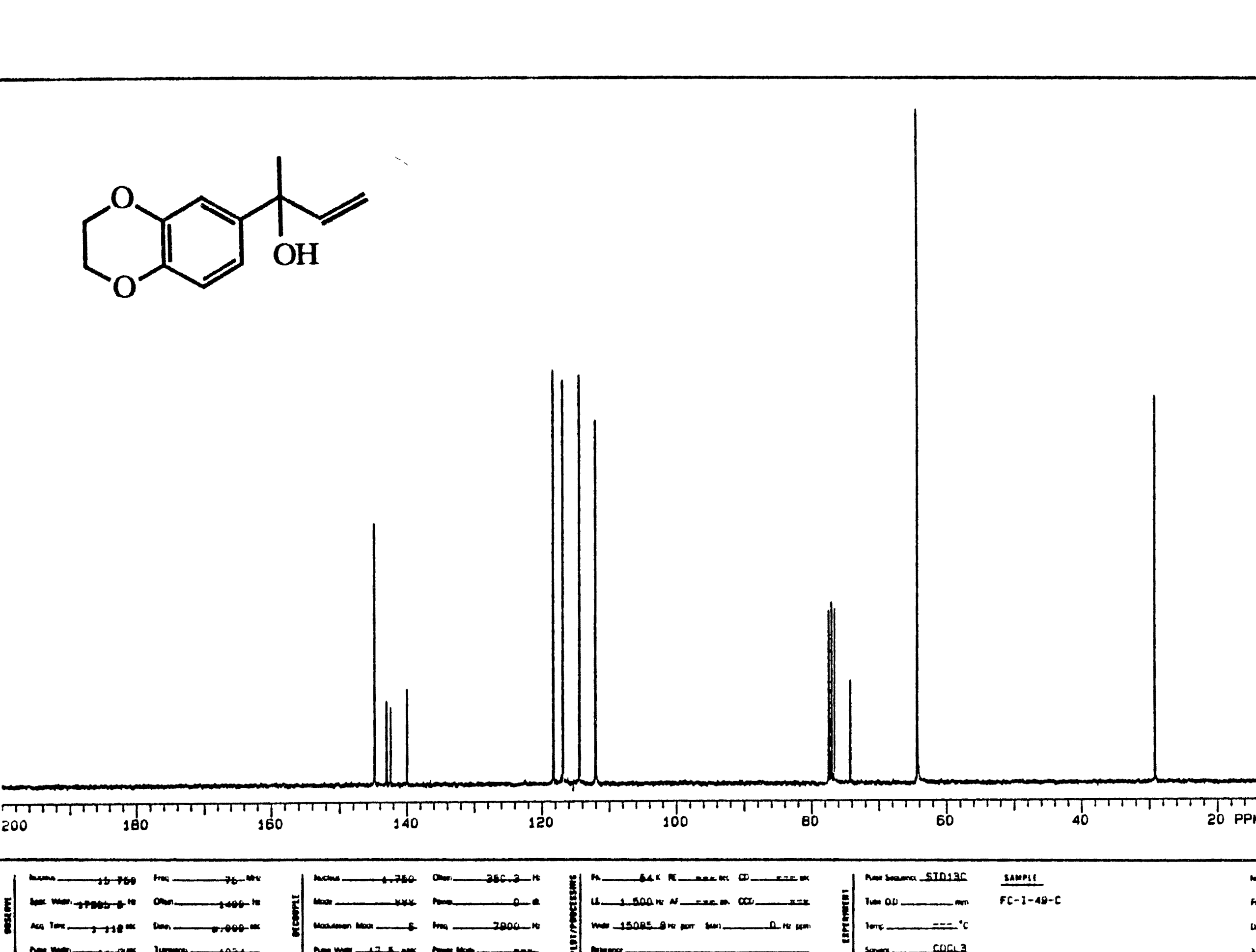
IR Spectrum of **73**

PLATE XVI



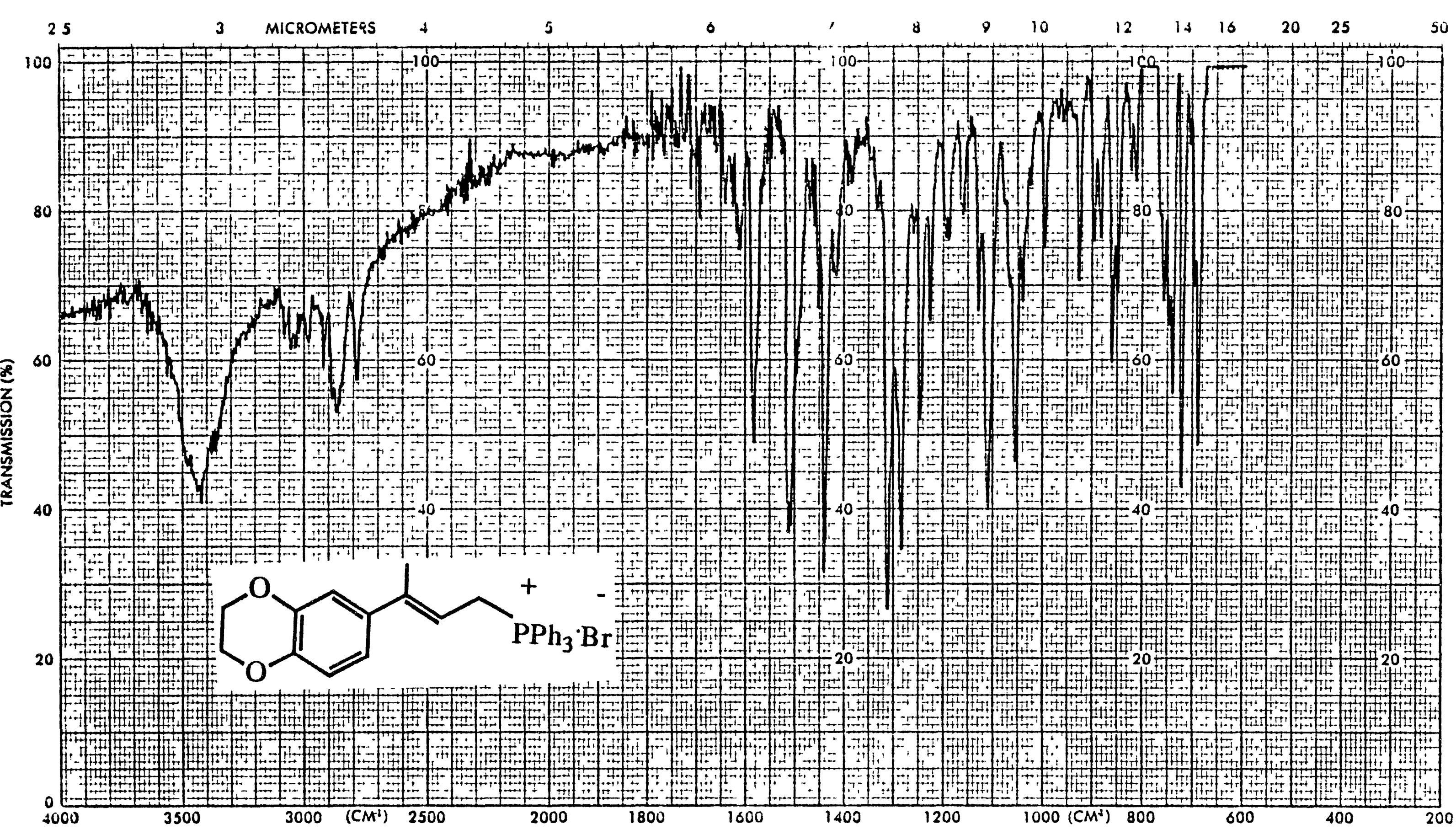
^{1}H Spectrum of **73**

PLATE XVII



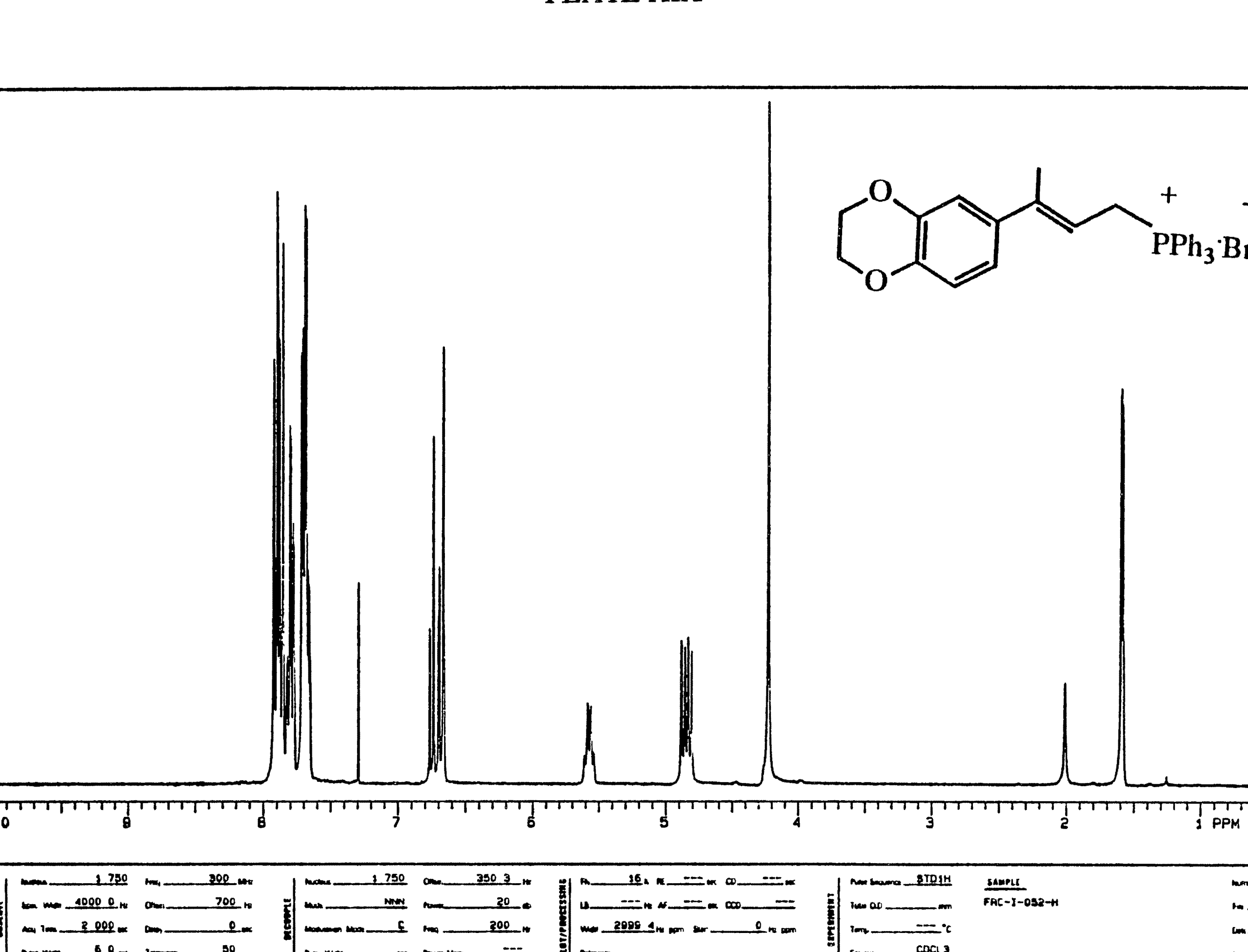
^{13}C Spectrum of **73**

PLATE XVIII



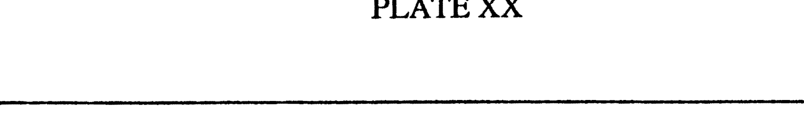
IR Spectrum of **74**

PLATE XIX



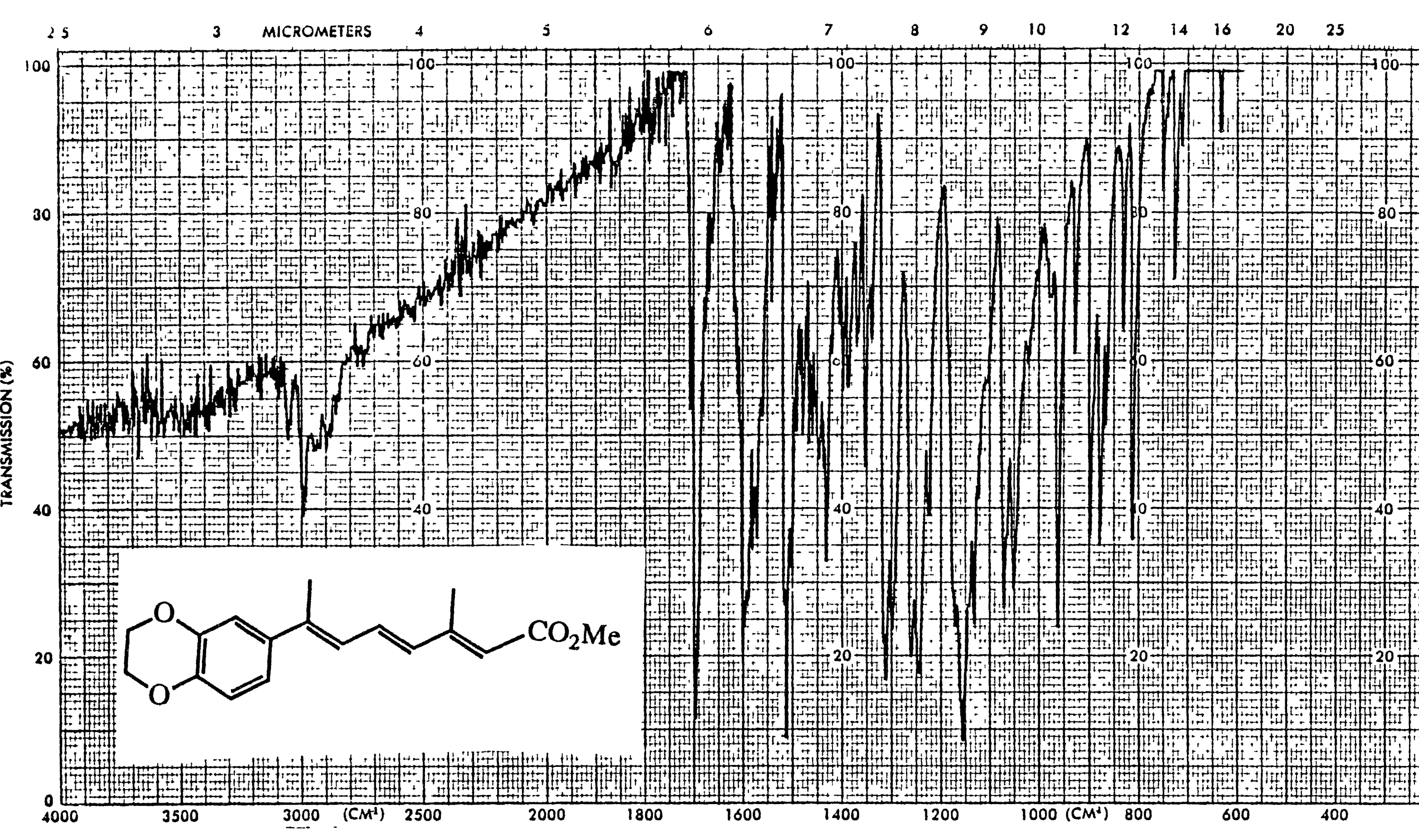
^1H Spectrum of **74**

PLATE XX



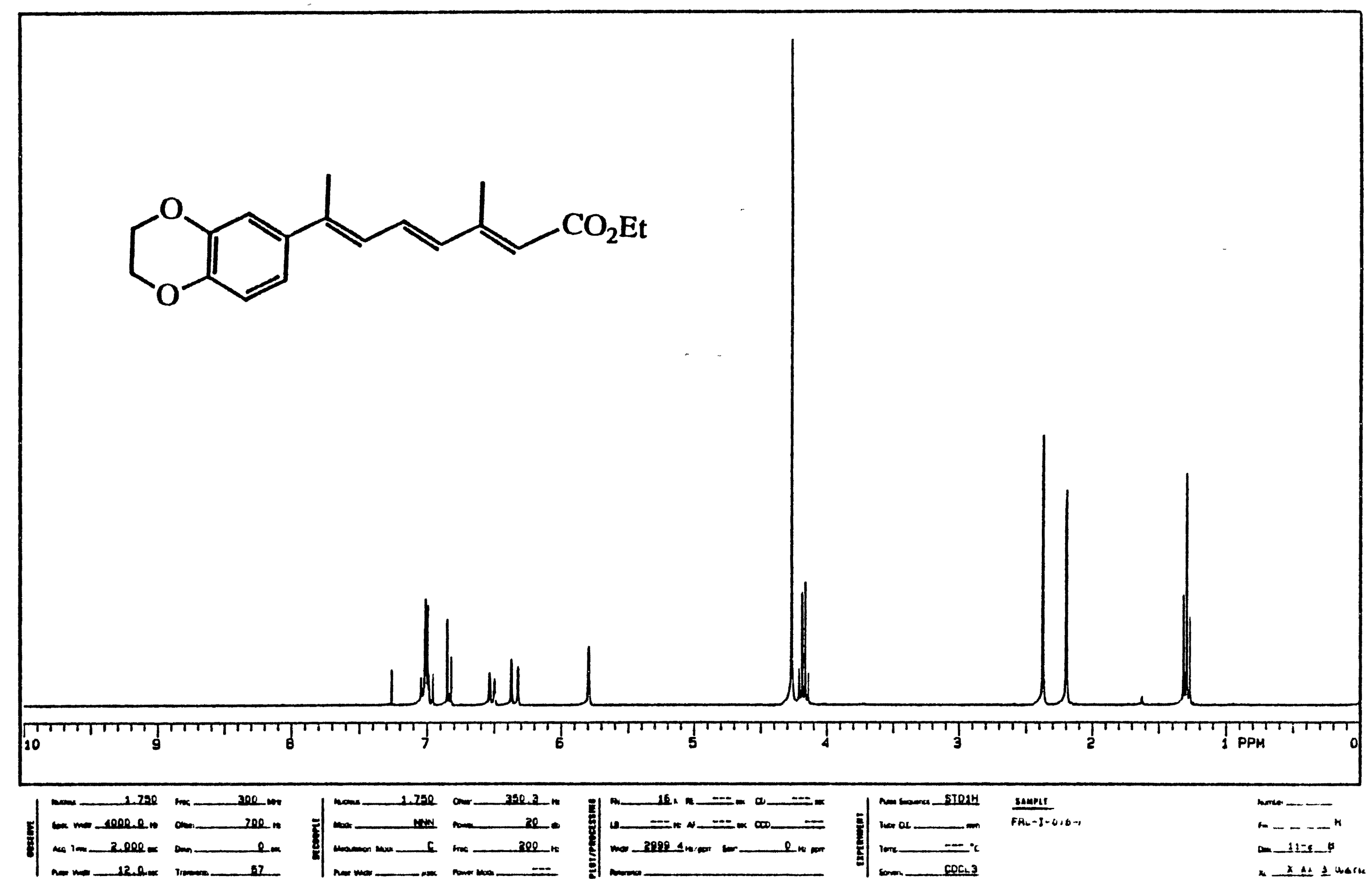
^{13}C Spectrum of 74

PLATE XXI



IR Spectrum of **66**

PLATE XXII

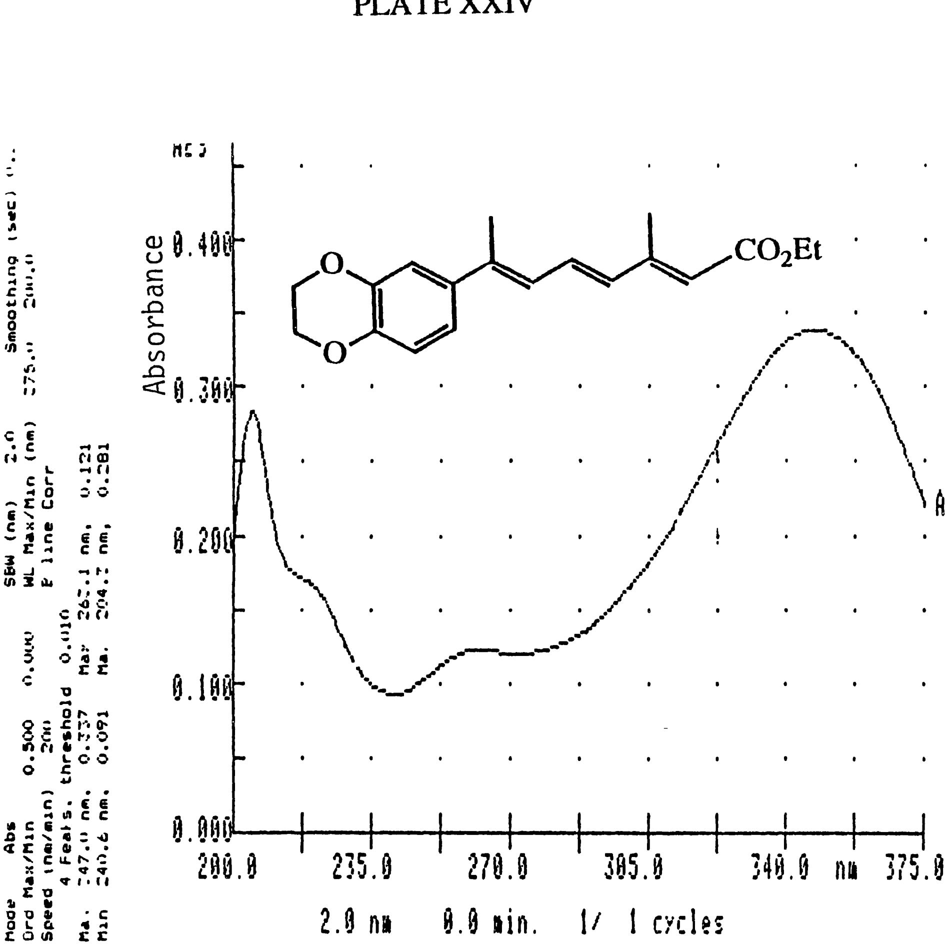


^{1}H Spectrum of **66**

PLATE XXIII

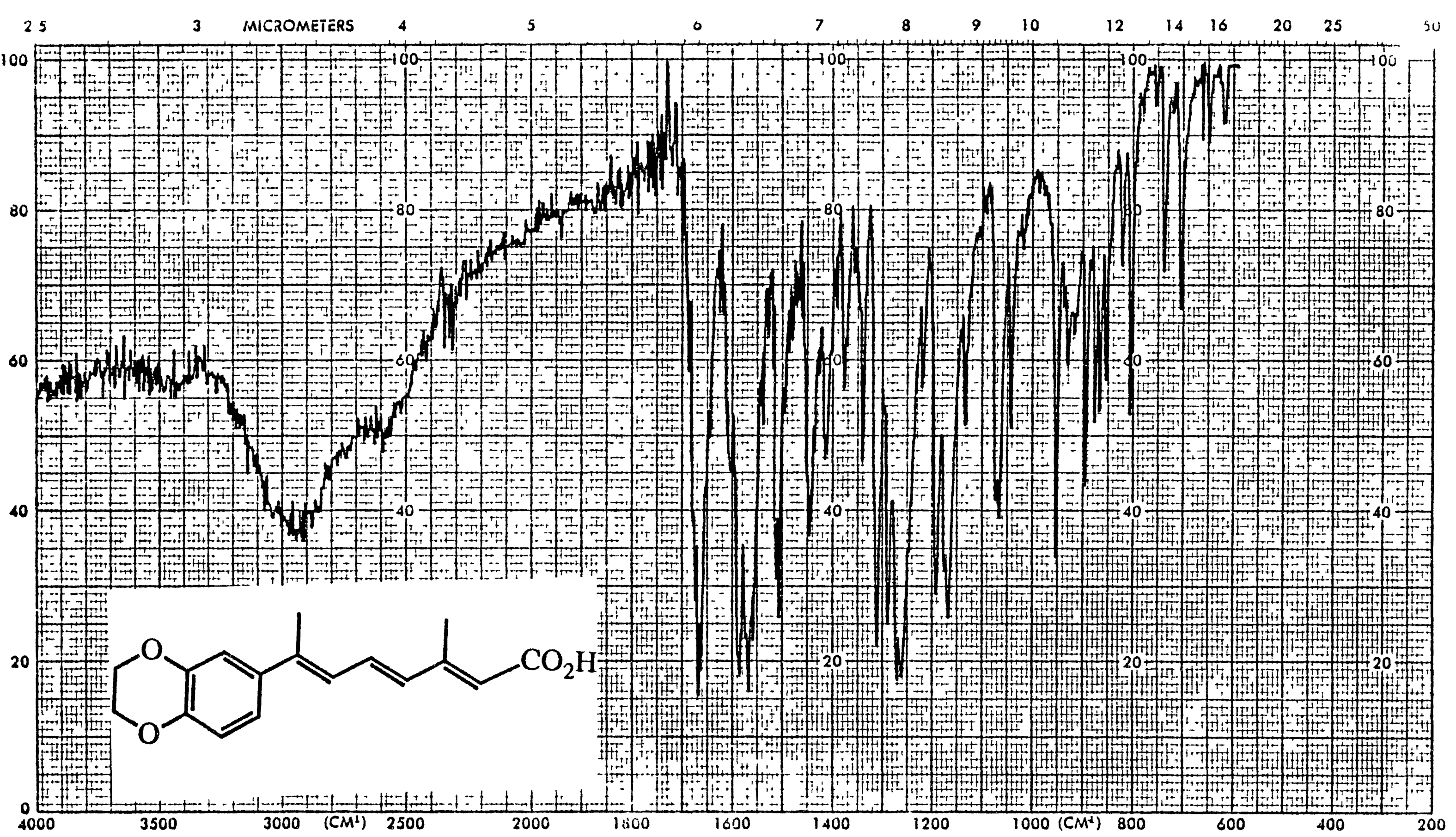
^{13}C Spectrum of **66**

PLATE XXIV



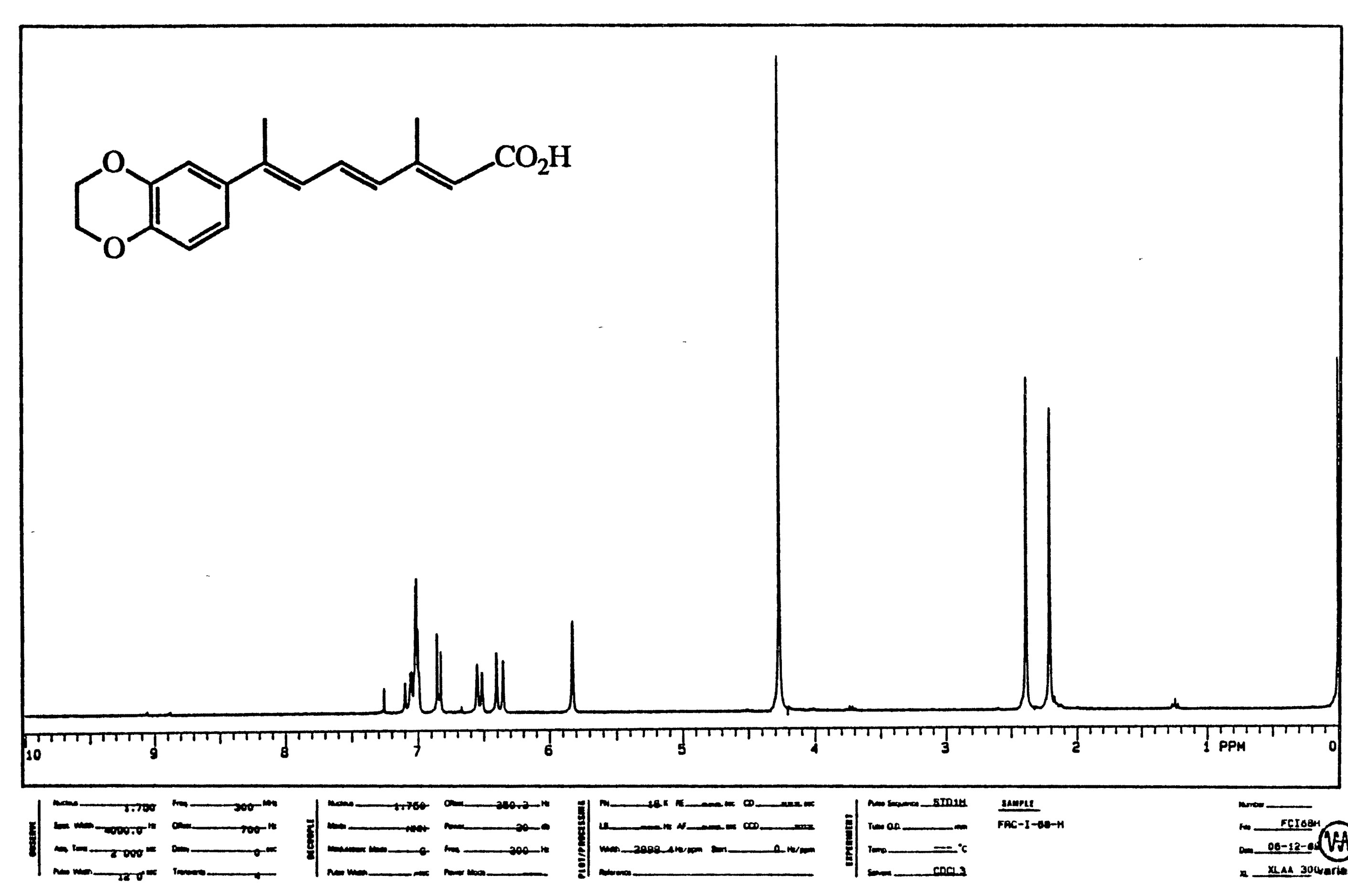
UV Spectrum of **66**

PLATE XXV



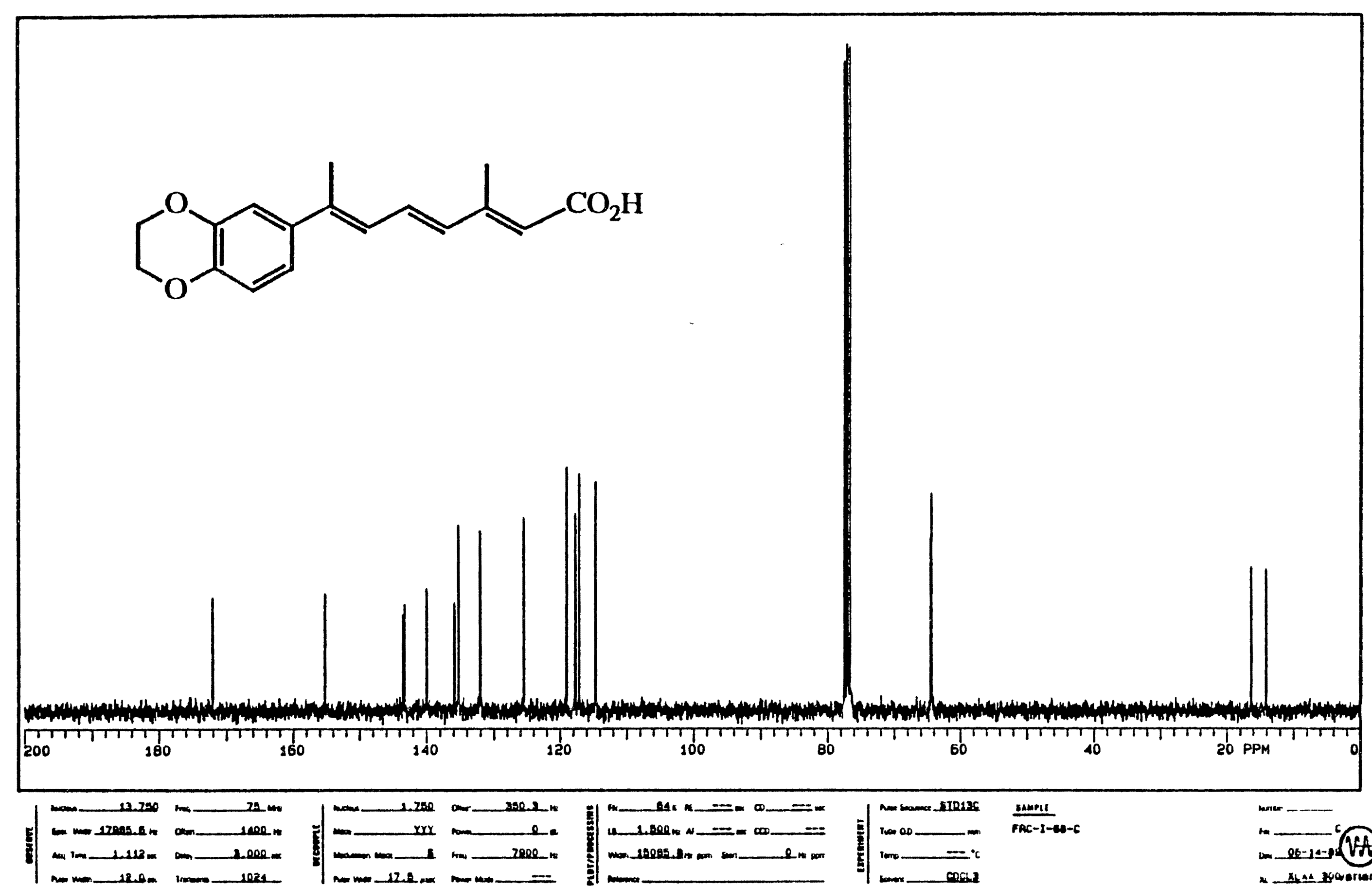
IR Spectrum of **67**

PLATE XXVI



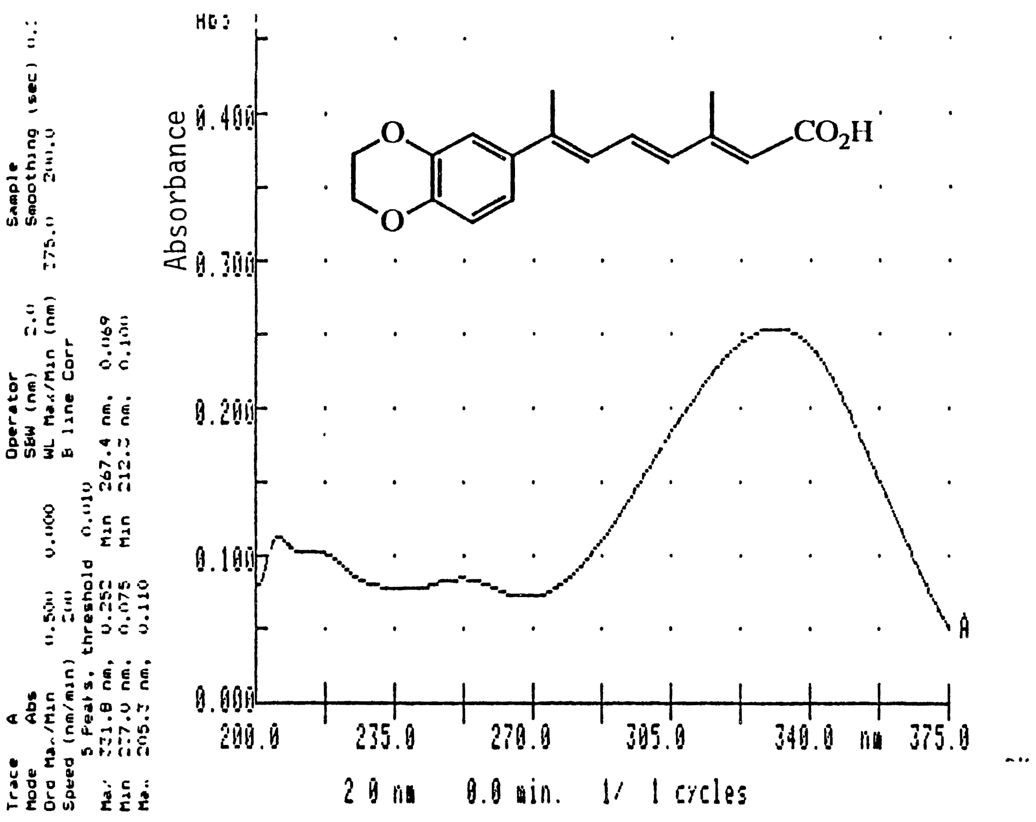
^{1}H Spectrum of **67**

PLATE XXVII



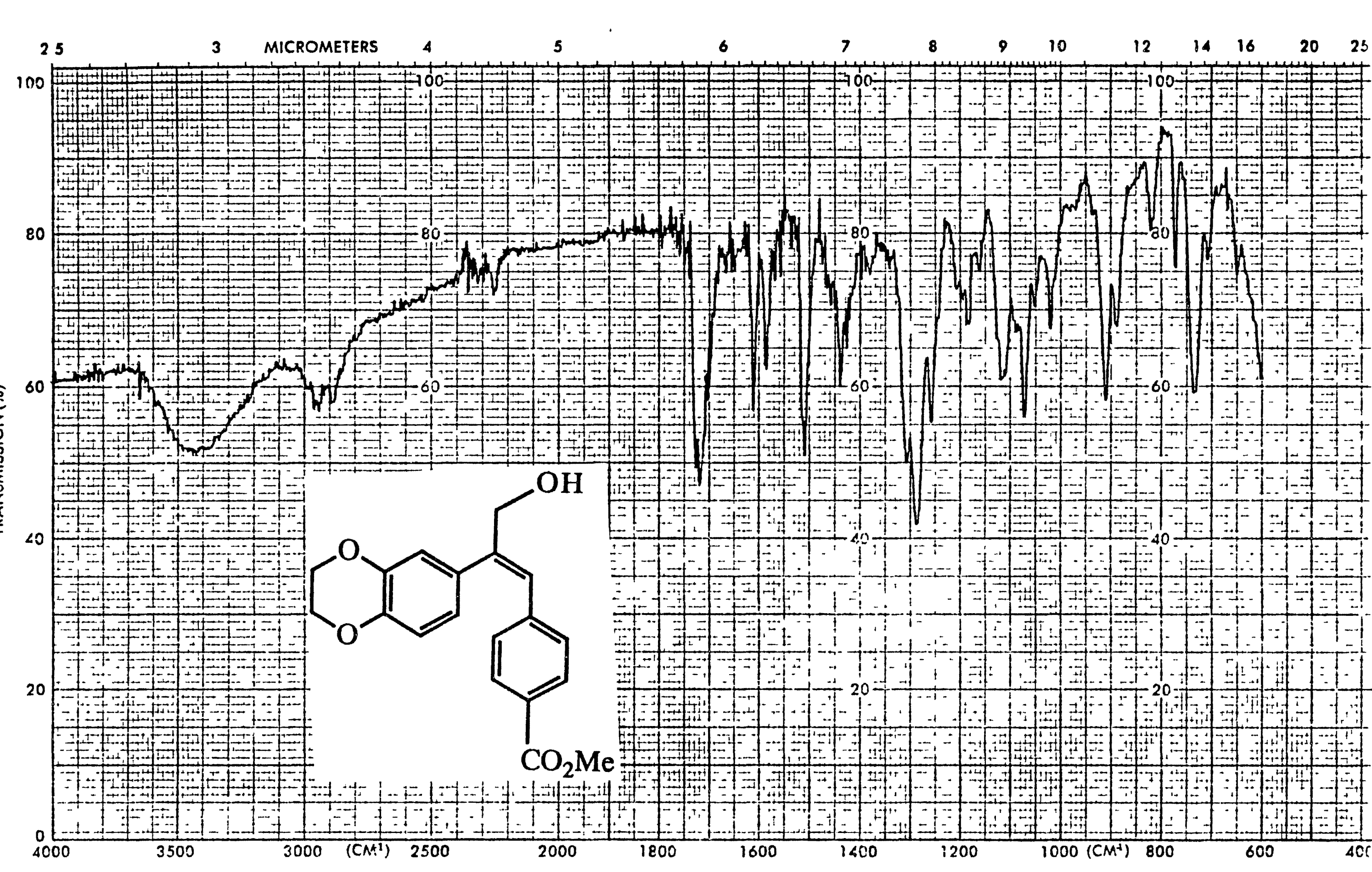
^{13}C Spectrum of **67**

PLATE XXVIII



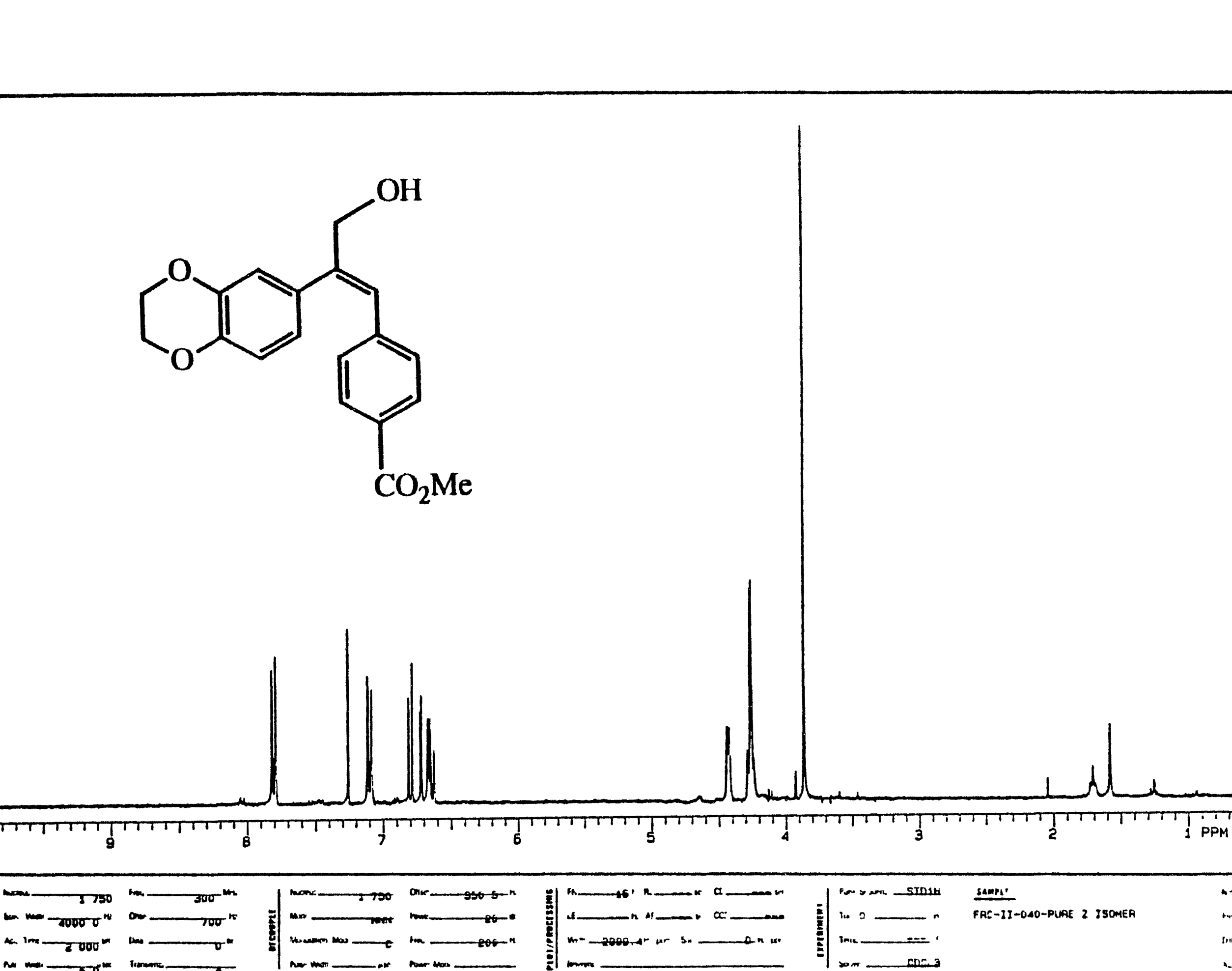
UV Spectrum of **67**

PLATE XXIX



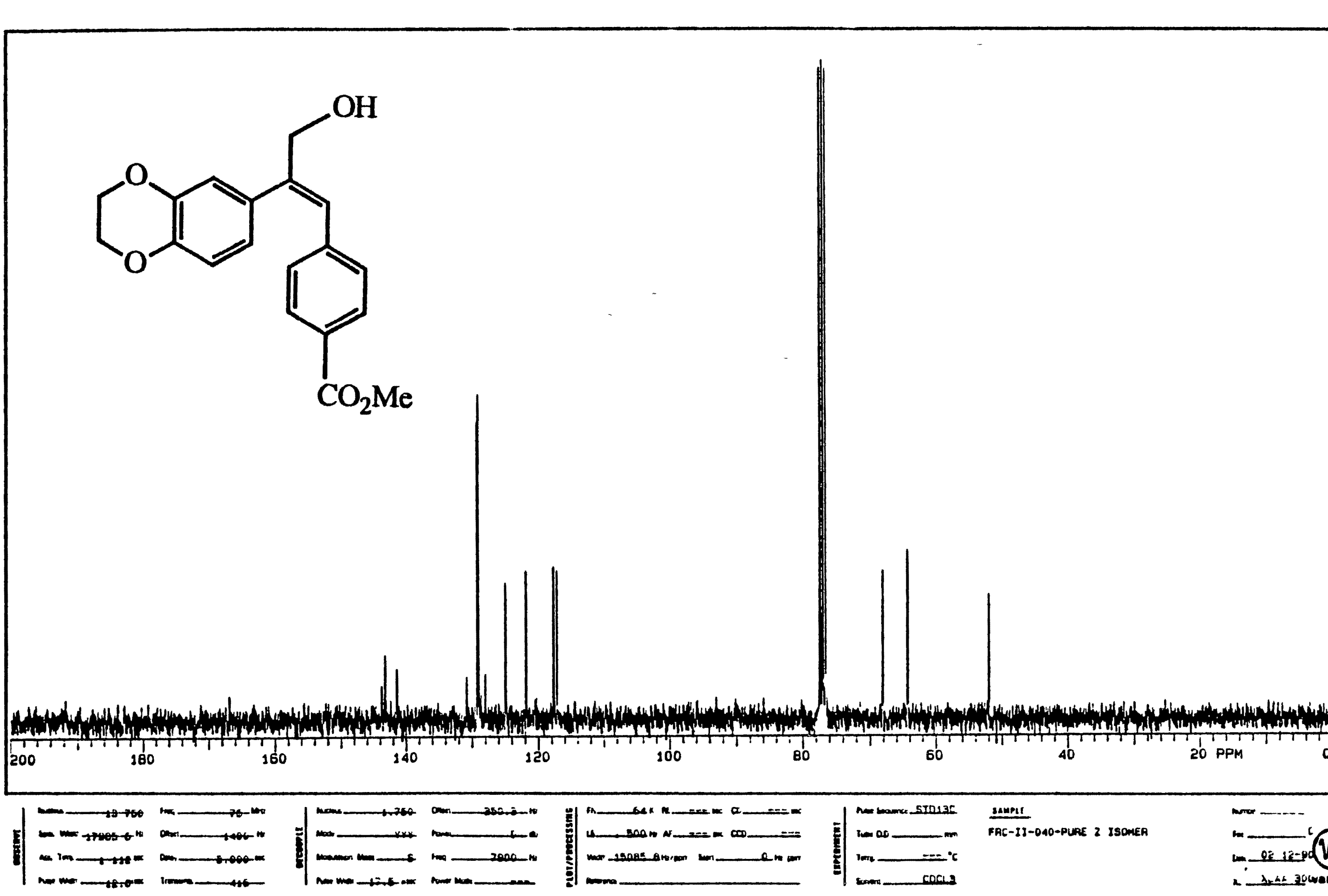
IR Spectrum of **68**

PLATE XXX



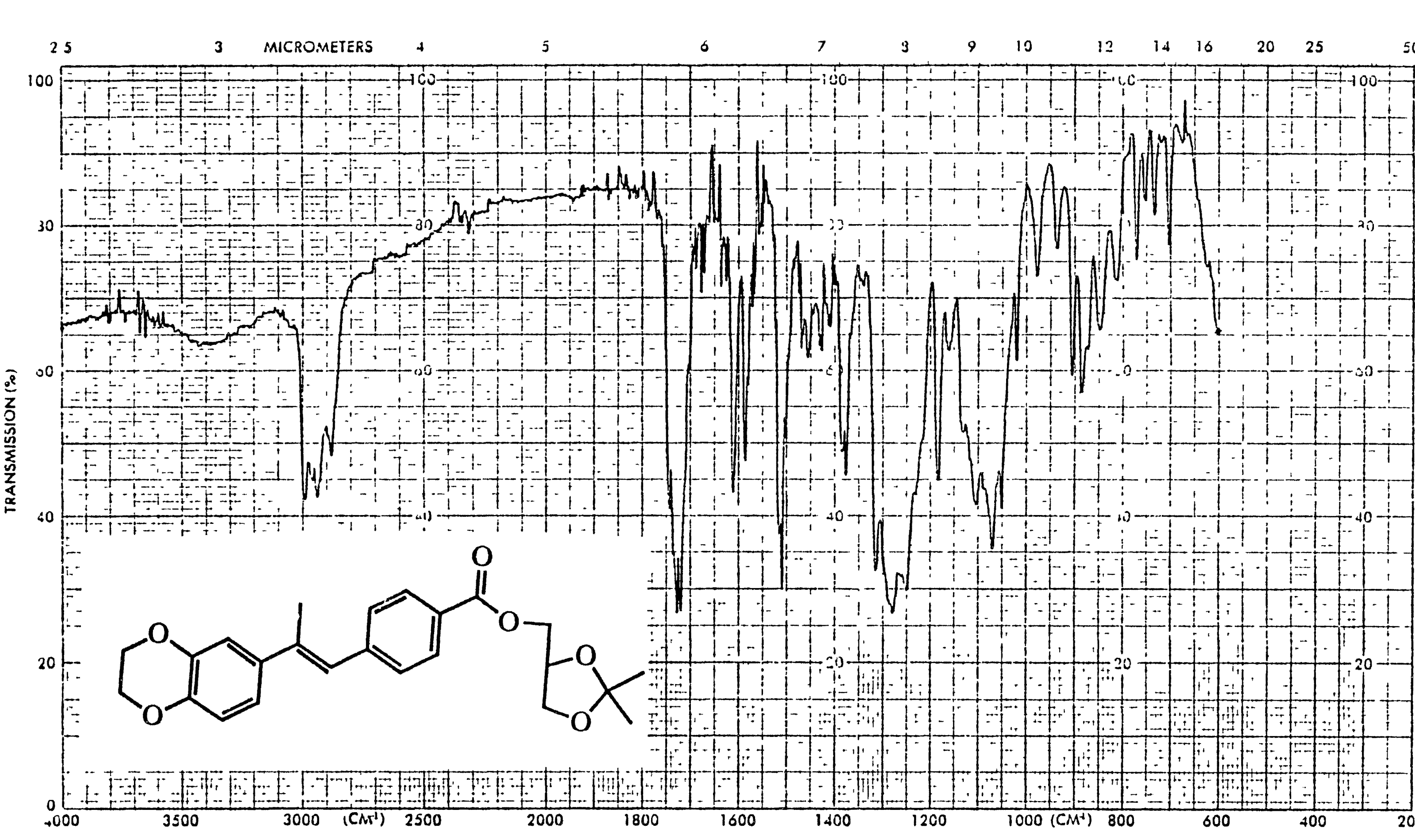
^1H Spectrum of **68**

PLATE XXXI



^{13}C Spectrum of **68**

PLATE XXXII

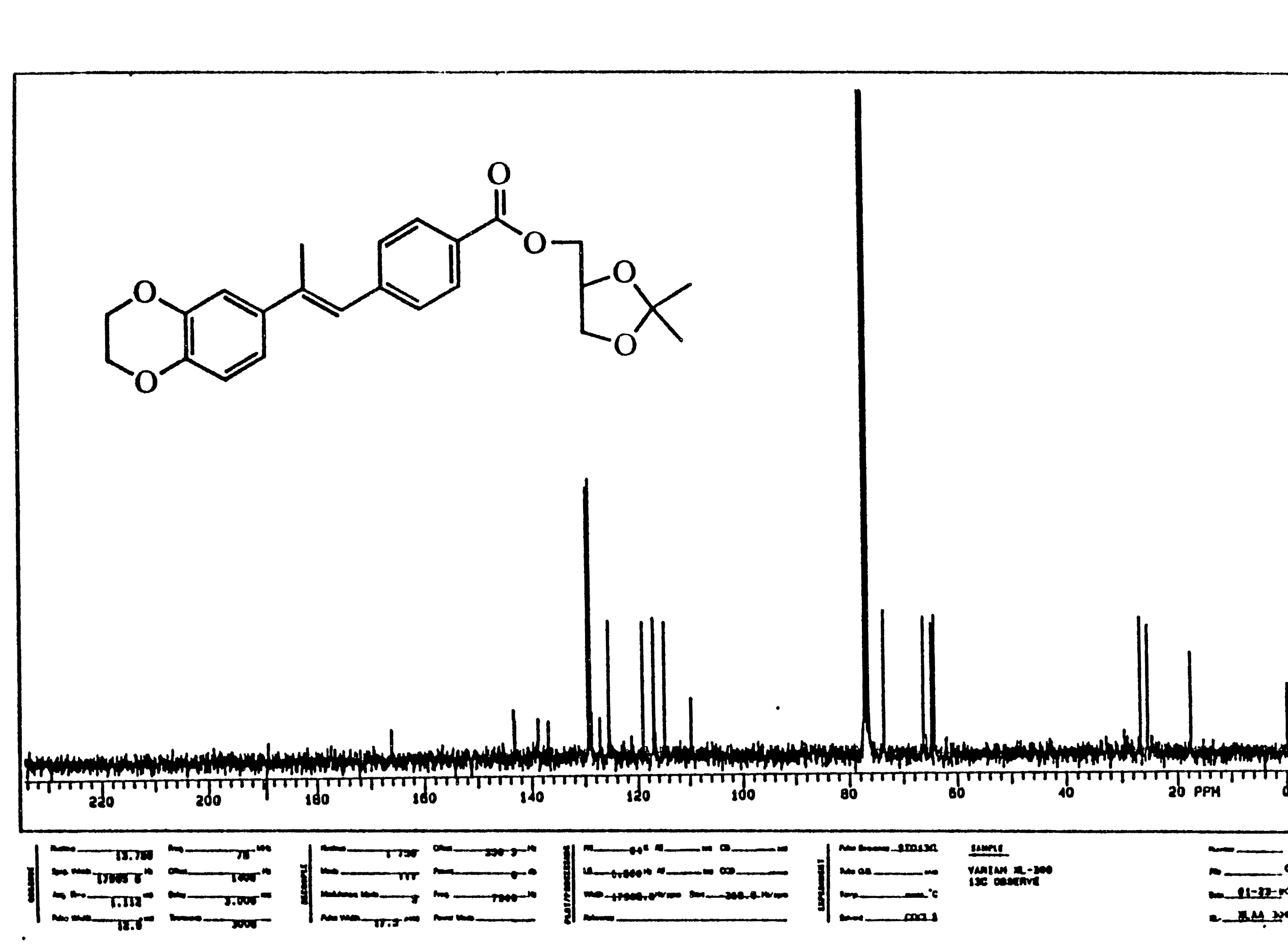


IR Spectrum of **69**

PLATE XXXIII

^1H Spectrum of **69**

PLATE XXXIV



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

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