THE SYNTHESIS AND THE BIOLOGICAL ACTIVITY

OF AN OXA-RETINOIC ACID

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CHAPTER I

HISTORICAL

Vitamin A and Related Compounds

The existence of an essential nutritional factor,
later to be called vitamin A, was assumed without proof,
and its physiological effects were described long before
the isolation of the vitamin itself and the determination
of its chemical structure. Dietary blindness, a disease
recognized in ancient Egypt, was shown in Denmark during
World War I to be a symptom of vitamin A dificiency.[1] The
Egyptians have recognized improvement from the effects of
night blindness after ingestion of liver, an organ that
stores vitamin A.[2]

Early attempts were made to isolate and identify
vitamin A from a milk, butter, and egg yolk.[2] In 1920,
two scientists, Drummond and Takahashi, independently were
able to succeed in partial purification of vitamin A from
from fish oil.[2,3] However it was not until 1931 when
Karrer and associates were able to determine the structure
of ($\underline{1}$) using a highly purified vitamin A from shark liver
oil.[4] Karrer and co-workers were also able to prepare
retinol esters, such as retinyl acetate, from the same

extracts of the shark liver oil.
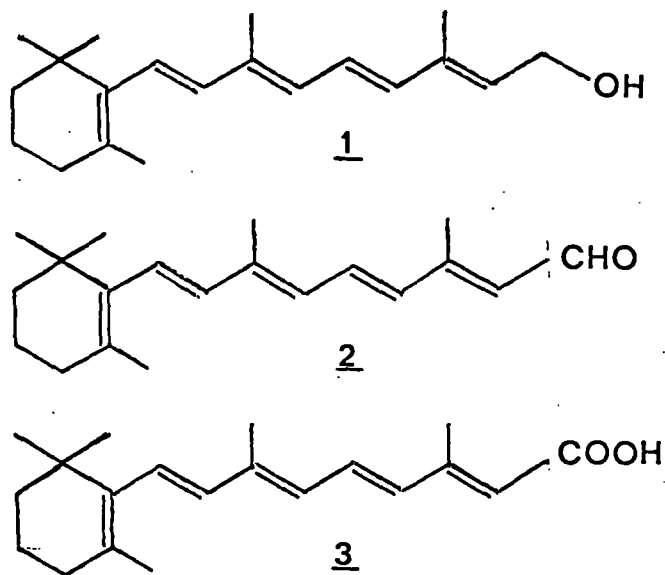
Synthetic Methods

The first industrial synthesis of retinoids were carried out by Isler and associates around 1947.[5] The most economically important processes today are those of Hoffmann-La Roche and BASF. At Hoffmann-La Roche, Isler and associates developed the first industrial synthesis of retinol (1) based on a reaction sequence they had used in 1947 to synthesize crystalline (1). The last C-C bond-forming step was a Grignard reaction between the C_{14} aldehyde (4) and the C_6 acetylene compound (5) to give the carbinol (6), which was converted to retinyl acetate (7).[2]
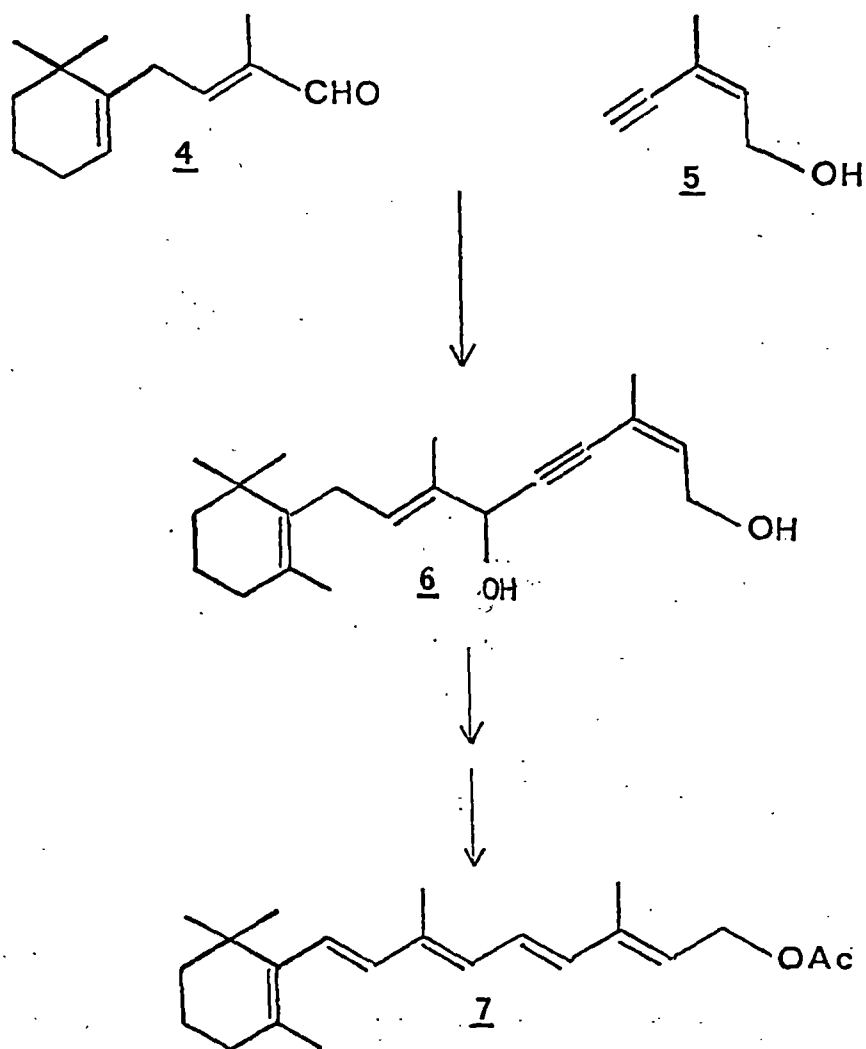
After the discovery of the Wittig reaction in 1953, Pommer and associates applied the Wittig reaction to the synthesis of retinoids. This work culminated in a novel industrial retinol synthesis at BASF laboratories in Ludwigshafen, Germany, central to which is the linking of the C_{15} phosphonium salt (8) to β-formylcrotyl acetate (9) to give a mixture of 11-cis-retinyl acetate (10) and all-trans retinyl acetate (7).[2]
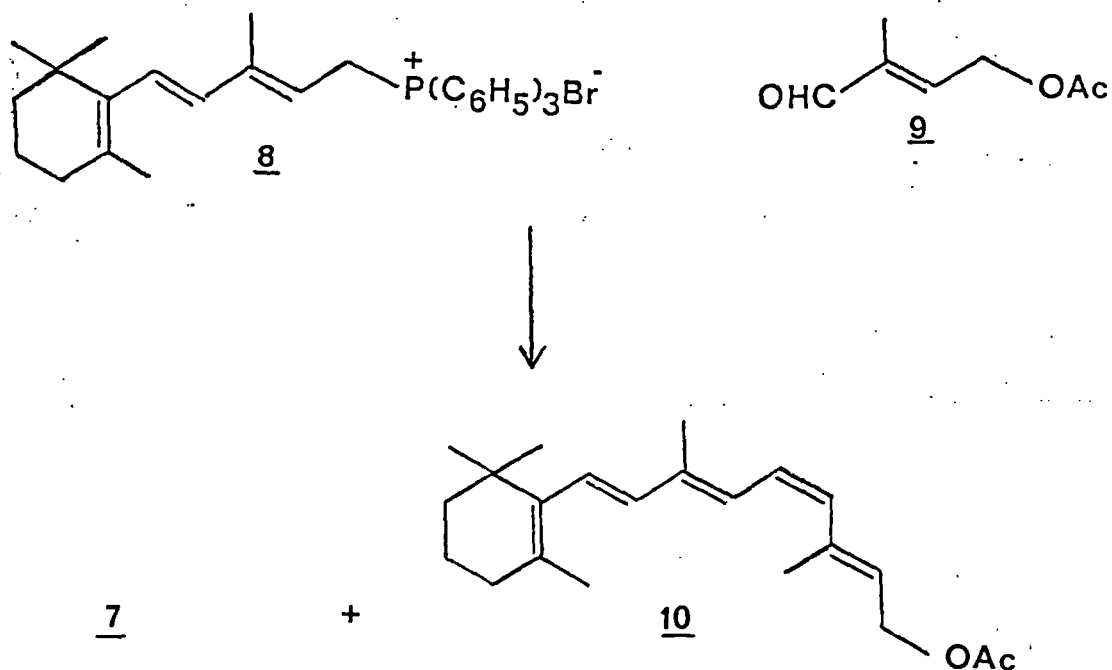
The Wittig reaction was not only economically advantageous for the synthesis of vitamin A, it was also employed in the synthesis of different carotenoids, compounds with aromas and flavors, juvenile hormones, and even prostaglandins.[2,6] Some advantages of the Wittig reaction are: 1) the reaction occurs in high yield; 2) high selectivity is possible; 3) there is no limit to the number of carbon atoms. Its major disadvantages are that the triphenylphosphine oxide by-product is sometimes difficult to separate[7] and stereoisomeric mixtures are produced.

Chemical Properties

Retinol (1) is chemically unstable and readily undergoes decomposition by the action of oxygen and heat.

In the presence of light, retinol (1) undergoes cis-trans isomerization, a fundamental reaction in the chemistry of vision. It is acid sensitive, but in the presence of a mild oxidizing agent such as manganese dioxide, it produces retinal (2) without cis-trans isomerization.

Retinal (2) is the aldehyde analog of (1) and the biosynthetic intermediate in the conversion of carotene to (1) in the intestinal mucosa of animals.[8] In 1933, Wald discovered that the "visual purple or rhodopsin" in the eye is a complex of a protein opsin and the polyene retinene, later identified as vitamin A aldehyde or retinal (2).[9]

Retinoic acid (3), like 1 and 2, is also susceptible to oxidation. In the presence of light, iodine, acid, and heat, 3, like 1 and 2, undergoes cis-trans isomerization.[10] Retinal (2) can be reduced to retinol with lithium aluminum hydride without isomerization of any double bond.[9]

Retinoids

About 50% of the total cancer deaths in the United States are due to malignancies in epithelial tissues, including lung, pancreas, colon, bladder, breast, and ovary.[9] Cytotoxic chemotherapy, surgery, and radiation are some of the techniques being employed to combat these malignancies in their acute stages. Recently, it has been suggested that the better approach to combat cancer would

be to treat the disease during its period of progression,
after the tissue has been exposed to a carcinogenic insult
but before the lesion produced has become invasive.[11]
One possible approach to this problem is the use of vitamin
A and compounds related to it called retinoids.

Retinoid is a general term that includes both the
naturally occurring compounds with vitamin A activity and
synthetic congeners with or without the biological activity
of retinol (1). Retinoid compounds may include
metabolites, oxidation products such as retinal (2) and
retinoic acid (3), reduction products, or derivatives
thereof, either synthetically or naturally derived, which
may or may not exhibit the various physiological activities
attributed to retinol (1).[9] During the IUPAC-IUC Joint
Commission on Biological Nomenclature (1982), it was
recommended that the term vitamin A should be used as a
general descriptor for retinoids exhibiting qualitatively
the biological activity of 1. This term should be used
in derived terms such as vitamin A deficiency, and vitamin
A antagonist.[2]

Activity of Retinoids

Retinoids are involved in the maintenance of a normal
epithelium in such tissues as the skin, lung, colon,
urinary bladder, and mammary gland. The beneficial effects
safety of retinoids must be proven first in test animals
before investigations in man can take place. Biological

methods of analysis are important tools for both the
practical development of new retinoids and for the analysis
of their mechanisms of action since they measure highly
specific cellular responses.

Bioassay methodology is of special importance in two
major areas of retinoid research: 1) To screen for new
retinoids that might be useful for prevention or treatment
of numerous diseases. Bioassay methodology can inform the
synthetic organic chemist that two retinoids which differ
by as little as cis-trans configuration around a double
bond or the location of a carboxyl group (ortho-, meta-,
or para-) on a benzene ring, could have surprisingly
different functional activity. 2) To begin to explore the
problem of the cellular mechanism of action of retinoids,
which still remains unsolved after more than 50 years since
the original discovery of their biological activity.
Bioassay systems make it possible to analyze how chemical
structures are transduced into biological function.
Knowledge of structure-function relationship in various
biological test systems by itself can provide important
information with respect to the mechanism of action.[9]

In vivo methods useful for biological analysis and
assay of retinoids include mouse papilloma test, the
ornithine decarboxylase assay, and others.[2,9] In vivo
assay methods are of particular importance for measuring
the biological activity of various metabolites of 1 and 3,
for screening of new retinoids for potential usefullness as

pharmacological agents, and for predicting the clinical utility of retinoids.[2,9] Some of the _in vivo_ methods and findings pertaining to it are described below.

Papilloma Test

This method, developed and extensively used for drug screening, measures the ability of retinoids to cause regression of papillomas and gives an estimate of the _in vivo_ toxicology of a retinoid. In the standard test system, the initiator 7,12-dimethylbenz(a)anthracene, DMBA, is applied twice at a 14-day interval to shaved dorsal skin of mice. A promoter, croton oil, is applied after 21 days at twice per week, and this treatment is continued for 3-8 months until animals develop multiple papillomas. At this point treatment with retinoids is begun either intraperitoneally or orally.[2]

Ornithine Decarboxylase (ODC) Activity

The induction of ornithine decarboxylase is one of the essential events in teh process of carcinogenesis based on the study of the biochemical mechanism of tumor formation in the skin.[12] Both natural and synthetic retinoids have been tested in this _in vivo/in vitro_ system.[13] The ODC assay is a highly sensitive _in vivo_ assay that gives significant responses to a single application of less than a nanomole (300 ng) of 3. It is the simplest _in vivo_ test because no special colony of animals is required

and the enzyme assay is not especially difficult. ODC
studies have indicated that TPA (11), 12-0-tetradecanoyl-
phorbol-13-acetate, the active principle of croton oil, is
a potent inducer of the enzyme ornithine decarboxylase
(ODC), and that retinoids can block this enzyme induction.
The ODC assay is performed as follows. The retinoid and
TPA are each dissolved in acetone and applied to the shaved
dorsal skin of mice. The mice are pretreated with an
appropriate dose of retinoid one hour before the applica-
tion of 17 nmol of TPA (11). The mice are sacrificed 4.5
hours after dosing with TPA, which is the time of maximum
ODC induction in the skin. Epidermal preparations, sepa-
rated by brief heat treatment, from three or four mice are
homogenized in neutral phosphate buffer containing 0.1 mM
pyridoxal phosphate and 0.1 mM EDTA and centrifuged to get
a clear extract.[2,14] The ODC activity of this extract is
determined by measuring the release of labeled CO_2 from
(^{14}C)-ornithine.[15] Determined in vivo, 13-cis-retinoic
acid (12) has activity that is comparable to its all-
trans isomer (3) when it is applied directly to the
skin. 13-cis-Retinoic acid (12) is the most useful of
all retinoids, to date, for systemic administration of a
variety of human skin diseases. Amide derivatives are
inactive in this test.[2]

CH₃(CH₂)₁₂CO₂ OAc
$CH_3(CH_2)_{12}CO_2$ OAc
CH₃ CH₃ CH₃
H3C OH H H
O OH OH OH

11

12 COOH
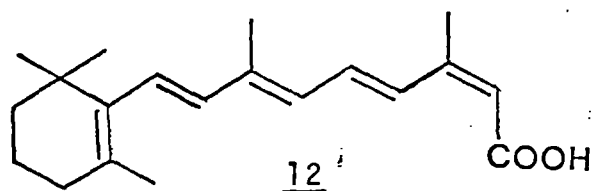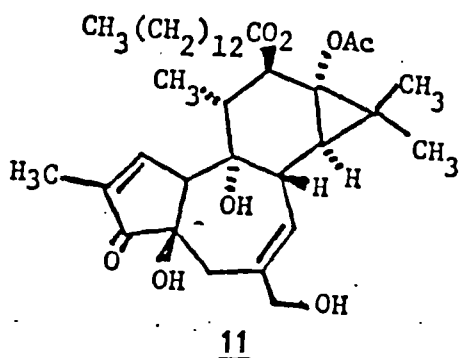
In vitro methods have recently been evaluated for the determination of the biological activity of retinoids. Advantages of in vitro methods are that they require smaller quantities of the compound for testing, and data can be obtained in a relatively short period of time. In vitro methods include a hamster tracheal organ culture, HL-60 leukemia cell, and others. Some of the methods and findings are described below.[2]

Hamster Tracheal Organ Culture Assay

The hamster tracheal organ culture assay is a highly sensitive, precise, and reproducible bioassay method which measures a physiological induction of differentiation in a serum free system that has been essentially depleted of endogenous retinoid.[9,14] This method has been utilized in the prevention of epithelial cancer by retinoids. The presence of dark purple keratohyaline granules and bright pink keratin are the histological end-points measured. Analogs are scored as "inactive" if both keratin and keratohyaline granules are seen and "active" if neither

keratine or keratohyaline granules are seen.[14]

The tracheal organ culture assay is an excellent method to study the biological and chemical properties of retinoids and particularly for measuring the structure-activity relationships. A complete dose-response curve can be obtained in a tracheal organ culture with as little as 1-10 ng sample of a highly active retinoid. Some disadvantages of this bioassay method are that it requires special animal care, and the method is definitely more expensive than other in vitro methods.[14,16]

Human Promyelocytic Leukemia
Cell Line HL-60 Assay

HL-60 cells can be induced to differentiate terminally to morphologically mature granulocytes by in vitro treatment with a wide variety of compounds, including sodium butyrate, hypoxanthine, dimethylsulfoxide, dimethylformamide, and actinomycin D.[2] The capacity of HL-60 cells to reduce nitroblue tetrazolium is the basis for the bioassay of retinoids in a serum free medium.

Retinoids are added to the HL-60 cells at the start of the assay. Ethanol is used as the vehicle. The HL-60 cells are incubated for 4 or 5 days. Differentiation is measured by nitroblue tetrazolium (NBT) of cytopsin slide preparations. Results are expressed as percentage of NBT positive cells. Approximately 4-8% of the cells will differentiate spontaneously in the absence of retinoid.[2]

Breitman reported that physiological amounts of
retinoic acid (3) would cause induction of differentia-
tion in HL-60 cells, the levels of 3 that are required
are one-thousandth to one-millionth of those of the other
inducers. Retinoic acid (3) at a concentration of 10^{-9}
M will induce approximately 20% of the HL-60 cells in a
culture to differentiate.[14,16]

<div align="center">

Problems Associated With

Natural Retinoids

</div>

The toxicity of retinoids is one of the major problems
when considering retinoids in cancer prevention. Altera-
tions of the skin, hair loss, desquamation, and headache
are among the symptoms observed in humans with excess
vitamin A. Takahashi observed loss of hair, emaciation,
paralysis of the hind legs, and death within several weeks
to mice and rats given crude concentrate of vitamin A
orally.[1,17] Sporn and associates have indicated that the
nature of the polar end of a homologous series of retinoids
is an important determinant of their toxicity.[18] Toxicity
can be diminished by the synthesis of analogs with less
polar end groups.[14] Thus carboxylic acid derivatives are
more toxic than their ester or amide analogs. Alcohols are
more toxic than their ether analogs.[14]

Another major concern is that retinoids may increase
the carcinogenic effect of certain substances in selected
systems. Experiments by Forbes in which hairless mice

received daily application of 0.01% or 0.001% retinoic acid

(3) in methanol and were exposed to ultraviolet irradia-

tion resulted in marked enhancement of tumor formation.

Under this condition, 3 enhanced utraviolet

tumorigenesis.[14]

Synthetic Retinoids

As mentioned earlier, a major problem associated with

natural retinoids is their inherent toxic properties.[2]

The use of both 3 and 7 in the chemoprevention of

cancer is severely limited by their toxicity when adminis-

tered in pharmacological doses.[14] Thus, it is necessary

to develop synthetic retinoids possessing pharmacological

properties significantly different from those of natural

retinoids so that toxicity is reduced while the preventive

activity is retained. One interesting property of some new

synthetic retinoids is their relatively high stability

compared with the classic retinoids, although dilute solu-

tions of these compounds still undergo isomerization under

the action of light to give a mixture containing cis and

trans isomers.[2]

Synthetic retinoids have been synthesized and tested

in various ways to determine their biological activity in

terms of growth, differentiation, and antipromoting

activities. In the papilloma test, a new synthetic

retinoical benzoic acid derivative TTNPB (13, tetrahydro-

tetramethylnapthalenylpropenylbenzoic acid) and its ethyl

ester have been found to be more active than 3.

13
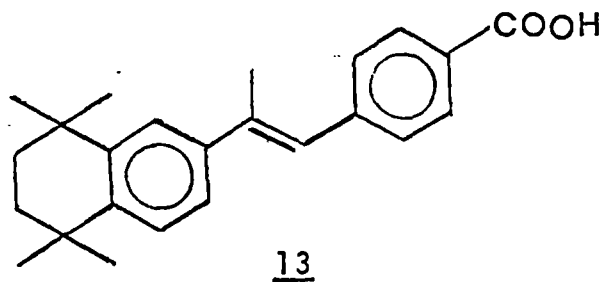
The synthetic analog that has been most extensively studied is the 13-cis isomer of retinoic acid (12). Compared to 3, 13 has a lower toxicity in both mice and rats.[1] In 1982, two synthetic retinoids were introduced into the clinical practice of dermatology. In the United States, Accutane, whose active agent is 12, was approved for the treatment of severe cystic acne. In Europe, etretinate (14), marketed as Tigason, is in use either alone or in combination with other agents for the treatment of psoriasis and related cutaneous disorders of keratinization.[14]

14
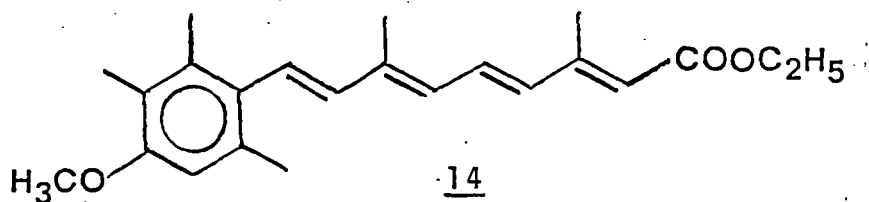
The main objective of our research was to synthesize the new retinoid compound 15 which, hopefully, would be more active than any natural retinoids but less toxic. We

hoped that by changing the basic retinoid structure, a
clinically useful compound could be synthesized that will
concentrate in specific target tissues.

15
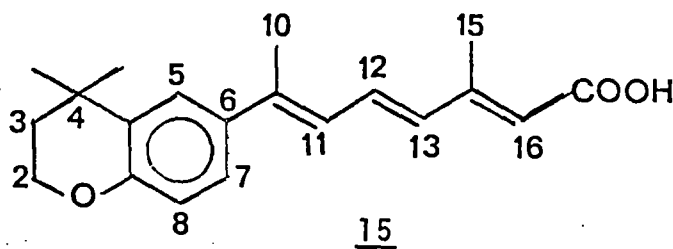
CHAPTER II

RESULTS AND DISCUSSION

Retinoids are rapidly oxidized or isomerized to altered products in the presence of light or oxidants. Because of their instability, all retinoid products were handled with extra caution. The oxa-retinoic acid (15) after recrystallization from absolute ethanol was carefully dried, sealed under a nitrogen atmosphere and kept in a freezer at $0^{\circ}C$ in an amber colored bottle. Considerable attention was paid during extraction. The separatory funnel was wrapped with an aluminum foil and most of the lights in the laboratory were turned off. The same precautions were observed during vacuum evaporation of the solvents used and during chromatographic analyses.

Methyl 3-phenoxypropanoate (17) and
2-Methyl-4-phenoxy-2-butanol (18)[19]

The ester (17) was prepared by boiling a solution of 3-phenoxypropanoic acid (16) in methanol for 4 h with p-toluenesulfonic acid as the catalyst. Reaction times were varied from as much as 48 h to as short as 4 h. All esters were vacuum distilled before proceeding to the next reaction. Percent yields, after vacuum distillation,

SCHEME 1. Preparation of the 4,4-Dimethylchroman ($\underline{19}$)[19]
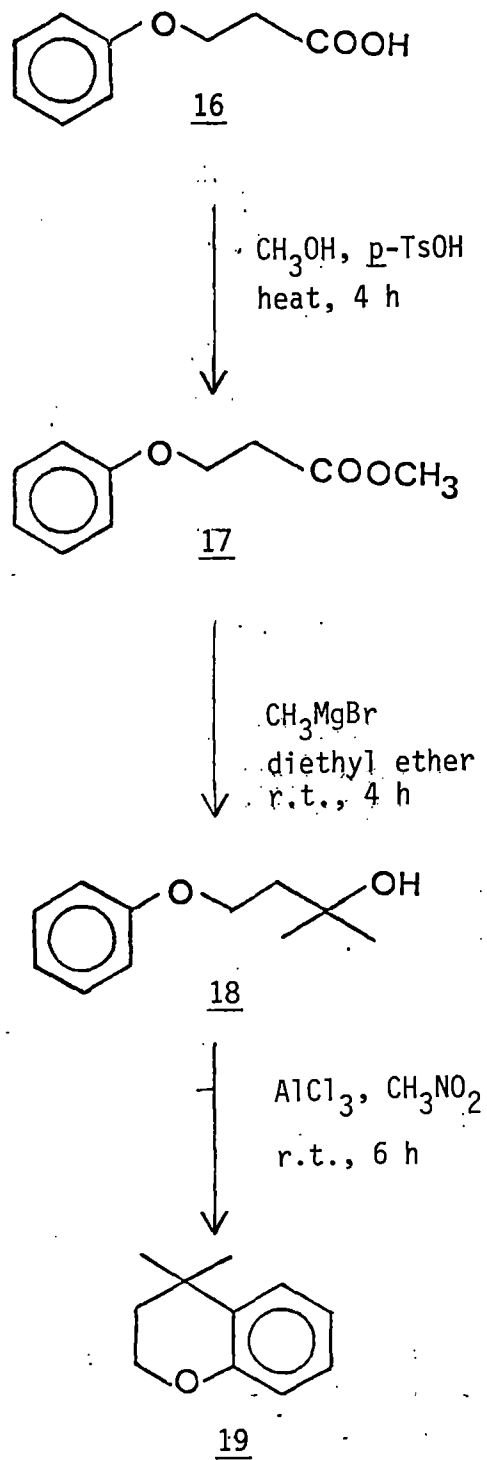
varied from 80% to 88%. A reaction time of 4 h was found to be the most suitable with yields around 88% after vacuum distillation. A Grignard reaction was employed to convert 17 to the tertiary alcohol (18). The tertiary alcohol (18) was prepared by adding methylmagnesium bromide in THF to an ether solution of 17 in a nitrogen atmosphere. Yields varied from as low as 30%, using smaller amounts of reagents but maintaining the same molar ratio, to as much as 80%, after vacuum distillation, using scaled up quantities. Ammonium chloride, a mild acid, was used as the quenching agent. Precautions were observed after vacuum distillation of 18, a tertiary alcohol which could dehydrate. Both 17 and 18 were found to be colorless liquids, stable for at least twelve months. Infrared, H-1 NMR, and C-13 NMR spectra were taken for all compounds.

<div style="text-align:center">

4,4-Dimethylchroman (19) and 6-Ethanoyl

4,4-Dimethylchroman (20)[19]

</div>

The dimethylchroman (19) was prepared by adding a solution of 18 in nitromethane to a solution of aluminum chloride in nitromethane. The addition of the first few drops of 18 to the aluminum chloride solution turned the solution cloudy. Upon the addition of all of the solution of 18 to the aluminum chloride solution, the color of the mixture was blood red. The reaction was followed by thin layer chromatography. After the third hour, a small amount of the starting material 18 was observed. During the

fourth hour of the reaction only one spot, corresponding to compound 19 was observed. The acetyldichroman (20), a yellowish liquid, was prepared by adding a nitromethane solution of anhydrous aluminum chloride to a solution of acetyl chloride and 19 in nitromethane. Different reaction times were tried, 6, 24, and 48 h, and yields obtained after vacuum distillation were low. Yields of 13, 30, 40, 44, and 48% were obtained. Generally, increased reaction time led to increase yield. The molar ratio of aluminum chloride: acetyl chloride: 19 was maintained at 1: 1: 1 in order to prevent further acylation at other sites. The reactions were conducted at room temperature only and the starting material 19, whose boiling point under vacuum is lower than 20, was recovered during vacuum distillation of 20. Generally, about a third of the original weight of the starting material 19 was recovered, though the percent yield calculations were based on the original amount of 19 used and not on the amount of 20 that reacted.

Formation of the Phosphonium Salt (22)

The alcohol (21) was prepared by adding a solution of vinyl magnesium bromide to a stirred solution of 20 in anhydrous ether. The product 21 was never purified and was converted to the phosphonium salt (22) right away. In one instance, 21 was kept in the refrigerator

Scheme 2: Preparation of the Phosphonium salt (22)
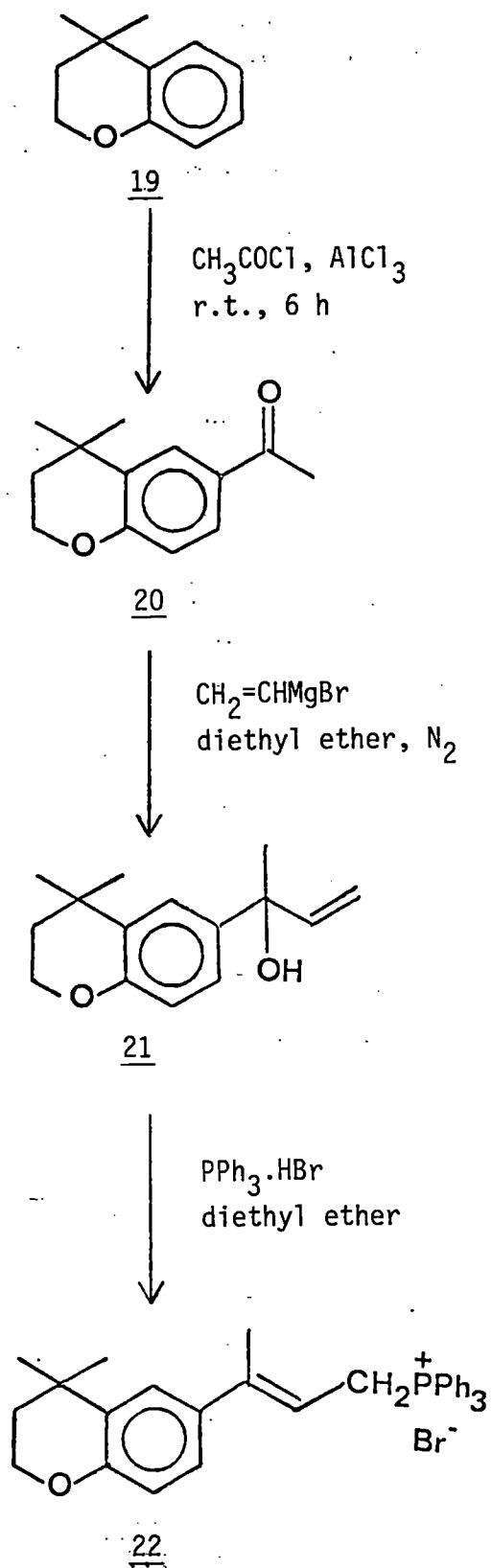
overnight and the crude, viscous, brown solution of 21

yielded clear colorless crystals on the sides of the

container which quickly liquified upon exposure to room

temperature. The phosphonium salt (22), was obtained from

the reaction of equal number of moles of the crude 21 and

triphenylphosphine hydrobromide.

Oxa-Retinoic Acid (15)

The Wittig reaction was used to prepare the retinoid

ester (24). The reaction of 22 with ethyl 3-formyl-2-

butenoate (23) plus a base, n-butyllithium or sodium

ethoxide, gave crude 24. Percent yields varied from

48-89%. Both H-1 and C-13 nmr of the crude retinoid ester

24 indicated the presence of triphenylphosphine oxide.

Thin layer chromatography of the crude 24 was performed

using petroleum ether:ethyl acetate (98:2) as the mobile

phase. Several spots were observed. Flash chromatography

of the retinoid ester (24) was done to get rid of the

triphenylphosphine oxide by product. C-13 and H-1 nmr of

the chromatographed 24 indicated a mixture of isomers.

The oxa-retinoid ester (24) was hydrolyzed to the oxa-

retinoic acid (15), as discussed in the experimental

section, and 15 was recrystallized from absolute ethanol.

H-1 and C-13 nmr revealed a single isomer of 15.

Repeated recrystallization of 15 from absolute ethanol

yielded the same all-trans isomer. The oxa-retinoic

acid (15), was obtained as yellow crystals and identified

SCHEME 3. Preparation of the Oxa-retinoic Acid (15)

1. n-BuLi or NaOEt

2. OHC—C(CH3)=CH—CO₂C₂H₅ 23

24

KOH, EtOH, H₂O

50°C, 4 h
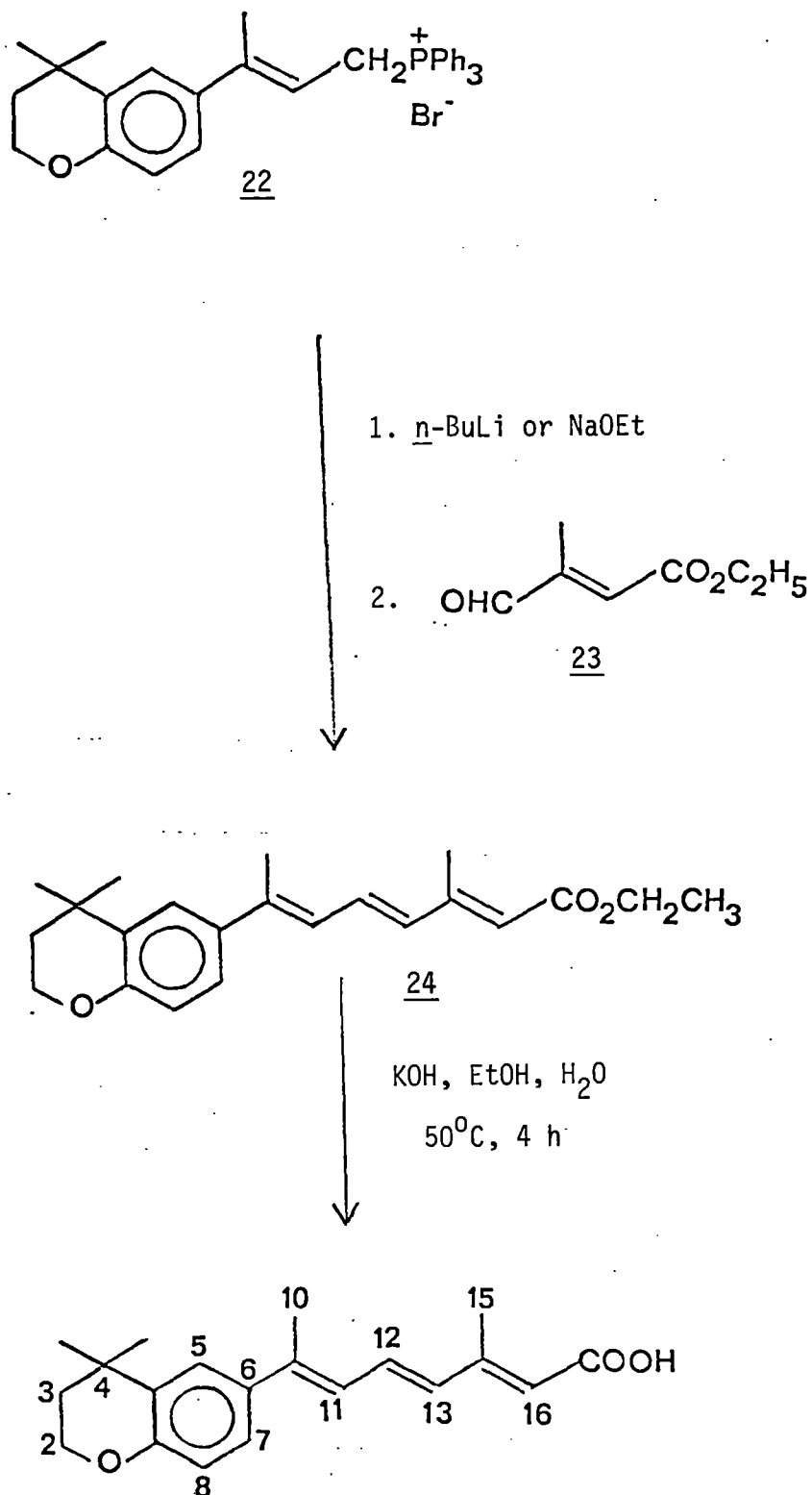
by its IR, UV, H-1 NMR, and C-13 NMR. The melting point, the yellow color, and the spectra did not change with further recrystallization. Hydrolysis of the ethyl oxa-retinoid ester (24) to the oxa-retinoic acid (15), as described in the experimental part, was carried out carefully under a nitrogen atmosphere and in a dark environment to prevent the oxidation of the oxa-retinoic acid (15). In our experiments, both chloroform and methylene chloride were used as the extracting solvents. The solution was first acidified with 6 $\underline{M}$ HCL before extraction with either chloroform or methylene chloride. Retinoids with a free carboxylic acid moiety show a selective extraction into the organic phase, depending on the pH of the extraction mixture.[2] At pH above 7, the retinoid remains in the aqueous layer, while acidification of the mixture allows the retinoid to be extracted into the organic phase. Isomerization during extraction has also been demonstrated.[2] Zile has observed an 11% isomerization of 3 to 12 after extraction of bile with 1-butanol.[2] Goodman has reported the isomerization of 12 to 3 within 5 minutes after exposure to standard flourescent light with a 75% (12): 25% (3) steady state being reached in 1-2 h.[2]

Polymeric Wittig Olefination

Polymeric reagents are polymers that carry functional groups.[20] In recent years there has been a great interest in polymer-supported reactions. Wittig reactions using

various alkyltriphenylphosphonium halides and aldehydes have been carried out successfully.[21]

Polymeric-supported Wittig reactions have the advantage of simplified work-up procedures, in particular the easy removal of the polymer-bound phosphine oxide by-product.[19] Another advantage is that the formation of by-products can be suppressed in some cases since intermediates, which are now more easily separated, can be removed.[7] Ethyl retinoate (ethyl ester of 3) have been prepared using polymer supported Wittig reagents, in 55% yield, without the need of chromatography, extraction, or crystallization to remove the usual triphenylphosphine oxide by-product.[22]

Polystyryldiphenylphosphine (27) and Poly-styryldiphenylphosphonium bromide (28)[23]

A 2% cross-linked polymer was used because there is a faster transport of reagents in and out of the polymer matrices. Highly cross-linked polymers have been reported to give poor yields, especially if the reactant and product molecules are large and therefore transport in and out of the polymer matrices is slow and difficult.

A 2% cross-linked bromopolystyrene (25) was treated with n-butyllithium in anhydrous toluene to give the lithiated product 26 under an argon atmosphere.

The product 26 was never isolated, but proof of its formation was based on the colors observed. The product

25

SCHEME 4. Preparation of the Polystyryldiphenylphos-
phonium bromide (28)
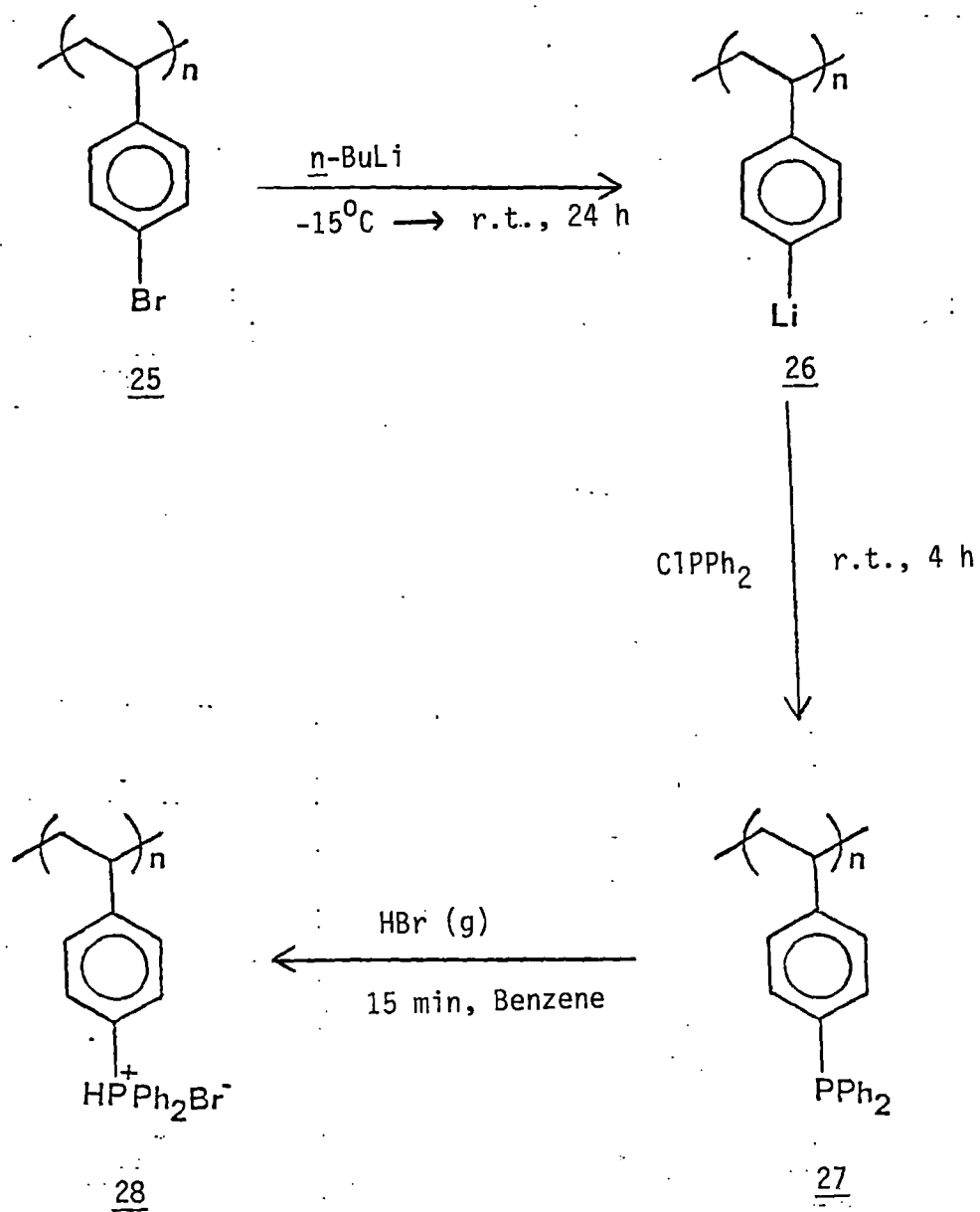
SCHEME 5. Polymer Supported Wittig Reactions
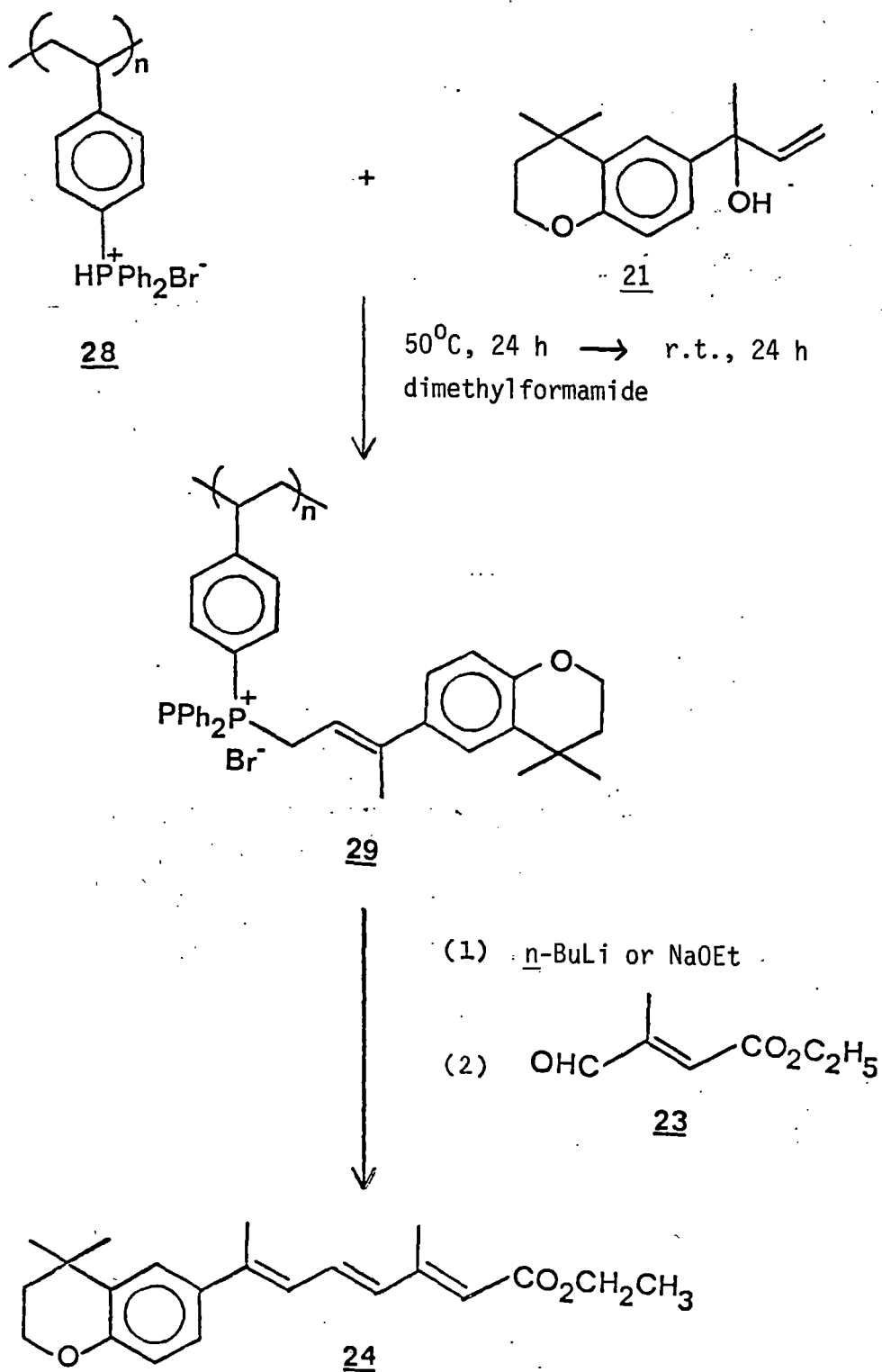
26 is brown in toluene and is colored red when THF is used as the solvent. Both colors were observed when the product was either suspended or washed with either toluene or THF. Upon washing the product 26, the solvent was changed to THF. Chlorodiphenylphosphine was slowly added to form polystryryldiphenylphosphine (27). The product 27 was converted right away to 28 by passing hydrogen bromide gas for 15 min to a suspension of 27 in dry benzene. Indication of the formation of 28 was confirmed by P-31 nmr analysis.

Polymer Supported Wittig Reactions

The reaction between the alcohol (21) and the polystyryldiphenylphosphine hydrobromide (28) was carried out in dimethylformamide with a yield of only 21%. One possible factor for the very low yield of this reaction is the size of 21. It is possible that due to its relatively large size, 21 may not have reached the reactive sites in the polymer matrices. The solvent used, DMF, maybe another factor for the relatively poor yield of this reaction. It may not have swelled the polymer properly. Improper stirring methods could be another factor. Polymeric Wittig reactions were also done under a dark environment. It has been reported that yields of polymer-bound reagents were highest with a 2% cross-linked support.[22] Two different bases, sodium ethoxide and n-butyllithium were used. The reactions were run in

anhydrous ether so that yield can be compared with non-polymeric Wittig reactions. The yield obtained when using sodium ethoxide was used as the base was 85%. Yield using n-butyllithium was 65%. Yields using poolymeric reagents were comparable to the non-polymeric Wittig reactions (68% using n-butyllithium and 89% using sodium ethoxide). The solvent used during polymeric Wittig reactions was ether, which is a relatively poor swelling solvent and thus could account for the slightly lower yields when compared to the non-polymeric reactions. The product 24 by the polymer route also indicated a mixture of isomers by nmr and hplc analyses. Thin layer chromatography and infrared results are the same as those of non-polymeric Wittig reactions.

<div align="center">Characterization of the Oxa-
retinoid ester (24)</div>

The oxa-retinoid ester (24) obtained from both polymeric and non-polymeric reactions indicated a mixture of isomers. Thin layer chromatography of the crude 24 in silica gel using ether: ethyl acetate (98:2) as the solvent system indicated the presence of a number of spots. The major spot (R_f value=0.68), was observed as a clear yellow spot when viewed under a visible light and was colored purple when viewed under an ultraviolet light. Another spot was observed at an R_f value of 0.83. It was later identified, using nmr and infrared techniques, to be that of the starting aldehyde ester (23). The other

starting material 22 and the triphenylphosphine oxide by-product were also detected by thin layer chromatography. R_f values for both were between 0.00 and 0.10.

Thin layer chromatography, using silica gel plate, of crude 24 in other solvent systems was also tried. An R_f value of 0.96 was observed for the oxa-retinoid ester when diethyl ether:ethyl acetate (98:2) was used as the solvent system. Using pure chloroform as the solvent, the R_f value was equal to approximately 0.87. In hexane:ethyl acetate (3:1), the R_f value observed was equal to 0.72. The retinoid estser (24) was easily observable since it appears as a yellow spot when observed by the unaided eye under a visible light.

Further characterization of the crude 24 was done to learn more about it. High performance liquid chromatography was used to characterize 24. Several solvent system combinations were tried. Flow rates were varied and a refractive index detector was used at first. A reverse phase column, made up of silica, was tried at first. In the reverse phase system, more polar compounds will elute first, and the system is not sensitive to the presence of water either in the sample or in the solvents. The result of the reverse phase hplc experiment is depicted in Figure 1.

The starting aldehyde ester (23) is responsible for the small peaks at 2.75 to 4.59 minutes, and the triphenyl-phosphine oxide by-product of the Wittig reaction appeared

Figure 1. Separation of crude oxa-retinoid ester (24)
           isomers by HPLC. Column: Whatman Partisil
           5-ODS-3; Solvent System: Methanol:0.01 M
           Ammonium Acetate (90:10); Detector: Waters
           R401 RI Detector

at 3.79 minutes. The two isomers of 24, which are probably the 12-cis 24 and the all-trans 24 came out at 10.58 and 11.28 minutes.

12-cis 24

CO2C2H5

all-trans 24
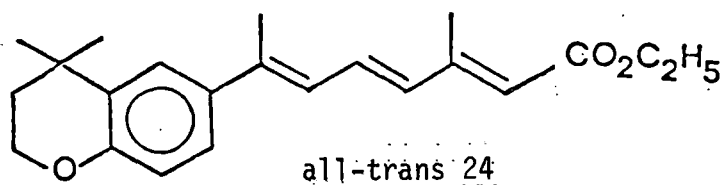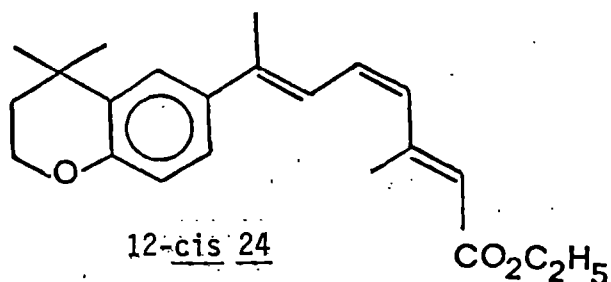
The fractions were collected in a dark room to prevent possible isomerization. H-1 and C-13 nmr of the fractions have never indicated a pure isomer. A possible explanation for this is isomerization during collection and extraction. The ultraviolet absorption of the collected fractions, from the HPLC experiments, were also determined. Absorption spectroscopy has been employed as an aid in the characterization of structure. For our oxa-retinoid ester (24), the first fraction exhibited a λ_{max} = 348 nm in methanol:0.01 M ammonium acetate (9:1). The second fraction had a λ_{max} = 356 nm. The mixture of the fractions exhibited a λ_{max} = 350 nm. These results indicate that the

first fraction is probably the 12-cis isomer of (24), since it has a more twisted double bond system and, therefore, will absorb at shorter wavelength.

Normal phase HPLC was also tried to separate the geometric isomers of 24. Retinoids that differ by isomeric configurations of their side chain have been separated by this method. Hexane with minor amounts of a more polar organic modifier has been chosen almost universally as the eluting solvent.[2]

The fractions collected from the reversed-phase HPLC experiments were the one reinjected in the normal phase experiments. The detector was changed to ultraviolet set at 313 nm. Most retinoids normally absorbs around 354 nm. The solvent system chosen was hexane:diethyl ether (90:10) for several reasons including: 1) the separation of the peaks is better, 2) the injection or run time is shorter, and 3) the solvent system chosen is easy to remove from the desired compounds.

Injection of the first fraction collected from the reversed phase experiments indicated the presence of at least htree major peaks (Figure 2b). The first peak, at 4.25 minutes, looked like a collection of several peaks. Two other peaks, 7.55 minutes and 8.59 minutes were observed. Both peaks were sharp and were probably the two major isomers of the oxa-retinoid ester (24).

The fractions at 7.55 and 8.59 minutes were collected and reinjected again to verify their purity. Each of the
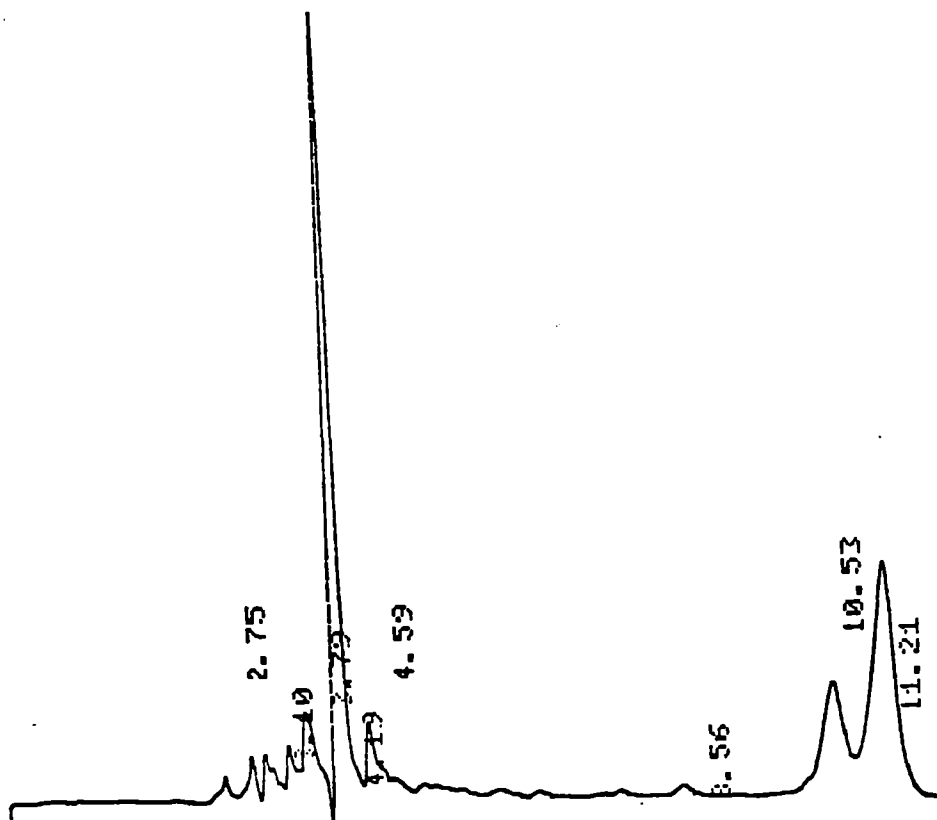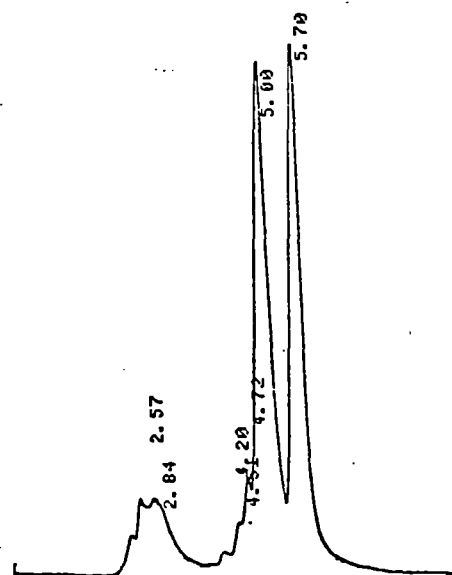
(a).   1.50 mL/min
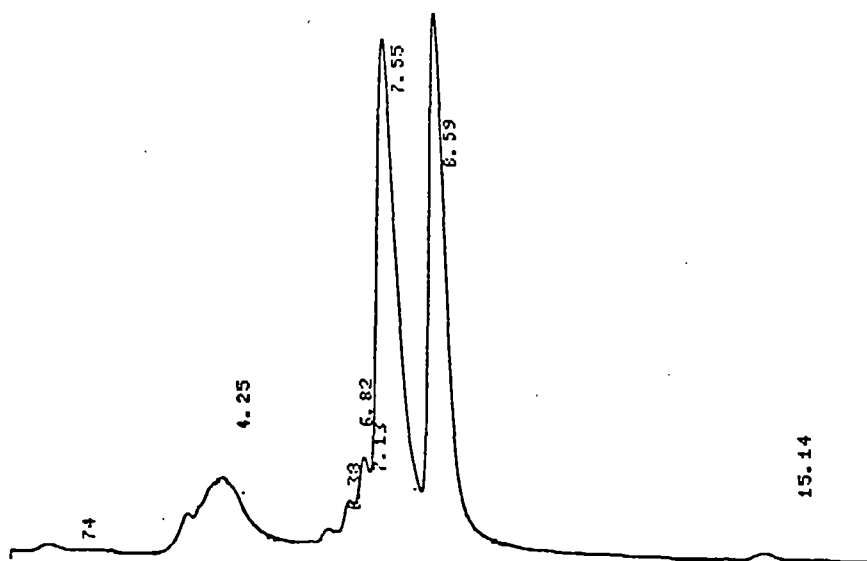
(b).   1.00 mL/min

Figure 2.   Separation of oxa-retinoid ester (24) isomers
            by HPLC.   Column: Whatman Partisil 5;
            Solvent System: Hexane:Diethyl Ether (90:10).
            Detector: Beckman Analytical UV Detector
            Model 153 at 313 nm.

collected fractions showed a single peak indicating that the fractions collected were pure. The samples were submitted for proton NMR, but the amount collected was not sufficient to obtain a good spectrum and it was not possible to distinguish many of the peaks from background noise.

In the normal phase liquid chromatography, the solvent system used was quite non-polar. It is expected that the more non-polar compound will come out first. It was expected that the trans-isomer, which is less polar than the cis-isomer, would come out first. To verify this statement, the ultraviolet absorption spectra of each pure isolated fraction was obtained. The λ_{max} of the first fraction in hexane was 346 nm and the second one has a λ_{max} = 335 nm. This supports our statement that the first fraction is probably the all-trans isomer or the less polar isomer and the second fraction is probably the more twisted 12-cis isomer, which is expected to absorb at a shorter wavelength than the all-trans isomer of 24.

<div style="text-align:center">

The Biological Activity of the Oxa-

retinoic acid (15) in the HL-60

Leukemia Cell Bioassay

</div>

Recently, the all-trans-retinoic acid (3) was found to be the most potent inducer of the granulocytic differentiation of HL-60 cells. Our compound, the oxa-

retinoic acid (15) was submitted for the HL-60 assay, and
the results of the assay are tabulated below. The results
with a newly synthesized retinoid, a sulfur containing
retinoid (30), closely related to 15, are included for
comparison.

ED_{50}, the median effective dose, is the amount of
retinoid required in achieving a half-maximal response.
The results of the HL-60 cell line bioassay indicate that

TABLE I

RESULTS OF THE HL-60 LEUKEMIA
CELL BIOASSAY

Retinoid	ED_{50}
Retinoic Acid (3)	41 nM
Oxa-retinoic Acid (15)	200 nM
Sulfur-retinoic Acid (30)	72 nM

15 is about five times less active than 3 and three
times less active than 30 in this bioassay.

In two differentiation assays, the HL-60 assay and the

F9 assay (an assay similar to the HL-60 assay), the new synthetic retinoid 13 is a potent inducer of differentiation. It had an ED_{50} approximately 100-fold lower than 3 in the F9 assay and an ED_{50} three-fold higher than 3 in the HL-60 assay. The substitution of an oxygen atom in the cyclohexyl ring of TTNPB (13) appears to be responsible for this large decrease in activity. This is consistent with the findings of Breitman that 15 is much less active than 30. However, substitution of a sulfur atom in the cyclohexyl ring of TTNPB (13) results in a large decrease in activity in the tracheal organ culture assay and in the ability to bind to the cytoplasmic retinoic acid binding protein.

Results have indicated that the most effective retinoid inducers of HL-60 differentiation process possess a carboxylic acid function at the terminal carbon. Substitution at the C-15 position of retinoic acid 3 resulted in essentially a complete loss of activity. Thus, retinol (1), retinal (2), and retinyl acetate (7), are all inactive in the HL-60 assay.

Inhibition of Ornithine Decar-

boxylase Activity

Our compound, the oxa-retinoic acid 15 was submitted and tested for its inhibition of the ODC activity. The results along with some newly synthesized retinoids are tabulated in Table II.

TABLE II

ORNITHINE DECARBOXYLASE ACTIVITY
OF VARIOUS RETINOIDS

Test System	Dose in nmol	ODC Activity
Acetone-TPA (11)		5.3 ± 0.7
trans-retinoic acid (3)	34	1.0 ± 0.1
Oxa-retinoic acid (15) + TPA 11	34	0.95 ± 0.06
31 + TPA (11)	34	1.7 ± 0.10
32 + TPA (11)	34	3.5 ± 0.20
33 + TPA (11)	34	1.5 ± 0.40

30

31

32

33
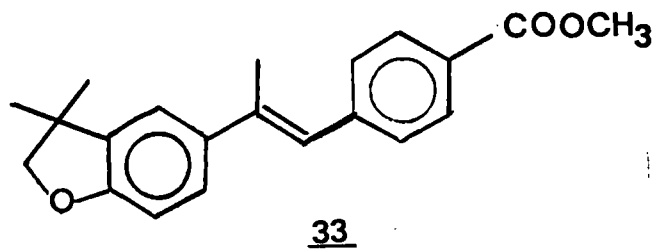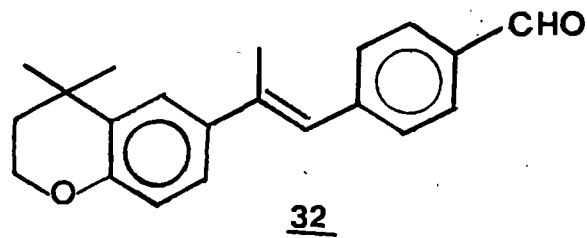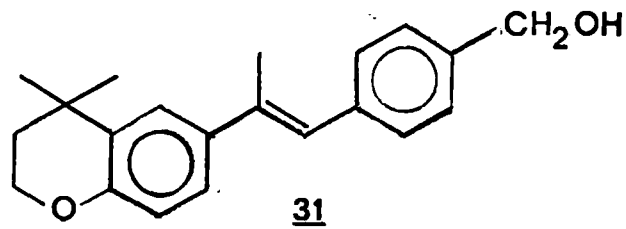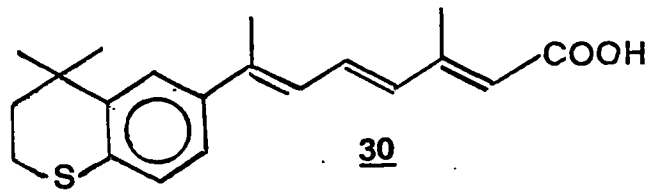
The ODC activity was based on the nmole of carbon dioxide released per 60 minutes per milligram protein. During the experiments 34 nmol each of the retinoids were used. The retinoic acid (3) inhibited ODC activity by 81% while the new synthetic retinoid, the oxa-retinoic acid (15), inhibited the ODC activity by 82%. The other compounds tested were not quite as active as 15 and 3. The alcoholic compound (31) inhibited the ODC activity by 68% while its aldehydic analog (32) gave an inhibition of only 34%. A similarly related compound 33 with an ester functionality was quite active too. It gave a 72% inhibition of the ODC activity. The results of the ODC assay for 15 indicates that this new retinoid is more active than 3. However, toxicity tests should be conducted for the oxa-retinoic acid (15) in order to appraise its usefulness as a potential drug. A number of new synthetic retinoids have proven useful in the prevention of cancers of various organs without noticeable appearance of hypervitaminosis syndrome, a problem associated with natural retinoids.

CHAPTER III

EXPERIMENTAL SECTION

General Information

All reactions were run under a nitrogen or argon
atmosphere. Evaporation of the solvent was performed on a
rotary evaporator with reduced pressure (water aspirator).
The starting material, 3-phenoxypropanoic acid (99% pure),
was purchased from Aldrich Chemical Company. Toluene,
tetrahydrofuran, N,N-dimethylformamide, and nitromethane
were distilled and dried prior to use. Solvents such as
methanol, ethanol, methylene chloride, acetonitrile,
diethyl ether, acetone, hexane, pentane, ethyl acetate,
petroleum ether (bp 45-60°C) and other chemicals such as
anhydrous aluminum chloride, acetyl chloride,
methylmagnesium bromide, vinylmagnesium bromide, and
triphenylphosphine were used without further purification.
Sodium ethoxide was prepared by dissolving a weighed amount
of freshly cut sodium in a measured volume of ethanol under
an argon atmosphere. The sodium ethoxide was standardized
with HCl solution using phenolhthalein as the indicator.
Triphenylphosphine hydrobromide was prepared by passing
hydrogen bromide into an ethanolic solution of

40

triphenylphosphine. Tetrahydrofuran was dried over sodium sulfate and distilled from the sodium ketyl of benzophenone under an argon atmosphere.

Analyses

Infrared spectra were run on a Perkin-Elmer model 681 IR spectrophotometer using KBr pellets for solids and NaCl plates for liquid samples. Proton and C-13 NMR spectra were obtained on a Varian XL-300 spectrometer operated at 300 MHz and 75.429 MHz respectively. Ultraviolet spectra were obtained on a Perkin-Elmer Lambda Array 3840 UV/VIS Spectrophotometer. Melting point data were performed on a MEL-TEMP melting point apparatus and are uncorrected. Gas chromatographic analysis data were obtained on a Hewlett-Packard Model 5840A instrument with a 6 ft x 0.125 in o.d. nickel column of 20% SE-30 on 80/100 mesh chromosorb Q and a thermal conductivity detector. Liquid chromtographic analyses were accomplished on a Waters HPLC Model 590 equipped with Waters Differential Refractometer R401 and Beckman Model 153 Analytical UV detectors. Thin-layer chromtographic analysis was done using Eastman silica gel plates. Analytical samples were submitted to Galbraith Laboratories Inc., Knoxville, Tennessee, and Huffmann Laboratories Inc., Wheatridge, Colorado.

General Procedures With Polymeric Reagents

Polymeric samples were washed with chloroform, ether,

methylene chloride, methanol, water, and ethanol. All
reactions were run in 3-neck flasks (round bottom) equipped
with an overhead stirrer, argon or nitrogen inlet, reflux
condenser, and serum stopper. Polymer samples were allowed
to swell in a solvent for one hour with stirring prior to a
reaction.

Methyl 3-phenoxypropanoate (17)[19]

A solution of 3-phenoxypropanoic acid (16, 25.0 g,
0.15 mole) and p-toluenesulfonic acid (0.008 mole) in 625
mL of methanol was refluxed for 4 h under a nitrogen
atmosphere. It was concentrated under vacuum, diluted with
distilled water (125 mL), and brine (200 mL), and dried
with anhydrous sodium sulfate. The solvent was removed
under vacuum. Vacuum distillation of the resulting
residual oil gave 23.95 g (88%) of 17 as a colorless
liquid: bp 79-87°C/0.32 mm Hg (lit.[19] bp 85°C/0.4 mm Hg);
IR (neat) 1750 cm^{-1} (C=O), 1375 cm^{-1} (CH$_3$); H-1 NMR δ
2.72 (t, 2H, CH$_2$CO$_2$CH$_3$), 3.64 (s, 3H, OCH$_3$),
4.16 (t, 2H, OCH$_2$), 6.84-6.92 [m, 3H, Ar-H (ortho and
para)], 7.20-7.25 [m, 2H, Ar-H (meta)]; C-13 NMR (CDCl$_3$)
ppm 34.3 (CH$_2$CO$_2$-), 51.7 (CH$_3$), 63.3 (OCH$_2$), Ar-C
[114.6 (ortho), 121.0 (para), 129.5 (meta), 158.5 (ipso)],
171.4 (C=O).

2-Methyl-4-phenoxy-2-butanol (18)[19]

A solution of methyl 3-phenoxypropanoate (17, 15.37 g,

0.0853 mole) in dry ether (60 mL) was added dropwise under
nitrogen to a stirred solution of methylmagnesium bromide
in tetrahydrofuran (1.29 $\underline{M}$, 200 mL, 0.26 mole). The
mixture was allowed to react for 4 h and was quenched with
saturated ammonium chloride. The supernatant liquid was
decanted and the residue was washed with dry ether (3 x 100
mL). The combined organic solution was dried over sodium
sulfate and the solvent was removed in vacuo. Vacuum
distillation gave 12.31 g (80%) of $\underline{18}$ as a colorless
liquid: bp 80-90°C/0.24 mm Hg; IR (neat) 3600-3200 cm^{-1}
(-OH); H-1 NMR ($CDCl_3$) δ 1.30 [(s, 6H, $(C\underline{H}_3)_2$], 1.98
(t, 2H, $ArOCH_2C\underline{H}_2$), 2.57 (s, 1H, -OH), 4.16 (t, 2H,
$Ar-OC\underline{H}_2$), 6.88-6.98 [m, 3H, Ar-H (ortho and para)],
7.25-7.31 [m, 2H, Ar-H (meta)]; C-13 NMR ($CDCl_3$) ppm 29.4
$C(\underline{C}H_3)_2$, 41.5 ($Ar{-}OCH_2\underline{C}H_2$), 65.0 ($Ar-O\underline{C}H_2CH_2$), 70.4
$[(CH_3)_2\underline{C}-OH]$, Ar-C [114.5 (ortho), 121.0 (para),
129.5 (meta), 158.5 (ipso)].

4,4-Dimethylchroman ($\underline{19}$)[19]

A solution of 2-methyl-4-phenoxy-2-butanol ($\underline{18}$, 15.30 g,
0.0849 mole) in nitromethane (100 mL) was added dropwise
under nitrogen to a stirred suspension of anhydrous
aluminum chloride (15.60 g, 0.012 mole) in nitromethane
(100 mL). After stirring at room temperature for 6 h, a
solution of 6 $\underline{M}$ HCl (160 mL) was added slowly. The layers
were separated and the organic layer was washed with water
(100 mL), saturated aqueous sodium bicarbonate (4 x 100

mL). After drying with sodium sulfate, the solvent was removed in vacuo. Vacuum distillation gave 10.83 g (79%) of 19 as a colorless liquid: bp 45°C-56°C/0.16 mm Hg; IR (neat) 2960 cm^{-1}, 2850 cm^{-1} (C-H), 3080-3020 cm^{-1} (C=CH); H-1 NMR (CDCl$_3$) δ 1.32 [s, 6H, (CH$_3$)$_2$], 1.80-1.84 [t, 2H, H(3)], 4.16-4.19 [t, 2H, H(2)], 6.77-7.27 (m, 4H, Ar-H); C-13 NMR (CDCl$_3$) ppm 30.4 [(CH$_3$)$_2$C], 31.0 [(CH$_3$)$_2$C], 37.6 (-OCH$_2$CH$_2$), 62.9 (-OCH$_2$CH$_2$), 116.8 C(8), 120.3 C(6), 126.8 C(5), 126.9 C(7), 131.5 C(5a), 153.4 C(8a).

6-Ethanoyl-4,4-dimethylchroman (20)[19]

Anhydrous aluminum chloride (3.40 g, 0.025 mole) in 22 mL of nitromethane was added in small portions to a solution of 4,4-dimethylchroman (19, 4.00 g, 0.025 mole) and acetyl chloride (2.00 g, 0.025 mole) in nitromethane (35 mL) under nitrogen. After stirring at room temperature for 6 h, 6 M HCl (35 mL) was slowly added, and the resulting mixture was stirred for 10 minutes. The mixture was diluted with ether (40 mL) and the layers were separated. The organic layer was washed with water (40 mL), saturated aqueous sodium bicarboante (4 x 30 mL), and brine (2 x 40 mL). After drying the solution over sodium sulfate, the solvent was removed under vacuum leaving a dark reddish brown oil. Vacuum distillation gave 2.42 g of 20 (48%) as a yellowish liquid; bp 97°C-100°C/0.035 mm Hg. A weighed amount of 20 (20 mg) was dissolved in 2 mL of anhydrous ether and 5 microliters (50 micrograms) of

this solution was injected onto a GC column. The results

showed 2 peaks, retention time (relative area) 4.41 min

(starting material 19, 3%) and 7.24 min (product 20,

97%). IR (neat) 1680 cm^{-1} (C=O); H-1 NMR δ (CDCl$_3$)

1.37 [s, 6H, (CH$_3$)$_2$], 1.83-1.87 [t, 2H, H(3)], 2.54 (s,

3H, CH$_3$C=O), 4.23-4.27 [t, 2H, H(2)], 6.80-6.82 [d, 1H,

H(8)], 7.67-7.70 [dd, 1H, H(7)], 7.95-7.96 [d, 1H, H(5)];

C-13 NMR (CDCl$_3$) ppm 26.3 (CH$_3$), 30.6 [(CH$_3$)$_2$C, 30.7

[(CH$_3$)$_2$C], 37.0 (OCH$_2$CH$_2$), 63.4 (OCH$_2$CH$_2$), 116.9

[C(8)], Ar-C (127.8, 128.2, 130.0, 131.6), 158.0 [C(8a)],

196.8 (C=O).

[(Triphenylphosphonium)-1-methyl-1-
propenyl-4,4-dimethylchroman-4,4-
dimethylchroman Bromide] (22)

A solution of the acetylchroman (20, 5.45 g, 26.67

mmole) in dry ether (20 mL) was added dropwise under

nitrogen to a stirred solution of vinyl magnesium bromide

(1.0 M, 40 mL, 40 mmole) in tetrahydrofuran. The mixture

was allowed to react for 3.5 h and was quenched with

saturated ammonium chloride. The supernatant liquid was

decanted and the residue was washed with dry ether (3 x 100

mL). The combined organic solution was dried over sodium

sulfate and the solvent was removed in vacuo to give 7.07 g

of 21. The product 21 was used in the next step

without purification. A solution of 21 (7.07 g, 30.47

mmole) in methanol (30 mL) was added to a cold solution of

triphenylphosphine hydrobromide (10.45 g, 30.47 mmole) in methanol (30 mL). The mixture was kept at room temperature for 2.5 h and poured slowly into ether (550 mL). The precipitated salt was filtered off and dried for 2 h under vacuum at room temperature to yield 9.14 g of $\underline{22}$, corresponding to a 61.5% yield. mp 246-249°C(dec); IR(KBr) 1667 cm^{-1} (C=C), 2975 cm^{-1} (C-H); H-1 NMR (CDCl$_3$) δ 1.29 [s, 6H, (CH$_3$)$_2$, 1.62 [s, 3H, CH$_3$ (10)], 1.76-1.84 [t, 2H, H(3)], 4.14-4.20 [t, 2H, H(2)], 4.75-4.83 [dd, 2H, H(12)], 5.54 [m, 1H, H(11)], 6.68-6.71 [d, 1H, H(8)], 6.88-6.91 [d, 1H, H(7)], 7.07 [s, 1H, H(5)], 7.60-8.00 [m, 15H, (Ph)$_3$].

Oxa-retinoic Acid ($\underline{15}$)

A solution of $\underline{n}$-butyllithium in hexane (8.20 mL, 1.31 $\underline{M}$, 10.77 mmole) was added dropwise under argon to a stirred suspension of the phsophonium salt ($\underline{22}$, 5.99 g, 10.77 mmole) in dry ether (100 mL). The resulting dark reddish brown mixture was stirred at room temperature for ten minutes. A solution of the ethyl 3-formyl-2-butenoate ($\underline{23}$, 1.55 g, 10.77 mmole) (Hoffmann-La Roche) in dry ether (30 mL) was added. After stirring at room temperature for 22 h, the mixture was filtered. The resulting solid was washed with ether (250 mL) and the combined filtrates were concentrated in vacuo to afford 2.40 g of crude $\underline{24}$ (68%). The ethyl oxa-retinoid ester ($\underline{24}$) was chromatographed on a silica gel (40 μm, J.T. Baker Co.)

and eluted with 2% ethyl acetate:98% petroleum ether to afford 24 as the middle fraction. Hydrolysis of 70 mg of the oxa-retinoid ester (24) was then effected with a potassium hydroxide solution (1.20 g KOH in 2.00 mL of water) and absolute ethanol (8 mL). The mixture was protected from light and stirred at 50°C for 4 h under nitrogen. The solution was cooled to 0°C, acidified with 6 M HCl and extracted with chloroform. The chloroform extracts were washed with brine, dried (MgSO$_4$), and evaporated. The resulting crystals of 15 were recrystallized from absolute ethanol to give 29 mg (45%) of 15, mp 191.5-192.5°C; IR(KBr) 3250-2500 cm^{-1} (OH), 1680 cm^{-1} (C=O); H-1 NMR (CDCl$_3$-DMSO-d$_6$) 1.35 [s, 6H, (CH$_3$)$_2$, 1.79-1.83 [t, 2H, H(3)], 2.20 [s, 3H, CH$_3$ (10 or 15)], 2.32 [s, 3H, CH$_3$(10 or 15)], 4.15-4.18 [t, 2H, H(2)], 5.76 [s, 1H, R-C(CH$_3$)=CHCO$_2$H], 6.40-6.45 [d, 1H, J= 11 Hz, H(11)], 6.53-6.57 [d, 1H, J= 15 Hz, H(13)], 6.68-6.71 [d, 1H, J= 9 Hz, H(8)], 6.96-7.05 [dd, 1H, J= 11, 15 Hz, H(12)], 7.19-7.23 [dd, 1H, J= 2, 9 Hz, H(7)], 7.41-7.42 [d, 1H, J= 2 Hz, H(5)]; C-13 NMR (CDCl$_3$-DMSO-d$_6$) 13.4 [C(15)], 16.0 [C(10)], 30.4 [C(4)], 30.8 [CH$_3$]$_2$], 37.2 [C(3)], 62.6 [C(2)], 116.5 [C(8 or 16)], 119.2 [C(8 or 16)], 123.9 [C(5, 7, or 11)], 124.3 [C(5, 7, or 11)], 124.6 [C(5, 7, or 11)], 130.9 [C(12)], 131.1 [C(6 or 9)], 134.0 [C(6 or 9)], 135.1 [C(13)], 139.1 [C(4a)], 151.5 [C(8a or 14)], 153.2 [C(8a or 14)], 168.0 [C(17)]; UV (absolute ethanol) λ_{max}= 341.8 nm, ε = 26000;

Thin layer chromatography, plate: Eastman 13181 silica gel, solvent: petroleum ether:ethanol:acetone (2:1:1), only one spot was observed, R_f 0.50; Anal.: Calcd. for $C_{20}H_{24}O_3$, C, 76.89; H, 7.74. Found: C, 76.79; H 7.90.

Oxa-retinoid Ester (24) By
Sodium Ethoxide Method

A solution of sodium ethoxide in ethanol (21.10 mL, 0.51 $\underline{M}$, 10.77 mmole) was added dropwise under nitrogen to a stirred suspension of the phosphonium salt (22, 5.99 g, 10.77 mmole) in methylene chloride (60 mL). The resulting mixture was stirred at room temperature for ten minutes. A solution of the ethyl 3-formyl-2-butenoate (23, 1.55 g, 10.77 mmole) (Hoffmann-La Roche) in methylene chloride (30 mL) was added slowly. After stirring at room temperature for 2 h, the mixture was diluted with hexane:ethyl acetate (7:3) (600 mL) and filtered. The filtrates were concentrated in vacuo to obtain 3.27 g of crude 24 (89%). The crude retinoid ester (24) was hydrolyzed to the oxa-retinoic acid (15) as described earlier.

Methyl-1-propenyl-4,4-dimethylchroman-
polystyrylphosphonium Bromide (29)

Polystyryldiphenylphosphonium bromide (28, 7.69 g, 5.71 meq Br) was added to a crude solution of 1-methyl-1-propenyl-4,4-dimethylchroman (21, 2.13 g, 9.17 mmole) in dimethylformamide (40.0 mL). The mixture was stirred under

nitrogen at 50 °C for 24 h and then at room temperature
for 24 h. The polymer was filtered and washed with toluene
(40 mL), methylene chloride (40 mL), and diethyl ether (3 x
40 mL). The sample was dried at 50 °C under vacuum for
20 h to give 7.91 g of 29 (21%). Anal. Found: P 3.64%
(1.18 meq P/g polymer); Br 5.93% (0.74 meq Br/g polymer).

Wittig Reaction Using Polymer Support
n-Butyllithium Method

Anhydrous diethyl ether (50 mL) was added to the
previously prepared 29 (3.83 g, 1.00 mmole) under a
nitrogen atmosphere. Using an addition funnel, n-butyl-
lithium (3.00 mL, 1.60 M, 4.80 mmole) was slowly added.
Upon addition of the n-butyllithium, the red-colored
solution was stirred for another 10 min. A solution of
ethyl 3-formyl-2-butenoate (23, 0.67 g, 4.72 mmole) in
ether (10 mL) was slowly added. After stirring for an
additional 24 h, the mixture was filtered. The solid that
had accumulated on the funnel was washed with diethyl ether
(150 mL) and the combined filtrates concentrated. Pentane
(10 mL) was added to the concentrate and the solution was
filtered in a filter unit (Millex-HV, 0.45 μm). The sol-
vent was evaporated by means of a vacuum rotavapor to give
219.2 mg of 24 (65%).

Wittig Reaction Using Polymer Support

Sodium Ethoxide Method

Anhydrous diethyl ether (50 mL) was added to the previously prepared 29 (3.86 g, 1.00 mmole) under nitrogen atmosphere. Sodium ethoxide (10.0 mL, 0.48 N, 4.83 mmole) was slowly added using an addition funnel. Upon addition of the sodium ethoxide, the solution was stirred for an additional 10 min. A solution of ethyl 3-formyl-2-butenoate (23, 0.67 g, 4.72 mmole) in ether was slowly added. After stirring for an additional 24 h, the mixture was filtered. The solid polymer was washed with diethyl ether and the combined filtrates were concentrated. The product 24 was redissolved in pentane and refiltered in a filter unit (Millex-HV, 0.45 μm) with the use of a disposable syringe. The solvent was evaporated to give 288.4 mg of 24 (85%).

Procedure for Reversed Phase High Performance Liquid Chromatography

About 50 mg of crude oxa-retinoid ester (24) was dissolved in 4.0 mL of either methanol or acetonitrile. The mixture was transferred to a vial with an appropriate cover. The mixture was shaken for 60 s and filtered through a filter unit (Millex-HV, 0.45 μm filter, Millipore Corporation, West Germany). During injections, a 20 μL

syringe (Hamilton Company, Reno, Nevada) was used. The
solutions were kept cold and the fractions collected were
kept in a covered black container filled with ice. Solvent
systems used were filtered in a millipore filter (0.45 μm
filter, Millipore Corporation) with the aid of a
filtration kit (Millipore Corporation, Bedford,
Massachusetts). The following parameters were used:
Column: Whatman Partisil 5-ODS-3; mobile phase,
methanol:0.01 M ammonium acetate (90:10); flow rate, 1.00
mL/min; injection loop, 20 μL; injection volume, 20 μL;
attenuation, 64x or NL 128x; detection, refractive index.

Procedure for Normal Phase High Performance Liquid Chromatography

The fractions were dissolved in HPLC grade hexane.
The same procedure as above was followed. The following
parameters were used. Column: Whatman Partisil 5 Silica;
mobile phase; hexane:diethyl ether (90:10); flow rate, 1.00
mL/min; injection loop, 20 μL; injection volume, 20 μL;
attenuation, 1.28; pressure, 400-600 psi; detection, ultra-
violet absorption at 313 nm.

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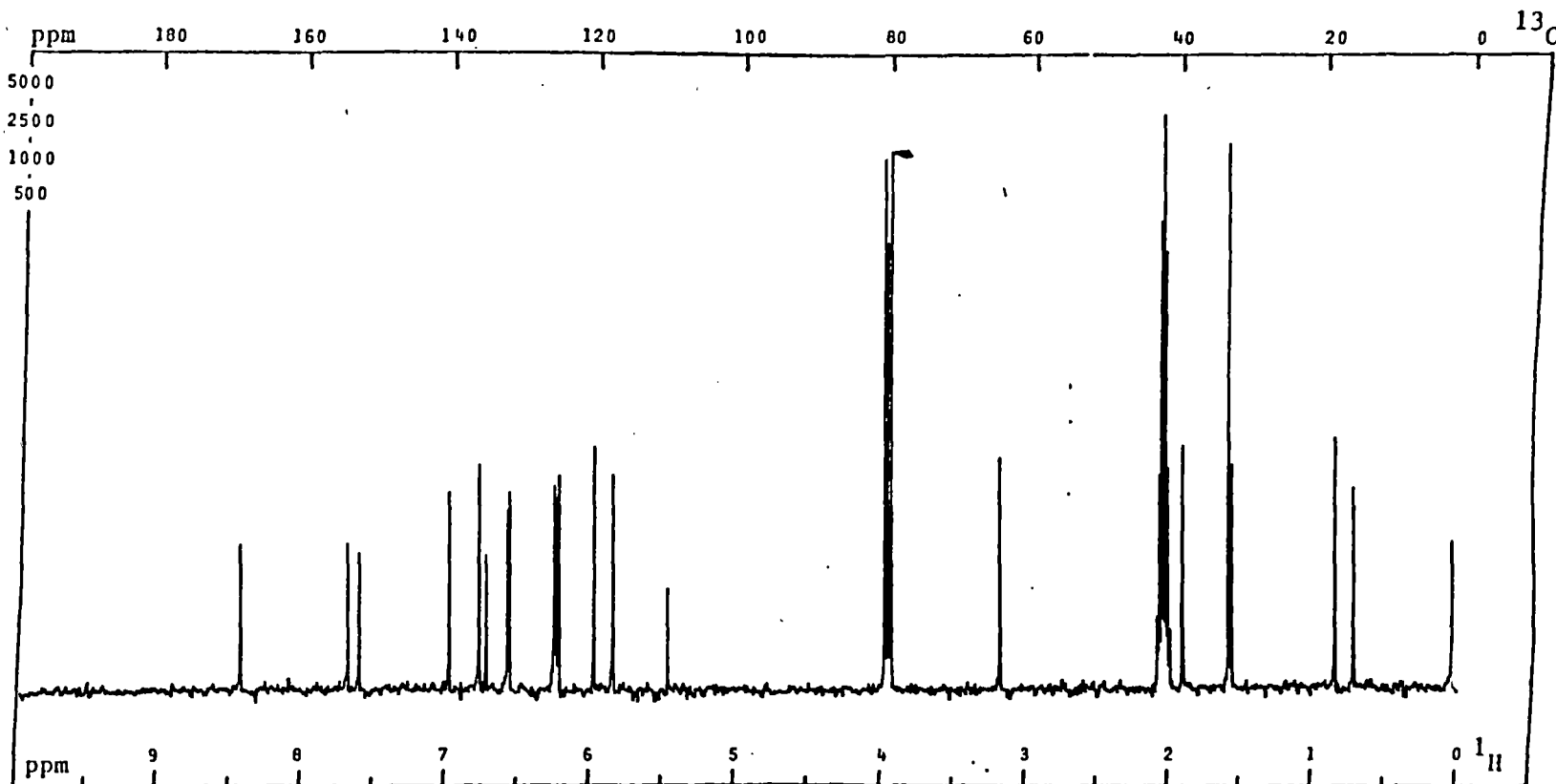   1653-1656 (1983).

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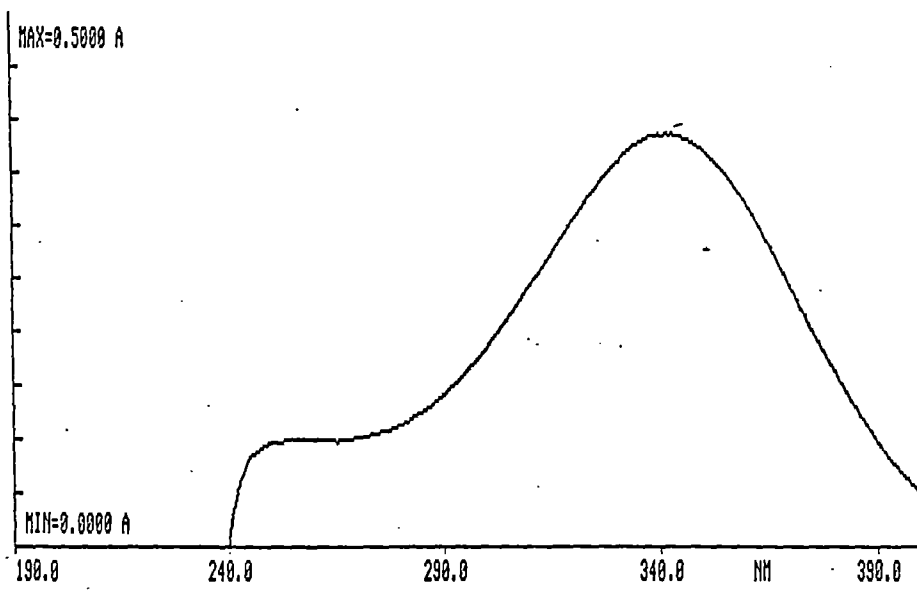23. Bernard, M., Ford, W. T., <u>J. Org. Chem.</u>, <u>48</u>, 326, (1983).

APPENDIX

Spectrum 1. H-1 NMR of Oxa-retinoic Acid (15)
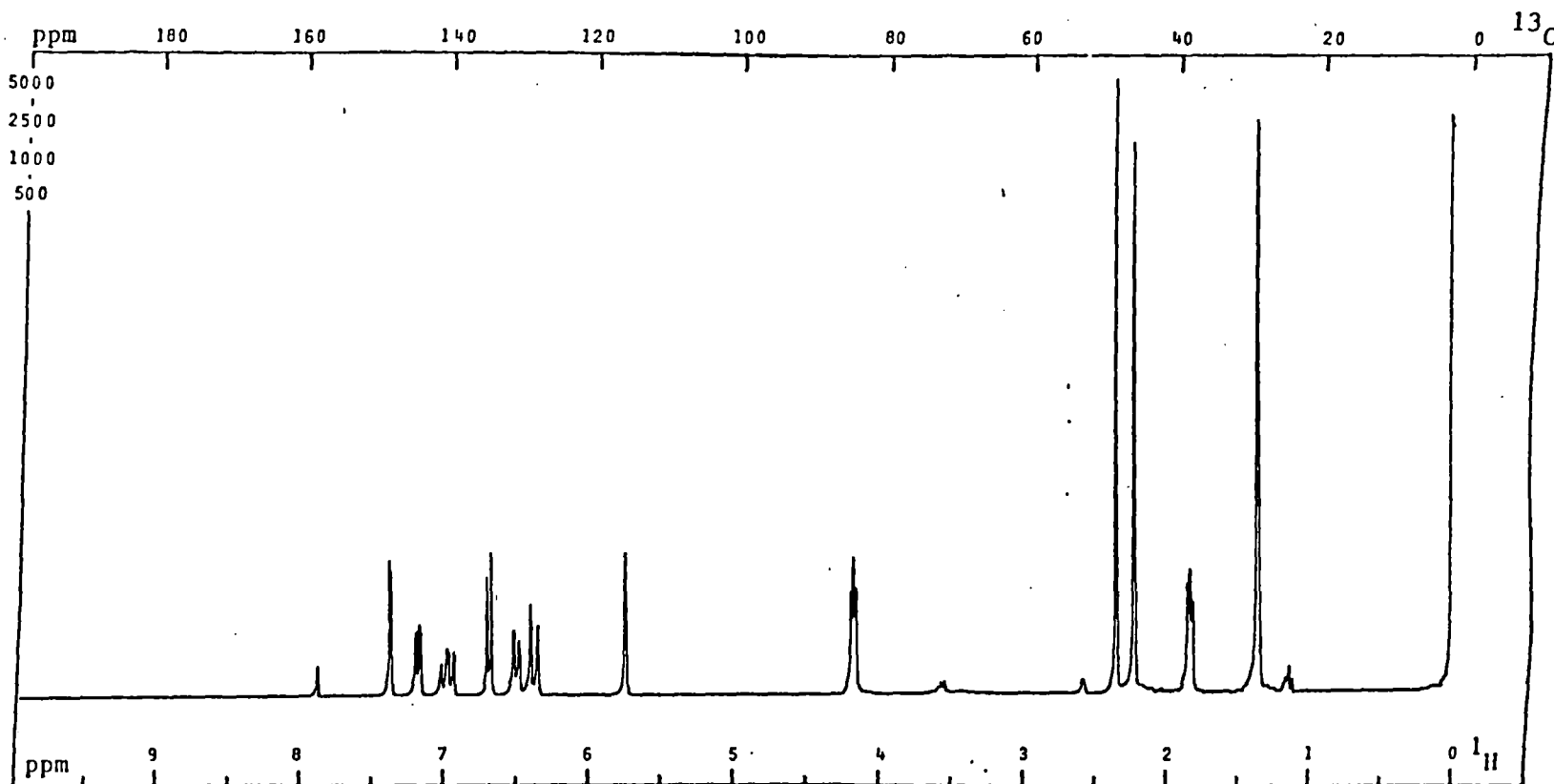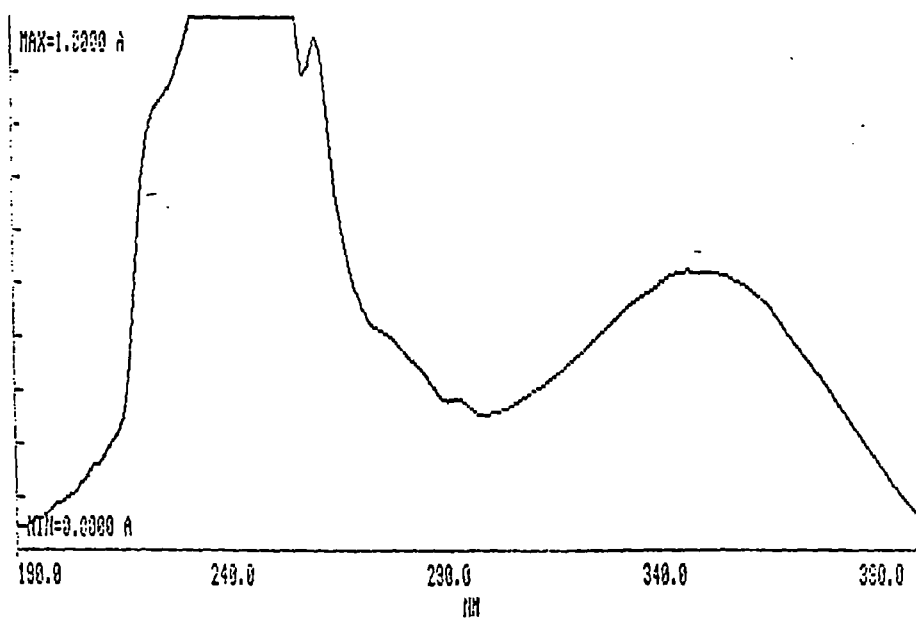
Spectrum 2.   C-13 NMR of Oxa-Retinoic Acid (15)

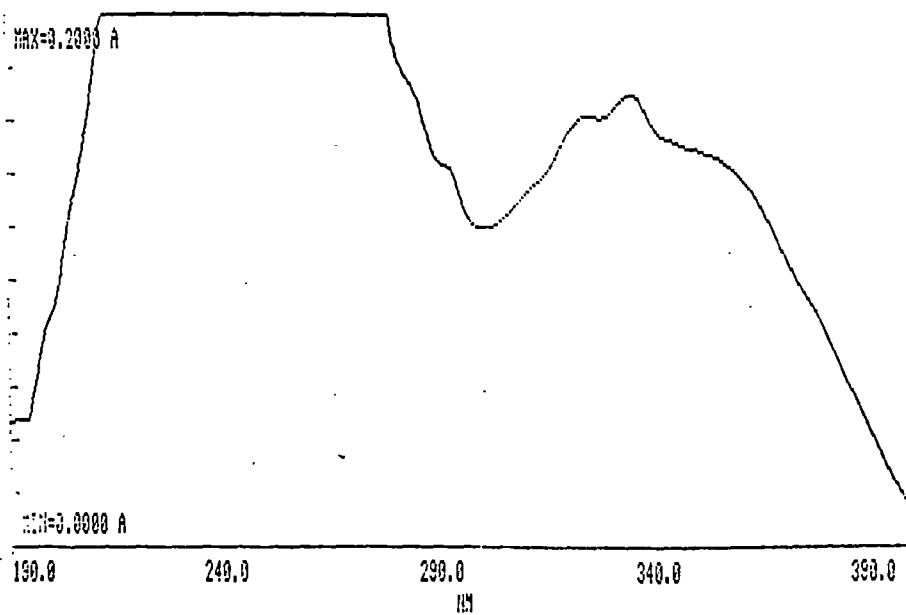PFT _ CW _ ;   Solvent: $CDCl_3$-DMSO ;   SF: MHz; WC: Hz;   T: 25 °C;   NT:

Size: K;   PW/RF: 12   μs/dB;   TO: Hz;   FB: Hz;   Lock: ^2H ;D1,D5:   s.

DC: Y, N ;   Gated Off:A or D ;   DO: Hz;   RF(Power):   W/dB;   NBW: Hz;   LB: Hz.

MAX=0.5000 A

MIN=0.0000 A

190.0   240.0   290.0   340.0   NM   390.0
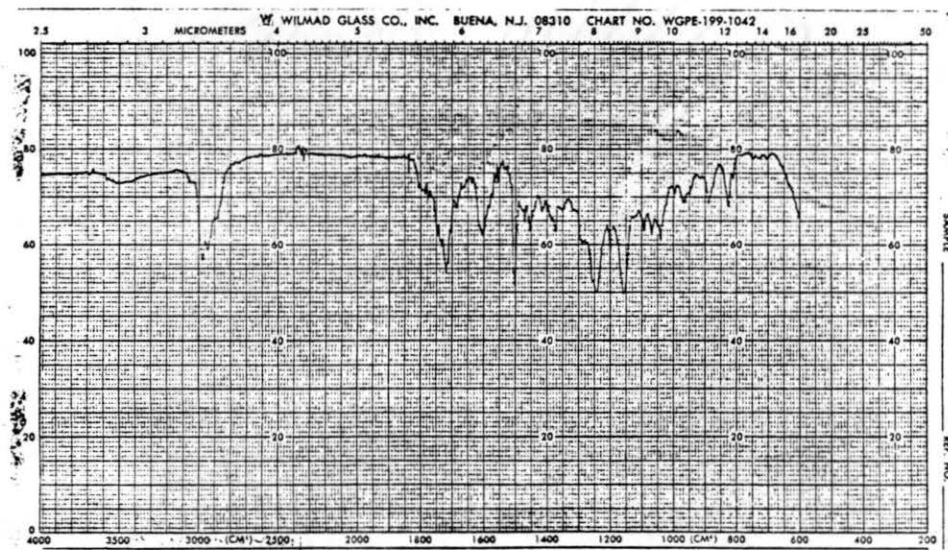
Spectrum 3.   UV Spectrum of 15

Spectrum 4. UV Spectrum of all-<u>trans</u> Oxa-
Retinoid Ester (<u>24</u>)

Spectrum 5.   UV Spectrum of 12-cis Oxa-
Retinoid Ester (24)

Spectrum 6. IR Spectrum of Oxa-retinoid Ester (24)

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VITA

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