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SYNTHESIS OF PROSTAGLANDIN ANTAGONISTS.
The Ohio State University,
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SYNTHESIS OF PROSTAGLANDIN ANTAGONISTS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Sunil V. Kakodkar, M. Phil.

* * * * *

The Ohio State University

1977

Reading Committee:
Donald T. Witiak, Ph.D.
Duane D. Miller, Ph.D.
Neil J. Lewis, Ph.D.
Timothy S. Gaginella, Ph.D.
John A. Secrist III, Ph.D.

Approved By

Adviser
College of Pharmacy
To my mother,

Mrs. Kamalabai V. Kakodkar
ACKNOWLEDGMENTS

I would like to express my personal gratitude and very sincere appreciation to my adviser, Professor Donald T. Witlak, for his guidance, encouragement and financial support throughout this study.

Thanks are also due to my fellow graduate student David Grattan for moral support during the moments of frustration.
VITA

July 19, 1942 .................. Born, Bombay, India

1958-1962 ..................... B.Sc. (Hons.), University of Bombay

1967-1970 ..................... M. Phil., University of London

1973-1977 ..................... Research Assistant, Department of Medicinal Chemistry, The Ohio State University, Columbus, Ohio

1971-1973 ..................... Senior Chemist, American Health Foundation, New York, N.Y.

1970-1971 ..................... Senior Chemist, Murphy Chemical Company, Wheathampstead, Herts, U.K.


1963-1967 ..................... Demonstrator in Chemistry, S.I.E.S. College of Arts and Sciences, Bombay, India

PUBLICATIONS


ABSTRACTS

G. Hite, M. S. Shen, D. T. Witiak and S. V. Kakodkar, The crystal structure of 2-Methyl-β-(3,4-methylenedioxyphenyl) aeraldehyde and β-phenyl-β-(3,4-methylenedioxyphenyl) aeraldehyde, presented to the Pittsburgh Diffraction Conference, Pa., November 9, 1973.

FIELDS OF STUDY

Major Field: Medicinal Organic Chemistry
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INTRODUCTION

Prostaglandin (PG) chemistry, biochemistry and pharmacology have been the subject of extensive investigation during recent years. The most common natural prostaglandins (figure 1) are named as derivatives of prostanoic acids, a C-20 straight chain acid having the C-8 and C-12 positions bonded so as to form a cyclopentane ring. These PGs have a double bond at C-13 and a 15-α-hydroxyl group. Each is known by a letter signifying a particular substitution of the ring and a subscript numeral denoting the number of double bonds in the side chains. These may occur at C-5 and C-17 in addition to the one at C-13. The subscript in PGFα refers to the 9α-hydroxyl group.

The term prostaglandin first appeared in literature in 1935 and was applied by von Euler\textsuperscript{1} to a new group of physiologically active substances extracted from sheep vesicular glands. Over the next 30 years, very few studies concerned with this new class of compounds were reported. However, after Bergstrom\textsuperscript{2} reported the structural characterization and techniques used to produce small amounts of prostaglandins biosynthetically, the literature has virtually exploded. Initial studies designed to elucidate the mechanism of the biosynthesis were reported independently by
Figure 1
Figure 2
Bergstrom$^{3,4}$ and Van Dorp$^{5,6}$, who showed that certain $\omega$-6-unsaturated fatty acids were biological precursors of prostaglandins. Extension of these studies, mainly by Samuellson and associates$^{7-10}$, has led to the proposed mechanism in figure 2. PGE$_1$ is formed biosynthetically by removal of hydrogen at O-9; PGF$_{1\alpha}$ is formed by reductive cleavage. The proposed biosynthetic pathway was suggested through the use of deuterium labelled precursors.$^{7,8,10,11}$

Prostaglandins are present in many mammalian tissue. However, the concentrations in most tissues are about 1µg/g of wet tissue; thus their isolation is difficult. Human and sheep seminal plasma exceed all other tissues in the number of prostaglandins present and in the total concentration found ($>10$µg/ml)$^{12-15}$.

The conformation of PGF$_{1\alpha}$ was reported by Abrahamsson$^{16}$ using X-ray crystallography on the tri-p-bromobenzoate methyl ester. Hoyland and Kier$^{17}$ calculated the preferred conformation of PGE$_1$ using the extended Hückel theory [The structure in the article is drawn incorrectly]. The calculated conformation is in agreement with the X-ray studies of Abrahamsson. Rabinowitz and co-workers$^{18}$ employed unit cell dimensions to calculate the preferred conformation of prostaglandins; it was shown that the calculated interaction energy between the terminal portion of the upper (α) and the lower (ω) chain decreased in the order PGE$_1$, PGF$_{1\alpha}$, PGA$_1$ and PGB$_1$. These authors also noted that the isolated rat uterus potency decreased in the same order and suggested that interaction between two chains results in increased potency.
Samuellson and co-workers along with Hamberg and Wilson have summarized the results of their studies on the catabolism of prostaglandins of the E and F series (figures 3 and 4). Thus, in man and most other mammals, the initial step involves oxidation of the 15-hydroxyl group by prostaglandin dehydrogenase, which has been isolated from swine lung and kidney, bovine lung and human placenta. The sequence PGE₁ — M-I — M-II — M-III was first demonstrated in guinea pig lung homogenates; the first two steps have been confirmed in man. M-II is the major blood plasma metabolite and M-V is the major urinary metabolite of PGE₂ in man. The presence of M-V indicates other common metabolic pathways including β-oxidation and sequential ω-oxidation. Recent studies by Granstrom on intravenously injected [9β-³H]-PGF₂α in female subjects showed 95% excretion of label in the urine within 6 hours. Twelve metabolites were isolated; several were identified (figure 4). Extensive degradation of the prostaglandin skeleton from the ω-end was observed in addition to the usual biosynthetic pathways previously discussed. Products result from ω-hydroxylation followed by oxidation to the α,ω-dioic acid. Sequential "β-oxidation" of the ω-end produced the six and four carbon chains observed. Metabolite M-VIII is particularly interesting; this compound was the first to be identified resulting from complete loss of the 15-oxygen function.

Actions of different prostaglandins are often dissimilar and sometimes opposed. These compounds have a wide spectrum of biological activity. They exhibit smooth muscle stimulant and
Figure 4
depressor activity, have peripheral vasodilator properties (except PGFs which are pressor and vasoconstrictor), inhibit lipolysis (only E prostaglandins have significant antilipolytic activity), platelet aggregation and gastric secretion. In these areas they are among the most potent compounds known.

The mechanism of action of prostaglandins is not completely understood. The first suggestion that prostaglandins may be related to cyclic AMP was noted by Steinberg who showed that PGE blocked the action of lipolytic agents which act by raising cyclic AMP levels in fat cells. This led to the concept that prostaglandins may be formed as a consequence of cyclic AMP action and that they function as negative feedback regulators. To the contrary, Butcher and Baird found that prostaglandins stimulate cyclic AMP formation in a number of tissues. These data indicate that the negative feedback mechanism is not a general phenomenon. The E prostaglandins and to a lesser extent the A and F types were potent when assessed for their ability to increase cyclic AMP formation. The order of potency in stimulating cyclic AMP formation paralleled the ability of these prostaglandins to stimulate progesterone formation. In cases where prostaglandins increase cyclic AMP levels investigators postulated that their effect involved stimulation of adenylate cyclase. However, certain prostaglandins diminish cyclic AMP levels. Therefore it is difficult to visualize an effect resulting from a direct action on adenyl cyclase since such a mechanism would involve inhibition of basal rates. The possibility that certain
prostaglandins may depress cyclic AMP levels by affecting the locus of phosphodiesterase activity is suggested by Amer and Marquis.\textsuperscript{28} Furthermore, Abdulla and McFarlane\textsuperscript{29} have shown that PGE\textsubscript{2} significantly increases platelet ADP by stimulating adenylate kinase. This observation is consistent with an effect of prostaglandins on ATP levels. Whether such a phenomenon is universal remains to be established. Indeed, the net effect of prostaglandins on cyclic AMP levels in a given cell, whether elevated or depressed, may be a reflection of differential sensitivity to their action on adenylate cyclase, phosphodiesterase and enzymes regulating substrate ATP levels. Based on available data, Kuehl\textsuperscript{30} has proposed that prostaglandins play an essential role in hormone action. However, the precise mechanism by which this is accomplished remains to be established. The sequential scheme (figure 5), wherein prostaglandins act as a membrane bound second messenger, requiring cyclic AMP to be the third or subsequent messenger, is compatible with most findings. Nevertheless, there are certain observations that are difficult to rationalize using this simple model. Much additional work remains to be carried out prior to elucidation of the precise relationship between prostaglandins and cyclic AMP in cells.

The different activities of the E and F prostaglandins have been the subject of recent investigations. Depending on the species, differences can be quantitative (human uterus), opposite in action (human bronchiole) or selective (PGF\textsubscript{2\alpha} in luteolysis; sheep and rats). Although studies by Kuehl and co-workers\textsuperscript{30,31} have shown that the F
HORMONE

↓

RECEPTOR

↓

E-type Prostaglandins

↓

ATP-regulating enzymes

Adenylyl Cyclase

↓

Phosphodiesterase

Cell Membrane

ATP

↓

Cyclic-AMP

↓

5'-AMP

Cyclic-AMP

↓

Physiological effect

Figure 5.
prostaglandins can mimic E prostaglandins in stimulating cyclic AMP formation and also bind to the PGE receptor, the high concentration required for this action led to the suggestion that these prostaglandins must act on a different receptor via a different mediator. All attempts to demonstrate antagonism between these two series of prostaglandins using contractile studies with isolated bronchioles and veins were unsuccessful when cyclic AMP levels were measured. The weak stimulatory action of F prostaglandins (less than 1/100th of PGE₁), on cyclic AMP levels likely do not represent their true function at the cell level. Rather, the high non-physiological concentration of the F prostaglandins required to elicit binding and stimulate cyclic AMP production is consistent with the proposal that E-receptors fail to discriminate at such high concentrations. However, in some instances PGF₂ɑ stimulates the contractile response to a greater extent than PGE₁ and thus its action cannot always be rationalized on the basis of weak binding to E-receptors or diminished ability to raise cyclic AMP levels. The inverse relationship shown to exist between cyclic AMP and cyclic GMP in response to the stimulators has led to a novel concept proposed by Goldberg and associates. Rather than attributing control of cell functions solely to bidirectional changes in cyclic AMP levels (i.e., increases or decreases), this hypothesis attributes an equally important role to cyclic GMP; this role is opposite to that of cyclic AMP. It was found that $10^{-5}$ M PGF₂ɑ caused a four fold increase in cyclic GMP levels in isolated rat
uterus preparations within 45 seconds. Earlier studies had shown oxytocin to induce a rapid increase in cyclic GMP levels in this tissue, with no further effect upon cyclic AMP levels. Thus, on the basis of these limited studies, it was proposed that cyclic GMP may be related to F prostaglandin activity much as cyclic AMP is related to E prostaglandin activity. More recent studies utilizing isolated bovine and canine veins have shown that the cyclic GMP/cyclic AMP ratio increases under the influence of PGF, whereas the reverse situation is observed for PGE. Since PGF causes contraction and PGE causes relaxation in this tissue, this finding is consistent with the concept that the opposing actions of E and F prostaglandins are expressed at the cyclic nucleotide level.

Prostaglandins are also thought to mediate the activity of luteinizing hormone (LH). Kuehl has proposed that events initiated by luteinizing hormone in promoting ovarian steroidogenesis involve intermediates in the sequence shown in Figure 6. In this scheme, luteinizing hormone causes the release of prostaglandins which in turn cause an increase in the concentration of cyclic AMP. Cyclic AMP subsequently activates a protein kinase by binding to and removing a regulator protein R from the actual catalytic protein C. This protein kinase catalyzes the enzymatic production of a phosphorylated protein which is essential for promoting the synthesis of progesterone.
**Figure 6.** Abbreviations: LH, luteinizing hormone; AA, arachidonic acid; ATP, adenosine triphosphate; R, regulatory subunit; C, cyclic AMP dependent kinase; P-protein, phosphorylated protein; C-AMP, cyclic AMP; C-GMP, cyclic GMP.
Prostaglandin antagonists could serve as useful molecular probes in the elucidation of the role of prostaglandin agonists in physiological and pharmacological processes. Further, prostaglandin antagonists may be useful therapeutic agents in the treatment of a variety of conditions including abortion, premature labor, diarrhea, cerebral vasospasm, fever, bone resorption, sickle cell anemia, obstructive lung disease, inflammation and glaucoma. Thus far, available antagonists have only weak activity. Their clinical utility has not been established.

The term "prostaglandin antagonist" is used here to denote a compound which selectively antagonizes the actions of prostaglandins at their site of action and not compounds, such as aspirin or indomethacin, which inhibit the biosynthesis of endogenous prostaglandins by blocking prostaglandin synthetase. Available antagonists belong to four chemically unrelated classes. These include the prostaglandin analogs (7-oxa-13-prostynoic acid; 7-OPya), dibenzoxazepine derivatives (SC-19220), phosphorylated polymers of phloretin (polyphtloretin phosphate; PPP) and sodium p-benzyl-4-[1-oxo-2-(4-chlorobenzyl)-3-phenylpropyl] phenyl phosphonate (N-0164).

Antagonists might be classified as either pharmacological or chemical. Pharmacological antagonists are thought to act by interfering with the formation of the agonist-receptor complex. Competitive antagonists compete with the agonist for the receptor and combine with it in much the same way as does the agonist, but the
formation of the antagonist-receptor complex is ineffective in producing a response; i.e., the antagonist has no intrinsic activity. Competitive antagonism is reversible. Increasing the concentration of agonist results in decreasing antagonist-receptor binding. Competitive antagonists produce a parallel displacement of the log dose response curve of the agonist to the right, without a change in maximum response. In non-competitive antagonism there is no mass action equilibrium between the antagonist and the agonist at the receptor and the effect is only determined by the concentration of the antagonist. When fully developed non-competitive antagonism cannot be overcome by increasing the concentration of the agonist. In the presence of a non-competitive antagonist, the slope of the log dose response curve will be reduced and the maximum response diminished in relation to the degree of non-competitive blockade established.

Chemical antagonists reduce or abolish the biological activity of agonist by direct chemical interaction with the agonist molecule. This type of antagonism can result in dose response curves similar to those seen with competitive antagonists. Thus, studies of dose response curves alone might not afford sufficient information to differentiate between the two types of antagonism. Differentiation can be made, however, since a competitive antagonist will be equally effective against different agonists acting on the same receptor. A chemical antagonist will be equally effective against a given agonist acting on different receptors.
One method employed to design antagonists involves synthesis of analogs of the agonist. Various analogs may have affinity for a receptor, but owing to structural modification no intrinsic activity. Therefore, such compounds may act as competitive antagonists. Fried and co-workers\textsuperscript{35} observed that a series of 7-oxa-prostaglandin analogs specifically inhibited smooth muscle stimulating properties of prostaglandins. Several racemic 7-oxa-7-prostaglandins, which had no oxygen function at C-9 and C-11 or at C-9, C-11 and C-15 were prepared. Various homologs including those having four-membered or six-membered cycloalkane rings were also synthesized. The degree of hydroxylation seemed to determine whether or not a given compound exhibited agonist or antagonist activity on the guinea pig ileum, rabbit jejunum and gerbil colon (Figure 7). Compounds having an oxygen function (hydroxyl or carbonyl) at C-9 and C-11 were agonists in all three systems whether or not such analogs contained a 15-hydroxyl group (compounds 28-32; Figure 7). Compounds without an oxygen function at C-9 and C-11 having a C-15 hydroxyl group (compounds 25-27; Figure 7) exhibited either agonist or antagonist activity against PGE\textsubscript{1}. Compounds with no oxygen function at C-9, 11 and 15 (compounds 33 and 34; Figure 7) were claimed to be pure antagonists, in all three of these pharmacological systems. The most selective antagonist found by Fried and co-workers\textsuperscript{35} was 7-oxa-13-prostynoic acid (Figure 8), which has been separated into its optical isomers. Activity for the two diastereomers has not been reported. Fried\textsuperscript{35} has reported that most compounds synthesized
Both Agonists and Antagonists

Figure 7
Figure 7 - continued

Pure Agonists
Figure 8.

exhibited little or no inhibition of contractions induced by histamine or acetylcholine on the guinea pig ileum. These data suggested that the various analogs were specific for prostaglandins (PGE₁) on this tissue. Little specificity was observed on the gerbil colon by analogs other than those that contained an acetylenic linkage in the 13,14-position. Acetylene analogs were found to antagonize PGF₁α at a concentration of 50 µg/ml on the gerbil colon. Flack tested compounds synthesized by the Fried group and demonstrated that 7-oxa-13-prostynoic acid was the only compound (3.25 x 10⁻⁵ M) which antagonized the contractions of isolated gerbil colon to prostaglandins E₁, E₂, F₁α and F₂α with little or no inhibition of responses to acetylcholine. He showed that the effect of this compound on the guinea pig ileum and rabbit jejunum was non-selective. Bennett and Posner confirmed that 7-O Pya was a non-selective antagonist which did not specifically inhibit prostaglandin effects in vitro on smooth muscle preparations of guinea pig ileum and colon and human stomach, ileum and colon. The
7-oxa analog produced contractions (a prostaglandin-like activity) on isolated rat stomach strips. These data were at variance with the observation by Fried\textsuperscript{35} showing that a hydroxyl group is required for agonist activity. At concentrations between 6 and 100 \( \mu \text{g/ml} \) or lower, 7-oxa-13-prostynoic acid caused no block of prostaglandin induced contractions of longitudinal muscle of the human gastric body or ileum and did not block the relaxant effect of PGE\textsubscript{2} on circular muscle from these organs. Higher concentrations reduced contractions produced by both prostaglandins and acetylcholine.

The most interesting action of 7-OPya involves inhibition of prostaglandin-induced cyclic AMP formation. Kuehl and co-workers\textsuperscript{38} investigated the interrelationship of luteinizing hormone (LH) and prostaglandins on excised mouse ovaries. They found that an increased formation of cyclic AMP was associated with increasing concentrations of PGE\textsubscript{1} and PGE\textsubscript{2}. 7-OPya (1.63 - 2.44 \( \times 10^{-4} \) M) antagonized this prostaglandin effect \( (K_1 = 6 \times \times 10^{-5} \) for PGE\textsubscript{1}; \( K_1 = 3 \times 10^{-5} \) for PGE\textsubscript{2} \) as well as the increase in cyclic AMP formation produced by LH \( (K_1 = 5 \times 10^{-5}) \). Lineweaver-Burke plots indicated competitive antagonism of both prostaglandins and LH. These experiments suggested that prostaglandins are involved in stimulation of cyclic AMP formation induced by LH. Channing\textsuperscript{39} found that 7-OPya inhibited human chorionic gonadotropin (HCG), LH and PGE\textsubscript{2} stimulation of morphological luteinization and progestin secretion in monkey granulosa cultures. 7-OPya (10\textsuperscript{-5}M) and its 15-hydroxyl analog have been shown to inhibit cyclic AMP formation induced by prostaglandins.
and thyroid stimulating hormone (TSH) in isolated thyroid cells and thyroid slices. Vale and co-workers demonstrated the inhibition of TRF, CRF and LRF in releasing their respective pituitary hormones in vitro by 7-OPya (3 x 10^-5 M). Reactions inhibited by 7-OPya are described in Figure 9. 7-OPya (3.25 x 10^-5 M to 1.63 x 10^-5 M) partially overcame the inhibitory effect of PGE_1 and

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Figure 9.

PGE_2 on epinephrine-induced lipolysis in isolated fat cells from rats and enhanced epinephrine-induced lipolysis in the absence of added prostaglandins. 7-OPya (1.2 x 10^-5 M) also reversed prostaglandin inhibition of antidiuretic hormone stimulated cyclic AMP production in isolated toad bladder. In contrast to their stimulating action on the formation of cyclic AMP in ovary, prostaglandins inhibit cyclic AMP formation when it is stimulated by vasopressin in the renal medulla. Marumo and Edelman examined the effects of 7-OPya (10^-7 M) on the adenyl cyclase system of the hamster renal medulla.
In their preparations this compound showed only prostaglandin-like activity; no prostaglandin antagonism was observed. Kuehl⁴⁸ has shown that 7-OPya has a weak, but dose related affinity for the lipocyte prostaglandin receptor, suggesting that it is truly acting as a prostaglandin antagonist. 7-Oxa-15-hydroxy-13-prostynoic acid was also observed to bind to this site. These two analogs displaced PGE₁ from the receptor as would be expected for a competitive antagonist although high concentrations were required. Miller and Magee⁴⁹ also found that 7-OPya (10 µg/3 ml) competed slightly with PGE₁ for specific prostaglandin binding sites from rat forestomach. Marazzi and co-workers⁵⁰ showed that 7-OPya (78.5 µM) inhibits the enzymatic degradation of prostaglandins by 15-hydroxy prostaglandin dehydrogenase. This reaction is inhibited in the reverse direction (15-keto prostaglandin to 15-hydroxy prostaglandin) as well as in the forward direction.⁵¹ 7-OPya was also reported to inhibit cyclic AMP dependent protein kinase²⁷ and synthesis of ovarian proteins (100 µg/ml).⁵² Ramwell and Johnson⁵³ found that this compound activated ATPase from platelets, mitochondria and erythrocytes. This analog also exhibited marked inhibitory effects on skeletal muscle adenylate kinase, whereas it appeared to have mixed inhibitory and potentiating effects on platelet and erythrocyte adenylate kinase.

Fried and co-workers³⁵ have described the resolution of racemic 7-OPya. Perhaps further study of optically pure compounds in a variety of biological systems will shed more light on the actions of this interesting group of compounds.
Coyne and Gusic synthesized a series of 1-acyl-2(10,11-dihydrotetral[2,1]-dihydro-1,4-oxazepin-10-carbonyl)hydrazines (Figure 10) which were shown to possess anticonvulsant and analgetic properties.

Various tricyclic drugs exhibit a broad range of blocking activity against a number of unrelated agonists. Chlorpromazine has been shown to block the actions of acetylcholine, epinephrine, histamine and serotonin. Imipramine blocks the actions of acetylcholine, histamine and serotonin. The more selective promethazine has antihistaminic activity and propantheline is mainly anticholinergic. Sanner proposed that alterations in the tricyclic nucleus may result in compounds that inhibit the actions of prostaglandins, and he first provided evidence that SC-19220 (36; Figure 10), did indeed block PGE$_2$-induced contractions on the isolated guinea pig ileum (1 - 50 μg/ml); no significant inhibition of contractions produced by bradykinin or acetylcholine was observed. Using concentrations ranging from 2.5 to 20 μg/ml, SC-19220 progressively shifted the dose response curve produced by PGE$_2$ to the
right without decreasing the maximum response. These data are consistent with a competitive antagonism mechanism. However, at concentrations about 20 µg/ml there was a decrease in the slope of the curve and a reduction of the maximum response indicating non-competitive antagonism occurred. Several other laboratories have also observed competitive antagonism by SC-19220 against PGE₂ on the guinea pig ileum²⁶,³⁷ (0.5 - 1.0 x 10⁻⁵ M), gerbil colon⁵⁷ (10⁻⁵ M), rat fundus strip³⁷,₅₈ (5 x 10⁻⁵ M) and guinea pig uterus (0.5 - 1.0 x 10⁻⁵ M).

Sanner⁵⁹ later reported prostaglandin antagonism by various analogs of SC-19220 on the isolated guinea pig ileum and rat stomach. For the n-butanoyl, i-butanoyl and n-hexanoyl analogs of SC-19220, it was observed that, as the length of the carbon chain increased, high antiprostaglandin potency was observed. However, these compounds were less selective against PGE₂. The isobutanoyl derivative was considerably less potent and less selective than the n-butanoyl analog.

SC-19220 also inhibited contractions produced by PGF₂α on circular muscle from guinea pig colon and rat fundus strips,³⁷ but did not reverse prostaglandin inhibition of gastric acid secretion even at concentrations of 250 µg/kg and 500µg/kg.⁶⁰ SC-19220 (15 µg/ml) also blocked the electrically induced contractions of isolated guinea pig ileum. This inhibition could be reversed by PGE₁ and PGE₂.⁶¹

It appears that prostaglandins facilitate release of acetylcholine at cholinergic nerve terminals. Also, PGE₁ and PGE₂ inhibit the release of norepinephrine from adrenergic nerves.
Therefore, these compounds inhibit responses produced by adrenergic nerve stimulation. Larger concentrations of E prostaglandins also potentiate responses produced by exogenous norepinephrine on the isolated guinea pig vas deferens. Potentiation of norepinephrine-induced responses were inhibited by SC-19220.\textsuperscript{62} However, this analog did not reverse prostaglandin inhibition of adrenergic nerve stimulation.\textsuperscript{62,63} SC-19220 did not antagonize prostaglandin responses on isolated human gastrointestinal muscle (7.5 x 10\textsuperscript{-6} M to 1.5 x 10\textsuperscript{-4} M). Sanner\textsuperscript{64} also observed no antagonism of PGE\textsubscript{2} on isolated human myometrium by SC-19220. Alternatively, Vanossin and co-workers\textsuperscript{65} have reported that SC-19220 inhibits contractions produced by PGE\textsubscript{1} and PGE\textsubscript{2} on longitudinal muscle from dog and human colons. These authors did not report whether the observed inhibition was specific for prostaglandins. Park and co-workers\textsuperscript{66} found that PGE\textsubscript{2}-induced responses of human umbilical vessels were inhibited by SC-19220 (1 x 10\textsuperscript{-4} M); serotonin was inhibited to a lesser extent. SC-19220, administered intraarterially (0.1-2 mg/kg), did not antagonize the vasodilator actions of PGE\textsubscript{1}, PGE\textsubscript{2} or PGA\textsubscript{2} in the femoral artery or the vasoconstrictor action of PGF\textsubscript{2\alpha} in the pulmonary artery of anesthetized dogs.\textsuperscript{67}

Kuehl and Humes\textsuperscript{48} have isolated a prostaglandin binding substance. A good correlation was observed between the ability of this protein to bind different prostaglandins and the relative potency of the prostaglandins when assessed for their ability to stimulate cyclic AMP formation. For these reasons, Kuehl and Humes\textsuperscript{48} concluded that this
substance is the prostaglandin receptor involved in prostaglandin stimulation of adenyl cyclase. SC-19220 did not bind to this substance. Similarly Marazzi and Matschinsky\textsuperscript{50} found that SC-19220 (370 \mu M) did not inhibit 15-hydroxy prostaglandin dehydrogenase. Radzialowski and Novak\textsuperscript{68} found that SC-19220 (1 \times 10^{-4} M to 1 \times 10^{-7} M) reversed the inhibitory effect of PGE\textsubscript{2} on epinephrine-induced lipolysis in isolated rat epididymal fat pads. The authors also reported that SC-19220 has no effect in the absence of PGE\textsubscript{2}. However, in another report, Iliano and Cuatrecasas\textsuperscript{45} observed that SC-19220 (35 \mu M) potentiates epinephrine in the absence of PGE\textsubscript{2}. On the isolated toad bladder, antidiuretic hormone (ADH)-induced increase in osmotic water flow, was inhibited by PGE\textsubscript{1}. SC-19220 did not inhibit this effect\textsuperscript{46} (10^{-5} M).

Polyphloretin phosphate (PPP) (Figure 11), a polyester of phloretin and phosphoric acid, was first synthesized by Diczfaluzy.\textsuperscript{69}
PPP is a potent inhibitor of a number of enzymes including phosphatases and hyaluronidase. The first evidence linking PPP and prostaglandins resulted from the study of the actions of prostaglandins on rabbit intraocular pressure by Beitch and Eakins. Interest in PPP resulted in part because this compound prevented rise in the intraocular pressure and the breakdown of the blood aqueous barriers in the rabbit eye. This increase in pressure normally is accompanied by ocular irritation and trauma. Since Ambache and co-workers had demonstrated that irin, a mixture of prostaglandins, was involved in the response of the rabbit eye to irritation, interaction of PPP with prostaglandins was investigated. Intraarterial infusions of PPP were found to antagonize both the rise in intraocular pressure and the accompanying increase in permeability of the blood aqueous barrier produced by PGE₂.

Eakins, using gel permeation chromatography, found that the most active material could be recovered from the latter fractions. These results led to the proposal that the biological effects of PPP may be attributed to the presence of small molecules. Various monomers and dimers of this compound were synthesized (Figure 12). The only compound which had a markedly higher potency than PPP, as judged by the Pₑ₂ values, was dl-4-phloretin phosphate. Eakins and Karim investigated the effect of PPP on prostaglandin antagonism in smooth muscle. On isolated gerbil colon the actions of PGF₁₀, but not PGE₁, were antagonized by PPP (5 - 10 µg/ml). However, upon further examination these investigators concluded that PPP (10 µg/ml)
The inhibitory effect of various compounds on contractions of the gerbil colon elicited by PGF$_{2\alpha}$. The calculations were made according to the method of Arunlakshna and Schild. The $P_{0.2} = \text{the negative log of the quantity of agonist needed to produce a response equivalent to a given control response.}$

was effective against both E and F prostaglandins. PPP did not affect responses produced by acetylcholine, bradykinin, angiotensin or serotonin. Thus, this polymer was judged to be a specific prostaglandin antagonist. PPP also produced stepwise shifts of prostaglandin dose response curves to the right with increasing PPP concentrations thus suggesting competitive antagonism. PPP also antagonized E$_2$ and F$_{2\alpha}$ in several other tissues, e.g., rabbit jejunum (2.5 - 30 $\mu$g/ml),$^{78,79}$ guinea pig colon (200 - 300 $\mu$g/ml),$^{37}$ rat colon (10 and 20 $\mu$g/ml; this preparation is more sensitive to the action of PGF$_{2\alpha}$ at high dose levels),$^{80}$ chick rectum,$^{81}$ human stomach and colon

<table>
<thead>
<tr>
<th>Compound</th>
<th>$P_{0.2}$</th>
<th>Slope</th>
<th>Relative potency at $P_{0.2}$ PPP = 1.0</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyphloretin phosphate (PPP)</td>
<td>2.4</td>
<td>-1.21</td>
<td>1.0</td>
<td>++</td>
</tr>
<tr>
<td>4'-Phloretin monophosphate</td>
<td>2.4</td>
<td>-1.30</td>
<td>1.0</td>
<td>++</td>
</tr>
<tr>
<td>4'-Phloretin monophosphate</td>
<td>2.6</td>
<td>-1.33</td>
<td>1.6</td>
<td>+++</td>
</tr>
<tr>
<td>Di-4'-phloretin phosphate</td>
<td>3.4</td>
<td>-1.36</td>
<td>8.9</td>
<td>+++</td>
</tr>
<tr>
<td>Di-4'-phloretin phosphate</td>
<td>2.7</td>
<td>-2.31</td>
<td>1.9</td>
<td></td>
</tr>
</tbody>
</table>

Table 1.
In most cases, however, the selectivity and effectiveness of PPP did not approach the selectivity and effectiveness of this agent on the gerbil colon. The inhibitory action of PPP on the gerbil colon was not due to its ability to inhibit various enzymes. The parent dihydrochalcone moiety, phloretin, was inactive. Some related polymeric phosphates (polyhydroquinone phosphate, polyphloroglucinol phosphate and polyestradiol phosphate) having enzyme inhibitory properties similar to PPP were observed not to be PG antagonists. Polystilbol phosphate (20 μg/ml), however, antagonized PGE₂ and PGF₂α on the gerbil colon, but was less selective than PPP. Polystilbol phosphate also antagonized angiotensin and to a lesser extent acetylcholine. Park and Dyer observed that PPP (100 μg/ml) on human isolated umbilical arteries shifted PGE₂ dose response curves to the right ninefold. Serotonin dose response curves were only shifted 3.5 fold. Although these investigators observed a greater inhibition of PGE₂ than serotonin, they concluded that PPP was not a specific antagonist of PGE₂ on this tissue. The effect of PPP on rat stomach muscle illustrates another interesting property of this compound. PPP (20 - 100 μg/ml) caused small dose dependent contractions of the rat fundus strip, but did not greatly affect the responses to E₂, F₂α and acetylcholine. Higher concentrations of PPP (200 - 2000 μg/ml) lowered the tone and reduced the effects of all three agonists. There are several reports concerning antagonism of vascular responses to F₂α prostaglandins. In guinea pigs and cats
F prostaglandins produce a pressor response and in rabbits they produce a depressor response. PPP antagonized both actions [40 - 80 mg/kg] for bronchoconstriction and 200 mg/kg for bronchodilation of F prostaglandins. However, PPP had no effect on the depressor effect of E₂ prostaglandins. Contrary to these reports, Levy and Lindner, did not observe antagonism of the depressor response produced by PGF₂α in two rabbits after intravenous administration of PPP (94 mg/kg). PPP did not inhibit the vasoconstrictor action of PGF₂α in the pulmonary artery and the vasodilator action of PGE₁, PGE₂ and PGA₂ in the femoral artery when administered intravenously (1 - 3 mg/kg) to dogs.

PPP (5 µg/ml) inhibited PGE₂ and TSH stimulation of adenylate cyclase on isolated bovine thyroid cells, but did not alter basal adenylate cyclase activity in these experiments. PPP (5 mg/ml) inhibited PGE₁- and LH-stimulated lactic acid production in prepubertal rat ovaries and reversed PGE₁ inhibition of osmotic water flow in toad bladders (50 µg/ml).

PPP did not bind to isolated "prostaglandin receptor sites" from rat lipocytes and did not compete with PGE₁ for prostaglandin binding sites from rat forestomach (10 µg/3 ml). PPP (1.96 µM) also inhibited the enzyme PGDH in a competitive manner. Studies by Kuehl and co-workers using PPP, revealed that this polymer (200 µg/ml) has no effect upon PGE₁-stimulated cyclic AMP formation in mouse ovaries or rabbit myometrium. Rather, PPP was shown to act at a site subsequent to cyclic AMP formation. This site was tentatively
identified as cyclic AMP-dependent protein kinase. Thus, PPP is not a true prostaglandin antagonist in these tissues. Clearly, PPP does not block the primary PGE$_1$ event. Furthermore, PPP (150 µg/ml) reduced the stimulation of progesterone formation by PGE$_1$.

Subsequent work by Kuehl and co-workers$^{30}$ showed that phloretin (50 µg/ml) itself inhibited PGE$_1$ stimulation of c-AMP formation in mouse ovaries. These investigators suggested that direct prostaglandin antagonism by polymers of phloretin may be related to the differential abilities of tissues to enzymatically dephosphorylate the compound to the parent phosphate-free analog. It therefore appears that the effect of PPP could be due to direct prostaglandin antagonism, inhibition of c-AMP-dependent protein kinase and inhibition of PGDH.

In 1976, Eakins, Rajadhyaksha and Schroer$^{92}$ reported that sodium p-benzyl 4-[1-oxo-2-(4-chlorobenzyl)-3-phenylpropyl]-phenyl phosphonate (N-0164; Figure 13) antagonizes contractions produced by PGE$_2$ and PGF$_{2\alpha}$ on isolated preparations of gerbil colon (1 - 3.8 x $10^{-6}$ M), rat stomach strip (0.19 - 3.8 x $10^{-6}$ M) and guinea pig gastrointestinal muscle. Blockade of responses on the rat stomach strip preparations to PGE$_2$ and PGF$_{2\alpha}$ by N-0164 appeared to be characteristically competitive. The antagonism was reversible and the dose-response curves in the presence of antagonist were parallel to those obtained in the absence of antagonist. Dose-response curves were shifted to the right without alteration of the maximum responses produced by either PGE$_2$ or PGF$_{2\alpha}$. However, the shift of the dose response curves to the right were not proportional to the increase in
Figure 12.

concentration of the antagonist. N-0164, administered by intraperitoneal injection (ED$_{50}$ = 55 mg/kg) prevented diarrhea induced by PGE$_2$ in mice. However, N-0164 was less effective orally (ED$_{50}$ = 105 mg/kg). N-0164 also inhibited chemically-induced irritation (croton oil and pyridine-ether) in the mouse ear (1% N-0164). It was concluded by these workers that N-0164 is a potent, partially selective prostaglandin antagonist on gerbil colon, rat stomach strip and guinea pig gastrointestinal muscle.

Ambache first observed the spasmolytic action of patulin (Figure 14) and other lactones. He found that patulin antagonized the effect of irin on the isolated hamster colon.$^{93}$ Since irin was later shown to be a mixture of prostaglandins, this is probably the first report of prostaglandin inhibition.$^{94,94}$ This action of
patulin was further investigated by Eliasson\textsuperscript{95} who reported that patulin antagonized contractions produced by a number of agonists on the isolated guinea pig ileum including histamine, pilocarpine, 5-hydroxytryptamine and acetylcholine. Therefore patulin is a nonspecific antagonist.

Collier and Sweatman\textsuperscript{96} found that sodium meclofenamate and sodium flufenamate (Figure 14) antagonized contractions of human bronchial muscle to PGF\textsubscript{2α} and also contractions of slow reacting substance in anaphylaxis (SRS-A). Tissue tone was also reduced,
but responses to acetylcholine were unaffected. Fenamates failed to antagonize the relaxations produced by PGE₁ and PGE₂ on the circular gut muscle. Other non-steroidal anti-inflammatory agents such as aspirin and phenylbutazone also antagonized contractions of the human isolated bronchial muscle produced by PGF₂α, but these synthetase inhibitors were less effective than fenamates. Indomethacin (10 - 160 μg/ml) was shown to inhibit contractions produced by serotonin, bradykinin and PGE₂ on isolated rat uterus and guinea pig ileum. This synthetase inhibitor was about four times more potent against PGE₂. By contrast, Vane and Eckenfels found that indomethacin (2 - 6 μg/ml) increased PGE₂-induced responses on isolated rat stomach strips.

Another early report concerning PG inhibition came from the laboratories of Vogt who observed partial inhibition by morphine of contractions of guinea pig ileum produced by darmstoff. Darmstoff also was later shown to be a mixture of prostaglandins. Jaques reported that morphine and certain related compounds, etonitrazene and nalorphine, are potent inhibitors of contractions induced by PGE₁ on the isolated guinea pig ileum.

Karim and Sharma found that intravenous infusion of ethanol (500 ml of 10% solution) inhibited uterine contractions in pregnant women, when the contractions were induced by PGE₂ and PGF₂α, but not when they were induced by oxytocin. Also, cerebral vasoconstriction produced by PGE₁ and PGF₂α in dogs or monkeys can be reversed or prevented by intracarotid infusion of ethanol in saline (0.08 - 0.5%)
but systemic injection of ethanol in saline did not diminish the sedative or tranquilizing action of PGE\textsubscript{1} or PGE\textsubscript{2} in rats. The sedative or tranquilizing action of PGE\textsubscript{1} or PGE\textsubscript{2} was thought to be due to reduced blood flow to the brain.\textsuperscript{103}

There are several unrelated substances which have been shown to inhibit certain prostaglandin actions. These include reserpine on the isolated guinea pig colon,\textsuperscript{104} isoproterenol and norepinephrine on the eye\textsuperscript{105} and isolated rat stomach,\textsuperscript{106} estradiol on luteolysis,\textsuperscript{107} valinomycin on the release of growth hormone,\textsuperscript{108} and progesterone on uterine contractions.\textsuperscript{109}

Prostaglandin antagonists have not been used therapeutically, but several possibilities have been suggested. The effect of prostaglandins on the eye has been the subject of an excellent review by Eakins.\textsuperscript{110} The effect of PPP on ocular responses to prostaglandins has been studied by several investigators. Beitch and Eakins\textsuperscript{70} have shown that close arterial infusion of PPP (10 mg/ml) markedly inhibited the rise in intraocular pressure normally seen after intracameral injection of 1 μg of PGE\textsubscript{2}. Similarly PPP reduced the high aqueous humor protein levels produced by prostaglandins. These findings were confirmed by Storr\textsuperscript{111} who showed that both close-arterial infusions and intravitreal injections of PPP inhibited the effect of PGE\textsubscript{1} on rabbit intraocular pressure. PPP was found to be most effective when given by subconjunctival injection in the rabbit.\textsuperscript{112} When applied topically, PPP inhibited the intraocular pressure rise in rabbits which is produced by PGE\textsubscript{2α}. Subconjunctival injections of PPP
inhibited intraocular pressure increases produced by PGE$_2$, PGF$_{2\alpha}$ and formaldehyde solution. Whereas PPP antagonized the ocular vasodilation produced by PGE$_1$ and PGE$_2$, this antagonism was non-specific. The same doses of PPP also antagonize the actions of isoproterenol and norepinephrine.

PGE$_2$ induced and potentiated sickling in vitro of erythrocytes from sickle cell disease patients in concentrations as low as 10 $\mu$g/ml.$^{113}$ Thus, these workers suggested that prostaglandin antagonists may play a role in the treatment of the disease. Rabinowitz and co-workers$^{114}$ have shown that 7-OPya (200 $\mu$g/ml), ouabain (0.1 mM) and oligomycin (5 $\mu$g/ml) modify the responses of the sickling erythrocytes to prostaglandins (PGE$_2$).

Intravenous administration of F prostaglandins cause cerebral vasoconstriction. E and A prostaglandins cause cerebral vasodilation.$^{115-119}$ Yamamoto and co-workers$^{102}$ have shown that intracarotid infusion of PGE$_1$ (0.5 $\mu$g/min) and PGF$_{2\alpha}$ (25 $\mu$g/min) caused constriction of epicerebral arteries in dogs. Ethanol in saline (0.08 - 0.5 %) inhibited these responses. For these reasons it was suggested that prostaglandins may be involved in cerebral vasospasm and that low concentrations of ethanol may prevent and relieve this condition. However, no other reports may be found concerning this type of antagonism. As little as 1 $\mu$g of PGE$_1$ exhibits a potent hyperthermic effect when injected into the cerebral ventricles of laboratory animals.$^{120-122}$ Fever has also been observed during F prostaglandin infusions in humans.$^{123}$ Vane$^{124}$ has proposed that the
antipyretic activity of aspirin-like drugs is due to inhibition of prostaglandin synthesis. For these reasons it might be expected that prostaglandin antagonists may also have antipyretic activity. The literature reveals no reports which either confirm or deny this hypothesis.

The actions of E and F prostaglandins on circular muscle of human, guinea pig and rat distal colon are opposite. In doses as low as 0.01 µg/ml, E compounds relax while F compounds contract the muscle. Bennett suggested that prostaglandins may play a physiological or pathological role in gut motility, but the effects on peristalsis are not very clear. Karim and co-workers have shown that administration of prostaglandins (especially PGE) can cause diarrhea in humans. The induction of diarrhea, due to prostaglandins, may either be due to their effects on intestinal smooth muscle activity, their effect on water and electrolyte transport, or a combination of both. Several groups have noted the effects of prostaglandins and those of cholera toxin on water and electrolyte movement and on adenyl cyclase activation. It also has been suggested that prostaglandin antagonists or inhibitors may be useful in the treatment of diarrhea caused by cholera or other bacterial toxins. Antagonism of cholera enterotoxin by anti-inflammatory agents in rats was described by Jacoby and Marshall. Bourne has observed that certain anti-inflammatory agents do not produce a detectable inhibition of cholera toxin-induced c-AMP increase in human leukocytes. Both PPP (50 - 100 mg/kg)
and SC-19220 (17 - 20 mg/kg) inhibit diarrhea produced in mice by intraperitoneal injection of PGE₂ (50 μg/kg).

Many studies have indicated that E prostaglandins are bronchodilators, whereas F prostaglandins are bronchoconstrictors. It has been suggested that in asthma, bronchospasm may be due to an overproduction of bronchoconstrictor PGF₂α at the expense of bronchodilators PGE₁ and PGE₂. It also has been suggested that in the lungs PGE may be converted to PGF. The usefulness of PGE₁ and PGE₂, both of which have bronchodilator properties, is limited by their irritant effects. Antagonism of F prostaglandins represents another approach to the treatment of obstructive lung disease. PPP (40 - 80 mg/kg) has been found to inhibit bronchoconstriction produced by PGF₂ in cats and guinea pigs. PPP (10 - 40 μg/ml) also antagonized the PGF₂α response on human isolated bronchial muscle and counteracted anaphylactic bronchoconstriction in guinea pigs. PPP had no effect on the bronchodilator response produced by E prostaglandins.

Current experimental evidence suggests that prostaglandins participate in the development of the inflammatory response. These compounds appear to have a central complex role in inflammation. While small local concentrations of prostaglandins may cause a direct inflammatory reaction in the involved tissue, large systemically administered doses seem to act by an indirect mechanism resulting in inhibition of experimentally induced inflammation. The anti-inflammatory activity of SC-19220 has been studied in rats. This compound was found to inhibit croton oil induced inflammation in rat ears when applied topically (400 μg). Fries demonstrated that PPP
has antiphlogistic activity in rat paws. Intradermal injection of 7-OPya caused blanching of erythema caused by ultraviolet irradiation of guinea pigs; indomethacin and aspirin produced similar effects in both humans and guinea pigs.

There is sufficient evidence to indicate that prostaglandins are involved during labor as natural uterine stimulants. Thus prostaglandin antagonists may be useful in preventing habitual abortion or premature labor. Aspirin (200 mg/kg/day) and indomethacin (2 mg/kg/day) inhibit prostaglandin formation in the uterus, reduce uterine motility and prolong parturition in rats. These drugs also prolonged mid-trimester saline abortions in humans. For these studies, aspirin was administered orally in doses of 650 mg every six hours for ten doses and indomethacin was administered orally in doses of 25 mg every six hours for eight doses. In rats, however, the treatment resulted in a high incidence of fetal deaths.

Tashjian presented evidence suggesting that the bone resorption stimulating substance from mouse fibrosarcoma cells is PGE\textsubscript{2}. Thus, prostaglandins may be responsible for the observed hypercalcemia found in cancer patients. Both PPP and SC-19220 inhibit prostaglandin induced bone resorption in the fetal rat bone assay at 20 µg/ml.

The future of prostaglandin antagonists lies in several directions. None of the major prostaglandin antagonists (7-OPya, SC-19220 or PPP) were found to inhibit relaxation of circular gastrointestinal muscle which is generally produced by E
prostaglandins. Also none of the antagonists thus far reported
inhibited all the actions of prostaglandins. Selective inhibition
is usually explained by invoking the notion of receptor difference,
but another explanation may be found by considering difference in
transport to the site of action. Whereas one might consider the
present generation of antagonists as prototypes for further work, it
seems to us that a new approach is desirable and this dissertation
describes such an approach.
Our research has been directed towards the development of a specific primary prostaglandin antagonist. In the simplest sense two methods are available which may provide direction to the search for an antagonist to a given agonist. One approach involves the random screening of a variety of compounds with regard to their activity in the given system. The other approach involves the synthesis or selection of a compound proposed to be an antagonist based upon a knowledge of the properties of the agonist. If a knowledge of the agonist structure-action relationship is available, such information may also aid in the rational design of antagonists. Further, one might consider antagonist design based on agonist-antagonist relationships in other systems. For example, biological effects of histamine and antihistamine are the result of physico-chemical interactions with receptor (H1-receptors) molecules in an organism. Modification of the structure of histamine does not give rise to antagonists, but rather to weaker agonists. Antihistamines have different structural requirements from histamine, but both have an aliphatic amino group which could exist as a cation at physiological pH. Presumably, this cation is capable of interacting with an anionic site on the receptor. Electrostatic forces of attraction of histamine and antihistamine to the receptor apparently outweigh
the importance of other forces that might be involved. For $H_1$-receptors, antihistamines probably do not occupy the exact receptor occupied by histamine, but do occupy the anionic site thus blocking the accessibility of the site to histamine. Secondary binding of the aromatic rings to extra-histamine receptor sites afford increased affinity of these antihistamines to the receptor.\textsuperscript{150}

![Histamine 46](image)

![Antihistamine 47](image)

Figure 15.

Just as the amino function is presumed to be necessary for binding of an antihistamine or histamine to the histamine receptor, it may well be that the acid function in PGE\textsubscript{1} is necessary for interaction with a cationic site of a prostaglandin receptor. The acid group exists in equilibrium with its anion at physiological pH. It has also been shown that long aliphatic chains and hydroxy groups at position 15 are necessary for prostaglandin agonist activity.\textsuperscript{151} Possibly these functions are also involved in receptor binding and are thus responsible for selective intrinsic activity.
Insertion of a diphenylmethyl ether function in place of the imidazole ring of histamine converts the molecule into an $H_1$-histamine antagonist. Similarly, introduction of one or more aromatic rings into a "prostaglandin-like" structure in place of the cyclopentyl-ol-one grouping in PGE$_1$, at an appropriate distance from the acid group, may afford an antagonist.

For comparison purposes, target intermediate 48 in Figure 16 is numbered in accord with expected prostaglandin nomenclature. The structural similarity between 2 and 48 thus becomes clear. The $\{C = C\}$ between the 13 and 14 positions of 2 coincides with the $\pi$ electrons of the aryl function at position 13 and 14 of 48. Furthermore, the keto function at 15 of 48 is potentially convertible to a 15-OH group having a juxtaposition related to the 15-OH group in agonist 2. The 5-membered methylenedioxy ring of 48 coincides with the cyclopentyl-ol-one system of 2. Analog 48 may exhibit antagonist properties to various prostaglandins and/or serve as intermediate for the synthesis
of many related analogs such as (49-53). Whereas this thesis considers structural analogs having n-propyl side chains, the synthetic procedures developed in these laboratories for (49-53) allows for the preparation and study of many related homologs containing various aliphatic and aryl-aliphatic substituents.

Thus, in this dissertation we propose to construct a variety of cyclic long chain aromatic carboxylic acids (49-53) as potential prostaglandin antagonists. We describe facile synthesis leading to the construction of such molecules. Although the biological results are not included in this dissertation, preliminary results obtained by Prof. Rahwan and his co-workers in the Division of Pharmacology indicate that this approach to drug design has been successful.
Figure 17
RESULTS AND DISCUSSION

A. Synthetic Aspects

Initially we employed the reaction sequence described in Figure 18 to synthesize compounds 49-53. Treatment of piperonal (54) with n-BuLi afforded 1-(3,4-methylenedioxyphenyl)pentan-1-ol (55) in 66.3% yield. The E-olefin 56 was obtained in 95% yield by dehydration of 55 using PTSA in refluxing dry benzene. Vinyl proton coupling $J = 15.5$ Hz established the E stereochemistry for 56. During the course of these investigations we observed that methylenedioxy substituted styrene analogs, in the presence of Vilsmeier-Haack reagent [POCl$_3$ + DMF] afford the previously unreported 2-substituted-3-dimethylamino-5,6 methylenedioxyindenes. This new reaction was termed the Vilsmeier-Haack cyclization.$^{152}$ A proposed mechanism for the formation of these aminoindenes is described in Figure 19. The mechanism involves electrophilic attack by 64 on the $\beta$-carbon of the styrene system to give cationic intermediate 65. Intermediate 65 undergoes thermally and sterically dependant proton loss to give (E)-66, which provides aldehydes of this configuration upon hydrolysis, and (Z)-66, which provides the correct juxtaposition of functional groups for cyclization to aminoindenes. Formation of (Z)-66 is favored at elevated temperature.
Figure 18 - continued
Treatment of isosafrole (56b) where $R' = \text{CH}_3$ with cooled (20°) Vilsmeier-Haack reagent is reported to afford corresponding aldehyde 70 in 24% yield. Under similar conditions we obtained aldehyde 70 in 48% yield. The E configuration of the aldehyde was substantiated by x-ray crystallography. Heating the isosafrole-vilsmeier reagent on a steam bath for 3 hours afforded the corresponding aminoidene (47%) and the aldehyde (23%). In contrast to this result, olefin 56a where $R' = \text{-CH}_2\text{-CH}_2\text{-CH}_3$ gave exclusively 62 in 71% yield. No aldehyde was detected. Application of first principles of conformational analysis to the formation of (E)-66 and (Z)-66 from 65 ($R' = \text{-CH}_3$) provides an explanation for the change in stearic course of the reaction leading to (E)-66 at the lower temperature and a mixture of (E)-66 and (Z)-66 at higher temperature. At lower temperatures minimization of nonbonded repulsions in 65 requires that product 66 develop mainly from 65-a (Figure 20), the thermodynamically more stable intermediate to give (E)-66 rather than 65-b which leads to (Z)-66. At higher temperatures the relative population of 65-b increases at the expense of 65-a and the product 66 contains more (Z)-66 than at lower temperatures. In contrast, treatment of 56-a where $R' = \text{-CH}_2\text{CH}_2\text{CH}_3$ at elevated temperatures produced only indene 69. No aldehyde was detected. Thus the stearic bulk of the n-propyl group in 65-b ($R' = \text{-CH}_2\text{CH}_2\text{CH}_3$) reinforces the thermal effect by increasing the relative population of 65-b relative to 65-a, lowers the yield of aldehyde precursor (E)-66 and increases the yield of indene precursor (Z)-66 relative to that obtained from
isosalafrole. The aminooindenes undergo alkaline hydrolysis to indanones in quantitative yield thus affording a new method for the preparation of 2-substituted indanones.

To further investigate the scope of this cyclization (E)-1-(3,4-methylenedioxyphenyl)-2-phenylethene was prepared according to the method of Tiffeneau and Levy. The corresponding aminooindene was obtained initially in 16% yield, although the yield was increased to 25% if the olefin was added directly to the previously heated (steam-bath) Vilsmeier-Haack reagent. Recovery of starting olefin averaged 70%. Alkaline hydrolysis of 2-phenyl-3-dimethylamino-5,6-methylenedioxyindene (71) afforded 2-phenyl-2-hydroxy-5,6-methylenedioxy-1-indanone (72) in 50% yield (Figure 21). This likely resulted from both hydrolysis and air oxidation in the alkaline medium. The mass spectrum showed a molecular ion at 268(35). The
alcoholic proton resonance at \( r \leq 6.22 \) was exchangeable with \( D_2O \). Unlike other indanones described previously, 72 did not form a 2,4-dinitrophenylhydrazone. Both hydrogen-bonded -OH stretching (3435 cm\(^{-1}\)) and \( \gamma = 0 \) stretching (1685 cm\(^{-1}\)) were observed in the infrared spectrum.

In contrast to the above olefin, 1-(3,4-methylenedioxyphenyl)-1-phenylethene (73) afforded 70% yield of olefin 74, but no aminoidene 75 was detected (Figure 22). The \( E \) geometry of the aldehyde was established by x-ray crystallography. The crystal conformation of 74 provided an explanation for the stearic course of the reaction. Since there is greater propensity for resonance interaction between the methylenedioxyphenyl ring system and the \( \alpha,\beta \) unsaturated aldehyde moiety, these groups are more nearly coplanar (25°) than are the phenyl and \( \alpha,\beta \)-unsaturated aldehyde function (63°). In the
intermediate carbonium ion 76 (Figure 23), the methylenedioxyphenyl and the contiguous ethyl units should more closely approach coplanarity, while the phenyl and the methylenedioxyphenethyl units should more closely approach 90°. Accordingly 76a which leads to aldehyde 74, is more thermodynamically stable than 76b which leads to indene (75). Since the effective steric bulk of the phenyl moiety in 76a is smaller than that of the methylenedioxyphenyl group of 76b in the direction of the ClCHN(CH₃)₂ unit.

The indanone 58 underwent facile Michael addition of ethyl acrylate to yield indanone ester (86%). This indanone ester served as a prostaglandin like intermediate, and was subjected to a multitude of reaction conditions designed to affect structural changes in the methylenedioxyaryl portion of the molecule.
Alkaline hydrolysis of the ester 52 gave acid 49 in quantitative yield. Acid 49 was reduced using the Clemmenson's reaction, affording 50 in 84% yield. Alternatively, reduction of 49 using NaBH₄ afforded two products (Figure 24). The cis-hydroxy acid
diastereomer 60a was isolated in 70% yield as the cyclized lactone 61, and was easily separated from trans-60, formed in 15% yield, by column chromatography (CHCl₃: MeOH 95:5).

The synthesis for benzyl-ether 52 involved cleavage of the methylenedioxy group of 49 affording catechol 62 in 71% yield. Treatment of 62 with K₂CO₃/acetone/O₂/H₂O/Br afforded the desired benzyl ether in 39% yield. Methylenedioxy cleavage posed considerable difficulty mainly owing to the harsh reaction conditions required and the facile air oxidation of the resulting catechol. Available methods for methylenedioxy cleavage using BCl₃ and PCl₃ were unsatisfactory.

Considerations by us of modifications utilized by Bick and Russel (summarized in Figure 25) led to the synthesis of 62 without isolation of intermediates. Product 62 required storage under N₂ since this

Figure 25.
compound was very sensitive to air oxidation and turned brown upon sudden exposure. Treatment of 62 under N₂ with anhydrous K₂CO₃/G₆H₅CH₂Br in refluxing acetone for 50 hrs. gave the dibenzyl ether ester, which was not isolated, but was immediately hydrolyzed to acid 52. NaBH₄ reduction of 52 afforded lactone 63 in 56.5% yield. No trans acid was detected.

Initial approach to the synthesis of 53 is outlined in Figure 26. Treatment of 50 with Ni-Al alloy¹⁶⁵ or Na/NH₃¹⁶⁶ was expected to yield 81. Condensation of 81 with α-bromoacetate in the presence of K₂CO₃ and acetone should yield 82. Alkaline hydrolysis of 82 followed by cyclization with PPA was expected to afford 53. Unfortunately conversion of 50 to 81 was unsuccessful and led only to isolation of starting material. In the case of 49 only complex mixtures were

\[
\begin{align*}
\text{COOH} & \xrightarrow{\text{Li}-\text{NH}_3} \text{COOH} \\
\text{Ni-Al alloy} & \xrightarrow{\text{50}} \text{Ni-Al alloy} \\
\text{COOH} & \xrightarrow{\text{K}_2\text{CO}_3/\text{Me}_2\text{CO}/} \text{COOH} \\
\text{Br-CH}_2-\text{O-CH}_3 & \xrightarrow{\text{10\% KOH}} \\
\text{COOH} & \xrightarrow{\text{PAA}} \text{COOH} \\
\end{align*}
\]

Figure 26.
produced under these conditions. Repetition of the work of Birch (no yields reported) using saffrole afforded p-allyl phenol in 16% yield; a substantial quantity of unidentified impurity was also produced. For these reasons the alternative synthetic procedure described in Figure 27 was pursued. Condensation of 6-methoxy-1-indanone 83 with dimethyl carbonate in presence of NaH afforded the carbomethoxy analog 84 in 95% yield. Attempts to prepare 2-alkyl substituted indanones from 83 via the enamine were unsuccessful owing to poor enamine yields (10 - 15% from 83). Alkylation of 84 using Na/n-PrI or NaOEt/n-PrI in EtOH only afforded poor yields (<5%) of 85. Alternatively, use of NaH/n-PrI in freshly distilled THF containing HMPA afforded 85 in 67% yield. In the absence of HMPA the yields were drastically lower. Hydrolysis and decarboxylation in HOAc: conc. HCl: H₂O (2.5:1.1) afforded indanone 86 in 78% yield. Anion formation using KOT-Bu in t-BuOH followed by Michael addition to methyl acrylate yielded indanone ester 87 isolated as crude material after column chromatography on silica gel-CHCl₃. Alkaline hydrolysis of ester 87 afforded Keto acid 88 in 54.3% yield based on 86. The poorer yields experienced in this Michael addition when compared to the conversion of methylenedioxy analog 58 to 59 may be a reflection of increased nucleophilicity of 58 owing to resonance interaction of the carbonyl function with the 5-CH₂-O- electron pair on oxygen.

Hydrogenation (Pd/C) of 87 in glacial HOAc at 65° for 3 hours afforded 89 in 56% yield. Concomitant ester hydrolysis and methyl
Figure 27
ether cleavage using BBr$_3$ afforded unstable phenol 81 which was not purified, but immediately heated with methyl bromoacetate in acetone containing K$_2$CO$_3$ to yield an oil which was hydrolyzed with 15% KOH to generate 82. The NMR spectra for 82 is in accordance with the assigned structure, but the viscous oil could not be purified by distillation owing to decomposition. Treatment of 82 with diazomethane gave analytically pure diester 90.
EXPERIMENTAL

A. Synthetic Procedures

Elemental analyses were performed by Clark Microanalytical Laboratory, Urbana, Illinois, and Galbraith Laboratories, Knoxville, Tennessee. Infrared spectra were recorded on a Perkin-Elmer Model 257 grating infrared spectrophotometer and Beckman IR4230. Gas-Liquid partition chromatography was performed using the F and M Scientific Model 402 high efficiency gas chromatograph. Nuclear magnetic resonance spectra were recorded on a Varian A-60A and Bruker HX-90E spectrophotometer. All melting points were taken with a Thomas Hoover capillary melting point apparatus and are uncorrected.

Ethyl 5,6-(Methyleneoxy)-1-oxo-2-propyl-2-indanpropionate (59).

To a solution of 2.18 g (0.01 mol) of 2-n-propyl-5,6-methyleneoxy-1-indanone (58) and 0.112 g (0.001 mol) of potassium-t-butoxide in 25 ml of t-Butanol was added dropwise with stirring and under a nitrogen atmosphere 1.2 g (0.012 mol) of ethyl acrylate. The resulting solution was stirred at room temperature for 90 minutes, acidified with HOAc, concentrated under reduced pressure and diluted with Et2O. The Et2O solution was washed twice with aqueous Na2CO3 solution, dried (Na2SO4) and concentrated under reduced pressure affording a viscous oil 2.78 g (87%) which was purified by distillation affording a colorless oil, bp 188°-190° C (0.15 mm).

IR 1690 and 1730 cm⁻¹; NMR (CDCl3) 7.08 (s, 1H, aromatic), 6.81 (s, 1H, aromatic), 6.05 (s, 2H, -O-CH2-O-), 4.09 (2H, q, -CH2-ester, J = 7 Hz), 2.89 (s, 2H, geminal), and (0.68-2.43 m, 14H, aliphatic; containing a triplet-CH3 of ester; J = 7 Hz).
A solution of NaOH (10 g) in 100 ml of MeOH: H₂O (15:85) was added to 1.66 g (0.005 mol) of ethyl 5,6-(methylenedioxy)-1-oxo-2-propyl-2-indanpropionate (49). The mixture was stirred at room temperature overnight, diluted with H₂O and extracted with two 100 ml portions of Et₂O. The aqueous layer was acidified with 2N HCl and extracted with three 100 ml portions of Et₂O. The combined Et₂O layers were dried (Na₂SO₄) and evaporated to give an oil. The oil was stirred overnight with hexane affording a pale yellow solid, which was crystallized from benzene-hexane yielding 1.4 g (93%) of white needles, mp 91-92°C. IR 3400, 1710 and 1693 cm⁻¹: NMR (CDCl₃) δ 9.07 (1H, -COOH exchangeable with D₂O), 7.1 (s, 1H, aromatic), 6.8 (s, 1H, aromatic), 6.05 (s, 2H, -O-CH₂-O-), 2.9 (s, 2H, geminal), and (0.66-2.5, m, 11H, aliphatic).

5,6-(Methylenedioxy)-2-propyl-2-indanpropionic Acid (50). A mixture of 20 g of mossy Zn, 1.5 g of HgCl₂, 2 ml of concentrated HCl and 20 ml of H₂O was stirred for 5 minutes. The aqueous solution was decanted and the amalgamated Zn was covered with 20 ml of H₂O and 16 ml of concentrated HCl. A solution of 1 g (0.0034 mol) of 5,6-(methylenedioxy)-1-oxo-2-propyl-2-indanpropionic acid (49) in 25 ml of sulfur-free toluene was added to the amalgamated Zn along with 2 ml
of glacial HOAc. The mixture was refluxed for 24 hours during which time 6 ml of concentrated HCl was added approximately every 5 hours. The solution was cooled to room temperature (the aqueous layer separated) and after dilution with H_2O was extracted with three 75 ml portions of Et_2O. The combined organic layers were washed with saturated NaCl solution, dried (Na_2SO_4), and concentrated under reduced pressure affording 0.8 g (84%) of a viscous oil, bp 193-194°C (0.05 mm), the purified distillate afforded white crystals, mp 81-82°C. IR 3580, 3300 and 1710 cm^{-1}; NMR (CDCl_3) δ 9.63 (s, 1H, -COOH), 6.56 (s, 2H, aromatic), 5.83 (s, 2H, -O-CH_2-O-), 2.63 (s, 4H benzylic), and 0.76-2.5 (m, 11H, aliphatic; two virtually symmetrical multiplets).


5,6-(Dihydroxy)-1-oxo-2-propyl-2-indanpropionic Acid (62). Dry benzene (90 ml) was added to a mixture of 1.16 g (0.004 mol) of 5,6-(methyleneedioxy)-1-oxo-2-propyl-2-indanpropionic acid (49) and 3.34 g (0.016 mol) of PCl_5. The resulting solution maintained under N_2 and heated at reflux for 9 hours. After evaporation to dryness, the residual oil was stirred with 150 ml of H_2O for 12-14 hours. The resulting suspension was refluxed on an oil bath for an additional 3 hours. The hot solution was filtered into a round bottom flask wrapped in aluminum foil and was stored overnight in the dark. Upon standing 0.8 g (71.4%) of white needles, mp 85-87°C were obtained. This product was extremely sensitive to light and decomposed
on standing. NMR (acetone-\(d_2\)) \(\delta 7.09\) (s, 1H, aromatic), \(6.93\) (s, 1H, aromatic), \(2.91\) (s, 2H, benzylic), and \(0.65-2.41\) (m complex, 11H, aliphatic) mass spectrum m/e (rel. intensity) \(163 (14), 164 (11), 175 (17), 176 (13), 177 (100), 178 (11), 189 (19), 218 (23), 236 (83), 237 (10), 261 (13), 278 (8).

Anal. Calcd for C\(_{15}\)H\(_{18}\)O\(_5\): C, 64.76; H, 6.51. Found: C, 64.47; H, 6.51.

5,6-(Dibenzylxoy)-1-oxo-2-propyl-2-indanpropionic Acid (52). To a solution of 0.6 g (0.0019 mol) of 5,6-(dihydroxy)-1-oxo-2-propyl-2-indanpropionic acid (62) and 1.0 g of K\(_2\)CO\(_3\) in 50 ml of dry acetone was added dropwise with stirring and under N\(_2\) 1.30 g (0.0073 mol) of benzyl bromide. The resulting mixture was refluxed under N\(_2\) for 50 hours, after which the solution was filtered to remove K\(_2\)CO\(_3\). The K\(_2\)CO\(_3\) solids were washed with acetone and the combined solutions were concentrated under reduced pressure affording an oil which was partitioned between benzene and 1N NaOH. The benzene layer was dried (Na\(_2\)SO\(_4\)) and concentrated under reduced pressure affording an oil which was hydrolyzed with 10% KOH solution in the usual manner. The oil obtained after reaction workup was stirred with n-hexane yielding a white solid. Recrystallization from benzene-hexane afforded 0.38 g (39%) of white crystals, mp 109-110°C. IR 3300, 1720 (broad) cm\(^{-1}\); NMR (CDCl\(_3\)) \(\delta 7.21-7.58\) (m, 12H, aromatic + one CO\(_2\)H), \(6.9\) (s, 1H, aromatic), \(5.21\) (s, 2H, -O-CH\(_2\)-O-), \(5.14\) (s, 2H, 0-CH\(_2\)-0-), \(2.87\) (b, 2H, indanone benzylic H) and \(0.66-2.5\) (m, 11H, aliphatic).
Anal. Calcd for C_{29}H_{30}O_5: C, 75.96; H, 6.59. Found: C, 75.84; H, 6.50.

5,6-Dibenzyloxy-1-hydroxy-2-propyl-2-indanpropionic Acid β-Lactone (63). To a solution of 0.458 g (0.001 mol) of 5,6-(dibenzyloxy)-1-oxo-2-propyl-2-indanpropionic acid (52) in 50 ml of 95% EtOH was added with stirring a solution of 0.38 g (0.01 mol) of NaBH₄ in 25 ml of H₂O. The mixture was stirred 18 hours at room temperature after which time 10 ml of 1N NaOH solution was added. The solution was concentrated under reduced pressure affording an oil which was chromatographed on silica gel -CHCl₃ yielding 0.25 g (56.5%) of a colorless oil. Crystallization from hexane afforded white crystals mp 72-73° C. IR 1730 cm⁻¹; NMR (CDCl₃) 90 mHz, δ 7.25 - 7.45 (m, 10H, aromatic), 7.00 (s, 1H, aromatic), 6.74 (s, 1H, aromatic), 5.34 (s, 1H), 5.12 (s, 4H, -benzyloxy), 2.85 (s, 2H, benzylc) and 0.98 - 2.44 (m, 11H, aliphatic).

Anal. Calcd for C_{29}H_{30}O_4: C, 78.7; H, 6.80. Found: C, 78.54; H, 7.16.

2-Carbomethoxy-6-methoxy-1-indanone (84). To a slurry of 3.4 g (0.04 mol; 57% emulsion) of NaH in 10 ml of dimethyl carbonate (distilled from NaH) was added with stirring under N₂ a solution of 6.48 g (0.018 mol) of 6-methoxy-1-indanone (83) in 70 ml of dimethyl carbonate. The mixture was refluxed for 3 hours after which time the excess NaH was decomposed by dropwise addition of ice cold H₂O followed by acidification with glacial HOAc. The solution was extracted with Et₂O and the Et₂O layer was concentrated under reduced
pressure affording an oil which was chromatographed on florisil-CHCl₃ yielding 8.4 g (95.6\%) of a yellow solid. A small portion of this solid was purified by recrystallizing from MeOH affording white crystals, mp 78-79°C. IR 1710 and 1740 cm⁻¹; NMR (CDCl₃) δ 7.03-7.51 (m, 3H, aromatic), 3.28-4.0 (m, 9H, 2H-3, H-2 and two sharp singlets -CO₂CH₃ and -OCH₃).


2-Carbomethoxy-2-propyl-6-methoxy-1-indanone (85). To a slurry of 0.8 g (0.018 mol; 57\% emulsion) of NaH in 10 ml of tetrahydrofuran (freshly distilled from LiAlH₄) was added with stirring under N₂ to a solution of 4.0 g (0.018 mol) of 2-carbomethoxy-6-methoxy-1-indanone (84) in 50-60 ml of THF. The mixture was heated at 60-70°C for 15 minutes and 3 ml of n-propyl iodide was added. The mixture was refluxed for 5 hours, cooled and 15 ml of hexamethylphosphoramide (highly carcinogenic) was added. The clear solution was stirred at room temperature 1 hour and refluxed overnight. The solution was concentrated under reduced pressure, partitioned several times between H₂O and Et₂O and the organic layer concentrated under reduced pressure affording a light brown oil which was chromatographed on florisil-CHCl₃ affording 3.18 g (66.8\%) of a yellow oil which was purified by microdistillation using a bath temperature of 116-117°C (0.05 mm). IR 1710 and 1745 cm⁻¹; NMR (CDCl₃) δ 7.08-7.5 (m, 3H, aromatic), 3.82 (s, 3H, CO₂CH₃), 3.68 (s, 3H, -OCH₃), 0.71-2.23 (m, 7H, aliphatic). Calcd. for the AB part (benzylc) δA 3.64, δB 3.03 with JAB = 17.4 Hz.
Anal. Calcd for C_{15}H_{18}O_4: C, 68.68; H, 6.91. Found: C, 68.91; H, 6.75.

2-Propyl-6-methoxy-1-indanone (86). A solution (25 ml) of concentrated HCl and H_2O (1:1) was added to a solution of 4.6 g (0.017 mol) of 2-propyl-2-carbomethoxy-6-methoxy-1-indanone (85) in 40 ml of glacial HOAc. The mixture was refluxed for 5 hours. Concentrated HCl (5 ml) was added and the solution was stirred overnight at room temperature. The reaction mixture was extracted with three 100 ml portions of Et_2O and the combined Et_2O layers were concentrated under reduced pressure affording a yellow oil which was chromatographed on silica gel-CHCl_3 yielding 2.8 g (78%) of oil which solidified under reduced pressure. A small portion of this oil was distilled bath temperature, 95-96°C (0.025 mm) affording a white solid, mp 50-51°C. IR 1710 cm^{-1}; NMR (CDCl_3) δ 7.03-7.46 (m, 3H, aromatic), 3.83 (s, 3H, -OCH_3), 2.45-3.43 (m, 3H, ABX pattern of 2H-3 and H-2) and 0.76-2.16 (m, 7H, aliphatic).

Anal. Calcd for C_{15}H_{16}O_2: C, 76.47; H, 7.84. Found: C, 76.64; H, 7.95.

6-Methoxy-1-oxo-2-propyl-2-indanpropionic Acid (88). To a solution of 2.04 g (0.01 mol) of 2-propyl-6-methoxy-1-indanone (86) and 0.112 g (0.001 mol) of potassium-t-butoxide in 25 ml of t-butanol was added dropwise with stirring under N_2. 1.03 g (0.012 mol) of methyl acrylate. The resulting solution was stirred at room temperature for 2 hours, acidified with HOAc, concentrated under reduced pressure and diluted with Et_2O. The ether extract was washed
twice with 10% Na₂CO₃ solution, dried (Na₂SO₄) and concentrated under reduced pressure affording 2.4 g of a viscous oil of methyl-6-(methoxy)-1-oxo-2-propyl-2-indanpropionate (87) which was not further purified, but used as such in the conversion to 88.

Hydrolysis of 87 using 15% methanolic KOH (80:20 H₂O: MeOH), afforded a yellow semisolid which was crystallized from benzene-hexane to yield 1.5 g (54.3%) of white needles, mp 96-97°C. IR 1740 and 1710 cm⁻¹; NMR (CDCl₃) δ 8.46 (1H, -COOH exchangeable with D₂O), 7.41-7.05 (m, 3H, aromatic), 3.82 (s, 3H, -OCH₃), 2.92 (b, 2H, -benzylic) and 0.66-2.5 (m, 11H, aliphatic).


Methyl-6-(methoxy)-2-propyl-2-indanpropionate (89). A mixture of 1.5 g (0.005 mol) of methyl-6-methoxy-1-oxo-2-propyl-2-indanpropionate (87) and 10% Pd-C (0.375 g) in 40 ml of glacial HOAc was hydrogenated at 65°C (49 p.s.i.) for 3 hours. After 3 hours hydrogen absorption ceased and the mixture was filtered. The residue was washed with 50 ml of MeOH and the combined filtrates were evaporated to afford a brown oil, which was chromatographed on silica gel-CHCl₃ to yield 0.8 g (56.33%) of a clear colorless oil. A small portion of this oil was purified by microdistillation. Bath temperature 124-126°C (0.025 mm). IR 1740 cm⁻¹; NMR (CDCl₃) δ 8.56-7.30 (m, 3H, aromatic), 3.79 (s, 3H, -CO₂CH₃), 3.68 (s, 3H, -OCH₃), 2.75 (b, 4H, benzylic) and 0.71-2.5 (m, 11H, aliphatic).

Anal. Calcd for C₁₇H₂₄O₅: C, 73.91; H, 8.69. Found: C, 73.55; H, 8.58.
Sodium Borohydride Reduction of 5,6-(Methylenedioxy)-1-oxo-2-propyl-2-indanpropionic Acid (49). To a solution of 2.0 g (0.0069 mol) of 5,6-(methylenedioxy)-1-oxo-2-propyl-2-indanpropionic acid (49) in 60-70 ml of 95% EtOH was added dropwise with stirring 2.65 g (0.07 mol) of NaBH₄ in 20 ml of H₂O. The mixture was stirred for 20 hours at room temperature after which time 20 ml of 1N NaOH solution was added. EtOH was removed under reduced pressure and the residual mixture was acidified using 1:1 conc. HCl: H₂O and extracted with Et₂O (2 x 70 ml). The Et₂O layer was dried (Na₂SO₄) and concentrated under reduced pressure to yield an oil (1.85 g) which was chromatographed on silica gel [CHCl₃: MeOH (96:4)]. Fractions were monitored by T.L.C. [CHCl₃: MeOH (96:4)].

A) 1-Hydroxy-5,6-(methylenedioxy)-2-propyl-2-indanpropionic Acid-Lactone (61) was obtained in the first 100-150 ml and was crystallized from hexane affording 1.3 g (70%) of white needles, mp 81-82°C. IR 1740 cm⁻¹; NMR (CDCl₃) δ 6.8 (s, 1H, aromatic), 6.6 (s, 1H, aromatic), 5.9 (s, 2H, -O-C₆H₄-O-), 5.3 (s, 1H), 2.8 (s, 2H, geminal) and (0.66-2.5, m, 11H, aliphatic).


B) 1-Hydroxy-5,6-(methylenedioxy)-2-propyl-2-indanpropionic Acid (60) was obtained in a second fraction and was crystallized from benzene affording 0.3 g (14.9%) of white needles, mp 107-108°C. IR 3400 and 1680 cm⁻¹; NMR (DMSO-d₆) δ 6.76 (s, 1H, aromatic), 6.66 (s, 1H, aromatic), 5.92 (s, 2H, -O-C₆H₄-O-), 4.54 (s, 1H, adjacent to -OH), 3.29 (b, -OH and C-OH) and a complex multiplet (0.84-2.76).
Anal. Calcd for C_{16}H_{20}O_5: C, 65.75; H, 6.85. Found: C, 65.60; H, 6.82.

Methyl 5-(Carboxymethoxymethoxy)-2-propyl-2-indanpropionate (90).

To a solution of 1.16 g (0.0042 mol) of methyl 6-(methoxy)-2-propyl-2-indanpropionate (89) in 80 ml of dry methylene chloride was added dropwise with stirring and under N\(_2\) 1.2 ml (0.0126 mol) of boron tribromide at -80\(^\circ\). The mixture was warmed to room temperature overnight. The excess boron tribromide was destroyed by careful addition of ice cold water. The mixture was concentrated under reduced pressure and the residual emulsion was partitioned between benzene and water. The benzene layer was dried (Na\(_2\)SO\(_4\)) and concentrated under reduced pressure affording an oil (0.9 g). The oil was dissolved in dry acetone and 1 g of K\(_2\)CO\(_3\) was added. The mixture was heated gently and 1 ml (0.011 mol) of \(\alpha\)-bromoacetate was added. The mixture was heated at reflux for 40-50 hours, after which time the solution was filtered. The K\(_2\)CO\(_3\) solids were washed with acetone and the combined filtrates were concentrated under reduced pressure affording an oil, which was hydrolyzed using a 15% KOH solution [MeOH: H\(_2\)O (20:80)] affording a viscous oil (81). Treatment of 81 with CH\(_2\)N\(_2\) followed by column chromatography on silica gel-CHCl\(_3\) afforded a colorless oil 0.5 g (35.7%) based on starting 82. A small portion of this oil was distilled bath temperature 158-162\(^\circ\)(0.025 mm) affording an analytically pure sample. IR 1740 and 1770 cm\(^{-1}\); NMR (CDCl\(_3\)) \(\delta\) 6.5-7.10 (m, 3H, aromatic), 4.57 (s, 2H, -O-CH\(_2\)-CO\(_2\)CH\(_3\)), 3.79 (s, 3H, -O-CH\(_2\)-CO\(_2\)CH\(_3\)), 3.64 (s, 3H, -CH\(_2\)-CH\(_2\)-CO\(_2\)CH\(_3\)) and 1.52-2.32 and 0.60-1.44 (m, 11H, aliphatic).
Anal. Calcd for $\text{C}_{19}\text{H}_{26}\text{O}_5$: C, 68.26; H, 7.78. Found: C, 68.36; H, 7.90.
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