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SYNTHESIS AND BIOLOGICAL ACTIVITY
OF NEW HYPOLIPIDEMIC AGENTS

DISSERTATION

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

By
Guiragos K. Poochikian, B.S., Pharmacy

The Ohio State University
1974

Reading Committee:
Dr. Dennis R. Feller
Dr. Duane D. Miller
Dr. Howard A. I. Newman
Dr. Donald T. Witiak

Approved by

Advisor
Department of Pharmacy
ACKNOWLEDGMENTS

The author wishes to express his gratitude and appreciation to the following individuals who have rendered assistance in the completion of this project:

Dr. Donald T. Witiak, under whose direction this study was undertaken, not only for his advice and guidance in scientific matters, but also for his unfailing optimism and sense of humor.

Dr. Dennis R. Feller, for his many helpful suggestions in the biological aspect of this work.

Dr. Howard A. Newman, for his collaboration in the hypolipidemic studies.

The National Heart and Lung Institute of The National Institute of Health, grant number HE-12740, for the financial support of this research.

The graduate students of my department for their helpful suggestions.

Lastly, my parents for many things.
VITA

1946 . . . . . . . . . . . . . . Born - Beirut, Lebanon

1970 . . . . . . . . . . B.S. Pharmacy, the American University of Beirut, Beirut, Lebanon.

1970-1971 . . . . . . . . Teaching Assistant; College of Pharmacy, The Ohio State University, Columbus, Ohio.

1971-1974 . . . . . . . . Research Associate, College of Pharmacy, The Ohio State University, Columbus, Ohio.

FIELD OF STUDY

Major Field: Medicinal Chemistry.

Advisor: Associate Professor Donald T. Witiak
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Atherosclerosis, a form of arteriosclerosis, is the most common arterial disease today. It is a disease of the intima of the arteries,\(^1\text{-}^2\) which develops progressively over a long period of time and becomes symptomatic only when it is far advanced. This disease, and associated embolisms, have now emerged as the primary causes of death in Europe, the United States and in many other regions. Atherosclerosis is caused by the formation of lipid-containing lesions (plaques) that proliferate into the arterial lumen, thus partially or completely blocking blood flow.\(^3\) The process of atherosclerosis progresses in irregular and episodic fashion over the individual's life span.\(^4\) The agent that initiates the fatty streak in humans is not yet identified. Geographic studies indicate that all persons, regardless of race or environment, develop fatty streaks in the aorta and to some degree in other large arteries.\(^5\) The agent that initiates the fatty streak may be different from the agent that converts the fatty streak into the potentially dangerous lesion, the fibrous plaque.

Several risk factors related to the atherosclerotic
lesion are evident: (1) Age has the strongest and most consistent association with lesions of all known factors. The average involvement with raised atherosclerotic lesions increases with age. (2) Coronary atherosclerosis is generally considered to be more extensive in men than women. (3) In addition to the difference in sex distribution of coronary atherosclerosis within white and black groups, there is less atherosclerosis in black populations than whites. (4) There is a positive relationship between serum cholesterol and narrowing of coronary arteries. (5) On the average, persons with hypertension or diabetes mellitus have consistently more coronary and aortic atherosclerosis than persons without hypertension or diabetes for all sex, age, race, or geographic location groups. (6) There is no consistent relationships between occupation, physical activity, and coronary atherosclerosis. (7) Severity of atherosclerosis in the coronary arteries and aorta is not associated strongly with obesity, body weight, or body height at death. However, obesity is thought to exert its effect through concomitant hypertension, diabetes, or hyperlipemia. (8) There is little evidence that water hardness or mineral content influence atherosclerosis. (9) Studies have shown consistent trends of more coronary and aortic atherosclerosis in heavy smokers when compared to non-smokers of similar age, sex, and race. (10) Evidence has implicated elevated plasma triglycerides or pre-beta-
lipoproteins as an additional risk factor for clinical atherosclerosis.\textsuperscript{14-16} Recently, it was suggested that pathogenesis of atherosclerosis can be traced to the action of xanthine oxidase, found in homogenized but unboiled cow's milk, on plasmalogens which are important components of the arterial wall. It is at the site of such tissue destruction that cholesterol deposition begins.\textsuperscript{17}

Atherosclerosis is a complex dynamic process that at any given moment represents the interaction between (a) components of the blood (constituents and those in transit) (b) hemodynamic forces, and (c) the structure and function of the arterial wall. What presents itself to us as atherosclerosis is the ultimate summation and outcome resulting from interplay of forces and elements of these three groups.

The atherosclerotic plaque in the tunica intima is characterized by lipid accumulation and a variable connective tissue reaction. Some lesions are largely fatty, whereas others are predominantly fibrous (sclerotic) in nature. It is generally accepted that the early lesions, known as fatty streaks, are more cellular and much of their lipid lies within these cells. However, the progress of the streak to the plaque remains unproven and the fate of this lesion has still not been unequivocally established.

Numerous pathogenetic theories have emerged which attempt to explain the mechanisms and features that may be involved in the development of atherosclerosis. Duguid\textsuperscript{18-19}
has suggested that encrustation of fine platelet deposits and films of fibrin on the endothelium provokes an inflammatory reaction that results in organization with fibrous tissue. Astrup,\textsuperscript{20} however, modified this view by supposing that local disorders in fibrinolysis may tip the balance towards encrustation and that vasoactive compounds liberated from the disintegrating platelets locally increase the permeability of the endothelium.\textsuperscript{21-22}

Hemodynamic aspects of atherogenesis also are important. Texon, et al.\textsuperscript{23} have hypothesized that atheromatous plaques appear in regions of low pressure because a suction action exerted on the surface endothelium eventually causes the layer to be selectively separated from adjacent tissue. This tearing action is thought to cause damage to the endothelium and the adjacent wall layers, with subsequent thickening of the intima and eventual plaque development.

The influence of wall shear stress levels on the integrity of the endothelium has been examined by Fry\textsuperscript{24} and others.\textsuperscript{25-26} It was suggested by Fry that deformation, swelling, and eventual erosion of the endothelium may occur at sites where the local wall shear stress is relatively high. In a subsequent study Fry\textsuperscript{27} showed that exposure of the endothelial surface to shear stresses could result in an increase in albumin flux into the intimal layer. This behavior corresponds to an apparent increase in wall permeability and implies that an increased flux of lipoproteins
can occur. Caro, et al.,\textsuperscript{28-29} however, found that early lesions tended to develop predominantly in regions of low wall shear. Possible reasons for the differing results have been discussed by Caro, et al.,\textsuperscript{29} who hypothesized that a series of events lead to a shear-dependent diffusional efflux of cholesterol from the intima to the blood stream rather than an influx of cholesterol directly from the blood to the wall as suggested by others.\textsuperscript{30-31}

Cholesterol accumulates in the internal elastic membrane during the development of atherosclerosis.\textsuperscript{32} It is interesting to note that rubber absorbs more dye when stretched\textsuperscript{33} and, correspondingly, arterial elastic tissue absorbs more liquid in its stressed stretched state.\textsuperscript{34} In addition, autoradiographic studies using radioactively labelled thymidine have shown that endothelial cells proliferate more rapidly around the orifices of branch vessels,\textsuperscript{35-36} and the dye exclusion test has indicated that endothelial permeability is increased at these sites in the normal aorta.\textsuperscript{37}

Atherosclerotic lesions are correlated directly with anticipated regions of local turbulence.\textsuperscript{38-39} Mitchell and Schwartz\textsuperscript{38} suggested that turbulent velocity fluctuates at branching sites and that this causes platelets and other particulate matter in the bloodstream to coalesce and eventually form mural thrombi or elevated plaques on the intima. They also assumed that agglomerates, which are formed in the
bloodstream, will eventually migrate to the intima and form a thrombus or elevated plaque. Wesolowski, et al.\textsuperscript{39} suggested that atherosclerotic lesions can develop in regions of turbulence as a result of (1) induced vibration of the arterial wall which may lead to eventual injury to the intima followed by a reparative process during which lesions can develop or (2) local increases in lateral (static) wall pressure which may injure the intima or lead to local lipid accumulation within the intima, suppression of lipid secretion from within the arterial wall, or both.

Fox and Hugh\textsuperscript{30} have proposed models which assume that local flow separation \textit{per se} has a causal effect on plaque development. The model proposed by Keller\textsuperscript{31} is similar to the one proposed by Fox and Hugh\textsuperscript{30} in that flow separation is regarded as the primary hemodynamic factor associated with lesion development. In this case, however, it is suggested that lesions develop because of mass transfer between constituents in the bloodstream and the arterial wall, with the intima acting as the selective filter. More specifically, the bloodstream is considered to contain particles of one or more species that are transported to the wall, but are unable to permeate the wall because of their relatively large size. Therefore, large concentrations of these particles may form in quiescent zones of separated flow where chemical reactions that give rise to the formation of atheromatous plaques can occur. This unidirectional mass
transfer concept differs from the one proposed by Caro, et al.\textsuperscript{29} which admits the possibility of both diffusional entry and efflux of mass from the intima.

Spain and Aristizabal\textsuperscript{40} observed that lipids implanted under the skin induce the formation of granulomas. Later studies showed that cholesterol and certain free fatty acids (FFA) are the most potent lipids for inducing sclerosis, while triglycerides and phospholipids are relatively harmless.\textsuperscript{41} Many investigations have shown that the predominant lipid in atheromatous accumulations is cholesterol, particularly in its esterified form. The proportion of cholesterol in ester form increases with the development of atherosclerosis.\textsuperscript{42} Triglycerides and phospholipids which are actively synthesized and metabolized by the arterial wall, however, seem to be only minor components of the ageing and atherosclerotic artery.\textsuperscript{26,43-44} The relative absence of triglycerides in atherosclerotic lesions probably reflects the activity of the arterial wall lipase.\textsuperscript{45}

Cholesteryl ester of atherosclerotic aortas is derived in part from plasma and in part by \textit{in situ} synthesis.\textsuperscript{46-47} Two pathways for cholesterol esterification in atherosclerotic arterial tissues have been reported:\textsuperscript{48-50} Hashimoto, et al.\textsuperscript{51} along with others\textsuperscript{48,52} have shown that acyl-CoA-cholesterol acyltransferase is the dominant, if not the only, enzyme responsible for cholesterol esterification and accumulation in the atheroma. This enzyme was shown\textsuperscript{51} to
be localized for the most part in the microsomes, although some of the activity was found in the mitochondrial frac­
tions of the atherosclerotic tissue. Recent studies\textsuperscript{53} have shown that esterification of cholesterol by atherosclerotic microsomes was much more active than that by normal micro­somes. This increased atherosclerotic microsomal activity relative to normal microsomal activity could arise from either higher concentration of the enzyme in or on the microsomes, or greater accessibility of the substrates to enzyme due to altered physical state of the microsomes. The cholesterol-esterifying system of atherosclerotic microsomes demonstrated preference toward some of the acyl-CoA esters tested. Incorporation into cholesteryl ester was greatest with oleyl-CoA followed by palmityl-CoA and least with linoleyl-CoA. The other pathway involved transesterifica­tion of cholesterol with the $\beta$-fatty acid of lecithin.\textsuperscript{48} However, Smith, et al.\textsuperscript{49} indicated that only a small amount of ester is formed locally, while the majority of the esters are derived by filtration from the plasma. Analysis of the atheroma lipid in plaques showed that approximately 80%  of the cholesterol ester was derived directly from plasma lipoprotein and only 20% from the disintegrating cells.\textsuperscript{54} This agrees with the findings of Dayton and Hashimoto.\textsuperscript{55} Having accepted the thesis that the major part of aortic cholesterol is derived from the circulation,\textsuperscript{50} one has to consider the routes of transport of this molecule.
Like most of the other serum lipids, cholesterol, both in its free and esterified form, is present in the circulation as part of lipoprotein particles. These particles have been subdivided into four major classes on their flotation characteristics: chylomicrons, very low-density lipoproteins (pre-beta-lipoproteins, VLDL), low density lipoproteins (beta-lipoproteins, LDL) and high density lipoproteins (alpha-lipoproteins, HDL). Since free or complexed LDL or apoprotein has been demonstrated in atheromatous plaques and fatty streaks, LDL and HDL particles are considered as the major carriers of serum cholesterol.

The mechanism by which the VLDL act as atherogenic agents is not clear, since such lipoproteins are too large (250-750 Å) to penetrate into the arterial intima. However, Zilversmit has proposed a mechanism of atherogenesis that attributes key roles not only to LDL, but also to VLDL and to chylomicrons. The proposal is partly based on the demonstration that, as a result of lipolysis in vitro or in vivo, VLDL are converted to LDL, and chylomicrons are degraded to relatively small cholesterol-rich remnants. It is likely that, under physiological conditions, VLDL and chylomicrons are degraded while they are absorbed to, or engulfed by, vascular endothelium. It has been reported that lipoprotein lipase, which catalyzes these degradations, is localized in capillary endothelium, adipose cells, myocardium, and skeletal muscle cells.
The amount of lipoprotein in normal intima is very highly correlated with the serum cholesterol concentration \( r = 0.965, p < 0.001 \). In addition, the amount of lipoprotein in the intima is highly dependent on the plasma lipoprotein level and blood pressure of the patient. It has been indicated that "plasma" must enter the intima as a unit, presumably by pinocytosis or by a leakage through the endothelial cell junctions. Once the unit of plasma has entered, the intima albumin might diffuse out more rapidly because of its lower molecular weight. Alternatively, lipoprotein might be specifically retarded by some form of reversible binding. However, it is not still clear whether transport of lipid into the arterial wall by lipophages (circulating lipid-laden monocytes), which is a feature of atheroma induced by high cholesterol diets in rabbits, plays any significant role in human atherogenesis.

Another important cause of cholesterol deposition in the arterial wall involves the fact that this sterol cannot be significantly metabolized therein, apart from esterification or possible slight degradation. The liver can excrete cholesterol and degrade it to water-soluble bile acids, but other tissues - apart from some endocrine glands - lack this capacity to deal with this damaging sterol. Cholesterol metabolism in the atheromatous intima is extremely sluggish. After intravenous injection of tritium-labelled cholesterol to rabbits already fed a
cholesterol-enriched diet for three months, the liver rapidly equilibrated with plasma. By contrast, the atheromatous aortic intima equilibrated slowly. Moreover, Adams and Morgan have shown that cholesterol deposits do not readily exchange with plasma cholesterol. Thus, it takes rather prolonged periods of time to remove sterols from the diseased arterial wall.

Through autoradiographic studies Adams and Morgan have proposed that there are at least two cholesterol pools in the normal artery and that a third arises at the moment atherosclerosis develops. Exchange and net transfer would be expected to be rapid between Pools 1 and 2, but cholesterol in Pool 3 would not appear to be in the correct physical state for exchange. Moreover, being extracellular it would be inaccessible to intracellular metabolic processes.
Pool 3 could receive increments of deposited cholesterol, but would lack the lipoprotein vehicle to enable it to discharge cholesterol back to plasma. When cholesterol becomes dissociated from lipoprotein in the normal intima and lesion, it would not be expected to readily exchange with cholesterol in plasma lipoproteins.\textsuperscript{73}

Whether the juvenile fatty streak is the precursor of large plaques, is a major controversy in atherosclerosis research. This may be true in experimental animals subjected to intermittent cholesterol feeding, but in man there is probably more evidence against this idea than in its favor. Topographically, Schwartz and Mitchell\textsuperscript{74} have shown that locations of the earliest fibrous plaques do not coincide with the locations of early fatty streaks. Epidemiologically, Strong, \textit{et al.}\textsuperscript{75} have shown that there is present much more extensive fatty streaking in young blacks than in young whites, although in older age groups there is minimal involvement with fibrous plaques in the blacks and very extensive involvement in the whites. Chemically, the cholesterol esters in fat-filled cells are grossly different from the cholesterol esters in the pool of amorphous atheroma lipid underlying fibrous plaques.\textsuperscript{54} Smith and Slater\textsuperscript{54} have indicated that the atheroma cholesterol ester is not derived from fat-filled cells. This agrees with the experimental findings of Day, \textit{et al.}\textsuperscript{76} who showed that there was no difference in the rate of removal of different cholesterol
Another theory of atherogenesis involves the lysosomes. Morphological studies have shown that increased numbers of lysosome-like structures are present both in human\textsuperscript{77} and in experimentally induced atherosclerosis.\textsuperscript{78} The increased levels of lysosomal enzymes in isolated aortic cells may therefore be associated with endocytosis by the smooth muscle cell of the large amounts of lipid within the arterial wall.\textsuperscript{79} A large part of the cholesterol in the atheromatous cells was intralysosomal, in contrast to cells isolated from control animals where most of the cholesterol appears to be associated with the plasma membrane. It is of interest that arteries, such as the internal mammary artery, that are relatively resistant to atherosclerosis, have significantly higher levels of acid hydrolase activity than those that are particularly prone to the disease, such as the coronary artery.\textsuperscript{80} Similarly, animals resistant to experimentally induced atherosclerosis have higher levels of these enzymes than do animals, such as the rabbit, which are particularly susceptible.\textsuperscript{81}

Peter, \textit{et al.}\textsuperscript{79} have observed that low-density lysosomes appear to be relatively deficient in cholesterol esterase. Werb and Cohn\textsuperscript{82} have shown that mouse peritoneal macrophages can accumulate large amounts of cholesterol ester as albumin complexes. However, before the macrophages could excrete the cholesterol, hydrolysis of the esters by a lysosomal
cholesterol esterase was necessary. A similar situation may apply to the smooth muscle cell of the aortic wall. In this case there is a congenital deficiency of lysosomal lipases. However, the precise role of the lysosome in the atherosclerotic process remains to be clarified.

Each of the pathogenetic theories proposed does not explain all features of atherosclerosis. Further, we do not think that these theories are exclusive of one another, but rather that they may be complementary to each other and each may have its place in the metabolic, morphological, and clinical aspects in the pathogenesis of atherosclerosis.
LITERATURE REVIEW AND OBJECTIVES

Subsequent to the initial demonstration by Anitschkow and Chalatow\(^a\) who showed that cholesterol administration can induce atherosclerosis in experimental animals and the more recent elucidation of hyperlipoproteinemia as a primary risk factor in atherosclerotic heart disease,\(^b\) the search for an effective and safe hypolipidemic agent has engaged the interests of medicinal chemists, biochemists, pharmacologists, and clinicians. The primary focus of attention has been on hypocholesterolemic drugs. However, before we can discuss the modes of action of these agents it is important to first consider the main features involved in the biosynthesis of cholesterol.

The Nobel Award efforts of Lynen\(^4\) and Bloch,\(^5\) as well as the work of many others,\(^6\) have resulted in the elucidation of the precise biochemical steps acquired for the formation of cholesterol. Approximately, 26 steps are known to be required to complete the conversion of acetyl-CoA (Ac-CoA) to cholesterol.\(^7\)-\(^8\) (Scheme I)

Scheme I, which has been discussed in detail in several textbooks\(^3\) is self explanatory. Because of their particular relevance to this thesis we emphasize the following points.
SCHEME I

\[
\begin{align*}
\text{CH}_3\text{SCoA} & \rightarrow \text{CH}_3\text{CH}_2\text{SCoA} & \text{CH}_3\text{SCoA} \\
\text{HO-C-C-H}_2\text{SCoA} & \rightarrow \text{HO-C-C-C}_2\text{OH} & 2 \text{ ATP} \\
\text{HO}_2\text{CCH}_2\text{H}_3 & \rightarrow \text{HO}_2\text{CCH}_2\text{H}_3 & \text{MVA} \\
\text{CH}_2\text{OPP-O-P-OH} & \rightarrow \text{CH}_2\text{OPP} & \text{IPP} \\
\text{CH}_2\text{C}_2\text{O}_2\text{OH} & \rightarrow \text{CH}_2\text{C}_2\text{OH} & \text{IPP} \\
\text{CH}_3\text{C}_2\text{O}_2\text{CH}_3 & \rightarrow \text{CH}_3\text{C}_2\text{O}_2\text{CH}_3 & \text{GPP} \\
\text{CH}_2\text{OPP} & \rightarrow \text{CH}_2\text{OPP} & \text{IPP} \\
\text{CH}_3\text{C}_2\text{C}_3\text{C}_2\text{N} & \rightarrow \text{CH}_3\text{C}_2\text{C}_3\text{C}_2\text{N} & \text{GPP}
\end{align*}
\]
The initial biosynthetic steps are reversible. Reduction of \( \beta \)-hydroxy-\( \beta \)-methylglutaryl-CoA (HMG-CoA) to mevalonic acid (MVA), which is the rate-limiting step,\(^9\) represents the first irreversible step in the cholesterol biosynthetic pathway. The physiological regulation of hepatic cholesterogenesis appears to be the result of a feedback inhibition of this step\(^9\) by cholesterol or a lipoprotein containing cholesterol by decreasing the amount of HMG-CoA reductase.\(^91-92\) However, in addition, White and Rudney\(^93\) have demonstrated a regulatory role for the reaction of Ac-CoA and acetoacetyl-CoA to form HMG-CoA catalyzed by HMG-CoA condensing enzyme. They indicated partial inhibition (50\%) of HMG-CoA condensing enzyme in cholesterol fed rats. Several pathways have been proposed between lanosterol and cholesterol, but the main pathway was concluded to be the one represented in Scheme I.

It was observed that different enzymatic steps are separately packaged within the cell.\(^94\) Some reactions seem to have representation in two cellular compartments.\(^95\) The condensation of acetyl-CoA with itself is carried out initially in the cytoplasm, but HMG-CoA reductase is found nearly exclusively in the microsomes.\(^94\) Most cellular cholesterol is associated with the microsomes.\(^96\) However, ketone body formation, which is actually a larger dispersal route of acetyl-CoA than cholesterol,\(^97\) is carried out in the mitochondria. Microsomal HMG-CoA reductase occupies the
central role in the control of the cholesterol formation.

Whereas all body tissues, with the exception of adult brain, have been shown to be capable of biosynthesizing cholesterol, two organs, namely the liver and small intestine, have been identified as the prime loci of the process. In addition to its central role in cholesterol biosynthesis (82% in the monkey), the liver also dominates cholesterol metabolism because it serves as the site for most cholesterol catabolism, is involved in the enterohepatic circulation of bile, and therefore in cholesterol absorption, and serves as a major endogenous source of plasma cholesterol. Recently Sodhi and Kudchodkar have suggested that increased cholesterol synthesis in hypertriglyceridemic subjects is due to increased synthetic activity of the liver, rather than an increase in the synthesis of cholesterol in the adipose tissue as was suggested by others. Gould, et al. and Frantz, et al. indicated that cholesterol synthesis by the liver seemed to vary inversely with the amount of cholesterol present in the liver. Thus, it appeared that cholesterol might be acting as a true end product "feedback" inhibitor of its own formation. However, cholesterogenesis which appears physiologically to represent an example of end product feedback inhibition, does not exhibit the biochemical characteristics of this process.

The specific biosynthetic step to be inhibited is important to consider in drug design; in the past far too
little emphasis was given to the potential consequences resulting from interference with this process. Inhibition prior to acetoacetyl-CoA may not be desirable because acetoacetyl-CoA is necessary for the tricarboxylic acid cycle (TCA), serves as precursor for fatty acid (FA) biosynthesis, and is intermediate to the production of HMG-CoA, which is the precursor of ketone bodies. Antimetabolites of acetoacetyl-CoA may inhibit FA production as well as HMG-CoA synthesis; thus MVA is the first intermediate which is selective for the biosynthesis of sterols and other isoprenoid units. It follows, therefore, that inhibition of HMG-CoA reductase, the enzyme that catalyzes the conversion of HMG-CoA to MVA, is the earliest rational place to control cholesterol biosynthesis. Blocking cholesterol biogenesis at this step would not result in a build-up of metabolites incapable of disposal by other catabolic pathways. Blocking enzymes after MVA formation requires essentially total enzymatic inhibition in order to effect cholesterol biosynthesis significantly. If the enzymes are blocked after squalene formation, steroid precursors to cholesterol may accumulate in atherosclerotic plaques, because the reactions following HMG-CoA production are irreversible. Such accumulation would result in serious toxic effects.

The major model for the study of the inhibition of cholesterol synthesis has been the fasting animal. Regen,
et al.\textsuperscript{113} have demonstrated a prompt decrease in the activity of microsomal reductase enzymes attributable to decreased enzyme synthesis in the fasted animal. Conversely, following reinstitution of feeding, cholesterogenesis takes a much longer time to return to normal levels.\textsuperscript{114} In addition, it has been shown that in the rat and mouse there is a large diurnal fluctuation (oscillation) in the rate of cholesterol synthesis. The range of minimum—maximum is four-to-five fold. The maximum occurs around midnight whereas the minimum is around 10 a.m.; most studies are carried out near 10 a.m. and consequently most of our knowledge of this process is at this time. It has been shown that changes in the diurnal cycle of cholesterol formation are mediated by alteration in the rate of synthesis of HMG-CoA reductase and not by changes in its activity.\textsuperscript{91—92} Diurnal rhythmicity has likewise been observed for hepatic fatty acid synthesis. However, fatty acid synthesis varies inversely with cholesterol synthesis, that is, it is highest in the morning and lowest in the evening.\textsuperscript{115—116}

Other situations in which hepatic cholesterogenesis is elevated seem to share the common feature of increased fat flow to the liver. Friedeman and Byers\textsuperscript{117} first reported that the detergent Triton-WR-1339 resulted in hyperlipidemia accompanied by increased liver cholesterol content, which was attributed by White and Rudney\textsuperscript{93} to the increased activity of HMG-CoA reductase. Thus, cholesterol formation is a
highly dynamic activity subject to control by many factors.

In the past, considerable emphasis was placed on the relationship of elevated serum cholesterol levels to atherosclerosis. Recent research, however, has taken into consideration serum lipoproteins; it is now established that their role in lipid transport and hence their involvement in the pathogenesis of atherosclerosis is of major importance. Frederickson and coworkers first classified hyperlipoproteinemia using electrophoresis into distinct phenotypes. Since each phenotype is not necessarily responsive to the same therapy, this method of classification has been extremely useful for the diagnosis and treatment of the disease state as well as beneficial to the search for new drugs which will lower selectively certain plasma lipid parameters.

Increased sophisticated biochemical methods and the availability of isotopic and enzymatic techniques have turned the direction of research towards the search for compounds which could affect a specific step in lipid synthesis, degradation, excretion, or transport. Several approaches have been used through which serum lipids may be lowered. These include (a) decreased ingestion of cholesterol, (b) decreased synthesis of cholesterol, (c) decreased absorption of cholesterol, (d) increased cholesterol catabolism, (e) inhibition of bile acid reabsorption, (f) inhibition of free fatty acid (FFA) release (lipolysis), (g) interference with lipoprotein synthesis, and (h) interference
with lipoprotein release. However, none of these approaches per se has yet produced a method of treatment that is completely satisfactory.

Controlling the diet alone, in most hyperlipemic patients, is insufficient as a means of maintaining low serum lipid levels. Moreover, many patients have difficulty adhering to dietary measures. The dietary approach is most beneficial when combined with other procedures. Thus, oral non-toxic hypolipemic agents should be very useful when treating coronary artery disease. For these reasons it is particularly desirable to design drugs capable not only of lowering serum sterol levels, but depending on the type of hyperlipidemia involved,\textsuperscript{118-119} effective in the selective lowering of abnormally high plasma lipid fractions.

Investigators have found that a variety of compounds lower serum cholesterol levels\textsuperscript{3} by inhibiting cholesterol biosynthesis\textsuperscript{120} or by influencing cholesterol metabolism in some other way.\textsuperscript{121} For example, various derivatives of mevalonate and biphenylvaleric acid inhibit the incorporation of acetate and/or mevalonate into cholesterol. Alkylenediamines, 1-(2-aminoethyl)-4-substituted-piperidines, dialkylaminoalkoxybenzenes, and certain maleamic acid derivatives inhibit the biosynthesis of cholesterol at various stages in the biosynthetic pathway. Nicotinic acid and its derivatives may work by blocking FFA release from adipose tissue,\textsuperscript{122} while estrogens have been proposed to exert their
effect by decreasing the concentrations of cholesterol attached to the $\beta$-lipoprotein fraction. On the other hand, thyroid hormones [thyroxine ($T_4$) and triiodothyronine ($T_3$)] cause increased conversion of cholesterol into bile acids. This increase in catabolism of the sterol overrides the increased cholesterogenesis caused by these hormones. Linoleic acid derivatives inhibit cholesterol absorption, while oral administration of neomycin and related antibiotics reduce human serum cholesterol levels by their action on the intestinal bacterial flora.

In 1962, Thorp and Waring reported that a combination of clofibrate [ethyl $\underline{\underline{\underline{\underline{\underline{\underline{C}}}}}}$-(4-chlorophenoxy)-$\underline{\underline{\underline{\underline{\underline{C}}}}}$-methyl-

![Chemical structure](image)

1 $R = C_2H_5$
2 $R = H$

propionate, CPIB, Atromid-S, 1] and androsterone produced a significant lowering of blood cholesterol and serum triglyceride levels in experimental animals. Subsequent studies showed that clofibrate was effective without added androsterone. It is now generally accepted that it is unnecessary to administer androsterone to produce an effective
lipid response, and thus Atromid-S has superceded the combination known as Atromid. Following these reports, the hypocholesterolemic and hypotriglyceridemic effects of \( \text{l} \) have been the subject of extensive investigation, but the mechanism of action of this drug is still not clear.

The ester \( \text{l} \) is rapidly hydrolyzed by tissue and serum esterases both \textit{in vivo} and \textit{in vitro} to \( \alpha-(4\text{-chlorophenoxy})-\alpha\text{-methylpropionic acid} \) \( \text{(2)} \), which is presumed to be the active drug.\(^{126}\) However, recently Yeh and Kabara\(^{129}\) have shown that \( \text{l} \) and \( \text{2} \) exhibit antagonistic effects in mitochondria \textit{in vitro}. The sodium salt of \( \text{2} \) competitively inhibited mitochondrial succinate oxidation while \( \text{l} \) noncompetitively stimulated mitochondrial respiration of succinate. On the other hand, when \( \alpha\text{-ketoglutarate} \) was used as the substrate, the effects of \( \text{l} \) and \( \text{2} \) were opposite to that of succinate. These observations suggest that \( \text{l} \) and \( \text{2} \) might be acting as independent entities at different sites in eliciting antagonistic effects in mitochondrial respiration. However, these results might not necessarily be applicable to the mode of action of clofibrate \textit{in vivo} since \( \text{l} \) is rapidly hydrolyzed to \( \text{2} \) by esterases.\(^{126}\)

Studies \textit{in vivo} and \textit{in vitro} with \( \text{l} \) or \( \text{2} \) suggest that the acid \( \text{2} \) may exert its effect by multiple modes of action. Many mechanisms of action have been proposed for the hypolipidemic effect of this compound; one possibility involves the Thyroxine (\( T_4 \)) release mechanism.
Before the $T_4$ displacement mechanism can be discussed in detail it is desirable to first consider the effect of $T_4$ on lipid metabolism. Hypothyroidism in humans results in the production of elevated concentrations of plasma cholesterol. This increase in the total plasma cholesterol, observed in myxedema, is due mainly to the amount of cholesterol found in the increased low-density and $\beta$-lipoproteins fractions. There is an absolute increase in the $\beta$-lipoprotein cholesterol as well as a relative increase in the proportion of total plasma cholesterol carried in these low-density lipoproteins. Treatment of such patients with thyroid hormones lowers the plasma cholesterol concentration to normal. It has been shown that thyroidectomized animals produce cholesterol more slowly than hyperthyroid animals; labeled cholesterol disappears at a slower rate from the blood of hypothyroid animals than it does from the blood of hyperthyroid animals. Similar effects are found in humans. Myxedematous patients cannot convert acetate-$2^{-14}C$ to cholesterol as rapidly as euthyroid individuals. Thyroid hormones, when administered to hypothyroid patients, induce a fall in serum cholesterol levels. As pointed out previously, the mechanism by which thyroid hormones lower blood serum cholesterol levels appears to involve both the biosynthesis and degradation of cholesterol. The hypocholesterolemia associated with $T_4$ administration is attributed to an increased conversion of cholesterol into bile.
acids as well as increased cholesterol and bile acid excretion. The increased rate of cholesterol biosynthesis induced by $T_4$ is attributed to an increase in HMG-CoA reductase activity and not to a direct effect on the enzyme level.

Robbins and Rall have shown that interaction between thyroid hormones and serum proteins greatly influences the distribution, degradation, excretion, and the action of $T_4$. There is considerable evidence suggesting that both the hypocholesterolemic effect and the rate of degradation of $T_4$ are functions of the concentration of unbound hormone. Three carrier proteins for $T_4$ have been identified: (a) thyroxine binding prealbumin (TBPA), (b) thyroxine binding globulin (TBG), and (c) serum albumin. Serum electrophoretic studies have shown that approximately 30 per cent of carrier proteins for $T_4$ are associated with TBPA, 60 per cent with TBG, and the remaining 10 per cent with serum albumin. The concentration of free thyroxine in normal human blood is extremely low; more than 99.8 per cent of total blood thyroxine is bound by proteins. Thus, any compound which decreases thyroxine binding to protein, either by changing the affinity constant or by reducing the concentration of unoccupied available binding sites, could increase the concentration of free thyroxine and hence change its rate of metabolism.

Like many other anions, compound 1, after undergoing
hydrolysis in vivo, binds to serum albumin and to serum proteins. Thus, it could theoretically compete with and displace other substances normally transported as protein complexes. Since the metabolic activity of T4 is related to the concentration of the unbound or "free" hormone rather than the total concentration in blood, such competition could increase the effective metabolic activity owing to increased free T4. According to the thyroxine displacement mechanism, 1 may undergo rapid in vivo hydrolysis144 to the acid 2 which exerts its effect, at least in part, by displacing T4 from the binding proteins145 in the plasma and the liver. The resulting hyperthyroid effect in the liver should increase lipid metabolism146 with concomitant lowering of serum lipid levels.147

Support for the displacement hypothesis comes from studies demonstrating that (a) thyroidectomy abolishes the hypolipidemic effect of clofibrate,148 (b) the maximal hypolipidemic effect of clofibrate corresponds to periods of maximal thyroid activity,128 (c) clofibrate produces a marked increase in liver mitochondrial α-glycerophosphate oxidase activity which is also seen after T4 administration,149-150 (d) T4 and clofibrate both decrease liver glycogen content146,148 and (e) clofibrate and physiologic quantities of T4 increase amino acid incorporation into protein by rat liver ribosomes within a few hours.147,151

Evidence against the displacement hypothesis comes from
the following: Chang, et al.\textsuperscript{152} and Musa, et al.\textsuperscript{153} were unable to demonstrate any interference with binding of T\textsubscript{4} to TBG, which is the primary carrier of T\textsubscript{4}. Two studies in man, those of McKerron, et al.\textsuperscript{154} and of Barbosa and Oliver,\textsuperscript{155} fail to reveal any significant effects on free (that is, effective) T\textsubscript{4} levels in man. Truswell and co-workers\textsuperscript{156} showed that in euthyroid individuals clofibrate produced no significant change in the glycine to taurine conjugate ratio of biliary bile acids whereas thyroid hormones (both L and D forms) decreased the glycine to taurine ratio in hypothyroid patients.

In any case, the effects of clofibrate cannot be attributed exclusively to a T\textsubscript{4}-like effect. For example, T\textsubscript{4} increases the rate of cholesterol synthesis, while clofibrate treatment inhibits it. Other drugs which increase T\textsubscript{4} accumulation in rat liver, e.g. novobiocin, aspirin, 2,4-dichlorophenoxacyetic acid, are not hypocholesterolemic.\textsuperscript{157} The efficacy of clofibrate treatment is dependent upon the type of hyperlipoproteinemia.\textsuperscript{118-119} Levy and co-workers\textsuperscript{158} found that type III (broad beta) hyperlipoproteinemia is most responsive to clofibrate treatment. Type III hyperlipoproteinemia involves an increased level of \( \beta \)-lipoproteins (\( S_f 0-20 \)) and pre-\( \beta \)-lipoproteins (\( S_f 20-400 \)).\textsuperscript{159} Clofibrate primarily reduces \( S_f 20-400 \) lipoprotein (LP) and increases \( S_f 0-20 \) LP, whereas T\textsubscript{4} primarily reduces \( S_f 0-20 \) LP.\textsuperscript{159-160} Finally, Strisower\textsuperscript{161} showed that adding T\textsubscript{4} to
clofibrate treatment did not enhance its effect on the $S_f$ 20-400 class. The effects of the two drugs appeared to be additive with respect to the $S_f$ 0-20 class, but not with respect to the $S_f$ 20-400 class.\textsuperscript{162}

Unlike thyroxine, clofibrate did not decrease the activity of hepatic alcohol dehydrogenase (ADH).\textsuperscript{163-164} Thus, the elimination of ethanol was significantly faster in clofibrate-treated rats than in normal controls\textsuperscript{165} or thyroxine-treated rats.\textsuperscript{163}

Westerfeld and his associates have extensively studied the effects of 1 on $T_4$ distribution in the rat.\textsuperscript{149-150,166-167} For example, 1 produced a hyperthyroid effect in the liver, with a concomitant increase in $\alpha\alpha$-glycerophosphate dehydrogenase (GPD) and malic enzyme.\textsuperscript{149,166} However, in subsequent studies\textsuperscript{150,167} the responses of the two enzymes were separated by 5,5-diphenyl-2-thiohydantion (DPTH) and imidazole. DPTH blocked the GPD response more than the malic enzyme response to $T_4$. Imidazole alone increased the quantity of malic enzyme and this effect was additive with the $T_4$ effect. In addition, changes in these enzyme activities did not appear to be involved with the clofibrate effect on the disposition of oral fat. Clofibrate had a greater effect on increased exogenous fat than did $T_4$ at a dosage which produced the same liver GPD and malic enzyme changes. Clofibrate completely prevented or corrected the fatty liver produced by feeding orotic acid.
to intact or thyroidectomized rats, while T\textsubscript{4} had only a small effect. Thus, the influence of 1 on lipid metabolism seems not to be mediated through its enhancement of a T\textsubscript{4} effect in the liver.

To further explore the mechanism of related hypolipemic compounds, Witiak, et al. studied the effects of D and L isomers on the ethyl \textsubscript{(\textgamma})-chlorophenoxy)propionate (3), the desmethyl isomers of clofibrate, as well as, other related conformationally constrained analogs on cholesterol biosynthesis\textsuperscript{168} and lipolysis\textsuperscript{169} and for their ability to displace albumin bound T\textsubscript{4} in vitro.\textsuperscript{172} The L(S)-(-) isomer of 3 and certain cyclic chloro analogs exhibited hypocholesterolemic activity in normocholesterolemic Swiss Webster rats, which compared favorably with the activity of clofibrate administered at similar doses. On the other hand, the D(R)- (+) isomer of 3 did not exhibit activity in this animal model. Further, the racemic mixture of 3 and the deschloro cyclic analogs did not exhibit significant hypocholesterolemic
activity in the Triton WR-1339 hyperlipemic Sprague-Dawley rat model. Studies using equilibrium dialysis techniques and a dye [2-(4'-hydroxybenzeneazo)benzoic acid], which mirrored the binding of \( T_4 \) to serum albumin, indicated that the D isomer of \( 3 \) was bound to a greater extent to rat plasma albumin than the corresponding L isomer, while all cyclic analogs (active and inactive) were bound to a similar extent. Furthermore, the primary binding constant (\( K_1 \)) for \( T_4 \) was much greater (approximately \( 10^9 \)) than the primary binding constant for clofibrate and related analogs. Since all of these compounds are structurally closely related to \( 1 \), Witiak and coworkers proposed that the hypolipemic activity of these agents would involve similar mechanisms. The lack of parallelism between albumin binding parameters and biological activity in vivo do not support the \( T_4 \) release hypothesis and in particular the suggestion that albumin binding studies in vitro could be employed to predict the hypocholesterolemic effect of a compound in vivo. If the \( T_4 \) release mechanism were to be operative, there would have been a positive correlation between the binding capacity of the drugs to albumin and their hypolipemic activity. Thus, while clofibrate seems to have some biological relationship to thyroid hormone action in tissues it seems reasonable, in light of binding data to consider the thyroxine displacement mechanism as an unlikely mode of action for clofibrate and related analogs.
A second theory regarding the mechanism of action of clofibrate which has received considerable attention involves inhibition of cholesterol biosynthesis. Clofibrate, when given to rats at 0.2 percent of the diet significantly decreases the secretion of adrenal steroids. Further, inhibition in vitro of steroidogenesis as assessed in terms of the reduction of adrenal steroids and the hypocholesterolemic effect is dose dependent.\textsuperscript{174} Witiak, et al.\textsuperscript{168,175} also investigated the effects of the D and L desmethyl analogs of 2, namely 3, on cholesterol biosynthesis in rat liver homogenate preparations. A parallelism existed between the hypocholesterolemic effect of these esters in vivo and inhibition of cholesterol biosynthesis of the corresponding acids in vitro. Similarly, the esters of various chloro substituted cyclic analogs of clofibrate returned serum cholesterol levels in hyperlipemic rats back to normal in vivo and the corresponding acids were shown to be effective inhibitors of cholesterol biosynthesis in vitro.\textsuperscript{176-177}

Thorp and Waring\textsuperscript{126} previously reported that 1 inhibits acetate incorporation into cholesterol by rat liver slices in vitro. Most evidence available leads one to conclude that the major site of action of clofibrate is between acetate and mevalonate. However, others have shown that high doses of clofibrate inhibit cholesterol biosynthesis after mevalonate.\textsuperscript{177-179}

Clofibrate appears to act, at least in part, by
inhibiting hepatic cholesterol synthesis at a relatively early stage.\textsuperscript{180} Burch and coworkers\textsuperscript{181-182} have reported that there is an increase in acetoacetyl-CoA deacylase activity in livers isolated from clofibrate treated rats. They suggest that the decreased sterol synthesis is due to a diversion of acetoacetyl-CoA to ketone body production. However, there is no evidence that this deacylase activity can become rate limiting.

Avoy, et al.\textsuperscript{180} showed that acetate-\textsuperscript{14}C incorporation into liver cholesterol in the presence of clofibrate in vivo or in vitro was markedly inhibited whereas MVA-\textsuperscript{14}C incorporation was unaffected. These results strongly suggest a site of action involving HMG-CoA reductase. White\textsuperscript{183} has presented more direct evidence; hepatic microsomal HMG-CoA reductase was shown to be specifically inhibited by clofibrate, but the activity of HMG-CoA condensing enzyme was not influenced. Therefore, in the presence of clofibrate, incorporation of pyruvate-\textsuperscript{14}C, but not MVA-2-\textsuperscript{14}C, into cholesterol was depressed; i.e. clofibrate effected the biosynthetic pathway prior to MVA formation. This proposal was confirmed when it was observed that clofibrate in vivo decreased incorporation of acetate-\textsuperscript{14}C, acetyl-CoA-\textsuperscript{14}C and HMG-CoA-\textsuperscript{14}C into MVA; i.e., the site of inhibition is beyond acetyl-CoA. Increased formation of acetoacetate is evidence that clofibrate inhibits HMG-CoA reductase. It is interesting to note that HMG, when administered to cholesterol fed
rabbits, significantly depressed the levels of cholesterol (total, free, and ester), phospholipids, triglycerides (TG), FFA, and total lipids. However, besides HMG-CoA reductase, other enzyme systems which are indirectly involved in cholesterol biosynthesis and metabolism have recently been shown to be affected by clofibrate treatment.

When administered to rats, clofibrate produces hepatomegaly and proliferation of the smooth endoplasmic reticulum (SER); the SER contains many of the enzymes necessary for the biosynthesis of cholesterol. Increase in liver size produced by is explained by an increase in size of the hepatocytes. Tchen and Bloch have shown that the enzyme which converts squalene into lanosterol requires NADPH and an activated form of molecular oxygen. The activated form of molecular oxygen probably involves cytochrome P-450. Therefore, cholesterol production may be enhanced by substances that accelerate oxidative microsomal drug metabolism and raise the level of cytochrome P-450. Substances that inhibit this process would be expected to decrease cholesterol levels. Cytochrome P-450, a microsomal haemoprotein, is reduced by NADPH-cytochrome c reductase in the presence of NADPH. The reduced form of cytochrome P-450 reacts with molecular O₂ and is also capable of binding to CO. Cytochrome P-450 functions to activate O₂ during steroid hydroxylation in the adrenal cortex and for the hydroxylation of drugs.
in the liver. Wada, et al. found that cholesterol biogenesis from $^{14}$C-acetate, $^{14}$C-mevalonate or $^{14}$C-squalene was inhibited by CO. They suggested that cytochrome P-450 is functional in cholesterol synthesis. However, they also showed that the inhibitory effect of CO on cholesterol synthesis was smaller than the effect of CO on inhibition of drug hydroxylation.

Atkin and his associates suggested that the normal hepatic concentration of cytochrome P-450 is not rate-limiting for cholesterol biosynthesis, while it is for the metabolism of drugs. The difference in interpretation of the results of Atkin, et al. and Wada, et al. was reconciled as follows. Only a small amount of normal cytochrome P-450 was rate-limiting in cholesterol synthesis, or, alternatively, a special form of cytochrome P-450 was required for cholesterol synthesis. Some evidence for the latter view was also found by Omura, et al.; affinity of CO for the drug-metabolizing enzyme system was different from its affinity for the cholesterol-synthesizing system. Furthermore, Atkin, et al. have found that n-propyl gallate and other compounds which act as inhibitors of drug hydroxylation at the 0.1mM concentration, have no effect on the cholesterol biosynthesis \textit{in vitro}. Recent results from our laboratories have demonstrated that clofibrate and related hypocholesterolemic chloro analogs increased cytochrome P-450 activity in rats. It is possible that
clofibrate is affecting more dominantly "cholesterol hydroxylating enzyme, cytochrome P-450" than "cholesterol synthesizing enzyme, cytochrome P-450."

Cholesterol is eliminated from the body in the faeces predominantly as bile acids and neutral steroids. Cholic acid (3,7,12-trihydroxycholanic acid) and chenodeoxycholic acid (3,7-dihydroxycholanic acid) are the major bile acids formed from cholesterol in the liver. A third bile acid found in human bile, namely deoxycholic acid (3,12-dihydroxycholanic acid) is a product of microbial dehydroxylation of cholic acid which occurs during its enterohepatic circulation. Bile acid formation in the liver is regulated by a negative feedback mechanism; interruption of the enterohepatic circulation results in a several-fold increase in bile synthesis. 7α-Hydroxylation of cholesterol is the first and rate-determining step in the conversion of cholesterol to bile acids. The enzyme, cholesterol 7α-hydroxylase, catalyzing this hydroxylation is found in the microsomal fraction. This process depends upon NADPH oxidation catalyzed by an electron transport system. A specific cytochrome P-450, possibly "cholesterol-hydroxylating enzyme, cytochrome P-450" independent from "drug hydroxylating enzyme, cytochrome P-450," has been proposed by Atkin, et al. to be involved in this electron transport system since it is not inducible by phenobarbital.

Several investigators have demonstrated that
bile acid formation is abnormally high in patients with hypertriglyceridemia. Kottke suggested that there exists a fundamental difference in cholesterol metabolism in patients with primary hypercholesteremia and patients with combined hypercholesteremia and hypertriglyceridemia. Einarsson showed that in hypertriglyceridemic patients the elevated bile acid formation was related to a stimulation of the cholic acid turnover. On the other hand, Miettinen and Mitchell, et al. found a subnormal faecal excretion of bile acids in hypercholesterolemic patients. In a subsequent study they interpreted the decrease in bile acid excretion in terms of reduced hepatic synthesis as well as reduced cholesterol catabolism.

Kritchevsky and Tepper have found that isolated mitochondria prepared from livers of rats fed clofibrate for 21 days oxidized cholesterol-26-14C more rapidly on an absolute basis than did normal mitochondria. This effect was related to the increase in liver protein content; rates of oxidation in normal and clofibrate treated rats were the same when considered in terms of nitrogen content. However, Einarsson, et al. have found no indication that clofibrate treatment results in an increased 70C-hydroxylation of cholesterol in rat liver. Thus, there is no conclusive evidence to accept the proposition that clofibrate lowers plasma cholesterol levels by stimulation of cholesterol metabolism and subsequently increased excretion of the bile
acids.

Another possible hypothesis, relating to the mode of action of clofibrate, involves the effects of this drug on the increased output of endogenous neutral steroids.\textsuperscript{212-215} Two groups of investigators have suggested that the existing sensitive and dynamic equilibrium between plasma cholesterol concentration and tissue pool size is distorted upon administration of clofibrate. The increased output of neutral steroids, which results upon clofibrate administration, was explained by various investigators\textsuperscript{214-216} in terms of an efflux of cholesterol from the tissue pool rather than the plasma (or plasma plus liver). Sodhi, \textit{et al.}\textsuperscript{215} suggested that mobilization of cholesterol from tissues likely was caused by a decrease in the plasma cholesterol concentration rather than by a direct effect of this hypocholesterolemic agent on tissues. These investigators also suggested that adipose tissue may not only be important for storage of tissue cholesterol, but may also be important for rapid mobilization of cholesterol.

While the hypocholesterolemic action of clofibrate is still not well understood, even less is known about the mechanism of action of this drug in lowering plasma triglyceride levels. The effect of clofibrate on plasma triglycerides is generally greater than its effect on cholesterol levels, but the effect is much more variable. Several mechanisms have been proposed to explain the hypotriglyceridemic
effect. Gould and his associates\textsuperscript{170,217-218} found that clofibrate increased the hepatic synthesis of triglycerides in the rat, but decreased their secretion into plasma. Alternatively, the results of Duncan, \textit{et al.},\textsuperscript{219} coupled with those of Spritz and Lieber,\textsuperscript{220} suggested that the long-term administration of clofibrate to rats depressed hepatic triglyceride synthesis. These observations indicated that a component of the mechanism of action of clofibrate may involve either an inhibition of the secretion of lipoproteins into plasma or an inhibition of the formation of lipoproteins in the liver. Other workers have also shown a decreased rate of release of triglycerides from the isolated perfused liver.\textsuperscript{177,221}

The critical importance of the carboxylation of acetyl-CoA to malonyl-CoA catalyzed by acetyl-CoA carboxylase (ACC) in fatty acid biosynthesis has long been recognized. Evidence\textsuperscript{220-223} suggests that this reaction is the rate-limiting step in the overall conversion of acetyl-CoA to fatty acids by the liver. Maragoudakis and his coworkers\textsuperscript{224-229} showed that reduction of total body lipids in clofibrate treated animals was associated with inhibition of ACC determined \textit{in vitro} after prefeeding. This reduction of total lipids was associated with a reversible binding of the drug to the enzyme protein. At a concentration of 1.25mM hydrolysis product 2 produced a 50% inhibition of labeled bicarbonate incorporation into malonyl-CoA by ACC.\textsuperscript{224} This
inhibition appears to be specific for ACC since no inhibition of a number of other enzymes (including fatty acid synthetase) could be demonstrated. The inhibition produced by 2 is competitive for acetyl-CoA and isocitrate and non-competitive for ATP and bicarbonate. Fatty acid synthetase activity was actually stimulated, but this effect should have little physiological significance since malonyl-CoA formation is the rate limiting step in the overall process. Zakim and coworkers studied the effect of clofibrate on human intestinal enzyme activities. They showed that the decrease in ACC activity is not the result of enzyme inhibition. Thus, further studies related to the inhibitory effect on ACC by clofibrate are needed before definite conclusions can be reached.

Several reports in the literature suggest that a component of the mechanism of action of clofibrate may involve an effect on carbohydrate metabolism. Maragoudakis, along with others, have indicated a decrease in the activity of lipogenic and glycolytic enzymes. These enzymes are involved in the conversion of carbohydrates to fatty acids. Depletion of cell glycogen during clofibrate therapy has been demonstrated in rat liver and in Tetrahymena pyriformis. This protozoan has many similarities to liver cells including a high capacity for gluconeogenesis. Presumably, the decrease in activity of glycolytic enzymes in the liver leads to a decrease in fatty acid synthesis and
thereby to a lower triglyceride level.

Clofibrate has also been shown to play an important role in the hepatic redox system. Ethanol oxidation in normal rats involves reduction of liver NAD to NADH. This change in NAD/NADH ratio can be observed as an increase in hepatic lactate/pyruvate and \( \alpha \)-glycerophosphate/dihydroxyacetone phosphate (\( \alpha \)-GP/DAP) ratios which chiefly indicate the redox state of the cytoplasmic compartment of the liver cell. In clofibrate treated rats, ethanol did not cause a significant rise in \( \alpha \)-GP, triglyceride and serum FFA concentrations; i.e. clofibrate treatment can inhibit the ethanol-induced change in the hepatic redox state in vivo as it can in the isolated liver. This further supports the hypothesis that clofibrate inhibits the production of acute ethanolic fatty liver, at least in part, by abolishing the ethanol-induced change in the hepatic redox state. In addition, Westerfeld, et al. and Periera and Holland have observed increased mitochondrial \( \alpha \)-glycerophosphate dehydrogenase (MGPD) activity in rats administered clofibrate. Thus, clofibrate may act by increasing the activity of "pacemaker" enzymes required for metabolism, and subsequently reduce the availability of \( \alpha \)-GP for triglyceride synthesis. The reduced rate of triglyceride synthesis limits the formation and secretion of plasma lipoproteins, which are the main lipid transport forms. Consequently, plasma cholesterol concentrations are also reduced. Thus,
it has been proposed that clofibrate produces its hypo-
lipemic action by a mechanism involving the induction of
synthesis of mitochondrial proteins, one of which is MGPD.

Another hypothesis put forward to explain the hypoli-
pidemic action of clofibrate involves its effect on de-
creased mobilization of FFA from adipose tissue triglycer-
ides.\textsuperscript{237-238} Such an inhibitory effect by clofibrate would
decrease hepatic uptake of FFA and this would be followed by
a reduction in the formation of plasma triglycerides in the
form of very low density lipoproteins. Therefore, clo-
fibrate, which has been shown to lower FFA levels \textit{in
vivo},\textsuperscript{238-239} has been proposed to work by reducing FFA out-
put from adipose tissue.\textsuperscript{237} Such inhibition has been ob-
served \textit{in vitro}.\textsuperscript{237} Conversely, other workers have reported
that clofibrate does not decrease serum FFA concentra-
tions.\textsuperscript{240} Furthermore, Hunninghake\textsuperscript{241} reported that clo-
fibrate inhibited adrenaline induced, but not the noradrena-
line or isoprenoline induced increase of plasma FFA levels.
Interestingly, clofibrate has been demonstrated to decrease
the rate of triglyceride lipolysis by lowering the intracel-
lular level of cyclic AMP.\textsuperscript{242} Cyclic AMP mediates the break-
down of triglycerides to FFA.\textsuperscript{243} Greene, \textit{et al.}\textsuperscript{244} have in-
dicated that the lipid-lowering action of clofibrate is
mediated through inhibition of adenyl cyclase. According
to Greene,\textsuperscript{244} it is not clear whether clofibrate has a spe-
cific action on adenyl cyclase, or whether it has a toxic
effect on the cell thereby resulting in decreased quantities of adenyl cyclase. In other words, the decreased cyclic AMP levels and decreased glycerol release might be a reflection of cell death.

Carlson, et al. speculate that clofibrate lowers cyclic AMP in adipose tissue, thus reducing triglyceride lipolysis. This in turn leads to decreased FFA levels, lowered uptake of FFA by the liver, decreased synthesis and secretion of VLDL by the liver, and decreased plasma triglyceride levels. However, other factors also should be considered. For example, Wing and Robinson have proposed that cyclic AMP levels and lipoprotein lipase activity (LPLA) in adipose tissue are reciprocally related. Thus, decreased cyclic AMP levels might promote increased LPLA concurrent with a decreasing activity of the hormone-sensitive triglyceride lipase. This hypothesis was corroborated by the work of Tolman, et al. and Greene, et al. Hence, it seems that there are at least two regulatory steps at which an effect of clofibrate on cyclic AMP levels in adipose tissue might contribute to lowered plasma triglycerides; i.e. decreased fat mobilizing lipolysis on the one hand and increased plasma triglyceride removal on the other hand.

The mechanisms of transport of cholesterol and triglycerides are complex and interrelated; both lipids are constituents of the lipoproteins. It might be assumed that
effecting triglyceride concentration in lipoproteins could indirectly effect cholesterol levels and vice versa. Thus, the enzyme lipoprotein lipase (LPL), which is a major enzyme involved in the transport of triglycerides from the blood to the tissues may also effect cholesterol metabolism by an indirect process. A negative correlation has been observed between serum triglyceride levels and adipose tissue LPLA in humans. It has been shown that the LPLA in adipose tissue is proportional to the rate of uptake of triglycerides; this enzyme has a significant role in controlling fat deposition in this tissue. Cenedella confirmed the suggestion that clofibrate increases adipose tissue LPLA which in turn lowers plasma triglyceride levels, in part, by increasing their clearance from plasma by peripheral tissues. This explains the observation of Sodhi, et al. who noticed an increase in plasma triglyceride fractional turnover rate.

Clearance of lipoproteins from plasma appears to involve a step-wise conversion of larger to smaller triglyceride-rich lipoproteins with the larger lipoprotein particles being cleared more rapidly than the smaller ones. On the other hand, Nestel and Austin reported increased rates of uptake in vivo of palmitate-labelled chylomicrons by epididymal fat pads from clofibrate-fed rats. This was not accompanied by a measurable increase in LPLA. They suggested clofibrate to have a primary effect on fatty acid
reesterification. Grafnetter and Geizerova showed that rat heart LPLA was markedly inhibited by clofibrate in vivo, as was the fat tissue activity in vitro. In agreement with this result, Whayne and Witiak have recently reported a dose-dependent inhibition of human serum LPL, guinea pig serum LPL, and rat heart LPL in vitro at concentrations which start four times greater than the therapeutic concentration (approximately 1.0 umol/ml) of clofibrate in vivo. If LPL is a link in the atherogenic process, it might be beneficial if LPLA in the serum and especially in the arterial wall were reduced. It has been shown that as a result of lipolysis in vitro or in vivo, pre-beta-lipoproteins are converted to beta-lipoproteins and that chylomicrons are degraded to relatively small cholesterol-rich remnants. Theoretically, this could be of significance to the prevention of the disease, since these fractions have been implicated as risk factors in atherosclerosis. Thus, the case for this mechanism is still preliminary and the data are conflicting.

Eaton has recently suggested that some of the factors regulating both hepatic triglyceride and lipoprotein synthesis may be hormonal in nature. For example, part of the hypolipemic action of clofibrate may be mediated by insulin and glucagon release. Insulin deficiency results in a marked depression in hepatic triglyceride production and secretion and lipoprotein formation is severely
impaired in the liver of the alloxan diabetic rat. The effect of glucagon appears to be opposite to the effect of insulin. Glucagon excess depresses triglyceride synthesis and inhibits protein formation in the liver. However, Reichl has suggested that factors other than the absolute concentration of insulin in the blood exert a considerable influence on LPLA and Berkowitz has reported that glucose tolerance is commonly improved by clofibrate therapy in "lipemic" human subjects. Thus, the data are preliminary and the effect deserves further investigation in order to determine whether the apparent hormonal effect of clofibrate has biological significance.

Other modes of action which have been suggested to contribute to the lipid lowering effect of clofibrate include reduction in fatty acid esterification and reduction of calcification and mucopolysaccharide content of the aorta. Clofibrate has also been observed to have some effect on the vascular wall. Robertson studied the effect of clofibrate on the incorporation of $^{3}$H-cholesterol in monolayer cultures of human cells, including aortic intimacies. He suggested that clofibrate may inhibit cholesterol uptake by its competitive affinity to cell membrane proteins, but has no effect on the excretion of cell cholesterol. Furthermore, there is evidence that clofibrate administration leads to decreased platelet adhesiveness. The effect of clofibrate on platelet stickiness is supposed
to be potentially an antithrombotic one. Clofibrate causes increased plasminogen and plasmin levels, with subsequent fall in plasma fibrinogen both in patients with ischaemic heart disease and in those with hypercholesterolemic xanthomatosis. The enzyme plasmin or fibrinolysin not only lyases fibrin; it also attacks factor V, factor VIII, and fibrinogen, with liberation from fibrinogen of substances that inhibit thrombin. Moreover, Gilbert and Mustard showed that clofibrate has a slight but significant prolongation on platelet survival time, which may represent a moderate change in clotting factors. They also indicated a decreased thromboplastin generation with clofibrate.

It has long been recognized that acyltransferases catalyze the reversible transfer of acyl groups from acyl-CoA to L-carnitine and thereby mediate the transport of activated fatty acids through the mitochondrial membrane. Recently, Solberg showed that clofibrate increases, to different extents, the activities of carnithine short-chain, carnithine long-chain and carnithine medium-chain acyltransferases in the liver. Clofibrate has also been shown to cause elevation (two fold) in liver cell catalase activity, due to increased catalase synthesis. Recently, Segal, et al. showed that the initial effect of clofibrate administration is on HDL synthesis and only subsequently on VLDL. Since rat HDL and VLDL contain subunit
polypeptides which are immunologically related,²⁸¹-²⁸² it was speculated that the primary effect of clofibrate was on these particular subunit proteins.

The fact that clofibrate, which is a relatively simple molecule, has profound influence on many enzymes and exhibits few observable toxic effects is remarkable. It is for these reasons that clofibrate is widely used clinically. However, despite the considerable volume of biological data available the mode of action of this drug still remains obscure. Thus, additional studies on the proposed modes of action of clofibrate and related analogs at the molecular level should contribute to a more conclusive understanding of these hypolipemic agents.

A large number of hypolipidemic compounds, which are structurally related to ¹, have been reported²⁸³-²⁸⁶ in the patent literature. However, pharmacological data are not available, and thus relatively little information is known generally to the scientific community about structure-activity relationships in this class of hypolipidemic compounds.

Results previously reported from our laboratories indicated that ethyl L-(-)-p-(4-chlorophenoxy)-α-propionate (³) exhibited significant hypocholesterolemic activity (P < 0.05) in normolipemic Swiss-Webster rats, while the D(R) enantiomorph was biologically inactive in such rats when administered for 12 days mixed with diet at 0.5%
concentration. Thus, some degree of stereoselectivity seems to exist. Furthermore, Kariya, et al.\textsuperscript{287} have shown that ethyl 5-chloroindole-2-carboxylate (5) lowered plasma cholesterol, without affecting plasma triglycerides, in immature and mature Sprague-Dawley rats and in Wistar rats when mixed with their diets.

In order to define minimum structural requirements for maximum, as well as, differential antilipemic properties related to clofibrate we set out to synthesize and biologically evaluate \textit{in vitro} and \textit{in vivo} a series of cyclic analogs. Ethyl 5-substituted-2,3-dihydro-2-benzofurancarboxylate (6, 7, 8) are conformationally constrained (nearly

\begin{align*}
6, R &= H \\
7, R &= Cl \\
8, R &= Phenyl
\end{align*}

\begin{align*}
9, R &= H \\
10, R &= Cl \\
11, R &= Phenyl
\end{align*}
rigid) analogs of 3, in which the methyl group is bonded to the phenyl ring ortho- to the phenolic oxygen. Since clofibrate is our model drug and in vivo is administered as the ethyl ester we also administered our compounds as esters, rather than as the corresponding carboxylic acid hydrolysis products. In addition, we have investigated the hydrolysis of these compounds by serum esterases in vitro to determine whether they undergo rapid hydrolysis to free acids as does clofibrate. Since previously reported cyclic analogs also undergo rapid hydrolysis in serum\textsuperscript{171} the resulting carboxylic acids, which are presumed to be the active hypolipemic agents, were utilized for studies in vitro. The hypolipidemic activities in vivo of the unsaturated ethyl 5-substituted-2-benzofurancarboxylates (9, 10, 11), which have no asymmetric center \( \alpha \) to the carbethoxy group, are also reported in this thesis.

Ethyl 2-methyl-5-chloro-2,3-dihydro-2-benzofurancarboxylate (12) is the asymmetric cyclic analog of clofibrate

and the synthesis and attempted purification of this
compound is also reported in this thesis. Compound 12 represents a new rigid analog of clofibrate in which one of the gem-dimethyl groups is bonded to the phenyl ring ortho- to the phenolic oxygen.

To further study the effect of another minor molecular modification on hypolipemic activity in vivo we introduced a carbonyl oxygen atom at the 3-position of the various ethyl 5-substituted-2,3-dihydro-2-benzofurancarboxylates (6, 7, 8); such a molecular modification affords the interesting 2-carbethoxy-5-substituted-3(2H)benzofuranone structure, specifically defined by compound 13, 14, and 15. These analogs also serve as intermediates in the synthesis of other potentially interesting new hypolipemic compounds. The synthesis and biological activity of many new compounds derived from these 3-oxo analogs will be discussed in this thesis.

\[
\begin{align*}
13, R &= H \\
14, R &= Cl \\
15, R &= \text{Phenyl}
\end{align*}
\]

and will provide the basis for several future publications.

To further study structural requirements for hypolipidemic activity and to determine whether carbethoxy or carboxy
substitution $\beta$ to the ether oxygen is necessary for biological activity the ketones 5-chloro-2-acetylbenzofuran (16) and 5-chloro-2,3-dihydro-2-acetylbenzofuran (17) were prepared. Such compounds do not contain the acidic carboxyl group and their distribution and metabolism are expected to be different than those observed for clofibrate or its related carboxylic acid analogs in vivo.

It is known that symmetrical analog 1 inhibits cholesterol biosynthesis in vitro at the HMG-CoA reductase stage. When one considers the structural similarities between antagonist 2 and substrate mevalonic acid (18) in light of
stereoselective antagonism observed for L(S)- and D(R)-desmethyl analogs it seemed reasonable to us to propose that homologs of 1 as well as 10 and 7 (namely ethyl (5-chloro-2-benzofuranyl)acetate (19) and ethyl (5-chloro-2,3-dihydro-2-benzofuranyl)acetate (20), respectively) would be particularly interesting to investigate. In addition, synthesis of such homologs would provide us with semirigid compounds enabling us to study the effect of the distance between the phenolic oxygen and the ester functions on hypolipidemic activity.

Further, analogs such as 13, 14 and 15 may serve as synthetic intermediates for the preparation of tricyclic lactones depicted by structures 21, 22, and 23. These tricyclic compounds, known as 9-substituted-2,3-dihydro-5H-1,4-dioxepino[6,5-b]benzofuran-5-ones, are analogs of clofibrate derived by bonding the β carbon of the ethyl group in the carbethoxy function to the benzofuran ring via an enol ether oxygen. Such compounds are of interest since in vivo they may undergo hydrolysis at a rate different than
the one observed for clofibrate. The resulting hypolipemic properties observed may also be different and be dependent upon the rate of hydrolysis. Similarly, compounds of the type illustrated by structure 24, 6-chloro-3,4,4a,9a-

![Chemical Structure](image)

\[ 21, \ R = \text{Cl} \]
\[ 22, \ R = \text{Phenyl} \]
\[ 23, \ R = \text{Phenoxy} \]

tetrahydro-1H-pyrano[3,4-b]benzofuran-1-one, have the \( \beta \) carbon of the ethyl function directly bonded to the 3-carbon of the dihydrobenzofuran ring. In such a system one can study geometrical structure-activity relationships of the two isomers on enzymatic hydrolysis rates, on in vivo hypolipemic activity, and on in vitro enzyme action.

Whereas clofibrate is useful as a hypotriglyceridemic agent and is effective mainly in type IV hyperlipoproteinemia, a compound which would also exhibit potent hypocholesterolemic properties would represent a major breakthrough in the treatment of atherosclerosis. For these reasons spirolactone 25, 5-chloro-4',5'-dihydrospiro[benzofuran-2(3H),3'(2H)-furan-2'-one is of interest. Because of its structural relationship to mevalonic acid lactone, such a
compound may have a greater affinity than clofibrate for HMG-CoA reductase and therefore enhanced hypocholesterolemic activity.

All of these compounds (21, 22, 23, 24, 25) possess conformationally restricted ester groups, and, for the reasons mentioned previously, are therefore of considerable theoretical and practical interest. Furthermore, the lactone functionality may provide the molecule with enhanced lipophilic character. Such compounds may selectively localize in adipose tissue, undergo hydrolysis in such tissue and subsequently exert their action in the hydrolyzed form. This approach might yield agents which are effective at lower doses since the compounds would selectively be taken up by tissues holding those enzymes important for regulating lipid metabolism. The enhanced concentration of such agents in lipid tissues followed by subsequent local hydrolysis may provide a greater concentration of the active hypolipemic
agent at the active critical site(s).

During the course of our synthetic investigations we also synthesized the photodimer of benzofuran, namely 26,

![Chemical Structure 26](image1)

(4b\textsubscript{oc}, 4c\textsubscript{oc}, 9b\textsubscript{oc}, 9c\textsubscript{oc})-diethyl 3,8-dichlorocyclobuta[1,2-b:3,4-b]bisbenzofuran-4c,9c(4bH,9bH)-dicarboxylate. We also discovered a synthesis for a new tetracyclic diacetal 27

![Chemical Structure 27](image2)
known as 2,8-dichloro-6,12-epoxy-6H,12H-dibenzod[b,f][1,5]dioxocin. The chemistry of both of these compounds as well as the selective biological activity of 26 is discussed in this thesis.
RESULTS AND DISCUSSION

A. SYNTHETIC APPROACHES

The synthetic route leading to the desired ethyl 5-substituted-2,3-dihydro-2-benzofurancarboxylates (6, 7, 8) is outlined in Scheme II. Appropriately substituted salicylaldehydes (28, 29, 30) served as starting material for the preparation of 6, 7, and 8. The intermediate ethyl 5-substituted-2-benzofurancarboxylates (9, 10, 11) were prepared by a modification of the procedure described by Tanaka.\(^{288}\) Reaction of 27, 28, and 29 with diethyl bromomalonate (31) in the presence of anhydrous potassium carbonate afforded 9,\(^{289}\) 10,\(^{290}\) and 11, respectively, in 65-75 percent yield. The 5-substituted-2-benzofurancarboxylic acids (32, 33, 34) were also isolated from the reaction mixture in 3-5 percent yield.

Base catalyzed hydrolysis of 9, 10, or 11 afforded the 5-substituted-2-benzofurancarboxylic acids 32,\(^{288,291}\) 33,\(^{292}\) and 34, respectively, in 60-80 percent yield. Reduction of 32, 33, and 34 with sodium amalgam according to the method described by Fredga\(^{293}\) afforded the 5-substituted-2,3-dihydro-2-benzofurancarboxylic acids (35,\(^{294}\)

59
Scheme II

\[
\begin{align*}
\text{R} & \quad \text{CHO} \\
\text{R} & \quad \text{BrCH(CO}_2\text{C}_2\text{H}_5)_2
\end{align*}
\overset{\text{K}_2\text{CO}_3}{\longrightarrow}
\]

28, \( R = \text{H} \)
29, \( R = \text{Cl} \)
30, \( R = \text{Phenyl} \)

\[
\begin{align*}
\text{R} & \quad \text{CO}_2\text{C}_2\text{H}_5 \\
\text{R} & \quad \text{CO}_2\text{H}
\end{align*}
\]

9, \( R = \text{H} \)
10, \( R = \text{Cl} \)
11, \( R = \text{Phenyl} \)

32, \( R = \text{H} \)
33, \( R = \text{Cl} \)
34, \( R = \text{Phenyl} \)

1. \( \text{Na(Hg)} \)
2. \( \text{H}_3\text{O}^+ \)

\[
\text{EtOH/H}^+ \quad \overset{\text{Toluene}}{\longrightarrow}
\]

35, \( R = \text{H} \)
36, \( R = \text{Cl} \)
37, \( R = \text{Phenyl} \)
in 80-85 percent yield. Complete reduction of the 2,3-double bond in benzofuran 34 required a 24 hour reaction time whereas reduction of 32 and 33 proceeded smoothly in 1.5 hours. Slow reduction of 34 may be ascribed to its sparingly soluble sodium salt in the aqueous solvent. When 95 percent ethanol was employed as the reaction solvent complete reduction occurred after 2 hours at room temperature. Attempted hydrogenation (50 psi) of 11 over 10 percent Pd-C in absolute ethanol in the presence of concentrated hydrochloric acid at room temperature only afforded starting 11. Conversely, hydrogenation (50 psi) of 11 over 10 percent Pd-C in acetic acid at 50° afforded a mixture, which based on nmr analysis contained desired analog 8 and ethyl 5-cyclohexyl-2,3-dihydro-2-benzofurancarboxylate.

Esters 6, 7, and 8 were readily prepared in 80-90 percent yield by refluxing acids 35, 36, and 37, respectively, in absolute ethanol and benzene (or toluene) in the presence of a catalytic amount of sulfuric acid. When all intermediates in Scheme II were not purified, the overall yield of ethyl 5-chloro-2,3-dihydro-2-benzofurancarboxylate (7) from 5-chlorosalicylaldehyde (29) was 50 percent.

Starting 5-phenylsalicylaldehyde (30) could not be prepared by use of a modified Gatterman reaction; p-phenylphenol (39) in the presence of zinc cyanide, hydrogen chloride gas (passed through concentrated H₂SO₄), and aluminum chloride in ethyl ether afforded no reaction. In
fact, this reaction, when applied to phenols or phenolic ethers, shows a very strong preference for para substitution as opposed to ortho or occasional meta substitution. If the para position of monohydric phenols is blocked, then the reaction may not proceed at all.\textsuperscript{298} Similarly, reaction of \textsuperscript{39} under Vilsmeier-Haack conditions\textsuperscript{299-300} failed; only starting phenol could be isolated. Under Reimer-Tiemann conditions\textsuperscript{301-302} \( p \)-phenylphenol (39) undergoes exothermic reaction affording aldehyde 30 which was separated from the reaction mixture by formation of the bisulfite addition compound. Acid hydrolysis of the separated adduct afforded 30 in 50 percent yield. To obtain this yield of 30 a 3 hour reaction time was required. Although the yield could not be increased further, shorter reaction time provided considerably decreased amounts of product.

Ethyl 2-methyl-5-chloro-2,3-dihydro-2-benzofurancarboxylate (12) could be prepared by methylation of ethyl 5-chloro-2,3-dihydro-2-benzofurancarboxylate (7) in the presence of lithium diisopropylamide, but thus far we
have not been able to separate pure 12 from starting 7. Reaction of 7 in the presence of lithium diisopropylamide, prepared in situ from dry diisopropylamine and n-BuLi in dry tetrahydrofuran at -78°C, with methyl iodide afforded after distillation, a 1:1 mixture of 7 and 12 based on nmr analysis. Both compounds exhibited the same Rf values on TLC using various solvent combinations (cyclohexane, ethyl acetate, petroleum ether, ethyl ether). Chromatography on a 125 cm x 2 cm silica gel column using ethyl ether:petroleum ether (1:2) as solvent afforded a 1:3 mixture of 7 and 12.

In order to prepare 5-chloro-4',5'-dihydrospiro[benzofuran-2(3H),3'(2'H)-furan]-2'-one (25), we anticipated that reaction of 5-chlorosalicylaldehyde (29) with α-bromo-γ-butyrolactone (40) in the presence of one mole of base would yield α-(4-chloro-2-formylphenoxy)-γ-butyrolactone [(41), Scheme III]. Subsequently, intramolecular aldol condensation should afford compound 42. If 42 could be isolated, mild oxidation might be expected to yield 5-chloro-4',5'-dihydrospiro[benzofuran-2(3H),3'(2'H)-furan]-.
2',3-dione (43), the ketone of which could be reductively removed under a variety of conditions. However, several attempts to prepare 25 from 29 and 40 via intermediate 41 proved unsuccessful. Reaction in sodium ethoxide in ethanol or sodium hydride in glyme or dimethylformamide yielded complex mixtures which could not be separated. Further, since Kawai et al. reported the preparation of 45 from salicylaldehyde (28) and ethyl α-bromo-α-phenylacetate (44) in the presence of anhydrous potassium carbonate in 2-butanone (unreported yield), we anticipated that 42 might be obtained from 29 and 40 under similar conditions.
Unexpectedly, we obtained a neutral compound 27, mp 172-
173°, which showed no hydroxyl or carbonyl stretching in
the infrared and failed to form a 2,4-dinitrophenylhydrazone
derivative in the cold. Nmr analysis showed a sharp proton
resonance singlet at δ 6.23 and a multiplet at δ 6.70-7.30
integrating in a 1:3 ratio.

The mass spectrum (Figure 1) shows the molecular ion
(M⁺) as the base peak. The fragmentation pattern and relative abundances of the peaks in the mass spectrum (Table 1)
indicate the presence of two chlorine atoms as well as certain structural features of the molecule. One of the more
significant ions is found at m/e 259 (M-C1) and results from
the loss of one chlorine atom. Loss of the chlorine atom is
claimed to be prominent when the halogen is attached to an
aromatic ring. Loss of mass units 29 (CHO) and 45 (CHO₂)
from the molecular ion should provide valuable structural
information. Cleavage of the diacetal rings in the M-35
mass ion followed by loss of CO affords the 231 mass ion
(Scheme IV).
Figure 1. - Mass spectrum (70 eV) of 2,8-dichloro-6,12-epoxy-6H,12H-benzo [b, f][1,5]dioxocin (27).
### TABLE 1

<table>
<thead>
<tr>
<th>Peak m/e</th>
<th>Relative Intensity</th>
<th>Fragmentation</th>
<th>Molecular Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>294</td>
<td>100.0</td>
<td>M</td>
<td>C\textsubscript{14}H\textsubscript{8}Cl\textsubscript{2}O\textsubscript{3}</td>
</tr>
<tr>
<td>296</td>
<td>40.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>298</td>
<td>6.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>265</td>
<td>10.6</td>
<td>M-CHO</td>
<td>C\textsubscript{13}H\textsubscript{7}Cl\textsubscript{2}O\textsubscript{2}</td>
</tr>
<tr>
<td>267</td>
<td>6.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>269</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>259</td>
<td>45.6</td>
<td>M-Cl</td>
<td>C\textsubscript{14}H\textsubscript{8}ClO\textsubscript{3}</td>
</tr>
<tr>
<td>261</td>
<td>15.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>249</td>
<td>20.1</td>
<td>M-CHO\textsubscript{2}</td>
<td>C\textsubscript{13}H\textsubscript{7}Cl\textsubscript{2}O</td>
</tr>
<tr>
<td>251</td>
<td>13.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>253</td>
<td>2.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>231</td>
<td>45.0</td>
<td>M-[Cl+CO]</td>
<td>C\textsubscript{13}H\textsubscript{8}ClO</td>
</tr>
<tr>
<td>233</td>
<td>15.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>155</td>
<td>14.9</td>
<td>231-C\textsubscript{6}H\textsubscript{4}</td>
<td>C\textsubscript{7}H\textsubscript{4}ClO\textsubscript{2}</td>
</tr>
<tr>
<td>157</td>
<td>5.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>154</td>
<td>87.9</td>
<td>M-C\textsubscript{7}H\textsubscript{5}ClO</td>
<td>C\textsubscript{7}H\textsubscript{3}ClO\textsubscript{2}</td>
</tr>
<tr>
<td>156</td>
<td>32.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>126</td>
<td>24.6</td>
<td>154-CO</td>
<td>C\textsubscript{6}H\textsubscript{3}ClO</td>
</tr>
<tr>
<td>128</td>
<td>8.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>46.4</td>
<td>259-C\textsubscript{8}H\textsubscript{5}ClO\textsubscript{3}</td>
<td>C\textsubscript{6}H\textsubscript{3}</td>
</tr>
<tr>
<td>63</td>
<td>19.7</td>
<td>231-C\textsubscript{8}H\textsubscript{5}ClO\textsubscript{2}</td>
<td>C\textsubscript{5}H\textsubscript{3}</td>
</tr>
</tbody>
</table>

The ion appearing at m/e 63 may be produced by path A from intermediate ion m/e 231. The ion of mass 155 which represents the monomer of compound 27 would result from ion m/e 231 via a loss of a neutral entity C\textsubscript{6}H\textsubscript{4}, 3-hexen-1,5-
diyne (path B, scheme IV). The abundant ion of mass 75 may arise from ion m/e 259 through the cleavage of the diacetal group resulting in the loss of neutral 5-chloro-2-0-formylbenzaldehyde (path C). The second most prominent ion (m/e 154) may arise from the base peak. Diacetal ring opening (scheme V) with subsequent hydrogen transfer via a four-membered transition state would yield ion m/e 154. As expected, the peak at m/e 154 eliminates CO to give mass ion 126.

These data coupled with elemental analysis support the molecular formula $C_{14}H_8Cl_2O_3$ for 27. Under other conditions
Scheme V

\[
\begin{align*}
\text{Scheme V} \\
\text{m/e 294} \\
\rightarrow \\
\text{m/e 126} \\
\rightarrow \\
\text{m/e 154}
\end{align*}
\]

(sodium ethoxide, sodium hydride) compound 27 could not be isolated. However, when 5-chlorosalicylaldehyde (29) was refluxed in 2-butanone in the presence of anhydrous potassium carbonate compound 27 was isolated in 40 percent yield. The structure for diacetal 27 was further substantiated by conversion in refluxing 6N hydrochloric acid to monomer 29 in 90 percent isolated yield.\(^{307}\) Compound 27, 2,8-dichloro-6,12-epoxy-6H,12H-dibenzo[bf][1,5]dioxocin, represents a new tetracyclic system containing a diacetal functionality prepared by a new synthetic technique.

A number of methods have been reported for the
preparation of acetals. Reaction of gem dihalides with metal alkoxides\textsuperscript{308} or ortho esters with lithium aluminum hydride\textsuperscript{309} or grignard reagent\textsuperscript{310} are several examples. Base catalyzed addition of alcohols to triple bonds has also been reported for the synthesis of acetals.\textsuperscript{311} The reaction rate decreases with increased steric hindrance (primary to a tertiary alcohols) and phenols require more severe conditions. Boron trifluoride and certain mercuric salts also catalyze the addition of alcohols to triple bonds.\textsuperscript{311} However, acetals are commonly formed reversibly by treatment of aldehydes with alcohols in the presence of acid catalysts. Neither the formation nor the degradation of diacetals (or acetals) is reported to be catalyzed by base.

Formation of 27 may be visualized as the base catalyzed intermolecular addition of the phenolic hydroxyl group of each of the two salicylaldehyde molecules to the aldehyde carbonyl of the other followed subsequent loss of a molecule of water. Thus, this base catalyzed reaction represents a novel method for the synthesis of the new tetracyclic
diacetal compound 27.

A second approach to the preparation of spirolactone 25 involved attempts to alkylate ethyl 5-chloro-2,3-dihydro-2-benzofurancarboxylate (7) with 2-bromoethanol (46) or 2-bromo-1-[2'-tetrahydropyranyl]hydroxy]ethane (47) in order to obtain the respective intermediates 48 or 49 (scheme VI).

Scheme VI

The protected tetrahydrophyranyl (THP) derivative 47, which was prepared in 90 percent yield by condensation of dihydrophyrany with 2-bromoethanol (46) in the presence of a catalytic amount of p-toluenesulfonic acid, was also used in the successful preparation of a new hypolipidemic agent discussed
later in this thesis. In this synthetic scheme we anticipated that ester hydrolysis, tetrahydropyranyl group cleavage, and subsequent lactone formation would yield 25. However, only starting material could be isolated when 7 was allowed to react with 46 or 47 under different conditions (sodium ethoxide in benzene or ethanol, sodium hydride in ethyl ether or glyme, lithium diethylamide in tetrahydropyran) and at various temperatures. Similarly, reaction of ethylene oxide with 7 in the presence of sodium ethoxide or sodium hydride in various solvents only afforded starting material. Reaction of ethylene oxide with 7 in the presence of lithium diethylamide in dry tetrahydropyran afforded a mixture consisting of 5-chloro-2,3-dihydro-2-benzofurancarboxylic acid (36, 20 percent), N,N-diethyl-5-chloro-2,3-dihydro-2-benzofurancarboxamide (50, 40 percent), and a minute quantity of unidentified compound.

Hine and Dalsin, 312 on the basis of kinetic studies, showed that the rate of carbanion formation is decreased when oxygen atoms are bonded to the carbon atom serving as the carbanion precursor. This is presumably due to the repulsion between the unshared electron pairs of the carbanion
Electron repulsion may be minimized by appropriate rotation around the carbon-oxygen bond in acyclic compounds. However, such rotation cannot take place in the dihydrobenzofuran series and for these reasons they are less reactive. Since the dihydrobenzofuran 7, did not readily undergo alkylation we turned our attention to alkylation of the more activated 3-keto analogs 13, 14, and 15.

The 5-substituted-2-carbethoxy-3(2H)-benzofuranones 13, 14, and 15 were prepared from the appropriately substituted salicylic acids 52, 53, and 54, respectively. Esterification of 52, 53, or 54 was achieved in 75-80 percent yield by refluxing azeotropically in absolute ethanol/
benzene containing a catalytic amount of concentrated sulfuric acid for 16 hr. 5-Phenylsalicylic acid (54) was prepared in over 90 percent yield from p-phenylphenol (39) under modified Kolbe-Schmidt reaction conditions.313

Williamson reaction of 55,514 56315 or 57 with ethyl bromoacetate in refluxing dry acetone316 over anhydrous potassium carbonate317 afforded the desired ethyl [4-substituted-2-carbethoxyphenoxy]acetates (58,317 59,318 60) in 70-80 percent yield (Scheme VII). Dieckman condensation of 58, 59, or 60 in sodium ethoxide and dry benzene316 afforded ethyl 5-substituted-2-carbethoxy-3(2H)-benzofuranones 13,318-319 14,319 or 15, respectively, in 80-90 percent yield. The yields reported here are greater than those reported by Schroeder, et al.319 owing to a longer reflux time. The optimum yield was obtained after refluxing for 15 hours. Infrared spectral analysis in chloroform showed carbonyl stretching at 1670 and 1730 cm⁻¹, as well as, hydroxyl stretching at 3350 cm⁻¹; apparently, these 3(2H)-benzofuranones exist in equilibrium with their respective enol forms (Scheme VII). Further, 14 only forms
a phenylhydrazone derivative after refluxing for 4 hr in the presence of concentrated hydrochloric acid. According to the method of Pasto and Johnson\textsuperscript{320} no phenylhydrazone was obtained.

When benzofuranone 14 was allowed to react with 2-bromo-1-[2-(2'-tetrahydropyranyl)hydroxy]ethane (47) in the presence of NaH in dry diglyme for 5 hours at 150° a 50 percent yield of unknown ethyl 5-chloro-3-[2-(2'-tetrahydropyranyl)hydroxyethoxy]-2-benzofuran carboxylate (61) was obtained (Scheme VIII). However, reaction of 14 with 2-bromoethanol (46) in the presence of sodium ethoxide yielded no reaction. Similarly, upon reaction of 14 with 46 in the
presence of sodium hydride in glyme or dioxane under different conditions (atmospheric, bomb, sealed tube) only starting material could be isolated. Further, reaction of 14 with 47 in the presence of sodium hydride and various solvents (glyme, dioxane) as well as under different reaction conditions (atmospheric, bomb, sealed-tube) failed to give any alkylated products. In no case could we isolate any
C-alkylated product. Unreported analog 61 was not isolated; upon base catalyzed hydrolysis of the ester function, followed by acid catalyzed hydrolysis of the tetrahydropyranyl protecting group, 61 yielded unknown hydroxy-acid 62 in 52 percent yield. The hydroxy-acid 62, 5-chlorc-3-(2-hydroxy-ethoxy)-2-benzofurancarboxylic acid, was converted to the desired compound 21 in 90 percent yield by refluxing in benzene containing a catalytic amount of p-toluenesulfonic acid. Enol-lactone 21, 9-chloro-2,3-dihydro-5H-1,4-dioxepino[6,5-b]benzofuran-5-one, failed to give a 2,4-dinitrophenylhydrazone derivative. The infrared spectrum of compound 21 showed only one carbonyl stretching band (1715 cm\(^{-1}\)); the 1680 cm\(^{-1}\) carbonyl band of 14 was absent. These data support a structural assignment resulting from O-alkylation. The mass spectrum of compound 21 is reproduced in figure 2. The relative intensity, major fragmentation ions, and molecular formulas are listed in table 2. The molecular ion (M\(^{+}\)) abundancy is high owing to the aromatic character of the compound. Loss of ethylene (C\(_2\)H\(_4\)) from the molecular ion gives rise to the ion m/e 210, while the m/e 194 peak arises via elimination of C\(_2\)H\(_4\)O from the 7-membered ring. The m/e 179 and 167 (base) peaks do not arise by simple bond cleavage; loss of the C\(_2\)H\(_3\)O\(_2\) and C\(_3\)H\(_3\)O fragments, respectively, requires the transfer of one hydrogen atom from the ethylenedioxy bridge to the benzofuran moiety. This could be confirmed by studying the mass spectrum of
Figure 2. - Mass spectrum (70 eV) of 9-chloro-2,3-dihydro-5H-1,4-dioxepino[6,5-b]benzofuran-5-one (21).
TABLE 2

RELATIVE INTENSITIES OF THE MAJOR PEAKS IN THE MASS SPECTRUM OF COMPOUND 21

<table>
<thead>
<tr>
<th>Peak m/e</th>
<th>Relative Intensity</th>
<th>Fragmentation</th>
<th>Molecular Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>238</td>
<td>82.2</td>
<td>M</td>
<td>C_{11}H_{7}ClO_{4}</td>
</tr>
<tr>
<td>240</td>
<td>29.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>210</td>
<td>3.1</td>
<td>M-C_2H_4</td>
<td>C_{9}H_{3}ClO_3</td>
</tr>
<tr>
<td>212</td>
<td>1.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>194</td>
<td>13.2</td>
<td>M-C_2H_4O</td>
<td></td>
</tr>
<tr>
<td>196</td>
<td>4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>179</td>
<td>10.6</td>
<td>M-C_2H_3O_2</td>
<td>C_{9}H_{4}ClO_2</td>
</tr>
<tr>
<td>167</td>
<td>100.0</td>
<td>M-C_3H_3O_2</td>
<td>C_{8}H_{4}ClO_2</td>
</tr>
<tr>
<td>169</td>
<td>33.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>139</td>
<td>11.5</td>
<td>167-CO</td>
<td>C_{7}H_{4}ClO</td>
</tr>
<tr>
<td>141</td>
<td>3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>138</td>
<td>38.1</td>
<td>167-CHO</td>
<td>C_{7}H_{3}ClO</td>
</tr>
<tr>
<td>140</td>
<td>13.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>111</td>
<td>6.8</td>
<td>139-CO</td>
<td>C_{6}H_{4}Cl</td>
</tr>
<tr>
<td>113</td>
<td>2.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>110</td>
<td>20.4</td>
<td>138-CO</td>
<td>C_{6}H_{3}Cl</td>
</tr>
<tr>
<td>112</td>
<td>7.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>23.5</td>
<td>110-Cl</td>
<td>C_{6}H_{3}</td>
</tr>
</tbody>
</table>

Compound 21, which possesses a deuterium labeled side chain. Further, loss of the fragment C\_2H\_3O\_2 in the mass spectrum substantiates an O-alkylated product as opposed to C-alkylated product. The expulsion of CHO from the base peak (m/e 167) gives rise to the ion m/e 138 (Scheme IX) which loses CO to give the ion m/e 110. The chlorine atom,
which is present in all these fragments, is ejected from the ion m/e 110 affording an ion of mass 75. This fragment does not contain chlorine, since there is no substantial peak at m/e 77. Similarly, successive losses of two carbon monoxide molecules from the base peak gives rise to m/e 139 and m/e 111 ions. The peak at m/e 75 may also be generated from the m/e 111 ion by loss of HCl.

Clofibrate, as pointed out in the introduction to this thesis, undergoes in vivo and enzymatic in vitro hydrolysis quantitatively within 5-10 minutes. The hydrolysis
product, free acid 2, is proposed to be the "active" hypolipemic agent. However, enzymatic hydrolysis studies in vitro on the very active hypolipemic compound, enol-lactone 21, showed 21 not to undergo hydrolysis more than 13 percent within 60 minutes (Table 3). These results coupled with the very high hypolipemic activity (favorably compared with clofibrate), may open new avenues and provide greater insight into the mechanism(s) of action of clofibrate and related analogs. Consequently, it may help in developing new leads for the design of antilipidemic drugs which may have great potential in the treatment of

### TABLE 3

**HYDROLYSIS OF CLOFIBRATE AND 9-CHLORO-2,3-DIHYDRO-5H-1,4-DIOXEPINO[6,5-b]BENZOFURAN-5-ONE (21) BY RAT PLASMA IN VITRO AT 37°**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Percent Hydrolysis ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Clofibrate: 81.44 ± 0.88&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>Clofibrate: 89.99 ± 1.25</td>
</tr>
<tr>
<td>30</td>
<td>Clofibrate: 91.56 ± 1.31</td>
</tr>
<tr>
<td>60</td>
<td>Clofibrate: 91.20 ± 1.18</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean ± SD; for four determinations.
atherosclerosis and related diseases.

Ethyl 5-chloro-2-methyl-2-carbethoxy-3(2H)-benzofuranone (65) represents another rigid analog of clofibrate, in which one of the gem-dimethyl groups is attached to the phenyl ring. The 3-keto functionality in compound 63 will serve in the construction of the lactone ring fused to the furan moiety of the dihydrobenzofuran. Owing to its structural similarities to mevalonic acid, we anticipate such an analog may be more active as a hypocholesterolemic agent than clofibrate because it would better inhibit the synthesis of cholesterol at the HMG-CoA reductase stage or between mevalonate and squalene, and also maintain the high hypotriglyceridemic properties of the clofibrate molecule.

Previous reports from our laboratories have reported the synthesis of minute quantities of the desired C-alkylated product 63, using sodium ethoxide and methyl iodide in a sealed tube. However, recent attempts in our laboratories to methylate 14 under basic conditions (sodium ethoxide, thallium ethoxide, lithium diisopropylamide) were
unsuccessful. Difficulty in achieving C-alkylation arises from the tendency of 14 to exist in equilibrium with the aromatized enol form which would lead to O-alkylation as is the case in dimethylsulfoxide. It also became evident that under refluxing conditions sodium ethoxide was probably facilitating nucleophilic cleavage of the alkylated benzo-furanone system leading to ring opening.

An alternative approach for the preparation of 63 involved Williamson reaction of ethyl 5-chlorosalicylate 59 with ethyl 2-bromopropionate 65 in the presence of anhydrous potassium carbonate. This afforded ethyl \( \text{O}-(4\text{-chloro}-2\text{-}
\begin{align*}
\text{Cl} & \quad \text{OH} \\
\text{CO}_2\text{C}_2\text{H}_5 & \quad + \quad \text{CH}_3 \\
\text{Br} & \quad \text{CHCH}_2\text{CO}_2\text{C}_2\text{H}_5 \\
\text{Cl} & \quad \text{OH} \\
\text{CO}_2\text{C}_2\text{H}_5 & \quad \text{CO}_2\text{Et}
\end{align*}

\text{carbethoxy})\text{propionate} (66) in over 90 percent yield. Base (sodium ethoxide or sodium hydride) catalyzed cyclization of 66 afforded a compound, mp 149-150°, to which structure 64 was assigned. The nmr analysis showed a singlet at \( \delta \) 1.62 integrating for three protons, a multiplet at \( \delta \) 7.10-8.25 integrating for three aromatic protons, and a carboxylic acid proton at \( \delta \) 9.30.
In order to pursue structure activity relationship studies of 2-acylbenzofuran derivatives, we also prepared 5-chloro-2-acetylbenzofuran (16) and 5-chloro-2,3-dihydro-2-acetylbenzofuran (17). Such compounds, along with their metabolites, may be of value in mechanism of action studies of clofibrate and related analogs. Compound 16 was prepared by modification of the procedure utilized by Royer, et al. Condensation of 5-chlorosalicylaldehyde (29) with freshly distilled chloroacetone (67) in 2-butanone in the presence of anhydrous potassium carbonate afforded the desired ketone 16 in 60 percent yield. Reduction of 16 with sodium-amalgam afforded 5-chloro-2,3-dihydro-2-acetylbenzo-
furan (17). Alternatively, reaction of 5-chloro-2,3-dihydro-2-benzofurancarboxylic acid (36) with methyl lithium afforded the identical compound 17.

The homologs of benzofuran 10 and particularly the selective hypocholesterolemic dihydrobenzofuran 7 are of special interest because of their close structural resemblance to mevalonic acid. The importance of blocking the cholesterol biosynthetic pathway by inhibiting HMG-CoA reductase is well known. Such analogs may act as competitive or noncompetitive antagonists of mevalonate or HMG-CoA reductase. The preparation of ethyl (5-chloro-2-benzofuranyl)acetate (19) and ethyl (5-chloro-2,3-dihydro-2-benzofuranyl)acetate (20) involved use of the Willgerodt-Kindler reaction. Reaction of 5-chloro-2-acetylbenzofuran (16) in the presence of sulfur and morpholine afforded

\[
\begin{align*}
\text{O} & \quad \text{CH}_3 \\
\text{Cl} & \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{CH}_2 \text{CO}_2 \\
\text{Cl} & \\
\end{align*}
\]

\[
\begin{align*}
16 & \quad 68 \\
\end{align*}
\]

crude \( \varphi \)- (5-chloro-2-benzofuranyl)acetic acid (79), which upon Fisher esterification should afford the desired benzofuran homolog ester 19. Similarly, 5-chloro-2,3-dihydro-2-acetylbenzofuran (17) may be converted to the 2,3-
dihydrobenzofuran homolog 20.

(Z)-Ethyl [4-chloro-2-(3-oxo-1-propenyl)phenoxy]acetate (72) will be utilized as the intermediate for the synthesis of 24 (Scheme X). Methyl triphenylphosphonium iodide (69)

Scheme X

\[
(C_6H_5)_3 + CH_3I \rightarrow (C_6H_5)_3PCH_3I^- 
\]

1. n-BuLi
2. HCO_2C_2H_5

was prepared in quantitative yield according to the procedure of Wittig and Schoellkopf\textsuperscript{327} who prepared methyl triphenylphosphonium bromide. Reaction of 69 with ethyl formate in the presence of n-butyl lithium afforded formylmethylenetriphenylphosphorane (70) in 60 percent yield.\textsuperscript{328} Ethyl 4-chloro-2-formylphenoxyacetate (71)
was prepared from 5-chlorosalicylaldehyde (29) and ethyl bromoacetate in the presence of sodium ethoxide in 60 percent yield. Consequently, reaction of 70 with 71 under Wittig reaction conditions afforded the desired unknown intermediate 72 in 77 percent yield. The coupling constant for the vinylic system showed cis coupling (J = 8 Hz), as expected for Wittig reactions. Internal Michael reaction of 72 should afford ethyl 5-chloro-2,3-dihydro-3-(2-oxoethyl)-2-benzofurancarboxylate (73). Sodium borohydride reduction of 73 and subsequent lactonization should yield the desired tricyclic lactone 24, namely 6-chloro-3,4,4a,9a-

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{CH}_2\text{COCH}_2\text{H}_5 & \quad \text{COCH}_2\text{H}_5 \\
\text{Cl} & \quad \text{Cl} \\
\text{CH=CHCHO} & \quad \text{CH=CHCHO} \\
\end{align*}
\]

72 73 24

tetrahydro-1H-pyrano[3,4-b]benzofuran-1-one.

The photodimerization of compounds with general structure 74a-c has been reported and is observed to proceed only in the presence of triplet sensitizers. However, Harpp and Heitner showed that sulfone 74d undergoes photodimerization by direct irradiation. For these reasons, we decided to study the unsensitized photochemical reaction of benzofuran 10, and subsequently evaluate in vivo the
resulting dimer for antilipemic activity in hyperlipemic rats.

Irradiation of ethyl 5-chloro-2-benzofurancarboxylate (10) in ethanol using a 450-W Hanovia medium-pressure mercury arc and a water-cooled Vycor immersion well resulted in the formation of a crystalline dimer [Mol. wt = 441 (Rast Camphor)] in 50 percent yield plus 40 percent starting material. The resulting dimer may have four possible stereoisomers: anti head-to-tail 75, anti head-to-head 76, syn head-to-head 77, and syn head-to-tail 26. Acenaphtylene-1-carboxylic acid, upon irradiation, has resulted in the formation of the syn head-to-tail photocyclization as the main product in 35 percent yield.335

The isolated dimer 26 was characterized in part by nmr analysis. The nmr showed a singlet at $\delta$ 4.67 integrating for two protons assigned to the cyclobutyl methine proton resonances. No vinylic proton resonances, observed for the monomer 10 at $\delta$ 7.21, could be detected in the dimer. The methyl ($\delta$ 1.33) triplet and methylene ($\delta$ 4.29) quartet
proton resonances confirmed the presence of two ethyl groups; carbonyl stretching in the infrared (1735 cm\(^{-1}\)) substantiated the presence of the carbethoxy functions. The mass spectrum showed a parent ion peak at m/e 448 (very low relative intensity) which is in agreement with the molecular formula C\(_{22}\)H\(_{18}\)Cl\(_2\)O\(_6\). Expulsion of one or two carbethoxy groups from the molecular ion generate respectively, ions of mass 375 and 302 (Figure 3). The relative abundances of the peaks at m/e 375 and 302 (Table 4) confirm the presence of two chlorine atoms. The presence of the m/e 239 ion may be explained by ejection of a chlorine atom with apparent concomitant loss of CO from the m/e 302 peak. The base ion peak (m/e 224) corresponds to the starting monomer \(\text{X}_0\), likely resulting from thermal decomposition of the dimer; upon pyrolysis (gentle flame) in a pyrex tube starting \(\text{X}_0\) was isolated in quantitative yield. Ions which arise from the monomer (m/e 224) dominate the 70 eV spectrum (Figure 3) of the dimer \(\text{X}_2\). The m/e 196 peak arises from the base peak (m/e 224), by a rearrangement of one hydrogen atom via a six-membered transition state with elimination of neutral ethylene\(^{336}\) (Scheme X). Formation of the m/e 179 ion may originate by two pathways; loss of ethoxide radical from the base peak (m/e 224), and/or loss of hydroxyl radical from the m/e 196 fragment. Both, loss of alkoxides from esters and loss of hydroxyls from acids are prominent operations in their respective classes.\(^{336}\) The ion of mass 152 is
Figure 3.- Mass spectrum (70 eV) of (4bâ€œ, 4câ€œ, 9bâ€œ, 9câ€œ)-diethyl 3,8-dichlorocyclobuta[1,2-b: 3,4-b']bisbenzofuran-4c, 9c(4â†’H, 9bH)-dicarboxylate (26).
TABLE 4
RELATIVE INTENSITIES OF THE MAJOR PEAKS
IN THE MASS SPECTRUM OF DIMER 26

<table>
<thead>
<tr>
<th>Peak m/e</th>
<th>Relative Intensity</th>
<th>Fragmentation</th>
<th>Molecular Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>448</td>
<td>0.1</td>
<td>M</td>
<td>C_{22}H_{18}Cl_{12}O_{6}</td>
</tr>
<tr>
<td>375</td>
<td>0.8</td>
<td>M-[CO_2C_2H_5]</td>
<td>C_{19}H_{13}Cl_{12}O_{4}</td>
</tr>
<tr>
<td>377</td>
<td>0.5</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>379</td>
<td>0.1</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>302</td>
<td>7.9</td>
<td>M-2[CO_2C_2H_5]</td>
<td>C_{16}H_{8}Cl_{12}O_{2}</td>
</tr>
<tr>
<td>304</td>
<td>5.0</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>306</td>
<td>1.2</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>239</td>
<td>6.2</td>
<td>M-2[Cl + CO]</td>
<td>C_{15}H_{8}ClO</td>
</tr>
<tr>
<td>241</td>
<td>2.1</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>224</td>
<td>100.0</td>
<td>M/2</td>
<td>C_{11}H_{19}ClO_{3}</td>
</tr>
<tr>
<td>226</td>
<td>52.9</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>196</td>
<td>42.1</td>
<td>224-[C_2H_4]</td>
<td>C_{9}H_{5}ClO_{3}</td>
</tr>
<tr>
<td>194</td>
<td>15.0</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>179</td>
<td>27.4</td>
<td>224-[OC_2H_5]</td>
<td>C_{9}H_{4}ClO_{2}</td>
</tr>
<tr>
<td>181</td>
<td>11.2</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>152</td>
<td>15.0</td>
<td>196-[CO_2]</td>
<td>C_{8}H_{5}ClO</td>
</tr>
<tr>
<td>154</td>
<td>4.7</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>123</td>
<td>8.8</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>3.8</td>
<td>179-[C_2O_2]</td>
<td>C_{7}H_{4}Cl</td>
</tr>
<tr>
<td>89</td>
<td>4.6</td>
<td>M</td>
<td></td>
</tr>
</tbody>
</table>

generated by the elimination of CO_2 from the ion m/e 196.
The appearance of the ions m/e 123 and 89 may be explained
as outlined in Scheme XI. Loss of CO from m/e 152 gives rise to benzocyclopropenyl structure (m/e 124) which upon ejection of a chlorine atom generates the ion m/e 89. This fragment does not contain chlorine atoms, since there is no substantial peak at m/e 91. Concomitantly, loss of hydrogen radical from ion m/e 124 will afford the dehydrotropylium structure m/e 123 as suggested by Willhalm and his co-workers.\textsuperscript{337} Besides eliminating CO, ion m/e 152 may undergo a pronounced loss of CHO to give m/e 123 (C\textsubscript{7}H\textsubscript{4}Cl\textsuperscript{+}). The loss of the m/e 29 fragment is suggestive of the behavior of furan\textsuperscript{338} and may conceivably proceed with the production
of the tropylium species. Finally, ion m/e 123 may also be formed by the rather unusual direct loss of C₂O₂ from the ion m/e 179.³³⁷
These data coupled with the elemental analyses and sharp mp (166-167°) confirm our postulate that the dimer is one of the four possibilities, namely stereoisomer 75, 76, 77, or 26. In order to determine the geometrical configuration for the dimer, a number of reactions were undertaken. cis- Diacid isomers derived from the cis-diester isomers 77 and 26 should form anhydrides. On the other hand, carboxylic acid derivatives of the anti isomers 75 and 76 having trans-diester functions should not undergo anhydride formation. Thus, base catalyzed hydrolysis of the isolated diester dimer afforded a diacid dimer 78 in 90 percent yield. Isolation of pure diacid dimer was confirmed by its mass spectrum and nmr analysis (Experimental Section).
Refluxing diacid 78 in acetic anhydride afforded the corresponding anhydride 79. Compound 79, \((4\text{bo} \angle, 4\text{co} \angle, 9\text{bo} \angle, 9\text{co} \angle) - 3,8\)-dichloro-4\text{bo}\text{H}, 9\text{bo}\text{H}, 4\text{c}, 9\text{c}-(\text{methanoxymethano})\text{cyclobuta}[1,2-b: 3,4-b']\text{bisbenzofuran}-11,13\text{-dione}, showed two carbonyl stretching vibrations at frequencies \((1820, 1740 \text{ cm}^{-1})\) attributable to a six-membered anhydride ring.\(^{339}\) The absorbance at the longer wavelength had the stronger intensity in agreement with the six-membered anhydride structural assignment. The nmr analysis showed no \(\text{D}_2\text{O}\) exchangeable protons. The mass spectrum is reproduced in figure 4. Table 5 outlines the relative intensities of the major ions, summary of the possible fragmentation route, and lists the molecular formulas of these fragments. The spectrum does not show peaks other than the base peak, of intensities greater than 12.9. Mass spectral analysis, coupled with infrared and nmr data support the anhydride structural assignment for
Figure 4.- Mass spectrum (70 eV) of \((4b\alpha, 4c\alpha, 9b\alpha, 9c\alpha)-3,8\text{-dichloro-}
4b\beta, 9b\beta-4c, 9c\beta\text{-}(methanoxymethano)cyclobuta[1,2-\text{b}: 3,4-\text{b'}]bisbenzo-
\text{furan-11,13-dione} (79).
<table>
<thead>
<tr>
<th>Peak m/e</th>
<th>Relative Intensity</th>
<th>Fragmentation</th>
<th>Molecular Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>374</td>
<td>2.6</td>
<td>M</td>
<td>C_{18}H_{8}Cl_{2}O_{5}</td>
</tr>
<tr>
<td>376</td>
<td>1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>302</td>
<td>3.3</td>
<td>M-C_{2}O_{3}</td>
<td>C_{16}H_{8}Cl_{2}O_{2}</td>
</tr>
<tr>
<td>304</td>
<td>2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>306</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>239</td>
<td>4.8</td>
<td>302-[CO + Cl]</td>
<td>C_{15}H_{8}ClO</td>
</tr>
<tr>
<td>241</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>224</td>
<td>12.9</td>
<td>M-C_{8}H_{3}ClO</td>
<td>C_{10}H_{5}ClO_{4}</td>
</tr>
<tr>
<td>226</td>
<td>4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>196</td>
<td>12.3</td>
<td>224-CO</td>
<td>C_{9}H_{5}ClO_{3}</td>
</tr>
<tr>
<td>198</td>
<td>3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>179</td>
<td>100.0</td>
<td>M-[C_{9}H_{4}ClO_{3}]</td>
<td>C_{9}H_{4}ClO</td>
</tr>
<tr>
<td>181</td>
<td>35.8</td>
<td>196-[OH]</td>
<td></td>
</tr>
<tr>
<td>152</td>
<td>6.1</td>
<td>196-[CO_{2}]</td>
<td>C_{8}H_{5}ClO</td>
</tr>
<tr>
<td>154</td>
<td>1.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>123</td>
<td>11.7</td>
<td>152-[CHO]</td>
<td>C_{7}H_{4}Cl</td>
</tr>
<tr>
<td>125</td>
<td>4.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The molecular ion (m/e 374) abundancy is relatively higher than that of the diester 26 or the diacid dimer 78. The m/e 302 peak arises by the elimination of the C_{2}O_{3} fragment which can only arise from an acid anhydride. The relative abundances of the peaks at m/e 302, 304, and 306 confirm the presence of two chlorine atoms (Table 5). Loss of chlorine and ejection of CO from the compound 79.
m/e 302 ion generates the m/e 239 ion. Formation of ion m/e 224 ($C_{10}H_5ClO_4$) likely takes place via a rearrangement of a hydrogen atom from the furan ring of benzofuran to the oxygen with subsequent loss of $C_8H_3ClO$. This rearrangement may take place as follows. Cleavage of the cyclobutane in the molecular ion (m/e 374) will afford the noncyclic anhydride $A$, which upon transfer of a vinylic hydrogen atom to the anhydride oxygen via a 5-membered transition state followed by release of chlorobenzofuran ($C_8H_3ClO$) affording ion $B$, will yield ion m/e 224 upon loss of CO. The ion m/e 224, upon loss of neutral CO, would afford the ion m/e 196. The base peak (m/e 179) may result from loss of $CO_2$ with
concomitant pyrolysis of the molecular ion. The ions at m/e 152 and 123 may arise via pathways similar to those depicted in Schemes X and XI.

To further prove that structure 79 is a 6-membered anhydride rather than 5-membered anhydride 80, 79 was refluxed with sodium phenoxide in benzene thereby affording the monophenyl ester 81 in 90 percent yield. This monoester showed only one exchangeable proton (D$_2$O) in the nmr and a singlet at $\delta$ 4.60 integrating for two protons. The two cyclobutane methine protons in the syn head-to-head monophenyl ester are expected to exhibit different chemical shifts since their chemical environments are not identical. On the other hand, the isomer 81 (4box, 4cox, 9box, 9cox)-monophenyl 3,8-dichlorocyclobuta[1,2-b:3,4-b']bisbenzofuran-4c,9c(4bH,9bH)-dicarboxylate, is expected to show the same proton resonance chemical shift for each of the magnetically
equivalent methine protons. Therefore, the infrared, nmr, and mass spectra data, as well as the chemical data are consistent with the structural assignment 26, (4b\(\infty\), 4c\(\infty\), 9b\(\infty\), 9c\(\infty\))-diethyl 3,8-dichlorocyclobuta[1,2-\(b\):3,4-\(b'\)]bis-benzofuran-4c,9c(4bH,9bH)-dicarboxylate, for the isolated dimer. To confirm these conclusions the diester photodimer has been submitted for X-ray structural analysis.

To date, there have been only a few published studies concerning the mechanism of photodimerization of compounds related to 10 (c.f. 74). However, a number of investigators\(^{341-347}\) have extensively studied photodimerization of coumarin \(^{82}\). Direct irradiation of coumarin in polar solvents produced syn head-to-head dimer \(^{83}^{341,344-345}\) and syn head-to-tail dimer \(^{84,347}\) while irradiation of coumarin in the presence of sensitizer led to the formation of anti head-to-head dimer \(^{85}^{344-345}\) and a trace of anti head-to-
The results of Morrison, et al.\textsuperscript{344-345} and Krauch, et al.\textsuperscript{347} have indicated that the preference in nonpolar solvents for formation of the anti dimers 85 and 86 is markedly reduced in solvents of high polarity ($\xi = 33$ (CH$_3$OH) to 38 (DMF and acetonitrile)); in fact, the reaction course becomes completely reversed in protic solvents (methanol and ethanol); formation of the syn dimers 83\textsuperscript{345} and 84\textsuperscript{347} are favored. Thus, polar solvents strongly favor the formation of syn dimers 83\textsuperscript{344-346} and 84;\textsuperscript{347} nonpolar solvents lead almost exclusively to anti dimer 85.\textsuperscript{345} Further, high dilution of monomer favored formation of 85; formation of 83 was favored at low temperatures.\textsuperscript{347} Also halocarbon
solvents (e.g. \( \text{CCl}_4 \)) enhance the formation of \( \text{85} \).\(^{345}\)

Morrison, et al.\(^{345}\) as well as Hammond, et al.\(^{343}\) explained these results by proposing that \( \text{83} \) and \( \text{84} \) arise from coumarin excimers\(^{348-349}\) which are derived from a singlet excited state (Scheme XII). That coumarin excimers are intermediates in the photodimerization process was confirmed through luminescence experiments.\(^{345}\) These complexes are sandwiched in a parallel plane configuration\(^{350-352}\) ultimately leading to the \text{syn} head-to-head dimer; rotation in the plane affords a juxtaposition of reaction sites leading to the \text{syn} head-to-tail dimer. In the absence of a triplet sensitizer the \text{syn} coumarin dimers \( \text{83} \) and \( \text{84} \) arise from attack of an excited coumarin singlet on ground state coumarin; in the presence of a triplet state sensitizer, the \text{anti} coumarin dimers \( \text{85} \) and \( \text{86} \) arise from interaction of excited triplet coumarin with ground state coumarin. Excimer formation is known to be favored at low temperatures and high concentrations.

Many investigators\(^{332-333,353-354}\) have observed that photodimerization of benzofuran only takes place in the
presence of triplet sensitizers. Several investigators have obtained differing results. For example, Krauch, et al. obtained head-to-head dimers while Takamatsu, et al. obtained head-to-tail dimers in the presence of triplet sensitizers. Furthermore, there are only a few reported photochemical additions involving the excited singlet state of benzofuran, although many addition reactions of benzofuran derivatives have been reported. In contrast, our results show that ethyl 5-chlorobenzofuran undergoes photodimerization by direct irradiation, affording a syn head-to-tail dimer as the major product (plus 6 percent polymer), which can be explained via the formation of benzofuran excimer intermediates in a manner similar to those proposed for the coumarin.

B. BIOLOGICAL STUDIES

The effects of clofibrate (1) and several of its analogs were tested on plasma cholesterol levels in control male Sprague-Dawley Purina chow-fed and Triton-induced hyperlipidemic rats. Such an animal model is predictive of hypolipemic activity in man. In Triton-hyperlipemic rats (Tables 6 and 7) clofibrate and certain analogs showed differing degrees of hypolipemic activity. The poorly controlled factor in these experiments was the variability in the Triton hyperlipemic rat model. This variability does not permit us to compare the absolute activities of
# TABLE 6

**EFFECT OF CLOFIBRATE AND RELATED ANALOGS ON PLASMA CHOLESTEROL LEVELS**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Control Group (I)</th>
<th>Drug-treated Control (II)</th>
<th>Triton Hyperlipemic (III)</th>
<th>Drug-treated Triton Hyperlipemic (IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clofibrate (1)</td>
<td>60.6±7.2(5)a</td>
<td>62.4±8.5</td>
<td>267±85</td>
<td>59.3±12b</td>
</tr>
<tr>
<td>Ethyl 2,3-dihydrobenzofuran-2-carboxylate(6)</td>
<td>54.2±7.5</td>
<td>59.2±6.7</td>
<td>142±25</td>
<td>142±32c</td>
</tr>
<tr>
<td>Ethyl 5-chloro-2,3-dihydrobenzofuran-2-carboxylate(7)</td>
<td>97.8±10.8</td>
<td>55.0±11.7d</td>
<td>177±63</td>
<td>93.0±19b</td>
</tr>
<tr>
<td>Ethyl 5-phenyl-2,3-dihydrobenzofuran-2-carboxylate(8)</td>
<td>87.8±11.3(10)</td>
<td>80.4±12.4(10)</td>
<td>164.7±36.8(10)</td>
<td>94.3±9.8(10)b</td>
</tr>
<tr>
<td>Ethyl 5-phenylbenzofuran-2-carboxylate(11)</td>
<td>77.0±7.8(10)</td>
<td>72.8±7.7(10)</td>
<td>178.2±56.4(10)</td>
<td>131.6±60.1(10)c</td>
</tr>
</tbody>
</table>

*aMean ± SD; six male Sprague-Dawley rats unless otherwise noted by number in the parentheses.*

*bStatistically significant P 0.05; comparison of groups III and IV.*

*cStatistically significant P 0.05; comparison of groups I and IV.*

*dStatistically significant P 0.05; comparison of groups I and II.*
<table>
<thead>
<tr>
<th>Compound</th>
<th>Control Group (I)</th>
<th>Drug-treated Control (II)</th>
<th>Triton Hyperlipemic (III)</th>
<th>Drug-treated Triton Hyperlipemic (IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl 5-phenyl-2-carbethoxy-3(2H)-benzofuranone (15)</td>
<td>71.9±13.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.0±14.6</td>
<td>196±57.3</td>
<td>149.2±53.4&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>9-Chloro-2,3-dihydro-5H-1,4-dioxiapino[6,5-b]benzofuran-5-one (21)</td>
<td>85.9±7.6</td>
<td>85.6±10.5</td>
<td>233.2±105.3</td>
<td>95.0±16.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5-Chloro-3-(2-hydroxyethoxy)-2-benzofuran-2-carboxylic acid (62)</td>
<td>84.5±3.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>84.0±5.5</td>
<td>122.4±8.9</td>
<td>112.6±13.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dimer 26</td>
<td>66.7±12.4</td>
<td>63.9±11.6</td>
<td>163.4±99.2</td>
<td>99.0±39.2&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean ± SD; ten male Sprague-Dawley rats.

<sup>b</sup>Statistically significant P 0.05; comparison of groups III and IV.

<sup>c</sup>Statistically significant P 0.05; comparison of groups I and IV.

<sup>d</sup>Half the regular dose (62.5 umol/Kg).
different compounds. However, to define the activities of these agents in lowering cholesterol or triglyceride levels in hyperlipidemic rats, groups I and IV and groups III and IV were tested for significant differences. A compound that significantly reduces either cholesterol or triglycerides in drug-treated Triton hyperlipemic group (IV) with respect to Triton-hyperlipemic group (III), as well as, reduces the level of the parameter under consideration back to normal (group I, i.e. there is no significant difference between groups I and IV) may be defined as one of the most active hypolipemic agents. On the other hand, a compound that shows a significant difference between groups III and IV and groups I and IV may be defined as a partially active agent. When analyzed in this manner only compounds 7, 8 and 21 compared most favorably with clofibrate; there was no statistically significant difference between the plasma cholesterol levels in group IV and control rats (groups I). However, when compound 21 was administered at half the regular dose (62.5 umol/Kg) it showed no effect on plasma cholesterol levels. Unlike clofibrate, ethyl 5-chloro-2,3-dihydro-2-benzofurancarboxylate (7) significantly lowered cholesterol levels in normolipemic rats (comparing groups I and II). Benzofuranone 15 and dimer 26 significantly lowered plasma cholesterol concentrations when compared with hyperlipemic controls (group III). However, analogs 15 and 26 did not reduce the cholesterol levels back to normal
(comparing groups I and IV). Compounds 6, 11 and 52 showed no hypocholesterolemic activity in this animal model.

Triglyceride concentrations were also examined in Purina chow-fed and Triton-induced hyperlipemic rats (Tables 8 and 9). In Triton-induced hyperlipidemic animals only enol-lactone 21 compared favorably with clofibrate, which reduced plasma triglyceride concentration in Triton hyperlipemic (group IV) rats back to normal (group I). However, compound 21 at half the regular dose also showed partial hypotriglyceridemic activity. This selective activity on triglyceride concentration is of considerable interest since 21, unlike clofibrate, does not undergo rapid hydrolysis in serum in vitro; its mode of action is under investigation and may be related to the enhanced lipophilic character of the molecule. Benzofuran 11 also showed only partial hypotriglyceridemic activity. All of the dihydrobenzofurans 6, 7 and 8 (Table 8) showed no effect on plasma triglyceride levels. Hence, it appears that the absence or presence of a bond at the 2,3 position in the benzofuran system is critical for activity. The dihydrobenzofurans exhibited selective hypocholesterolemic activity, while the benzofurans exhibited only hypotriglyceridemic properties. Dimer 26 also showed selective hypotriglyceridemic activity. This specific activity may arise from the metabolism of dimer 26 to monomer 10 which is a selective hypotriglyceridemic agent. Compound 15, which is in equilibrium with its enol tautomer exhibits
<table>
<thead>
<tr>
<th>Compound</th>
<th>Control Group (I)</th>
<th>Drug-treated Control (II)</th>
<th>Triton Hyperlipemic (III)</th>
<th>Drug-treated Triton Hyperlipemic (IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clofibrate (1)</td>
<td>20.9±6.2(5)</td>
<td>30.5±8.2</td>
<td>774±329</td>
<td>27.6±15b</td>
</tr>
<tr>
<td>Ethyl 2,3-dihydrobenzofuran-2-carboxylate (6)</td>
<td>31.2±19</td>
<td>22.1±6.6</td>
<td>127±50</td>
<td>128±88c</td>
</tr>
<tr>
<td>Ethyl 5-chloro-2,3-dihydrobenzofuran-2-carboxylate (7)</td>
<td>56.4±29</td>
<td>34.3±8.7</td>
<td>162±121</td>
<td>109±59c</td>
</tr>
<tr>
<td>Ethyl 5-phenyl-2,3-dihydrobenzofuran-2-carboxylate (8)</td>
<td>18.7±6.4(10)</td>
<td>18.1±6.3(10)</td>
<td>108.5±28.3(10)</td>
<td>90.1±24.9(10)c</td>
</tr>
<tr>
<td>Ethyl 5-phenylbenzofuran-2-carboxylate (11)</td>
<td>18.1±2.6(10)</td>
<td>23.7±9.3(10)</td>
<td>132.2±99.2(10)</td>
<td>51.2±46.6(10)b,c</td>
</tr>
</tbody>
</table>

*Mean ± SD; six male Sprague-Dawley rats unless otherwise noted by number in the parentheses.

bStatistically significant P 0.05; comparison of groups III and IV.

CStatistically significant P 0.05; comparison of groups I and IV.
### Table 9

**EFFECTS OF NEW HYPOLIPEMIC AGENTS ON PLASMA TRIGLYCERIDE LEVELS**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Control Group (I)</th>
<th>Drug-treated Control (II)</th>
<th>Triton Hyperlipemic (III)</th>
<th>Drug-treated Triton Hyperlipemic (IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl 5-phenyl-2-carbethoxy-3(2H)-benzofuranone (15)</td>
<td>27.1±8.2^a</td>
<td>40.3±13.0</td>
<td>184.7±100</td>
<td>78.7±70.0^b,c</td>
</tr>
<tr>
<td>9-Chloro-2,3-dihydro-5H-1,4-dioxepino[6,5-b] benzofuran-5-one (21)</td>
<td>24.7±9.6</td>
<td>27.1±8.0</td>
<td>204.7±69.9</td>
<td>35.0±32.8^b</td>
</tr>
<tr>
<td>5-Chloro-3-(2-hydroxyethoxy)-2-benzofuran-3-carboxylic acid (62)</td>
<td>19.9±6.2^d</td>
<td>21.3±4.8</td>
<td>100.2±13.7</td>
<td>34.3±10.1^b,c</td>
</tr>
<tr>
<td>Dimer 26</td>
<td>23.6±5.5</td>
<td>24.3±4.5</td>
<td>188.4±114.2</td>
<td>33.0±11.2^b,c</td>
</tr>
</tbody>
</table>

^aMean ± SD; ten male Sprague-Dawley rats.

^bStatistically significant P  0.05; comparison of groups III and IV.

^cStatistically significant P  0.05; comparison of groups I and IV.

^dHalf the regular dose (62.5 umol/Kg).
only partial hypotriglyceridemic properties, while hydroxy-
acid 62 (Table 9) showed no effect on plasma triglyceride
levels. None of the compounds including clofibrate reduced
plasma triglyceride levels in normolipemic rats (II).

Clofibrate has its greatest effect in the treatment of
endogenous hypertriglyceridemias (Types III and IV); generally clofibrate lowers serum triglycerides more than
serum cholesterol. Thus, the selective hypolipemic
agents discussed in this thesis may have potential use in
the treatment of different hyperlipoproteinemias. Although further work needs to be carried out, dihydrobenzo-
furans 7 and 8 may be very beneficial for the treatment of
Type IIa hyperlipoproteinemia which is characterized by an
increase in the plasma \( \beta \)-lipoprotein levels with concomi-
tant increased serum cholesterol. On the other hand, benzo-
furan 11, low doses of enol-lactone 21, and particularly
dimer 26 may be used for the treatment of Type IV hyper-
lipoproteinemia (commonest type), which is characterized by
increased pre-\( \beta \)-lipoprotein levels and enhanced serum tri-
glycerides; these compounds may also have limited use in the
rare Type III as well as Type V hyperprebetalipoproteinemia.

Enol-lactone 21, of all the compounds tested, compared
most favorably with clofibrate in reducing both plasma cho-
lesterol and triglyceride levels, while the hydrolysis prod-
uct 62 is biologically inactive in this animal model. It is
known that clofibrate (1) is rapidly hydrolyzed by tissue
and serum esterases both in vivo and in vitro to the free acid 2, which is presumed to be the "active" agent. Further, studies in our laboratories have shown that a parallelism existed between the hypocholesterolemic effect of esters in vivo and inhibition of cholesterol biosynthesis of the corresponding acids in vitro. Similarly, while the esters of cyclic analogs of clofibrate returned serum cholesterol levels in hyperlipemic rats to normal in vivo; the corresponding acids were equally effective inhibitors of cholesterol biosynthesis in vitro. In addition, esters of clofibrate analogs, like 1, also undergo rapid hydrolysis by rat plasma in vitro. Consequently, we studied the hydrolytic stability of the very active hypolipemic agent 21 in rat plasma in vitro. To our knowledge compound 21 is the first hypolipidemic agent which contains a lactone functionality; all others related to clofibrate are either acyclic esters or amides. The enzymatic hydrolytic studies of clofibrate and lactone 21 by rat plasma in vitro at 37° are presented in Table 3. Compound 21 underwent less than 13 percent hydrolysis in one hour to the inactive hydrolysis product 62. Therefore, any differences observed in hypolipemic activities between different clofibrate analogs (cyclic and acyclic) as well as enol-lactone 21 cannot be attributed to the differences in rates of hydrolysis to the carboxylic acids. Conversely, if enzymatic hydrolysis is in fact contributing to
the hypolipemic properties of these compounds, than lactone 21 may be visualized as a prodrug having a longer duration of action than clofibrate which has a half-life of 12 hours in the blood. Further work is necessary; the reason why hydrolysis product 62 is inactive may be due to its lack of oral absorption or because of a different partition coefficient it does not reach the site of action.

As part of an extensive investigation designed to probe the nature of biochemical sites which are either blocked or stimulated in vivo by administration of several of the cyclic clofibrate analogs, we studied the effect of chronic administration of these analogs on various parameters of hepatic drug metabolism. In recent years, many compounds have been shown to stimulate the metabolism of drugs, foreign compounds, and normal body constituents by inducing the activity of the hepatic microsomal monooxygenase enzyme system. Preliminary experiments in our laboratories have indicated that alterations in hepatic protein and drug metabolism induced by chronic administration of clofibrate occurred within a 7-day period. For these reasons a 7-day dosing schedule was used for all compounds tested. The effect of pretreatment with phenobarbital (Pb), (type I and II substrate inducer) clofibrate and dihydrobenzofurans 6 and 7 on liver weight, liver/body weight, and microsomal protein in male Harlan Wistar rats is summarized in Table 10. Pretreatment with Pb, 1, and 7 (0.40 mmol/Kg) significantly
TABLE 10
EFFECT OF PRETREATMENT WITH PHENOBARBITAL AND ANALOGS 1, 6 AND 7
ON LIVER WEIGHT, LIVER/BODY AND MICROSOMAL PROTEIN IN MALE RATS

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose(^a) (mmol/kg)</th>
<th>Liver Weight (g)(^b)</th>
<th>Liver/Body Weight (%)(^b)</th>
<th>Microsomal Protein (mg/g-liver)(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Test</td>
<td>Control</td>
<td>Test</td>
</tr>
<tr>
<td>Pheno-barbital</td>
<td>0.16</td>
<td>7.70±0.37</td>
<td>9.80±0.40(^d)</td>
<td>4.39±0.13</td>
</tr>
<tr>
<td>1</td>
<td>0.40</td>
<td>5.53±0.50</td>
<td>5.90±0.27</td>
<td>4.82±0.13</td>
</tr>
<tr>
<td>0.80</td>
<td>7.72±0.27</td>
<td>7.08±0.34</td>
<td>4.96±0.18</td>
<td>4.94±0.10</td>
</tr>
<tr>
<td>7</td>
<td>0.40</td>
<td>4.85±0.52</td>
<td>4.18±0.69</td>
<td>4.57±0.25</td>
</tr>
<tr>
<td>0.80</td>
<td>5.42±0.92</td>
<td>5.96±0.96</td>
<td>4.24±0.28</td>
<td>4.63±0.54</td>
</tr>
<tr>
<td>6</td>
<td>0.40</td>
<td>3.91±0.39</td>
<td>3.73±0.57</td>
<td>4.06±0.29</td>
</tr>
<tr>
<td>0.80</td>
<td>5.14±0.37</td>
<td>5.27±1.03</td>
<td>4.09±0.28</td>
<td>3.92±0.21</td>
</tr>
</tbody>
</table>

\(^a\)Compounds were given orally in coconut oil once daily for seven days.

\(^b\)Values represent the mean ± S.E. of n = 6. **Control** refers to coconut oil pretreatment and **Test** refers to drug pretreated animals.

\(^c\)Values represent the mean ± S.E. of n = 3.

\(^d\)Significantly different from control (P < 0.05).
increased the liver microsomal protein content. It is interesting to note that only 7 increased microsomal protein at the lower dose. On the other hand, only Pb induced an increase in the liver weight and liver/body weight.

The effect of pretreatment with Pb, 1, 6 and 7 on ethylmorphine (Type I substrate) N-demethylase, aniline (Type II substrate) hydroxylase, and cytochrome P-450 in liver microsomes is presented in Table 11. The microsomal rates of ethylmorphine N-demethylation and cytochrome P-450 content were increased by prior administration of Pb or 1 while aniline hydroxylation was elevated only by Pb pre-treatment. Thus, clofibrate pretreatment enhanced the hepatic microsomal metabolism of type I substrates, but not of type II substrates. Chlorodihydrobenzofuran 7 possessed all of the inductive properties of 1 and in addition exhibited an enhancement of aniline hydroxylation. However, the deschlorodihydrobenzofuran 6 was unable to produce any changes in specific activities of the hepatic drug metabolizing enzyme systems, cytochrome P-450, microsomal protein, or liver weight after chronic administration. The elevations in cytochrome P-450 in liver of hypocholesterolemic chlorodihydrobenzofuran pretreated male rats may be related to an increased catabolism of cholesterol and thereby contribute, in part, to the hypocholesterolemic properties of this class of compounds.

In addition to the potential clinical significance of
TABLE 11

EFFECT OF PRETREATMENT WITH PHENOBARBITAL AND ANALOGS 1, 6, AND 7 ON ETHYLMORPHINE N-DEMETHYLASE, ANILINE HYDROXYLASE AND CYTOCHROME P-450 IN LIVER MICROSOMES FROM MALE RATS

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mmol/kg)</th>
<th>Ethylmorphine N-Demethylase (nmol CH$_2$O/mg/12 min)</th>
<th>Aniline Hydroxylase (n mol/mg/15 min)</th>
<th>Cytochrome P-450 ($O.D.$ 450-490/mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Test</td>
<td>Control Test</td>
<td>Control Test</td>
<td>Control Test</td>
</tr>
<tr>
<td>Pheno-barbital</td>
<td>0.16</td>
<td>48.9±7.1</td>
<td>116.5±8.9</td>
<td>16.4±0.8</td>
</tr>
<tr>
<td>1</td>
<td>0.40</td>
<td>57.2±2.4</td>
<td>77.8±4.5</td>
<td>28.9±0.2</td>
</tr>
<tr>
<td>0.80</td>
<td></td>
<td>55.5±1.2</td>
<td>76.4±0.9</td>
<td>12.6±2.2</td>
</tr>
<tr>
<td>7</td>
<td>0.40</td>
<td>58.8±5.0</td>
<td>90.0±3.8</td>
<td>36.0±3.0</td>
</tr>
<tr>
<td>0.80</td>
<td></td>
<td>50.0±5.0</td>
<td>65.0±3.8</td>
<td>24.0±3.0</td>
</tr>
<tr>
<td>6</td>
<td>0.40</td>
<td>63.8±2.0</td>
<td>67.5±2.5</td>
<td>-----</td>
</tr>
<tr>
<td>0.80</td>
<td></td>
<td>45.0±1.4</td>
<td>45.5±2.5</td>
<td>16.2±1.2</td>
</tr>
</tbody>
</table>

$^a$Compounds were given orally in coconut oil once daily for seven days.

$^b$Values represent the mean ± S.E. of n = 3.

$^c$Significantly different from control (P ≤ 0.05).
these studies, the differential effects of the various com-
pounds on different parameters (serum cholesterol levels,
serum triglyceride levels, hepatic drug metabolism, hydroly-
sis rates) may be discussed with respect to structural re-
quirements for maximum biological activity. Removal of the
substituent (Cl, phenyl) from the benzofuran system afforded
inactive compounds in hyperlipemic rats. Similarly, the
presence of a chloro group is required for the inductive
properties possessed by these analogs. The qualitative
and/or quantitative differences in biological effect between
chloro and deschloro analogs discussed in this thesis would
not appear to be due to differential rates of enzymatic
hydrolysis or to the ionization constants of the free car-
boxylic acids. One physical parameter which could con-
tribute to the overall differential effects observed between
chloro and deschloro analogs is their lipid solubility. There seems to be a favorable correlation between lipid
solubility and the ability to produce enzyme induction.
However, there was no apparent correlation of log P values
with differential hypolipemic activity. It seems, there-
fore, that the analogs studied exert their acute effects in
the hyperlipemia rat model by other mechanisms.

Another interesting structure-activity relationship ob-
served for these benzofuran systems involves their differ-
ential hypolipemic activity. The benzofuran derivatives 11,
15, and 26 showed only hypotriglyceridemic activity, while
2,3-dihydrobenzofurans 7 and 8 were only hypcholesterolemic in action. These differential activities may be attributed to differences in lipid solubility, selective enzyme induction, selective action at certain enzymes involved in lipid metabolism, biotransformation, deposition, distribution, and many other possibilities. Further experimentation is necessary in order to explain these observed differences in their hypolipemic actions.
EXPERIMENTAL

A. SYNTHETIC PROCEDURES

Melting points were taken using a Thomas-Hoover melting point apparatus. Infrared spectra were obtained with a Perkin-Elmer Model 257 grating spectrophotometer. The nmr spectra were recorded with a Varian A-60 nmr spectrometer at 60 MHz using TMS as an internal reference. Gas chromatographs were taken using an F and M model 402 gas chromatograph equipped with a flame ionization detector and glass columns. Mass spectra were determined using a DuPont model 21-491 instrument via the direct inlet mode at 70 eV. Chemical analyses were determined by Clark Microanalytical Laboratory, Urbana, Illinois.

Ethyl 2-Benzofurancarboxylate (9) was prepared according to the method of Kurkudar and Rao. A mixture of salicylaldehyde (28, 9.92 g, 0.08 mol), diethyl bromomalonate (31, 12.69 g, 0.05 mol), anhydrous \( \text{K}_2\text{CO}_3 \) (16 g, 0.11 mol) and 2-butanone (160 ml) were refluxed for 7 hr. The solvent was removed under reduced pressure, cooled and poured into \( \text{H}_2\text{O} \) (200 ml). The solution was extracted with \( \text{Et}_2\text{O} \). The
Et₂O layer was washed with dilute NaOH solution, H₂O, dried (Na₂SO₄), filtered and solvent removed under reduced pressure. Distillation of the resulting liquid afforded 9.9 g (65%) of a colorless liquid (9) bp 99-100° (0.005 mm) Lit.²⁸⁹ bp 275° (720 mm). Nmr (CDCl₃), δ 1.15 (t, 3H, CH₃), 4.2 (q, 2H, methylene), 6.70-7.55 (m, 4H aromatic and 1H vinylic).

2-Benzofurancarboxylic Acid (32).- Ethyl 2-benzofuran-carboxylate (9, 3.0 g, 0.015 mol) was hydrolyzed utilizing 10% alcoholic KOH (90 ml) by heating on a steam-bath for 1 hr. The EtOH was removed by distillation and the residue diluted with H₂O and extracted with Et₂O. Acidification of the aqueous portion with dilute H₂SO₄ afforded crude 2-benzofurancarboxylic acid which was recrystallized from benzene affording 1.4 g (60%) white crystals mp 192-193°. Lit.²⁸⁸ mp 192-193°. Nmr (acetone-d₆), δ 7.20-7.83 (m, 4H, aromatic, and 1H vinylic), 10.53 (broad, 1H, carboxylic).

2,3-Dihydro-2-benzofurancarboxylic Acid (35) was prepared according to a procedure described by Fredga.²⁹³ 2-Benzofurancarboxylic acid (32, 1.85 g, 0.01 mol) was added to a 10% aqueous NaOH solution (35 ml). A sparingly soluble sodium salt separated. Sodium amalgam, prepared from Na (0.69 g, 0.03 g-atom) and Hg (24.1 g, 0.12 g-atom),³⁵⁹ was added with stirring during 20 min. The mixture was stirred for 1.5 hr and allowed to stand overnight. The Hg was
separated and the solution filtered. Acidification of the aqueous solution with 10% $\text{H}_2\text{SO}_4$ precipitated the desired acid. Filtration and recrystallization from benzene afforded 1.5 g (80%) white solid mp 116.5-116.8° Lit$^{294}$ mp 116.5°. Nmr (acetone-$d_6$), $\delta$ 3.37-3.92 (m, 2H, methylene protons), 5.05-5.40 (q, 1H, methine proton), 10.50 (s, 1H, carboxyl proton), 6.75-7.42 (m, 4H, aromatic protons).

**Ethyl 2,3-Dihydro-2-benzofurancarboxylate (6)** was prepared by refluxing 2,3-dihydro-2-benzofurancarboxylic acid, (35, 1.05 g, 0.006 mol), absolute EtOH (25 ml), toluene (12 ml) and concentrated $\text{H}_2\text{SO}_4$ (0.5 ml) for 4 hr using a Dean-Stark trap to remove $\text{H}_2\text{O}$. The reaction mixture was cooled and washed with $\text{H}_2\text{O}$, saturated NaHCO$_3$ solution and finally with $\text{H}_2\text{O}$. The organic layer was separated. The aqueous portion was extracted with Et$_2$O and the organic layers were combined, dried (Na$_2$SO$_4$), filtered and the solvent removed under reduced pressure. The residual liquid, after distillation, afforded the desired product 6 (1.06 g, 92%). bp 78° (0.002 mm). Lit$^{360}$ bp 273°. Nmr (CDCl$_3$), $\delta$ 1.0-1.3 (t, 3H, CH$_3$), 3.16-3.48 (n, 2H, furan CH$_2$), 3.90-4.35 (q, 2H, ester CH$_2$), 4.88-5.28 (q, 1H, methine proton), 6.65-7.3 (m, 4H, aromatic).

**Attempted Formation of 4',5'-Dihydrospiropbenzofuran-2(3H),3'(2'H)-furan-2'-one with Ethylene Oxide and Ethyl 2,3-Dihydro-2-benzofurancarboxylate (6).**- The procedure
described by Glickman and Cope\textsuperscript{361} was utilized in which ethyl 2,3-dihydro-2-benzofurancarboxylate (6, 1.93 g, 0.01 mol) was added to NaOEt prepared from Na (0.23 g, 0.01 g-atom) and absolute EtOH (4 ml). The solution was cooled (0°C); ethylene oxide (0.50 g, 0.01 mol) dissolved in absolute EtOH (2 ml) was added slowly to the cooled solution. The solution was mixed for one hr and then poured into H\textsubscript{2}O and extracted with Et\textsubscript{2}O. The Et\textsubscript{2}O layer was dried (Na\textsubscript{2}SO\textsubscript{4}), filtered and the solvent removed under reduced pressure. The resultant solid was recrystallized from benzene affording 1.5 g (92%) white solid mp 115.5-116° identical in all respects to 2,3-dihydro-2-benzofurancarboxylic acid (35).

**Attempted Formation of 4',5'-Dihydropiro[benzofuran-2(3H),3'(2'H)-furan-2'-one With Ethylene Oxide and Ethyl 2,3-Dihydro-2-benzofurancarboxylate (6) Under Pressure.**

Sodium (0.23 g, 0.01 g-atom) was dissolved in absolute EtOH (5 ml) and transferred to a precooled Parr stainless steel bomb. To this solution was added ethyl 2,3-dihydro-2-benzofurancarboxylate (6, 1.93 g, 0.01 mol) followed by ethylene oxide (1.8 g, 0.04 mol) dissolved in 5 ml of absolute EtOH. The bomb was closed and shaken intermittently while warming to room temperature and subsequent heating to 45-50° over a 20 hr period. After cooling, the solid was dissolved in H\textsubscript{2}O, acidified, and extracted with Et\textsubscript{2}O. The Et\textsubscript{2}O solution was dried (Na\textsubscript{2}SO\textsubscript{4}), filtered and the solvent
removed under reduced pressure. The resulting solid was re-
crystallized from benzene affording white microcrystals mp
115-116° identical in all respects to 2,3-dihydro-2-benzo-
furancarboxylic acid (35).

Ethyl 5-Chloro-2-benzofurancarboxylate (10).—A mixture
of 5-chlorosalicylaldehyde (29, 50 gm, 0.32 mol), diethyl
bromomalonate (31, 50 gm, 0.21 mol), anhydrous K₂CO₃ (65 g,
0.47 mol) and 2-butanone (800 ml) were refluxed for 7 hr.
The solvent was removed under reduced pressure, cooled and
poured into H₂O (600 ml). The aqueous solution was ex-
tracted with Et₂O. The Et₂O layer was washed with dilute
NaOH solution, H₂O, dried (Na₂SO₄), filtered, and concen-
trated under reduced pressure. The resulting solid was re-
crystallized from EtOH affording 50.6 g (70%) white crys-
tals, mp 64.5-65.5°. Lit²⁹⁰ mp 65°. Nmr (CDCl₃), δ 1.18-
1.52 (t, 3H, CH₃), 4.18-4.62 (q, 2H, CH₂), 7.06-7.72 (m,
3H aromatic and 1H vinylic).

5-Chloro-2-benzofurancarboxylic Acid (33).—Ethyl 5-
chloro-2-benzofurancarboxylate (10, 18 g, 0.08 mol) in 10%
alcoholic KOH (450 ml) was refluxed for 1 hr. The reaction
mixture was concentrated and the residue was poured into
H₂O and washed with Et₂O. Acidification of the aqueous
fraction with dilute HCl afforded a solid which was recrystal-
lized from benzene yielding 12.5 g (80%) white solid mp
258-259°. Lit²⁹⁰ mp 258°. Nmr (acetone-d₆), δ 7.10-7.63
5-Chloro-2,3-dihydro-2-benzofurancarboxylic Acid (36).- 5-Chloro-2-benzofurancarboxylic acid (33, 11.8 g, 0.06 mol) was added to a 10% aqueous NaOH solution (200 ml). Sodium amalgam, prepared from Na (3.7 g, 0.16 g-atom) and Hg (145 g, 0.75 g-atom), was added with stirring during 20 min. The solution was stirred for 2 hr and then allowed to stand overnight. After separation of the Hg, the aqueous solution was acidified, and then extracted with Et₂O. The Et₂O solution was dried (Na₂SO₄), filtered and concentrated affording, after crystallization from benzene, 10.1 g (85%) of desired product (36), mp 115-116°. Nmr (d₆-acetone), δ 3.10-4.10 (m, 2H, CH₃) 5.08-5.51 (q, 1H, methine proton) 6.67-7.33 (m, 3H, aromatic), 10.77 (s, 1H, acid proton).

Anal. calcd. for C₉H₇ClO₃: C, 54.45; H, 3.62; Cl, 17.80. Found: C, 54.71; H, 3.73; Cl, 17.51.

Ethyl 5-Chloro-2,3-dihydro-2-benzofurancarboxylate (7).- 5-Chloro-2,3-dihydro-2-benzofurancarboxylic acid (36, 9.9 g, 0.05 mol), absolute EtOH (250 ml), toluene (100 ml) and concentrated H₂SO₄ (4 ml) were heated at refluxed for 7 hr using a Dean-Stark trap to remove H₂O. The reaction mixture was concentrated and the residue was dissolved in Et₂O. The Et₂O layer was washed with saturated NaHCO₃ solution and H₂O. The aqueous portion was extracted with Et₂O and the combined organic layers were dried (Na₂SO₄), filtered and
the solvent was removed under reduced pressure. The resid­
ual liquid, upon distillation, afforded 10.2 g (50%) of the
desired ester 7, bp 90-91° (0.05 mm). Nmr (CDCl₃), δ 1.12-
1.46 (t, 3H, CH₃), 3.25-3.60 (m, 2H, furan CH₂), 4.06-4.50
(q, 2H, ester CH₂), 5.02-5.42 (q, 1H, methine proton), 6.68-
7.27 (m, 3H, aromatic).

Anal. calcd. for C₁₁H₁₁ClO₃: C, 58.40; H, 4.91; Cl, 15.63. Found: C, 58.31; H, 4.72; Cl, 15.54.

5-Phenylsalicylaldehyde (30).- p-Phenylphenol (39, 22
g, 0.13 mol) was dissolved in 95% EtOH; a solution of NaOH
(40 g, 1 mol) in H₂O (80 ml) was rapidly added. The solu-
tion was heated to 75-80°, at which point CHCl₃ (20 ml) was
added dropwise. The addition was at such a rate that
gentle refluxing was maintained (added during one hour).
After formation of a dark-red color (generally 5-15 min)
further heating was unnecessary. Stirring was continued
for 3 hr after all the CHCl₃ was added. After cooling, the
EtOH and excess CHCl₃ were removed under reduced pressure
and the resulting residue was cooled, poured into cold H₂O,
acidified with dropwise addition of HCl, and extracted with
Et₂O. The Et₂O solution was concentrated under reduced
pressure and the residue was poured into 2 volumes of satu-
rated NaHSO₃ solution and shaken vigorously (on a shaker)
for 45 min. The semisolid (paste) bisulfite addition com-
pound was allowed to stand for one hr, filtered in the
dark and washed with small portions of EtOH and Et₂O (to remove the phenol). The bisulfite addition compound was decomposed with dilute H₂SO₄ by warming on a water-bath for 30 min. The cooled mixture was extracted with Et₂O, dried (Na₂SO₄), filtered and the solvent removed under reduced pressure. The residue was treated with activated charcoal and recrystallized from EtOH/H₂O affording 12.4 g (48%) yellow crystals mp 98-99°. Nmr (CDCl₃), δ 7.20-7.85 (m, 8H, aromatic), 9.90 (s, 1H, aldehydic proton), 10.95 (s, 1H, phenolic). Ir (CHCl₃) 1375, 1660, 2750, 2860, 3350 cm⁻¹.

Anal. calcd. for C₁₃H₁₀O₂: C, 78.77; H, 5.09. Found: C, 78.49; H, 5.02.

Ethyl 5-Phenyl-2-benzofurancarboxylate (11).- A mixture of 5-phenylsalicylaldehyde (30, 0.99 g, 0.005 mol), diethyl bromomalonate (31, 0.96 g, 0.004 mol), anhydrous K₂CO₃ (1.25 g, 0.009 mol) and 2-butanolone (20 ml) were refluxed for 10 hr. The solvent was removed under reduced pressure. The residue was cooled, poured into H₂O (100 ml) and extracted with Et₂O. The organic layer was washed with cold 5% NaOH solution, followed by H₂O, dried (Na₂SO₄), filtered and concentrated under reduced pressure. The solid residue was recrystallized from EtOH affording 0.95 g (73%) white crystals mp 109-110°. Ir (CHCl₃) 1730 cm⁻¹. Nmr (CDCl₃), δ 1.35 (t, 3H, CH₃), 4.38 (q, 4H, CH₂), 7.20-7.85 (m, 3H
aromatic, 1H vinylic).

**Anal. calcd. for C_{17}H_{14}O_{3}:** C, 76.69; H, 5.26. **Found:** C, 76.56; H, 5.44.

5-Phenyl-2-benzofurancarboxylic Acid (34).- Ethyl 5-phenyl-2-benzofurancarboxylate (11, 1.3 g, 0.0048 mol) was added to 50 ml of 10% ethanolic KOH and refluxed for 4 hr. The solvent was removed under reduced pressure and the residue was washed with Et$_2$O. The basic solution was acidified with dilute HCl and extracted with Et$_2$O. The Et$_2$O layer was washed with dilute NaHCO$_3$ solution. The aqueous layer was reacidified with dilute HCl and extracted with Et$_2$O. The Et$_2$O extract was dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure. The residue was crystallized from EtOH affording 1.1 g (95%) of a white solid mp 220-221. IR (KBr) 1690 cm$^{-1}$. NMR (DMSO-d$_6$), $\delta$ 7.20-8.15 (m, 8H aromatic, 1H vinylic), 12.35 (broad, 1H, carboxylic).

**Anal. calcd. for C$_{15}$H$_{10}$O$_3$:** C, 75.62; H, 4.23. **Found:** C, 75.73; H, 4.44.

5-Phenyl-2,3-dihydro-2-benzofurancarboxylic Acid (37).- 5-Phenyl-2-benzofurancarboxylic acid (34, 5.0 g, 0.021 mol) was added to 10% aqueous NaOH solution (90 ml). The sodium salt separated. Sodium amalgam, prepared from Na (1.5 g, 0.065 g-atom) and Hg (50 g, 0.25 g-atom), was added with stirring during one hr. The mixture was stirred for 24 hr and left to stand with the amalgam for an additional 24 hr.
The Hg was separated and the solution neutralized with dilute HCl and extracted with Et₂O. The Et₂O solution was washed with dilute NaHCO₃ solution. The aqueous portion was reacidified with dilute HCl, extracted with Et₂O, and the Et₂O solution was dried (Na₂SO₄), filtered and concentrated under reduced pressure. The residual solid was recrystallized from EtOH affording 4.8 g (95%) of white crystals mp 186-187. Nmr (DMSO-d₆), δ 2.90-3.80 (m, 2H, CHg), 4.90-5.40 (dd, 1H, methine), 6.0-6.5 (broad, 1H, carboxylic), 6.72-7.90 (m, 8H, aromatic).


Ethyl 5-Phenyl-2,3-dihydro-2-benzofurancarboxylate (8). -
5-Phenyl-2,3-dihydro-2-benzofurancarboxylic acid (37, 2.5 g, 0.01 mol) was dissolved in absolute EtOH (60 ml) and dry benzene (20 ml) in the presence of concentrated H₂SO₄ (2 ml) and refluxed for 15 hr. A Dean-Stark trap was used to remove H₂O. The solution was concentrated, cooled, poured into H₂O and extracted with Et₂O. The Et₂O layer was washed with cold 5% NaHCO₃ and cold H₂O. The aqueous portion was reextracted with Et₂O and the combined Et₂O extracts were dried (Na₂SO₄), filtered and concentrated under reduced pressure. The resulting oil was distilled affording 2.65 g (95%) colorless liquid bp 180-181° (0.35 mm). Nmr (CDCl₃), δ 1.16 (t, 3H, CH₃), 3.18-3.48 (m, 2H, benzylic
CH\(_2\), 4.12 (q, 2H, ester CH\(_2\)), 5.05 (dd, 1H, methine),
6.70-7.55 (m, 8H, aromatic).

Anal. calcd. for C\(_{17}\)H\(_{16}\)O\(_3\): C, 76.10; H, 6.01. Found:
C, 76.46; H, 6.09.

Attempted hydrogenation of Ethyl 5-Phenyl-2-benzofuran-
carboxylate (11).- Ethyl 5-phenyl-2-benzofuran carboxylate
(11, 0.65 g, 0.003 mol) was dissolved in glacial HOAc (15
ml) to which 10% Pd-C (50 mg) was added. The mixture was
shaken at 60° under H\(_2\) at 50 psi for 5 hr. After cooling,
the solution was filtered and the solvent was removed under
reduced pressure. The residue was dissolved in Et\(_2\)O, washed
with 5% NaHCO\(_3\) solution, dried (Na\(_2\)SO\(_4\)), filtered, and con­
centrated under reduced pressure. Nmr analysis indicated
the residue to be a mixture consisting of ethyl 5-cyclohexyl-
2,3-dihydro-2-benzofuran carboxylate and ethyl 5-phenyl-
2,3-dihydro-2-benzofuran carboxylate (8).

Ethyl 2-Methyl-5-chloro-2,3-dihydro-2-benzofuran carboxy-
late (12).- Diisopropylamine (1.01 g, 0.01 mol) dissolved
in dry THF (7 ml) was cooled to 0° under dry N\(_2\), and n-BuLi
in hexane (4.6 ml, 0.01 mol of a 2.2 M solution) was added.
After mixing for 15 min, at 0°. The solution (containing
approximately 0.4 M Li diethylamide) was cooled in a dry
ice-acetone bath and ethyl 5-chloro-2,3-dihydro-2-benzo-
furan carboxylate (7, 2.26 g, 0.01 mol), dissolved in dry
THF (25 ml), was added dropwise. The reaction mixture was
stirred for 15 min, and CH$_3$I (4.2 g, 0.03 mol), diluted in dry THF (10 ml), was added. The reaction mixture was warmed to room temperature and stirred for 2 hr and subsequently terminated by dropwise addition of H$_2$O. The mixture was extracted with Et$_2$O and the Et$_2$O layer was dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure. The residual oil was distilled affording a colorless liquid bp 110-112° (0.20 mm). Nmr analysis showed the product to be a mixture of 60% 7 and 40% 12. Tlc showed similar R$_f$ values for compounds 7 and 12. Column chromatography using a long silica-gel column and Et$_2$O:Pet-ether (1:2) yielded a mixture of 33% 7 and 66% 12. Nmr (CDCl$_3$), $\delta$ 1.26 (t, 3H, ester CH$_3$), 1.66 (s, 3H, CH$_3$), 3.12-3.80 (m, 2H, benzylic CH$_2$), 4.22 (q, 2H, ester CH$_2$), 6.70-7.55 (m, 3H, aromatic).

Ethyl Salicylate (55) was prepared by Fisher esterification of salicylic acid (52), bp 55-56° (0.02 mm). Lit$^{314}$ 104-108 (12 mm).

Ethyl 2-Carbethoxyphenoxoacetate (58).- A procedure described by Armarego$^{317}$ was used in which ethyl salicylate (55, 8.2 g, 0.05 mol) in dry acetone$^{316}$ (50 ml), ethyl bromoacetate (9.5 g, 0.06 mol) and anhydrous K$_2$CO$_3$ (25 g, 0.18 mol) were refluxed for 6 hr. After cooling, the acetone solution was filtered and concentrated under reduced pressure. The residual oil was dissolved in Et$_2$O and washed with cold dilute NaOH solution and H$_2$O, dried (Na$_2$SO$_4$),
filtered, and the solvent removed under reduced pressure. Distillation of the resulting liquid afforded 11.1 gm (90%) of bp 125-126° (0.05 mm) Lit\textsuperscript{317} bp 190-193° (25 mm). Nmr (CDCl\textsubscript{3}), $\delta$ 1.12-1.56 (m, 6H, 2 CH\textsubscript{3} groups), 4.04-4.62 (n, 4H, 2 ester methylene groups), 4.74 (s, 2H, O-CH$_2$-O) 6.87-7.80 (m, 4H, aromatic).

2-Carbethoxyphenoxyacetate-3(2H)-benzofuranone (13) was prepared by the method described by Schroeder and co-workers.\textsuperscript{319} Ethyl 2-carbethoxyacetate (58, 7.5 g, 0.03 mol) was added dropwise with stirring to NaOEt (0.03 mol) in 40 ml of dry benzene.\textsuperscript{316} The reaction solidified in approximately 20 min; 50 ml of absolute EtOH was added and stirring was continued with refluxing for 4 hr. After cooling, the reaction mixture was poured into H$_2$O and made alkaline to litmus with dilute NaOH solution. The organic layer was removed and dilute HCl was added to the aqueous portion, which was subsequently extracted with Et$_2$O. The Et$_2$O layer was dried (Na$_2$SO$_4$), filtered and the solvent was removed under reduced pressure. Upon cooling, a solid formed which was recrystallized from EtOH yielding crystals mp 60-61°. Lit mp 60-62°\textsuperscript{319} and 66°\textsuperscript{294}. Nmr (CDCl\textsubscript{3}), $\delta$ 1.30-1.56 (t, 3H, CH\textsubscript{3}) 4.24-4.78 (q, 2H, CH\textsubscript{2}), 6.92-7.70 (m, 5H, 4 aromatic and 1 methine).

Ethyl 5-Chlorosalicylate (56) was prepared by Fisher esterification of 5-chlorosalicylic acid (53), bp 78-79°
Ethyl-4-Chloro-2-carbethoxyphenoxyacetate (59).- A procedure described by Armarego\textsuperscript{317} was followed in which ethyl bromoacetate (25.05 g, 0.15 mol) was added to a solution of anhydrous K\textsubscript{2}CO\textsubscript{3} (62 g, 0.45 mol) and ethyl 5-chlorosalicylate (56, 25.07 g, 0.125 mol) in dry acetone\textsuperscript{316} (200 ml). The reaction mixture was refluxed for 15 hr, cooled, filtered and the acetone was removed under reduced pressure. The residue was dissolved in Et\textsubscript{2}O, and the Et\textsubscript{2}O solution was washed with cold dilute NaOH solution followed by H\textsubscript{2}O, dried (Na\textsubscript{2}SO\textsubscript{4}) and concentrated under reduced pressure. The resulting solid was recrystallized from EtOH affording 27.9 g (78%) of product 59, mp 52-53°. Lit\textsuperscript{315} mp 43-45°. Nmr (CDCl\textsubscript{3}), δ 1.10-1.55 (2 triplets, 6H, 2 CH\textsubscript{3}) 4.02-4.60 (2 quartets, 4H, 2 CH\textsubscript{2}) 4.75 (s, 2H, OCH\textsubscript{2}C), 6.80-7.82 (m, 3H, aromatic).

Ethyl 5-Chloro-2-carbethoxy-3(2H)-benzofuranone (14).- Ethyl 4-chloro-2-carbethoxyphenoxyacetate (59, 8.58 g, 0.03 mol), dissolved in dry benzene (40 ml),\textsuperscript{316} was added dropwise with stirring to NaOEt (2.1 g, 0.03 mol) in 40 ml of dry benzene. Stirring was continued under reflux for 16 hr. After cooling to room temperature, the reaction mixture was poured, with stirring, into H\textsubscript{2}O (200 ml) and made alkaline to litmus with dilute NaOH solution. The aqueous layer was made acidic with dilute HCl and extracted with Et\textsubscript{2}O. The
organic layer was dried (Na$_2$SO$_4$), filtered and the solvent removed under reduced pressure. The resulting solid was recrystallized from EtOH affording 6.4 g (88%) of white crystals, mp 128-129°. Lit$^{319}$ mp 126-127°. Nmr (CDCl$_3$), $\delta$ 1.28-1.58 (t, 3H, CH$_3$), 4.25-4.68 (q, 2H, CH$_2$), 7.10-7.74 (m, 3H aromatic and 1H methine).

**Ethyl 5-Chloro-3-(phenylhydrazone)-2-benzofurancarboxylate.** Ethyl 5-chloro-2-carbethoxy-3(2H)-benzofuranone (14, 0.72 g, 0.003 mol) was refluxed with phenylhydrazine (0.33 g, 0.003 mol) and concentrated HCl (2 drops) in absolute EtOH (20 ml) for 4 hr. The cooled reaction mixture was poured into H$_2$O and extracted with Et$_2$O. The organic layer was washed with 25 ml portions of saturated NaHCO$_3$ solution, H$_2$O, dried (Na$_2$SO$_4$), filtered and solvent was removed under reduced pressure. Recrystallization from EtOH afforded 0.45 g (45%) crystals, mp 135-136°. Nmr (CDCl$_3$), $\delta$ 1.43 (t, 3H, CH$_3$), 4.45 (q, 2H, CH$_2$), 6.04 (s, 1H, NH), 6.8-7.7 (m, 8H, aromatic), 8.03 (s, 1H, methine).

5-Phenylsalicylic Acid (54) was prepared according to the method described by Vorozhtsov, et al.$^{313}$ 4-Phenylphenol (39, 17 g, 0.1 mol) was mixed thoroughly in a mortar with anhydrous K$_2$CO$_3$ (69 g, 0.5 mol) and transformed to a stainless steel bomb. CO$_2$ (30 atm) was added through an inlet and the mixture was heated in an oven at 200° for 5 hr with intermittent shaking of the bomb. After cooling,
the pressure was slowly released and the content was dissolved in H$_2$O and washed with Et$_2$O. The aqueous portion was acidified with HCl, extracted with Et$_2$O, and the Et$_2$O extract was dried (Na$_2$SO$_4$), and concentrated under reduced pressure affording 19.3 g (90%) solid which was recrystallized from petroleum ether/benzene affording crystals mp 214-215°, Lit$^{313}$ mp 215-216°. Nmr (d$_6$-acetone), $\delta$ 6.75-8.24 (m, 8H, aromatic), 10.22-10.80 (wide multiplet, 2H, acidic and phenolic protons).

**Ethyl 5-Phenylsalicylate (57).** - 5-Phenylsalicylic acid (54, 10.7 g, 0.05 mol), absolute EtOH (200 ml), toluene (80 ml), and concentrated H$_2$SO$_4$ (4 ml) were refluxed for 20 hr using a Dean-Stark trap to remove H$_2$O. After cooling to room temperature, the excess solvent was removed under reduced pressure and the residue was poured into cold H$_2$O and extracted with Et$_2$O. The organic layer was washed with 5% cold NaHCO$_3$ solution, H$_2$O, dried (Na$_2$SO$_4$), filtered and the solvent was removed under reduced pressure. The residual oil solidified on standing and was recrystallized from EtOH affording 7.3 g (60%) white crystals mp 50-51°. Nmr (CDCl$_3$), $\delta$ 1.30 (t, 3H, CH$_3$), 4.30 (q, 2H, CH$_2$), 6.48-8.12 (m, 8H, aromatic), 10.78 (s, 1H, phenolic).

Anal. calcd. for C$_{15}$H$_{14}$O$_3$: C, 74.76; H, 5.83. Found: C, 74.37; H, 6.05.

**Ethyl 4-Phenyl-2-carbethoxyphenoxyacetate (60).** - A pro-
procedure described by Armarego was followed in which ethyl 5-phenylsalicylate (57, 6.05 g, 0.03 mol), ethyl bromoacetate (4.6 g, 0.03 mol), and anhydrous K$_2$CO$_3$ (13.8 g, 0.1 mol) were refluxed in dry acetone (30 ml) for 20 hr. After cooling, the acetone solution was filtered from the inorganic salt and the solvent was removed under reduced pressure. The residual oil was extracted (Et$_2$O) and the Et$_2$O layer washed with cold dilute NaOH solution and H$_2$O. The Et$_2$O layer was dried (Na$_2$SO$_4$), and the solvent was removed under reduced pressure. The resulting oil solidified on cooling and was recrystallized from EtOH affording 5.6 g (68%) crystals mp 53-54°. Nmr (CDCl$_3$), $\delta$ 1.08-1.50 (m, 6H, 2 CH$_3$), 4.02-4.58 (m, 4H, 2 ester CH$_2$), 4.70 (s, 2H, O-CH$_2$-C-), 6.80-8.10 (m, 8H, aromatic).

Anal. calcd. for C$_{19}$H$_{20}$O$_5$: C, 69.51; H, 6.09. Found: C, 69.69; H, 6.21.

Ethyl 5-Phenyl-2-carbethoxy-3(2H)-benzofuranone (15).- A method utilized by Schroeder and co-workers was used in which ethyl 4-phenyl-2-carbethoxyphenoxyacetate (60, 3.28 g, 0.01 mol) was dissolved in dry benzene (50 ml) and added dropwise with stirring to NaOEt (0.23 g, 0.01 g-atoms of Na in 15 ml dry EtOH). The mixture was refluxed for 10 hr, cooled, poured into cold H$_2$O, acidified with dilute HCl, and extracted with Et$_2$O. The organic layer was dried (Na$_2$SO$_4$), filtered and the solvent was removed under
reduced pressure. The resultant solid was recrystallized from EtOH affording 1.8 g (68%) crystals mp 129.5-130.5°. Nmr (CDCl₃), δ 1.42 (t, 3H, CH₃), 4.48 (q, 2H, CH₂), 7.05-8.10 (m, 8H aromatic and 1H methine).

Anal. calcd. for C₁₇H₁₄O₄: C, 72.34; H, 4.96. Found: C, 72.22; H, 5.15.

Attempted Reduction of Ethyl 5-Phenyl-2-carbethoxy-3(2H)-benzofuranone (15) Under Clemmensen Reduction Conditions.- A mixture of 12.5 g of mossy Zn and 1.25 g of Hg₂Cl₂ was used to prepare the Zn(Hg) according to the method described by Martin.362 To the freshly prepared Zn(Hg) was added concentrated HCl (1.50 ml) and H₂O (15 ml). The solution was stirred for 10 min and the supernatant was decanted. Concentrated HCl (20 ml), H₂O (20 ml) and ethyl 5-phenyl-2-carbethoxy-3(2H)-benzofuranone (15, 1.8 g, 0.006 mol) were added to the residue. The resulting mixture was stirred at room temperature for 24 hr and 18% HCl (50 ml) was added with stirring. After stirring at room temperature for additional 20 hr, 50 ml of 18% HCl was added and the mixture was heated on a steam bath for 2 hr. Finally, the mixture was heated at reflux for 1.5 hr. After cooling to room temperature, the liquid was decanted from the Zn(Hg). The Zn(Hg) and the aqueous mixture was extracted with Et₂O and the combined Et₂O solutions were washed with saturated NaHCO₃ solution. The aqueous layer was acidified with
dilute HCl, extracted (Et$_2$O), dried (Na$_2$SO$_4$), and concentrated under reduced pressure. Recrystallization from EtOH, afforded 0.07 g (5%) of white solid mp 184-186°. Nmr (acetone-d$_6$), $\delta$ 2.90-3.30 (m, 2H, benzylic), 5.10-5.40 (dd, 1H, methine) 6.80-7.70 (m, 8H, aromatic), 10.95 (broad, 1H, carboxyl).

**Ethyl ω-(4-Chloro-2-carbethoxyphenoxy)propionate** (66).- A mixture of ethyl 5-chlorosalicylate (56, 10 g, 0.05 mol), ω-bromopropionate (65, 11 g, 0.06 mol) and anhydrous K$_2$CO$_3$ (25 g, 0.18 mol) in dry acetone (100 ml) was refluxed for 15 hr. The cooled reaction mixture was filtered and the solvent was removed under reduced pressure. The residue was dissolved in Et$_2$O, and the Et$_2$O solution was washed with 5% NaOH solution followed by H$_2$O, dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure. Distillation of the resulting oily residue afforded 14.7 g (98%) of ethyl ω-(4-chloro-2-carbethoxyphenoxy)propionate (66) bp 135-136° (0.13 mm). Nmr (CDCl$_3$), $\delta$ 1.19 (t, 3H, O-CH(CH$_3$)CO$_2$CH$_2$CH$_3$), 1.34 (t, 3H, Phenyl-CO$_2$CH$_2$CH$_3$), 1.60 (d, 3H, propyl CH$_3$), 4.18 (q, 2H, O-CH(CH$_3$)CO$_2$CH$_2$CH$_3$), 4.33 (q, 2H, Phenyl-CO$_2$CH$_2$CH$_3$) 4.76 (q, 1H, methine), 6.8-7.8 (m, 3H, aromatic).

Anal. calcd. for C$_{14}$H$_{17}$ClO$_5$: C, 55.9; H, 5.7; Cl, 11.8. Found: C, 56.0; H, 5.7; Cl, 11.9.

5-Chloro-2-acetylbenzofuran (16).- A mixture of
5-chlorosalicylaldehyde (29, 5.75 g, 0.036 mol), freshly distilled chloroacetone (67, 4.35 g, 0.048 mol), and anhydrous K$_2$CO$_3$ (12 g, 0.09 mol) in 2-butanone (60 ml) was refluxed for 12 hr. The solvent was removed and the residue was poured into H$_2$O and extracted with Et$_2$O. The Et$_2$O solution was dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The dark-colored residue was dissolved in a minimum amount of EtOH; petroleum ether (30-60°) was added slowly until the solution started to become cloudy. The cloudy solution was allowed to stand for 12 hr and the supernatant was decanted and concentrated under reduced pressure. The resulting white solid was recrystallized from absolute EtOH affording 4.2 g (60%) crystals mp 96-97.0. Nmr (CDCl$_3$), $\delta$ 2.58 (s, 3H, CH$_3$), 7.20-7.74 (m, 3H aromatic, 1H vinylic).

Anal. calcd. for C$_{10}$H$_7$ClO$_2$: C, 61.71; H, 3.62; Cl, 18.21. Found: C, 61.58; H, 3.67; Cl, 17.94.

Ethyl (5-chloro-2-benzofuranyl)acetate (19) was prepared according to the procedure of Bisagni and Royer. A mixture of 5-chloro-2-acetylbenzofuran (16, 1.95 g, 0.01 mol), sulfur (0.5 g, 0.015 g-atom), and morpholine (1.3 g, 0.015 mol) were refluxed for 8 hr. The reaction mixture was cooled and diluted with HCl. After 18 hr the supernatant was decanted and the residue heated 6 hr with 10% ethanolic NaOH solution. The mixture was diluted with H$_2$O, acidified
with dilute HCl and extracted with Et$_2$O. The organic layer was dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure, affording crude 19. Nmr (CDCl$_3$), $\delta$ 3.83 (s, 2H, CH$_2$), 6.90-7.70 (m, 3H aromatic, 1H vinylic), 8.23 (broad, 1H, carboxylic).

5-Chloro-2,3-dihydro-2-acetylbenzofuran (17).- Methyl lithium (12 ml, 0.021 mol of a 1.8 M Et$_2$O solution) diluted with dry Et$_2$O was added dropwise to a stirring solution of 5-chloro-2,3-dihydro-2-benzofurancarboxylic acid (36, 1.5 g, 0.0075 mol) dissolved in 20 ml dry Et$_2$O, in a 3-necked round-bottom flask which is equipped with a magnetic stirrer, condenser, dropping funnel under N$_2$ atmosphere. As CH$_3$Li was added, CH$_4$ gas was evolved. Initially the acid precipitates out of Et$_2$O solution as the lithium salt. However, as the reaction progresses, and the reaction mixture is heated the acid returns to solution. After gentle refluxing for 6 hr, the mixture was cooled, poured over ice, neutralized with dilute HCl and extracted with Et$_2$O. The Et$_2$O layer was washed with saturated NaHCO$_3$ solution, H$_2$O, dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The residual oil upon distillation, bp 110-111$^\circ$ (1.35 mm), afforded 1.0 g (70%) colorless oil 17. Nmr (CDCl$_3$), $\delta$ 2.16 (s, 3H, CH$_3$), 2.84-3.70 (m, 2H, CH$_2$), 4.90 (dd, 1H, methine), 6.5-7.1 (m, 3H, aromatic).

Reaction of Ethyl 5-Chlorosalicylate (59) with $\beta$-Bromo-
Y-butyrolactone (40) in the Presence of Anhydrous K₂CO₃.- Ethyl 5-chlorosalicylate (50, 10 g, 0.05 mol) and occ-bromo-
Y-butyrolactone (40, 9.9 g, 0.06 mol) were refluxed in the
presence of anhydrous K₂CO₃ (20 g, 0.15 mol) and dry ace-
tone (100 ml) for 6 hr. The cooled reaction mixture was
filtered and the precipitate was washed with acetone. The
organic solution was concentrated under reduced pressure,
cooled poured into cold H₂O (solution A) and washed with
Et₂O (solution B).

A. 2-(2-Carbethoxy-4-chlorophenoxy)-4-hydroxybutanoic
acid.- The aqueous layer was acidified with dilute HCl, and
extracted with Et₂O. The Et₂O layer was dried (Na₂SO₄),
filtered and concentrated under reduced pressure. The re-
sulting solid was recrystallized from EtOH/Pet-ether afford-
ing 3.7 g (26%) white solid, mp 122-123. Nmr (DMSO-d₆),
1.15 (t, 3H, CH₃), 1.50-2.10 (m, 2H, CH₂), 3.33-3.68 (m,
2H, CH₂OH), 4.16 (q, 2H, ester CH₂) 4.75 (t, 1H, methine
proton), 6.70-7.55 (m, 3H, aromatic), 8.90 (broad, 2H,
carboxyl and hydroxyl protons).

Anal. calcd. for C₁₃H₁₀ClO₆:  C, 51.57; H, 4.97; Cl,
11.59. Found:  C, ; H, 5.04; Cl, 11.87.

B. occ-[4-Chloro-2-carbethoxyphenoxy]-Y-butyrolactone.-
The Et₂O layer was washed twice with cold 10% NaOH solution
(solution C). The Et₂O layer was dried (Na₂SO₄), filtered
and solvent removed under reduced pressure, affording 1.5 g
(11%) of oil. Ir (neat) 1730, 1780 cm⁻¹. Nmr (CDCl₃),
δ 1.37 (t, 3H, CH₃), 2.23-2.85 (m, 2H, β-CH₂), 4.34 (q, 2H, ester CH₂), 4.15-4.80 (m, 2H, γ-CH₂), 5.05 (t, 1H, α-CH), 7.03-7.95 (m, 3H, aromatic).

c. α-[4-Chloro-2-carboxyphenoxy]-γ-butyrolactone.-
The NaOH solution was acidified with dilute HCl and extracted with Et₂O, dried (Na₂SO₄), filtered and the solvent was removed under reduced pressure. The resulting solid was recrystallized from EtOH-Petroleum ether (30-60°) affording 6.1 g (48%) white crystals mp 157-158°. IR (nujol) 1715, 1780 cm⁻¹. Nmr (DMSO-d₆), δ 2.13-2.75 (m, 2H, β-CH₂), 4.20-4.57 (m, 2H, γ-CH₂), 5.40 (dd, 1H, α-CH), 7.30-7.80 (m, 3H, aromatic), 12.8 (broad, 1H, carboxylic).

Anal. calcd. for C₁₁H₉ClO₅: C, 51.47; H, 3.53; Cl, 13.81. Found: C, 51.07; H, 3.66; Cl, 13.49.

α-[4-Chloro-2-carbethoxyphenoxy]-γ-butyrolactone.-
α-[4-Chloro-2-carbethoxyphenoxy]-γ-butyrolactone (4 g, 0.016 mol), dissolved in absolute EtOH (100 ml) and dry benzene (50 ml), was refluxed for 10 hr in the presence of concentrated H₂SO₄ (8 ml). A Dean-Stark trap was used to remove H₂O. After removing the excess EtOH and benzene, the cooled residue was poured into H₂O and extracted with Et₂O. The organic layer was washed with saturated NaHCO₃ solution followed by H₂O, dried (Na₂SO₄), filtered, and the solvent was removed under reduced pressure. The residue was distilled affording 3.60 g (85%) of colorless oil bp 190-191°.
(0.25 mm). Ir (neat) 1730, 1780 cm\(^{-1}\). Nmr (CDCl\(_3\)), \(\delta\) 1.35 (t, 3H, CH\(_3\)), 2.25-2.90 (m, 2H, \(-\beta-\text{CH}_2\)), 4.34 (q, 2H, ester CH\(_2\)), 4.10-4.75 (m, 2H, \(-\gamma-\text{CH}_2\)), 5.03 (t, 1H, \(-\alpha-\text{CH}\)), 7.10-7.85 (m, 3H, aromatic).

2-Bromo-1-(2\'-tetrahydropyranyl)hydroxyethane (47).- Dihydropyran (9.3 g, 0.11 mol) was added slowly to 2-bromoethanol (46, 12.5 g, 0.1 mol) in the presence of a catalytic amount of \(p\)-toluenesulfonic acid at 0\(^\circ\). The reaction mixture was warmed to room temperature and stirred for one hr. Distillation [bp 67-68\(^\circ\) (0.70 mm)] of the mixture afforded 18.8 g (90\%) of a colorless liquid. Nmr (neat), \(\delta\) 1.3-1.9 (6H, m, 3 tetrahydropyran methylene groups remote to the ring oxygen), 3.38-4.22 (6H, m, 2 methylene groups of the alkyl chain and one methylene adjacent to the tetrahydropyran ring), and 4.56-4.72 (1H, m, methine proton).

Anal. calcd. for C\(_7\)H\(_{13}\)O\(_2\)Br: C, 40.21; H, 6.27; Br, 38.23. Found: C, 40.50; H, 6.37; Br, 38.61.

5-Chloro-3-(2'-hydroxyethoxy)-2-benzofurancarboxylic Acid (61).- NaH emulsion (1.1 g of 57\% NaH in mineral oil, 0.022 mol of NaH) purchased from Ventron Chemical Co. was washed with n-hexane and transferred with dry diglyme into a 3-necked flask fitted with a \(N_2\) inlet, condenser, and a dropping funnel. To this solution was added dropwise ethyl 5-chloro-2-carbethoxy-3(2H)-benzofuranone (14, 4.1 g, 0.02
mol) dissolved in diglyme. The mixture was heated with stirring to 75° and 2-bromo-1-(2'-tetrahydropyranyl)hydroxy] ethane (47, 6.32 g, 0.03 mol) was added dropwise. The mixture was heated at 150° for 6 hr. The solution was cooled to room temperature, the solvent removed under reduced pressure and the residue poured into H_2O (100 ml) and extracted with Et_2O. The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure. Glpc showed 3 unresolved peaks which could not be separated by column chromatography. Therefore, the mixture was treated with 10% ethanolic KOH at reflux for 1 hr. After cooling, the mixture was acidified with 25% H_2SO_4 and refluxed for 10 min. The solution was cooled to room temperature, extracted with Et_2O, dried (Na_2SO_4), filtered, and the solvent removed under reduced pressure. The residue was recrystallized from Et_2O, affording 2.66 g (51.9%) crystals, mp 192-193°. Nmr (d_6-acetone), 6 3.87 (t, 2H, CH_2CH_2OH), 4.55 (t, 2H, -OCH_2CH_2OH), 6.20 (broad, 2H, acidic, hydroxyl), 7.50-7.95 (m, 3H, aromatic). Principle ir bands at v_{max}^{nujol} 3260, 1680 cm^{-1}.

Anal. calcd. for C_{11}H_{9}O_{5}Cl: C, 51.48; H, 3.53; Cl, 13.81. Found: C, 51.53; H, 3.79; Cl, 13.74.

9-Chloro-2,3-dihydro-5H-1,4-dioxepino[6,5-b]benzofuran-5-one (21).- 5-Chloro-3-(2'-hydroxyethoxy)-2-benzofuran-carboxylic acid (61, 2.57 g, 0.1 mol) was dissolved in dry
benzene (200 ml). P-TsOH (200 mg) was added and the solution was refluxed for 1.5 hr under N\textsubscript{2} using a Dean-Stark apparatus to remove H\textsubscript{2}O. The solution was cooled to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in Et\textsubscript{2}O and the Et\textsubscript{2}O solution was washed with saturated NaHCO\textsubscript{3} solution followed by H\textsubscript{2}O. The Et\textsubscript{2}O extract was dried (Na\textsubscript{2}SO\textsubscript{4}), filtered, and concentrated under reduced pressure affording 2.1 g (90\%) crystals mp 201-202\(^\circ\). Nmr (d\textsubscript{6}-DMSO), \(\delta\) 4.50-4.90 (m, 4H, lactone methylenes), 7.40-7.95 (m, 3H, aromatic). Principle ir band at \(\nu_{\text{max}}^{\text{nujol}}\) 1715 cm\(^{-1}\). Mass spectrum (70 eV) m/e (rel. intensity) 138 (38.1), 140 (13.4), 167 (100), 169 (33.8), 238 (82.2), 240 (29.3). UV max isoctane:ethanol (95:5) = 229 (\(\epsilon\) = 54154).

Anal. calcd. for C\textsubscript{11}H\textsubscript{12}O\textsubscript{3}Cl: C, 55.35; H, 2.95; Cl, 14.85. Found: C, 55.34; H, 3.19; Cl, 15.03.

Methyltriphenylphosphonium Iodide (69) was prepared according to the procedure described by Wittig and Schoellkopf. A solution of triphenylphosphine (55 g, 0.21 mol), dissolved in dry benzene (45 ml),\textsuperscript{116} was placed in a pressure bottle. The bottle was cooled in an ice-salt mixture and CH\textsubscript{3}I (28 g, 0.29 mol) was added. The bottle was sealed, allowed to stand at room temperature for 2 days, and then opened. The solid was collected by means of suction filtration (with aid of hot benzene, 500 ml) and dried
in a vacuum oven at 100° over P₂O₅. White solid (74 g, 99%) mp 185-186° was isolated.

Formylmethylenetriphenylphosphorane (70) was prepared according to the method of Trippett and Walker in 60% yield. Mp (from acetone) 185-187° (decomp); Lit mp 186-187° (decomp).

Ethyl 4-Chloro-2-formylphenoxyacetate (71).- 5-Chlorosalicylaldehyde (29, 7.83 g, 0.05 mol) dissolved in dry benzene (50 ml) was added dropwise under N₂ to NaOEt (3.74 g, 0.055 mol). After the formation of the phenoxide salt, ethyl bromoacetate (9.2 g, 0.055 mol) was added dropwise and the solution was refluxed for 6 hr. The reaction mixture was cooled and concentrated under reduced pressure. The residue was poured into H₂O and extracted with Et₂O. The organic layer was washed with cold 10% NaOH solution, H₂O, dried (Na₂SO₄), filtered and the solvent removed under reduced pressure. Distillation afforded 7.2 g (60%) of a viscous oil, bp 139-140° (0.020 mm), which solidified on standing. The resulting solid was recrystallized from EtOH affording white crystals mp 52-53°. Ir (neat) 2725, 2830, 1740, 1685 cm⁻¹. Nmr (CDCl₃), δ 1.28 (t, 3H, CH₃), 4.27 (q, 2H, ester CH₂) 4.75 (s, 2H, O-CH₂), 6.80-7.85 (m, 3H, aromatic), 10.54 (s, 1H, aldehydic).

Anal. calcd. for C₁₁H₁₁ClO₄: C, 54.53; H, 4.54; Cl, 14.69. Found: C, 54.23; H, 4.51; Cl, 15.08.
(Z)-Ethyl [4-Chloro-2-(3-oxo-1-propenyl)phenoxy]acetate (72).- A solution of ethyl 4-chloro-2-formylphenoxyacetate (71, 1.7 g, 0.007 mol) and formylmethylenetriphenylphosphorane (70, 2.28 g, 0.0075 mol) in dry benzene (50 ml) was refluxed for 24 hr; the solvent was removed under reduced pressure and the residue extracted with Et₂O. The Et₂O layer was allowed to stand overnight during which time the triphenylphosphine oxide (1.45 g, mp 154-155°) precipitated. Evaporation of the supernatant Et₂O solution afforded a solid which after recrystallization from EtOH yielded 1.5 g (77%) white crystals mp 58-59°. Nmr (CDCl₃), 6 1.31 (t, 3H, CH₃), 4.29 (q, 2H, ester CH₂), 4.72 (s, 2H, O-CH₂-CO₂), 6.75 (q, 2H, J = 8Hz, vinylic), 7.15-8.00 (m, 3H, aromatic), 9.68 (d, 1H, J = 7Hz, aldehydic).

Anal. calcd. for C₁₃H₁₃O₄Cl: C, 58.11; H, 4.88; Cl, 13.19. Found: C, 58.12; H, 5.06; Cl, 13.07.

(4bOc,4cOc,9bOc,9cOc)-Diethyl 3,8-dichlorocyclobuta-1,2-b:3,4-b'bisbenzofuran-4c,9c(4bH,9bH)-dicarboxylate (26).- Ethyl 5-chloro-2-benzofuranacarboxylate (10, 5.2 g, 0.023 mol) dissolved in 350 ml EtOH was irradiated using a 450-W Hanovia medium-pressure mercury arc and a water-cooled Vycor immersion well. Argon was passed through the solution during a 14 hr irradiation period. Compound 26 partly crystallized on the walls of the container. The crystals were separated and the solution was concentrated under reduced
pressure. The dimer 26 was selectively recrystallized from starting monomer 10 using absolute EtOH. The combined yield of dimer mp 165-166° was 2.6 g (50%). Nmr (CDCl₃), δ 1.33 (t, 6H, 2CH₃), 4.29 (q, 4H, 2CH₂), 4.67 (s, 2H, 2CH), 6.60-7.20 (m, 6H, aromatic). V_max (CHCl₃) 1735 cm⁻¹. Mass spectrum (70 eV) m/e (rel. intensity) 152 (15), 179 (28), 196 (42), 224 (100), 448 (1).

Anal. calcd. for C₂₂H₁₈O₆Cl₂: C, 58.81; H, 4.04; Cl, 15.78; Mol. wt. 448 g/m. Found: C, 58.56; H, 4.11; Cl, 15.45; Mol. wt. (rast Camphor) 441 g/m.

(4bOC, 4cOC, 9bOC, 9cOC)-3,8-Dichlorocyclobuta[1,2-b: 3,4-b']bisbenzofuran-4c,9c(4bH,9bH)-dicarboxylic acid (78).- 4bOC, 4cOC, 9bOC, 9cOC-Diethyl 3,8-dichlorocyclobuta[1,2-b: 3,4-b']bisbenzofuran-4c,9c(4bH,9bH)-dicarboxylate (26, 1.14 g, 0.003 mol) in 10% ethanolic KOH (30 ml) was refluxed for 2 hr. The solution was concentrated, cooled, poured into H₂O, and washed with Et₂O. The aqueous layer was acidified with dilute HCl and extracted with Et₂O. The Et₂O layer was dried (Na₂SO₄), filtered and concentrated under reduced pressure. The residue was recrystallized from H₂O and dried under reduced pressure (to remove entrapped Et₂O) thereby affording 1.03 g (95%) of pure white solid mp 252-254°. Nmr (DMSO-d₆), δ 4.78 (s, 2H, cyclobutane protons), 6.65-7.34 (m, 6H, aromatic), 13.15 (broad, 2H, carboxylic). Mass spectrum (70 eV) m/e (rel. intensity) 179 (17.3), 196.
Anal. calcd. for C_{18}H_{10}Cl_{2}O_{6}: C, 54.98; H, 2.55; Cl, 18.06. Found: C, 54.76; H, 2.74; Cl, 17.90.

(4bOC, 4cOC, 9bOC, 9cOC)-3,8-Dichloro-4bH,9bH-4c,9c-
(methanoxymethano)cyclobuta-[1,2-b:3,4-b']bisbenzofuran-11,13-
dione (79).- (4bOC, 4cOC, 9bOC, 9cOC)-3,8-Dichloro-
cyclobuta[1,2-b:3,4-b']bisbenzofuran-4c,9c(4bH,9bH)-dicar-
boxylic acid (78, 0.39 g, 0.001 mol) was added to AC_{2}O (2 ml) and warmed to dissolve the acid. The solution was re-
fluaxed for 1 hr, the solvent was removed under reduced pres-
sure, and the residue was dissolved in Et_{2}O. The Et_{2}O so-
lution was washed with cold 5% NaHCO_{3} solution followed by
cold H_{2}O, dried (Na_{2}SO_{4}), filtered and concentrated under
reduced pressure to yield 0.094 g (25%) of white solid mp
231-233°; ir (nujol) 1820, 1740 cm^{-1}. Nmr (DMSO-d_{6}), \delta
4.68 (s, 2H, cyclobutane protons), 6.68-7.72 (m, 6H,
aromatic). Mass spectrum (70 eV) m/e (rel. intensity) 179
(100), 181 (35.8), 196 (12.3), 224 (12.9), 374 (2.6).

(4bOC, 4cOC, 9bOC, 9cOC)-Monophenyl 3,8-Dichlorocyclo-
buta[1,2-b:3,4-b']bisbenzofuran-4c,9c(4bH,9bH)-dicarboxylate
(81).- (4bOC, 4cOC, 9bOC, 9cOC)-3,8-Dichloro-4bH-9bH-4c,9c,
(methanoxymethano)cyclobuta-[1,2-b:3,4-b']bisbenzofuran-
11,13-dione (79, 0.175 g, 0.47 mmol) dissolved in 10 ml
absolute EtOH was added to sodium phenoxide (0.055 g, 0.47
mmol) prepared from phenol (0.045 g, 0.47 mmol) and NaOEt
(0.032 g, 0.48 mm). The solution was refluxed for 2 hr, concentrated, cooled and dissolved in Et₂O. The Et₂O layer was washed with 5% NaHCO₃ solution. The aqueous layer was acidified with dilute HCl, extracted with Et₂O, and the Et₂O extract was dried (Na₂SO₄), filtered and concentrated under reduced pressure affording 0.20 g (90%) of white solid, mp 238-240°. Nmr (DMSO-d₆), δ 4.60 (s, 2H, cyclobutane protons), 6.40-7.30 (m, 11H, aromatic), 8.60 (broad, 1H, carboxylic).

2,8-Dichloro-6,12-epoxy-6H,12H,Dibenzo[b,f][1,5]dioxocin (27).- 5-Chlorosalicylaldehyde (29, 3.2 g, 0.02 m) was refluxed in 2-butanone (30 ml) in the presence of anhydrous K₂CO₃ (8 g, 0.06 m) for 5 hr. The solvent was removed and the residue was poured into H₂O and extracted with Et₂O. The Et₂O layer was washed with cold 10% NaOH solution, dried (Na₂SO₄), filtered, and concentrated under reduced pressure. The residual solid was recrystallized from EtOH affording 1.0 g (35%) white crystals mp 173-173.5°. Nmr (CDCl₃), δ 6.23 (s, 2H, methine), 6.70-7.30 (m, 6H, aromatic). Mass spectrum (70 eV) m/e (rel. intensity) 126 (25), 154 (88), 231 (45) 249 (20), 259 (46), 265 (11), 294 (100).

Anal. calcd. for C₁₄H₈Cl₂O₃: C, 56.98; H, 2.71; Cl, 24.03. Found: C, 57.32; H, 3.08; Cl, 23.60.
B. BIOLOGICAL METHODS

(a) Hypolipemic Activity.- The compounds were tested in a hyperlipemic rat model in which the hyperlipemia was induced by ip injection of Triton WR-1339 (oxyethylated tertiaryoctylphenolformaldehyde polymer, Ruger Chemical Co., Philadelphia, Pa.). Male albino rats (Sprague-Dawley) were housed in groups of five and were fed Purina laboratory chow and water ad libitum for a 2 week stabilizing period. After this period, the rats were redistributed by weight into four experimental groups of ten rats each, housed in groups of five. At random two experimental groups, weighing 260-280 g per rat, were fasted for 24 hr and then injected ip with 225 mg Triton per kilogram dissolved in 0.15 M NaCl to give a concentration of 62.5 mg/ml. The two control groups of comparable weight were also fasted and received 2 ml only of the vehicle (0.25% aqueous methyl cellulose), whereas the remaining groups received test compounds in vehicle. Compounds were dispersed in the vehicle at concentrations of 0.0083 mmol/ml, providing a total screening dose of 0.125 mmol/Kg (for 270 + 10 g rats) in 4 ml. Each rat received two 2 ml doses of gastric intubation. The first dose was administered immediately after the Triton injection and the second dose was administered 20 hr later. Fasting was continued during the post-Triton period.

At 43 hr after Triton administration the rats were
anesthetized with ethyl ether; blood was drawn from the abdominal aorta and added to ethylenediaminetetraacetic acid (EDTA, 0.9 mg/ml); and plasma was obtained after centrifugation at 500 x g for 10 min. Plasma triglyceride was determined by the method of Eggstein; plasma cholesterol was analyzed by the method of Holub and Galli. Significant differences in the plasma cholesterol and triglyceride concentrations between drug-treated Triton hyperlipemic (group IV) and control (group I) as well as between drug-treated (group IV) and hyperlipemic control (group III) groups were determined by student t tests on logarithms of individual data to allow pooling of variances.

(b) Hydrolysis of 9-Chloro-2,3-dihydro-5H-1,4-dioxepino[6,5-b]benzofuran-5-one (21) by rat plasma in vitro.- The hydrolysis of 21 by rat plasma in vitro at 37° was determined according to the method of Barrett and Thorp.

Blood was collected from the abdominal aorta of anesthetized Sprague-Dawley rats (180 - 200 g) in Vacutainer tubes containing a small amount of EDTA. The blood was immediately centrifuged for 15 minutes in an IEC centrifuge and the supernatant plasma was separated and pooled. The lactone 21 was dissolved in DMSO to make a 0.05M solution.

Incubation System.- One tenth ml of the 0.05M solution of Lactone 21 in DMSO was added to 8 ml of rat plasma and the mixture was incubated at 37° on a Dubnoff metabolic
shaker. Samples (1.0 ml) were removed at various times (0, 5, 10, 30 and 60 minutes) for determination of the presence of parent ester.

**Assay Procedure.** - The 1.0 ml sample was placed in a 10 ml glass-stoppered centrifuge tube. One half ml of 3N HCl and 5.0 ml of an isooctane-absolute EtOH mixture (95:5, v:v) were added to the tube. The tube was stoppered, shaken by hand and allowed to stand. After the layers separated, the UV absorption of the extract was determined against a blank treated similar to that of the sample, but not containing lactone 21. An aliquot of this isooctane-EtOH extract was then treated with an equal volume of 2% NaHCO$_3$ solution to remove the free acid. The amount of free acid was determined by observing the reduction in the UV absorption of the organic phase. The wavelength used for the analysis of compound 21 was UV $\text{isooctane:EtOH (95:5) max } 229 (\lambda = 54154)$.

**Enzyme Induction Studies.** - Male Harlan Wistar rats (60 - 130 g) were given each drug dissolved in coconut oil (0.1-0.2 ml) at two dose levels (0.4 mmole/Kg or 0.8 mmole/Kg daily) twice daily for seven consecutive days. Phenobarbital (40 mg/Kg) was used as an internal control. All animals were allowed free access to food and water and were fasted overnight following the last dose. Experiments were initiated 14 - 16 hr after the final dose.
Liver Preparation.- Animals were sacrificed, the livers excised, weighed and homogenized with a glass-teflon homogenizer in 4 volumes of 0.02 M Tris-HCl buffer, pH 7.4, containing 1.15% KCl. The homogenates were centrifuged at 9000 x g for 20 min at 4° in a refrigerated centrifuge. The 9000 x g supernatant was carefully removed and recentrifuged at 105000 x g for 60 minutes in a Model L Beckman ultracentrifuge. The resulting microsomal pellet was resuspended in 0.02 M Tris-HCl-KCl buffer solution, pH 7.4, and stored on ice until further use.

Enzyme Assays.- Incubations were carried out with the microsomal fractions as follows: Reaction mixtures consisted of 150 μmoles Tris-HCl buffer, pH 7.4, a NADPH-generating system (2.4 μmoles NADP+, 2 E.U. glucose-6-phosphate dehydrogenase, 15 μmoles MgCl₂, and 15 μmoles glucose-6-phosphate), 5 mg microsomal protein and substrate in a final volume of 3.0 ml. Concentrations of substrates used were 10 μmoles aniline and 5 μmoles ethylmorphine. After the addition of the appropriate substrate, the reaction mixtures were incubated in a metabolic shaker at 37° with shaking.

Assay Methods.- Ethylmorphine N-demethylation was assayed by the method of Nash which was used to estimate the amount of formaldehyde formed from ethylmorphine. Hydroxylation of aniline to p-aminophenol was assayed by the method described by Kato and Gillette.

Microsomal
protein content was estimated by the method of Lowry, et al.\textsuperscript{368} Cytochrome P-450 was determined by the procedure of Omura and Sato.\textsuperscript{369} Statistical comparisons were made using the student "t" test.


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