Diuretic Agents

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FOREWORD

The ACS SYMPOSIUM SERIES was founded in 1974 to provide a medium for publishing symposia quickly in book form. The format of the SERIES parallels that of the continuing ADVANCES IN CHEMISTRY SERIES except that in order to save time the papers are not typeset but are reproduced as they are submitted by the authors in camera-ready form. As a further means of saving time, the papers are not edited or reviewed except by the symposium chairman, who becomes editor of the book. Papers published in the ACS SYMPOSIUM SERIES are original contributions not published elsewhere in whole or major part and include reports of research as well as reviews since symposia may embrace both types of presentation.

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PREFACE

Some scientists believe that man is a brain surrounded by cells of secondary significance while others are persuaded that the heart takes preeminence. Of course, neither is correct; man consists of two magnificent organs called kidneys which are imbedded in tissues of lesser importance. Seriously, the kidney provides a pervasive, homeostatic regulation of the entire internal environment of the body; more specifically, kidney function modulates electrolyte and fluid balances, the economy of nutrients and drugs, and the formation and elimination of metabolites. Because the kidney is involved either directly or indirectly with nearly every cellular process, the ability of this organ to function properly under conditions of stress, age, or disease becomes progressively more important and, ultimately often becomes the limiting factor in the survival of the body itself. Since water and electrolyte are the major constituents of terrestrial life, organs that control their transport are of vital importance.

Since the beginning of modern medicine, the discovery and use of drugs which control renal function have been a major goal. Only a small portion of what may be considered to be the full potential of this field has been realized. Future achievements in this area are certain to come, albeit with many birth pangs.

The first drugs developed for the control of renal function were the diuretics which also were among the first synthetic agents to be introduced into medicine. Although modern diuretic therapy is not yet three decades old, a series of prominent milestones mark its history, and this history is still being made. The outstanding and continuing record of the development of novel diuretic agents has greatly enhanced the progress of the basic science relating to kidney function. This fundamental knowledge has, in turn, permitted the discovery of even more unique diuretics. Most importantly, these drugs are as safe and sophisticated as any that are in our present medical armamentarium.

Mercurial agents constituted the first chemical class of the synthetic diuretics, and these drugs possessed many commendable attributes, including a good electrolyte excretion profile, high potency, and uricosuric activity. Within a few years, the carbonic anhydrase inhibitors were discovered which provided a more practical therapy. The advent of the thiazides opened the door to a deluge of structurally and functionally related sulfonamide diuretics. The loop diuretics, as represented by

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furosemide and ethacrynic acid, constituted the next major breakthrough. Finally, the antikaliuretic saluretics, i.e., aldactone, triamterene, and amiloride, entered the arena.

During their short history in medicine, the demand for and the use of diuretics has increased dramatically. It is estimated that the world-wide use of diuretics involved ten billion patient days in 1977 at a cost of $765 million. If the use of diuretics increases at its present rate, it will double within the next five or six years. Interestingly, the use of diuretics for the treatment of hypertension is growing at a faster rate than that for edema. Another obvious trend is the shift away from the use of potassium-losing diuretics to that of the antikaliuretic diuretics, either alone or in combination.

Research activity and the resultant major "breakthroughs" in the diuretic field have occurred sporadically and, if the current signs are valid, we are in the midst of a new phase of intense research activity. Although a number of safe and effective diuretics are available, they each have inherent deficiencies which impart to them a significant obsolescence liability. The most prevalent problems include hypokalemia, hyperglycemia, and hyperuricemia. In addition, most diuretics belong to a very small group of chemical or structural classes. As a result, they have similar mechanisms of action and side effects. The diuretics of the future must be structurally novel and mechanistically unique and, more importantly, they must obviate many of the side effects that are characteristic of the agents currently in use.

The rapid advances that have been made in the sciences which are basic to the understanding of water and electrolyte transport have permitted a more sophisticated approach to the design of new diuretics. It also has allowed the early examination of new leads for their site and mechanism of action. Progress in the biochemistry of electrolyte transport has been noteworthy in recent years, especially in regard to the role of renal hormones. Likewise, advances in micropuncture and a variety of other techniques have provided tools which are useful for the routine evaluation and recognition of truly novel renal agents.

It has been 15 years since deStevens' classic monograph (*1*) on the medicinal chemistry of diuretics appeared. Therefore, a symposium (*2*) was organized which was designed to review the progress that had occurred in the intervening years. Since the symposium was limited to only a one-half-day session, it was not possible to include reports from every institution where major discoveries had been made. The interest generated by the symposium suggested that the papers be published. In order to present a more complete picture of the innovative research in this field, both from the standpoint of the basic science and of the practical agents available at the clinical level, four more papers were added to those of the original symposium.

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Since the main purpose of this monograph was to describe the recent advances in the medicinal chemistry of diuretics, the emphasis was placed on the fundamental contributions of the medicinal chemists, i.e., drug design and structure–activity relationships. The authors of this monograph and the institutions that they represent have made particularly noteworthy contributions to renal research and many of them have been associated with a long history of outstanding work.

On the academic side, it seemed only appropriate to include the recent work on the prostaglandin and kallikrein–kinin systems. The role of these renal hormones is extremely complex and currently in a state of refinement, but it is of such importance that it demands consideration by all scientists involved in renal research.

Examination of the history and current status of the diuretic field not only permits but encourages extrapolation into the future. Such extrapolation suggests that significant diuretics which will emerge will have novel structures, unique mechanisms, and new sites of action. More importantly, new types of renal agents will be discovered which will provide for the prophylaxis and treatment of renal disorders where no drug is currently available.

Acknowledgments—Many people have contributed to the planning and programming of the diuretic symposium and to the writing of this book. The authors of each paper have been extremely cooperative and helpful, not only in providing their contribution, but in integrating their unit into the whole. Much editorial assistance and advice were provided by E. H. Blaine, M. G. Bock, S. J. deSolms, R. L. Smith, A. K. Willard, and O. W. Woltersdorf, Jr. I am indebted to Florence Berg for checking and editing the bibliographies of many of the manuscripts.

I am most grateful to my secretary, C. F. Slobodzian, for the exceptional job she did in typing and proofreading each draft of many of these manuscripts and of the final draft of most of the others. She also provided invaluable assistance in the voluminous correspondence required in organizing the symposium and writing the book.

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Prostaglandins and Renal Function: Implications for the Activity of Diuretic Agents

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Prostaglandins are primarily local or tissue hormones which act at or near their sites of synthesis and are synthesized on demand as they are not stored (1). In the kidney, as in other tissues, prostaglandins serve primarily a defensive function, although they may contribute to the maintenance of renal function under physiological conditions. Furosemide, ethacrynic acid and bumetanide, the most potent of the diuretic agents, can cause a precipitous decline in renal function, particularly in the sodium depleted subject; a prostaglandin response evoked in response to the "loop diuretic" may maintain renal function in the face of this challenge (2). The capacity of the kidney to respond to a stimulus which depresses renal function by increasing prostaglandin synthesis was first shown during administration of a vasoconstrictor agent such as angiotensin or norepinephrine (3). Release of prostaglandins coincided with restoration of renal blood flow and urine flow despite continued administration of either angiotensin II or norepinephrine.

STRESS EVOKED RENAL PROSTAGLANDIN RESPONSE

A prostaglandin mechanism seems important to the regulation of the renal circulation when the latter is compromised by an acute insult or chronic disease. For example, activation of the renin-angiotensin system by either hemorrhage (4), laparotomy (5) or a "loop diuretic" can increase synthesis of prostaglandins by the kidney; the concentration of PGE_2 in renal venous blood increased by as much as fifteen-fold during surgical stress and was closely correlated with the level of plasma renin activity (5). Thus, under acute stress the activities of the renin-angiotensin and prostaglandin systems within the kidney appear to be coupled. The contribution of a prostaglandin mechanism to the support of the renal circulation in the acutely stressed dog may be uncovered by administration of indomethacin, an inhibitor of prostaglandin synthesis (5). A large reduction in renal blood flow occurred rapidly in response to indomethacin, despite an

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attendant increase in renal perfusion pressure. There was a simultaneous decline in renal efflux of PGE_2 which was proportional to the reduction in renal blood flow. This study demonstrated that in the animal subjected to acute stress, the renal circulation was supported by a major prostaglandin component, withdrawal of which resulted in decreased renal blood flow, particularly that fraction to the inner cortex and medulla (7).

PROSTAGLANDIN RELATED EFFECTS ON RENAL BLOOD FLOW

The pattern of distribution of blood flow within the kidney may affect salt and water excretion; for example, increased blood flow to the medulla can lower the tonicity of the medullary interstitium and, thereby, diminish the capacity to concentrate urine, resulting in increased water excretion. Changes in prostaglandin synthesis, whether resulting from inhibition by aspirin-like drugs, or increases induced by either acute stress (5), infusion of arachidonic acid (8), or administration of a loop diuretic (2), are likely to be reflected primarily by alterations of that portion of renal blood flow which supplies the medulla and will be reflected by decreased or increased blood flow to the inner and mid cortex, as measured by the distribution of radioactive microspheres within the cortex. This effect of altered prostaglandin synthesis on zonal distribution of renal blood flow arises from two factors. First, stratification of prostaglandin synthetase intrarenally is opposite to that of renin; the greatest prostaglandin synthetic capacity is in the papilla and medulla, the least in the renal cortex (9). It should be noted that the apparent difference in prostaglandin biosynthetic capacity between the renal cortex and medulla may be related, in part, to the presence within the cortex of an inhibitor of cyclooxygenase (10). Second, the inner cortical and medullary circulations are continuous, as the afferent arterioles of the inner cortex extend into the medulla, giving rise to the vasa recta (11). Therefore, changes in prostaglandin synthesis in the inner medulla will have secondary effects on blood flow to the outer medulla and inner and mid cortex because of the morphological unity of these vascular structures. A possible clinical correlation of these findings is the nephropathy of analgesic abuse. Nanra et al have proposed that "analgesic-nephropathy" is due to medullary ischemia secondary to reduced synthesis of one or more vasodilator prostaglandin(s) (12), such as PGE_2 or PGI_2. Further, elevated tissue levels of PGE_2, the presumed agent of enhanced renomedullary blood flow, should result from inhibition of PGE-9-ketoreductase. Furosemide and ethacrynic acid have been shown to inhibit this enzyme and should thereby promote increased renal blood flow to the medulla (13).

The evidence for a prostaglandin mechanism participating in the regulation of the intrarenal distribution of blood flow was first obtained in the isolated blood-perfused kidney of the dog

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(14) - and later in the conscious rabbit (15). One or more renal prostaglandin(s), primarily PGE_2, is responsible for mediating increases in blood flow to the renal medulla in response to stimuli as diverse as surgical trauma (5), hemorrhagic hypotension (4), salt loading (16), and loop diuretic agents (17). Those interventions which increase prostaglandin production, even though they may reduce total renal blood flow, can increase blood flow to the renal medulla. A balanced mechanism seems to regulate the distribution of renal blood flow: a prostaglandin mechanism increases blood flow to the inner cortex and medulla, and one of the components of the renin-angiotensin system, angiotensin I, probably has a major intrarenal role effecting decreases in blood flow to the medulla (18). This action on the intrarenal distribution of blood flow may be unique for angiotensin I, as angiotensin II usually results in reduction in renal blood flow to all zones (18). It should be noted that high doses of angiotensin II, which can stimulate prostaglandin synthesis, may cause an increase in medullary blood flow despite a decline in total renal blood flow. As diuretic agents have the capacity to activate the renin-angiotensin system consequent to reduction of extracellular fluid volume, some of the effects on renal hemodynamics may operate through this mechanism.

In contrast to its effect on the surgically-stressed anesthetized dog, indomethacin did not affect renal blood flow in the conscious resting dog, even in doses having major toxic effects (5). This finding supports the proposal that, under physiological conditions, those mechanisms involving renal prostaglandins are quiescent, requiring a noxious stimulus to be activated. This proposal also is in agreement with the general conclusion that prostaglandins subserve a defensive function, and that their release from an organ represents synthesis on demand, as prostaglandins are not stored (1). Although this conclusion appears valid for many tissues, it fails to explain the basal efflux, albeit low, of prostaglandins from kidneys of the conscious resting dog, which is unaffected by high doses of indomethacin (5). Further, in the conscious rabbit (15) and perhaps in resting man (19), inhibition of prostaglandin synthesis has been shown to result in increased vascular resistance. In the conscious rabbit, indomethacin increased renal vascular resistance two-fold, associated with a shift of renal blood flow to the outer cortex (15). Thus, the activity of intrarenal prostaglandin mechanisms in the conscious animal under physiological conditions may vary with the species. Nasjletti et al (20) have obtained evidence that the release of prostaglandins from the kidney under resting conditions is determined, in large part, by the activity of the renal kallikrein-kinin system.

PROSTAGLANDIN BIOSYNTHETIC CAPACITY OF RENAL TISSUES

An alternative explanation for the failure of indomethacin

to affect renal blood flow in the conscious resting dog derives from possible differences in accessibility of aspirin-like compounds to prostaglandin synthetase, perhaps reflecting variation in metabolism or distribution of the inhibitor. Another explanation is that the cyclooxygenase varies in its susceptibility to aspirin-like drugs depending on the tissue and species; this seems less likely (21). Thus, the question of access of indomethacin to its site of action, as well as species and tissue differences in the effects of indomethacin on the prostaglandin synthesizing machinery, must be kept in mind. This consideration leads to an important observation; viz., the capacity to synthesize prostaglandins is distributed widely among the cellular elements of the kidney. Cyclooxygenase is present in at least three different tissues in the kidney. The interstitial cells of the renal medulla were the first to be shown to have the capacity to synthesize prostaglandins (21). Also, cyclooxygenase was shown to be localized in the cells lining the distal nephron and collecting ducts (22); this location accords with the known interrelationships of prostaglandins and ADH (23). (Figure 1). For example, increased urinary concentrating ability in response to ADH occurred after treatment with indomethacin (24). Prostaglandins of the E series have been shown to blunt the effects of ADH (23) and favor the excretion of free water. A prostaglandin mechanism may contribute to the action of those diuretic agents which increase levels of PGE_2 in the renal medulla. The latter could be effected by an action of the diuretic agent either on synthesis of prostaglandins, or on the enzyme 15-hydroxyprostaglandin dehydrogenase which degrades PGE_2 or by inhibition of PGE-9-ketoreductase which transforms PGE_2 to $PGF_{2\alpha}$. Indeed, furosemide may have effects on each of these mechanisms; it increases prostaglandin synthesis by promoting arachidonic acid delivery to the cyclooxygenase (25) and inhibits both the dehydrogenase and reductase (13). The $NADP^+$-dependent form of the dehydrogenase has been suggested to be identical to the PGE-9-ketoreductase (26).

POSSIBLE CIRCULATING PROSTAGLANDINS

There is little evidence that prostaglandins function as circulating hormones. An exception to this was thought to be PGA_2 which, when infused intravenously, was not destroyed on passage across the pulmonary circulation (31). However, in all probability, PGA_2 is an artifact resulting from spontaneous breakdown of PGE_2 during extraction and purification of tissues or plasma; recent studies based on highly sensitive and specific mass spectrometric methods did not detect PGA_2 in the blood (32). Recently, PGI_2 has been suggested to function as a circulating hormone because its vasodepressor activity is undiminished by passage across the lung (33).

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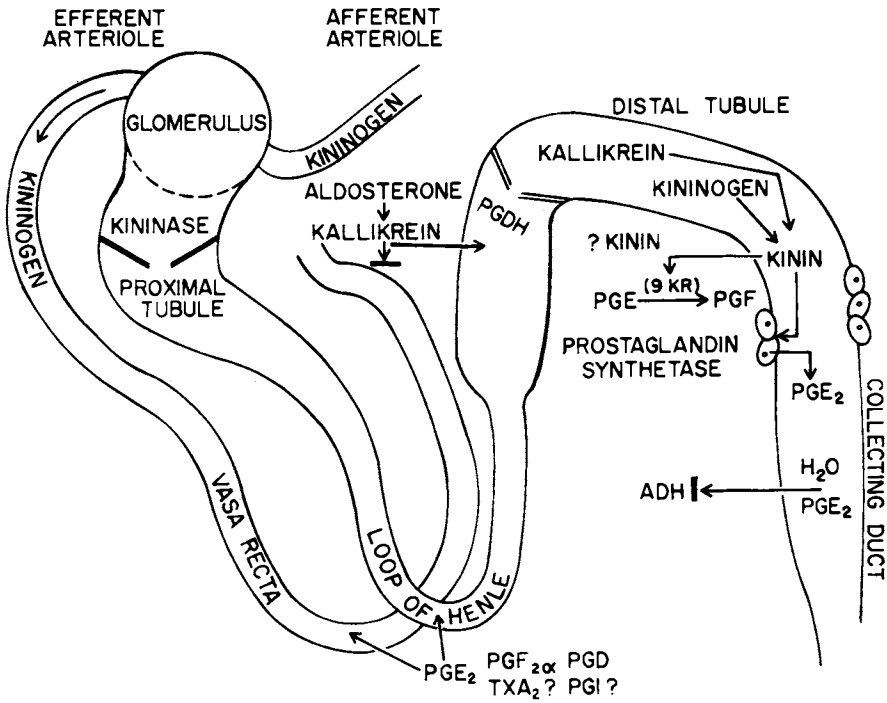

Figure 1. Prostaglandin–kinin interaction in the nephron.

The generation of kinins in the distal nephron and collecting ducts results in the release of prostaglandins which inhibit the effect of ADH and thereby participate in the excretion of solute-free water. Prostaglandin-15-hydroxydehydrogenase (PGHD), PGE-9-ketoreductase (9 KR).

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RENAL ANATOMICAL COMPARTMENTS: PROSTAGLANDINS AND RENAL FUNCTION

Although cyclooxygenase is present in many tissues within the kidney, the major products of arachidonic acid metabolism, be they PGI_2, PGE_2, PGD_2, $PGF_{2\alpha}$, or TXA_2 (Figure 2), may be tissue specific and, consequently, their effects may be primarily restricted to one compartment, such as the vascular, tubular or interstitial. Thus, prostacyclin, a major product of arachidonic acid metabolism within the blood vessel wall (34), which together with other prostaglandins may affect the activity of the renin-angiotensin system, is possibly destroyed locally by the abundant prostaglandin dehydrogenases of the vascular tissues (27). The renin-angiotensin system is primarily restricted to the vascular compartment as is prostacyclin. This is in contrast to kallikrein-kinin and PGE_2 which are mainly associated with the urinary and interstitial compartments. Thus, the presence of prostaglandin synthetase within one or more cellular elements lining the urinary compartment, particularly the distal nephron and collecting ducts (22), facilitates the interaction of prostaglandins with kinins and ADH. For example, entry of kallikrein into the distal tubules, and subsequent formation of kinins, results in release of one or more prostaglandins by kinins from sites of prostaglandin generation along the collecting ducts. Inhibition of the effects of ADH can occur, then, in response to the kinin-mediated generation of prostaglandins in the distal nephron; this results in the excretion of solute-free water. A recent study by Weber et al (36) indicates that the activity of a major prostaglandin metabolizing enzyme (37), PGE-9-ketoreductase, which converts PGE_2 to $PGF_{2\alpha}$, is influenced by salt intake. Thus, reabsorption of water is facilitated by increased activity of this enzyme, which has the effect of lowering levels of PGE_2 intrarenally by favoring formation of $PGF_{2\alpha}$. As $PGF_{2\alpha}$, unlike PGE_2, does not inhibit ADH, increased activity of PGE-9-ketoreductase will facilitate reabsorption in water. The "loop diuretic" agents have already been noted to be capable of inhibiting the activity of this enzyme. It should be noted that kinins, in addition to promoting prostaglandin synthesis, are also capable of increasing the activity of PGE-9-ketoreductase (38), and that these effects may be crucial to the ability of kinins to alter excretion of solute-free water as affected by the state of sodium balance. As inhibition of prostaglandin synthesis has been shown to prevent increased free water generation induced by bradykinin (39), a prostaglandin mechanism appears to be necessary for this effect of the kinin (Figure 1).

PROSTAGLANDINS AND SALT EXCRETION

The concept of segregation of cyclooxygenases within several functional compartments of the kidney and different prostaglandins arising from these compartments is useful for

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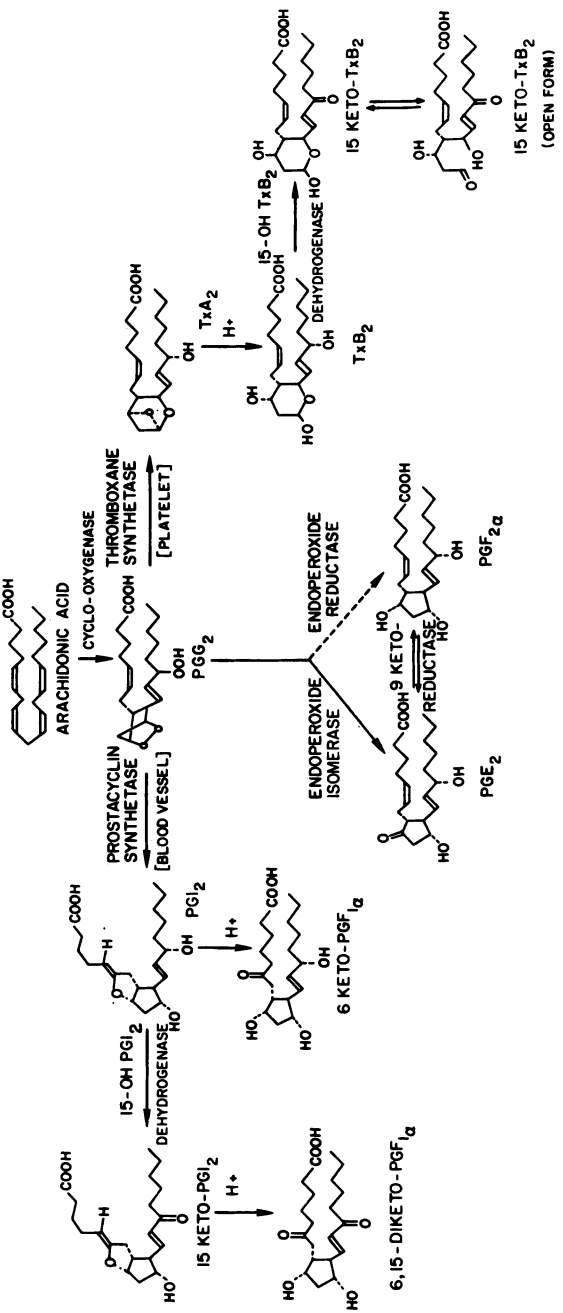

Figure 2. Metabolism of arachidonic acid by the prostaglandin synthetase complex. Major products of vascular tissues are prostacyclin (PGI_2) and PGE_2; a major product of blood platelets is thromboxane A_2 (TxA_2)

interpreting the variable effects of one or more prostaglandin mechanisms on salt excretion. The natriuretic effect of either the principal renal prostaglandin, PGE_2, or its precursor, arachidonic acid, cannot be dissociated easily from its effects on the renal circulation. In the only *in vivo* study which examined the effect of PGE_2 on tubular function uncomplicated by vascular effects, Kauker, using micro-injection techniques, demonstrated that intraluminal injection of PGE_2 in rats resulted in inhibition of sodium reabsorption (40). Further, Stokes and Kokko demonstrated an inhibitory effect of PGE_2 on sodium transport in isolated perfused renal collecting tubules of rabbits pretreated with mineralocorticoids (41). In conscious rats, Nasjletti et al (20) demonstrated that mineralocorticoid treatment not only increased kallikrein excretion, but also enhanced excretion of PGE_2 by two- to three-fold. Augmented excretion of kallikrein and PGE_2 in these rats was associated with escape from the salt and water retaining effects of mineralocorticoids. In the rat, inhibition of prostaglandin synthesis also results in increased concentration of sodium chloride in the renal medulla (42). The latter suggests that exaggerated tubular reabsorption of sodium in the ascending limb of the loop of Henle results from eliminating a prostaglandin mechanism which promotes salt excretion. On the other hand, in the conscious dog undergoing a water-induced diuresis, both indomethacin and meclofenamate have been reported to increase sodium excretion (43). A possible prostaglandin mechanism which prevents demonstration of the direct natriuretic action of bradykinin was described by McGiff et al in the blood-perfused isolated canine kidney (39). Thus, a natriuretic action of bradykinin was not shown until prostaglandin synthesis was inhibited by indomethacin. These seemingly discrepant studies may be reconciled if it is recognized that the experimental conditions determine not only the level of prostaglandin activity, but also the major species of prostaglandins produced within the urinary compartment. As these vary, the effects of indomethacin, which also alters prostaglandin metabolizing enzymes (13), will depend on the level and profile of prostaglandins produced under a given set of conditions. This, in turn, is related to the state of salt and water balance, the degree of stress occasioned by anesthesia and surgery, the activity of other hormonal systems, the "intrinsic" activity of the cyclooxygenase as determined by natural inhibitors and activators, and, finally, the species being studied. These general considerations force the conclusion that the products of cyclooxygenases in the various compartments within the kidney may vary with experimental conditions, as well as in health and in disease. For example, thromboxane, a powerful vasoconstrictor; is not normally synthesized by the kidney. However, when renal function is disturbed, as by acute ureteral ligation, thromboxane synthesis may occur (44). Its production may contribute to the late increase in renal vascular resistance in response to

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ureteral obstruction (45).

Changes in extracellular potassium concentration can also affect renal prostaglandin synthesis (46). As urinary kallikrein concentrations have been positively correlated with excretion of potassium, but not sodium (47), the possibility of a potassium-dependent interaction of prostaglandins with kallikrein-kinins should be considered. Thus, induction of potassium deficiency has been shown to result in enhanced renal prostaglandin synthesis (48). Hyposthenuria associated with potassium deficiency, then, may be related to inhibition of the effects of ADH (23) consequent to increased production of PGE_2 or a related prostaglandin.

SUMMARY

Those diuretic agents such as furosemide which have as their primary sites of action the ascending limb of the loop of Henle and the cortical collecting ducts, where they have a primary effect on chloride transport (49), can be shown to have major effects not only on the renin-angiotensin system (6), but also on the kallikrein-kinin (50) and prostaglandin systems (13). There is evidence suggesting that their diuretic action may be related partially to an effect on the vasodilator-diuretic system of the kidney, the kallikrein-kinin-prostaglandin system. Thus, aspirin-like compounds have been shown to blunt the diuretic action of furosemide (51), although this effect of antiinflammatory acids is complicated by their inhibition of the organic acid secretory system. Integrity of the latter may be required for access of these diuretic agents to their active sites. Further, furosemide and ethacrynic acid have been shown to inhibit two of the major prostaglandin catabolizing enzymes, prostaglandin-15-hydroxydehydrogenase and PGE-9-ketoreductase (13). Their effects on these enzymes may result in increased levels of PGE_2 and PGI_2 which may then contribute to vasodilator-diuretic mechanisms. The design of agents which have major effects on prostaglandin metabolism is well underway and has already resulted in novel diuretic agents (52).

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2

Structure-Activity Relationships of Aminobenzoic Acid Diuretics and Related Compounds (*1*)

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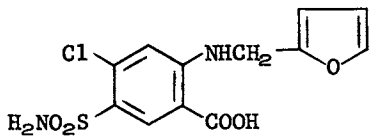
In a series of papers (2-10) during the nineteen-seventies, we presented some of our results based on a more or less systematic structural alteration of the sulfamoylbenzoic acid diuretics in order to elucidate the structural requirements for high-ceiling diuretic activity.

When we started this work, for reasons previously discussed (3), the only existing high-ceiling sulfonamide diuretics were the N-substituted 4-chloro-5-sulfamoylanthranilic acids represented by furosemide (Formula 1). Although the diuretic characteristics of furosemide were quite different from those of the thiazides or the thiazide-type diuretics, thereby reflecting a different site of action in the nephron, it still shared structural features with the latter compounds. Thus, furosemide fit the structural requirements for diuretic activity of the benzenesulfonamides and related bicyclic compounds originally proposed by Sprague (11). He predicted that an unsubstituted sulfamoyl group, an activating group represented mainly by chloro and trifluoromethyl and an electronegative substituent (which might be part of a condensed ring system) should be present in the molecule and have the mutual positions reflected in furosemide.



1

Our first approach was to move the substituted amino function (which is not part of Sprague's predictions) to the 3-position, with almost complete retention of diuretic activity (2). More surprisingly, replacement of the chlorine atom by various other groups in both the 2-substituted (4, 5) and the 3-substituted

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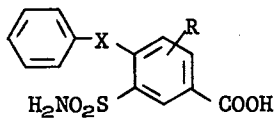
(3, 6, 7, 8) sulfamoylbenzoic acid diuretics, was found to enhance the diuretic potency. The most potent compounds (Formula 2) were those in which an unsubstituted or substituted phenyl group was linked to the 4-position by a group X, which represents NH, O, S, SO, SO_2, CH_2 or CO. These 4-substituents are not merely new "activating groups" in the sense that Sprague described, since replacement of chloro by phenoxy or phenylthio in some selected thiazides and thiazide-type diuretics led to compounds which were not diuretic (12). The predictions of Sprague, although still of value as far as the thiazide-type diuretics were concerned, were found not to account fully for the structural requirements of the high-ceiling diuretics.



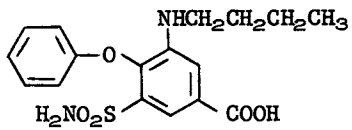
2

X = NH, O, S, SO, SO_2, CH_2, CO

R = NHR^1, OR^1, SR^1, CH_2R^1 in which R^1 preferably is n-butyl, benzyl, furylmethyl or thienylmethyl

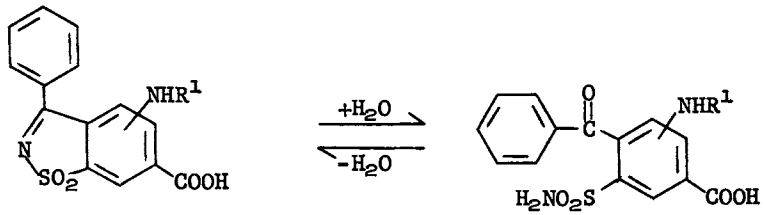
When placed in the 3-position, many variations of the substituent R in formula 2 contribute to high potency. However, when located in the 2-position, the only active compounds are those where R is restricted to a substituted amino function, preferably the 2-furylmethylamino group.

Bumetanide (Formula 3) was selected for further investigation and shown (13) to be 40 to 60 times more potent than furosemide; this was a level of potency exhibited in the dog assay by many compounds of formula 2. Its utility in clinical practice has since been established (14).



3

The compounds of formula 2, in which X represents carbonyl and R a substituted amino function, are not isolable due to a pH-dependent equilibrium in aqueous solution. At physiological pH, the equilibrium favors the cyclodehydration products, i.e., the benzisothiazole dioxides of formula 4. It has been suggested (5, 6), however, that the potent diuretic activity (one fourth to one tenth of that of bumetanide) observed after administration of these benzisothiazoles to dogs involves the interaction of the corresponding 4-benzoylsulfamoylbenzoic acids (Formula 5) with

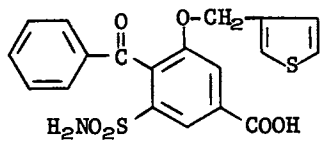


4 5

the receptor which is possible because of the dynamic equilibration in plasma. Thus, the potency of the benzisothiazole dioxides (Formula 4) appears to be due to this remarkable property. For the corresponding 3-alkoxy- or 3-alkylthio-4-benzoyl-5-sulfamoylbenzoic acids, the equilibrium is totally to the side of the benzoyl compound (8); this might explain why compound 6 (Table I) is one of the most potent, high-ceiling diuretics that we synthesized. Since this compound shows significant diuretic activity in the dog assay after an oral dose of only 1 µg/kg, it is 5 to 10 times more potent than bumetanide (8).

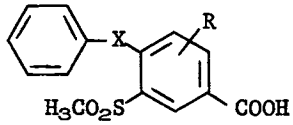
TABLE I

Urinary Excretion in a 3 hr. Period following I.V. Administration of Compound 6 to Dogs, Expressed for Vol. in ml/kg and for Electrolytes in mEq/kg.



6

Treatment mg/kg	Vol.	Na^+	K^+	Cl^-
control	1	0.10	0.16	0.08
0.001	4	0.7	0.28	0.7
0.01	23	2.5	0.53	3.5
0.1	38	4.4	0.97	4.9

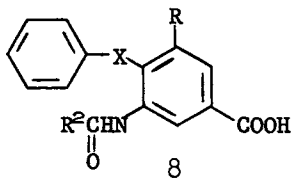


7

X = NH, O, S, CO

R = NHR^1, OR^1, SR^1 in which R^1 preferably is n-butyl, benzyl or furylmethyl

Another discovery was that active diuretics of the type represented by formula 7 could be obtained when the sulfamoyl group was replaced by the sterically similar mesyl group (9). Various 3-substituents contribute to the comparatively high potency of these compounds; whereas, virtually the only 2-substituent allowed is the 2-furylmethylamino group. These observations encouraged the investigation of the influence of other substituents in the 5-position. A suitably substituted amino function, preferably a formamido group in place of the sulfamoyl group (Formula 8), led to active diuretics (10).



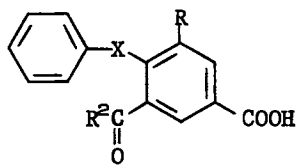
8

X = O, S, CH_2, CO

R = NHR^1, OR^1, SR^1, CH_2R^1 in which R^1 preferably is n-butyl, benzyl, furylmethyl or thienylmethyl.

R^2 = H, lower alkyl, NH_2, NH-lower alkyl, lower alkoxy

Table II shows the data obtained with one of the most potent compounds with this particular structural alteration. Compound 9 was found to be approximately one tenth as potent as bumetanide. Disappointingly, the electrolyte excretion pattern was still similar to that of bumetanide. The corresponding 2,4-disubstituted-5-acylaminobenzoic acids were, however, inactive.



10

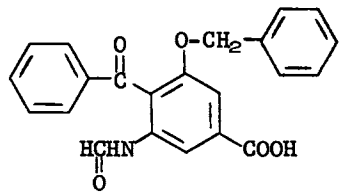
X = NH, O, S, CO

R = NHR^1, OR^1, SR^1 in which R^1 preferably is n-butyl or benzyl

R^2 = OH, NH_2, NH-lower alkyl

TABLE II

Urinary Excretion (Average of 3 Values) in a 3 hr. Period following I.V. Administration of Compound 9 to Dogs, Expressed for Vol. in ml/kg and for Electrolytes in mEq/kg.



9

Treatment mg/kg	Vol.	Na^+	K^+	Cl^-
control	1	0.10	0.16	0.08
0.1	9	1.0	0.24	1.3
0.25	18	2.1	0.41	2.6
1.0	22	2.5	0.49	3.1
5.0	32	3.7	0.83	4.5

In Diuretic Agents; Cragoe, E.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

In addition, isophthalic acid derivatives of formula 10 were prepared (15). Some of them exerted moderate diuretic activity; however, again, the characteristic electrolyte excretion profile of bumetanide was observed.

The above brief survey of previously reported results of structural modifications in benzoic acid diuretics reveals that our efforts had been directed toward compounds in which the carboxylic acid function remained intact. If such a function is present in biologically active molecules, its acidic character usually has, for known reasons, an effect on activity and/or potency. Therefore, it was considered to be of interest to investigate whether a carboxyl function is a prerequisite for high-ceiling diuretic activity. Consequently, derivatives in which this group was replaced by other functions were synthesized.

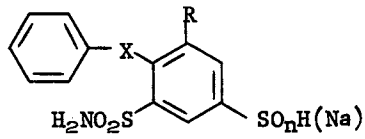
3,4-Disubstituted-5-sulfamoylbenzenesulfinic acids and sulfonic acids of formula 11 (Table III) were prepared from the corresponding carboxylic acids. Conversion of their azides by means of a Curtius degradation furnished the corresponding anilines. The latter derivatives were converted to the sulfonyl chlorides via diazotization and the Meerwein-reaction. These were subsequently hydrolyzed to sulfonic acids or reduced to sulfinic acids. It can be seen from Table III that these rather strong acids possess potent diuretic activity. Their diuretic profile is quite similar to that of bumetanide; however, they have a shorter duration of action. The corresponding 2,4,5-trisubstituted acids were not investigated since, 15 years previously, the sulfinic acid analogue of furosemide had been found by us to be devoid of activity at a dose of 10 mg/kg in the dog assay. In contrast, 3-butylamino-4-chloro-5-sulfamoylbenzenesulfinic acid was found to be diuretic at a dose of 1 mg/kg.

A series of 3,4-disubstituted-5-sulfamoylbenzylamines was synthesized (16). In the dog assay, many of these compounds exhibited high-ceiling diuretic activity in the range of potency from that of furosemide to that of bumetanide. Therefore, an extended series of benzylamines of formula 12 (see Table IV) having the 3-, 4-, and 5-substituents of bumetanide was prepared in order to elucidate the influence of the N-substituent(s) in the benzylamine moiety on diuretic activity. Selected members of this series are tabulated in Table IV. It appears that many R^2 and R^3 substituents, such as hydrogen, lower straight chain alkyl, allyl, benzyl, furylmethyl, pyridylmethyl and unsubstituted as well as suitably substituted phenyl, contribute to high potency. Disubstitution, as exemplified by N,N-dimethyl, N,N-diethyl and some N-alkyl, N-hydroxyethyl derivatives, also is an allowed structural modification. In contrast, incorporation of a nitrogen atom in a ring system to provide derivatives of piperidine, morpholine and N'-substituted piperazine results in compounds which are inactive at the doses tested. Likewise, substitution with branched alkyl, acyl, carbamoyl or N-substituted carbamoyl groups generally decreases or abolishes diuretic activity.

In Diuretic Agents; Cragoe, E.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

TABLE III

Urinary Excretion in a 6 hr. Period following I.V. Administration to Dogs of 1 mg/kg Expressed for Vol. in ml/kg and for Electrolytes in mEq/kg.



11

X	R	n	Vol.	Na^+	K^+	Cl^-
Control (extreme values from 108 exp.)			0.3-4.2	0.05 - 0.45	0.06 - 0.30	0.06 - 0.28
0	NHC_4H_9	2	37	4.1	1.02	5.3
0	$NHCH_2C_6H_5$	2	48	4.7	1.05	4.5
0	SC_4H_9	2	29	4.0	1.15	1.7
CH_2	OC_4H_9	2	34	3.0	1.31	5.2
0	NHC_4H_9	3	22	2.6	0.62	3.1
0	$NHCH_2C_6H_5$	3	48	4.3	0.91	6.6
0		3	35	3.3	0.91	4.1

In Diuretic Agents; Cragoe, E.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

Various 3- and 4-substituents, found in the series of benzoic acids of formula 2 to contribute to high-ceiling potency, were incorporated in a series of compounds in which the variation of the benzylamine moiety was restricted to selected N-substituents. Since the influence of these N-substituents did not deviate from that observed in the series of bumetanide analogues, only the results obtained with compounds of formula 13 having the benzylamino nitrogen atom substituted by phenyl are given in Table V. As previously observed with the benzoic acid diuretics of formula 2, groups such as R^1O, R^1S or R^1NH (where R^1 is n-butyl, benzyl, furylmethyl or thienylmethyl) placed in the 3-position enhanced the diuretic activity. Likewise, phenyl attached to the 4-position through the well-known links, NH, O, S or CH_2, also was found to contribute to the potent high-ceiling diuretic activity of the benzylamine series.

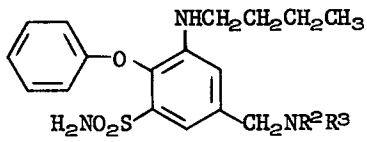
A number of 2,4-disubstituted-5-sulfamoylbenzylamines having as the 2-substituent the outstanding 2-furylmethylamino group of furosemide and related anthranilic acids were found to be inactive. This lack of activity might well be due to an inadmissible structural modification. However, after administration of selected 3-substituted benzylamines to dogs, the presence of the corresponding benzoic acids in the urine has been demonstrated qualitatively. Consequently, the question arises as to whether the diuretic response following treatment with 3-substituted benzylamines should be explained solely on the basis of metabolic transformation to the corresponding benzoic acid diuretics of formula 2. The marked difference in diuretic activity between the 2-substituted and the 3-substituted benzylamines could be due only to the repressed metabolism of the 2-derivatives due to the steric effect of the ortho-substituent. On the other hand, the above mentioned effects on diuretic activity may be accounted for by the similar differences between the 2- and the 3-substituted derivatives in the 5-acylaminobenzoic series and between the 2- and 3-substituted-4-chloro-5-sulfamoylbenzenesulfinic acid series. Furthermore, the inconsistency between the observed diuretic activities and the rate at which the subject benzylamines (Table IV) would be expected to be metabolized, might support the view that the 3-substituted benzylamines possess intrinsic diuretic activity.

The only conclusions we dare to draw from the results of our research during more than a decade in the exciting field of diuretics are that numerous structural alterations of disubstituted sulfamoylbenzoic acid diuretics led to compounds with highly potent high-ceiling diuretic activity, and that the 3,4-disubstituted members of this series are less sensitive to such alterations than are the 2,4-disubstituted members. Further exploration in this area might still, in our opinion, uncover benzene ring substituents or substitution patterns leading to compounds with high-ceiling diuretic activity.

Acknowledgment - The authors are greatly indebted to the

TABLE IV

Urinary Excretion (Mean of 2 Values) in a 6 hr. Period following I.V. (when marked with an asterisk p.o.) Administration to Dogs of 1 mg/kg, Expressed for Vol. in ml/kg and for Electrolytes in mEq/kg.



12

R^2	R^3	Vol.	Na^+	K^+	Cl^-
Control (extreme values from 108 exp.)		0.3 - 4.2	0.05 - 0.45	0.06 - 0.30	0.06 - 0.28
H	H	23	4.5	0.81	2.6
methyl	H	19	1.5	0.35	2.4
ethyl	H	17	2.0	0.48	2.4
n-propyl	H	*17	1.9	0.67	2.6
isopropyl	H	12	0.8	0.32	0.7
allyl	H	29	3.5	1.3	3.7
$CH_2C(CH_3)=CH_2$	H	20	1.4	0.35	2.1
n-butyl	H	8	1.4	0.42	1.6
isobutyl	H	3	0.4	0.30	0.2
sec-butyl	H	5	0.4	0.09	0.4
tert-butyl	H	*7	1.1	0.27	1.2
isopentyl	H	3	0.3	0.13	0.2
n-hexyl	H	9	1.1	0.75	0.6
CH_2CH_2OH	H	3	0.3	0.18	0.3
$CH_2CHOHCH_3$	H	9	1.0	0.39	1.1
$CH_2CH_2NEt_2$	H	1	0.1	0.05	0.1
methyl	methyl	21	2.1	0.48	2.6
ethyl	ethyl	10	1.0	0.37	1.2
n-propyl	n-propyl	*4	0.5	0.21	0.6
methyl	CH_2CH_2OH	3	0.5	0.18	0.4
ethyl	CH_2CH_2OH	13	1.3	0.32	1.9
isopropyl	CH_2CH_2OH	15	1.5	0.47	2.0
$CH_2C_6H_5$	H	33	3.1	1.02	4.5
$CH_2C_6H_4$-4-Me	H	3	0.4	0.16	0.5
$CH_2C_6H_4$-3-Cl	H	*11	1.1	0.24	1.4

In Diuretic Agents; Cragoe, E.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

Table IV (Continued)

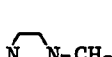
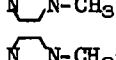
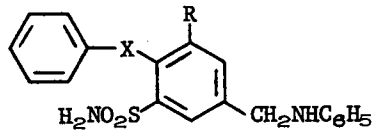
R_2	R_3	Vol.	Na^+	K^+	Cl^-
$CH_2C_6H_4$-3-OH	H	7	0.8	0.22	0.6
$CH_2C_6H_4$-4-OMe	H	11	0.8	0.25	1.3
$CH_2C_6H_5$	methyl	6	0.8	0.40	1.0
CH_2- 	H	19	2.8	0.86	1.9
CH_2- 	H	16	1.0	0.31	1.1
CH_2- 	H	25	2.6	0.67	2.9
CH_2- 	H	16	1.7	0.46	2.1
CH_2- 	H	2	0.3	0.11	0.2
CH_2- 	methyl	7	0.6	0.38	0.7
CH_2- 	methyl	7	0.9	0.26	0.9
C_6H_5	H	33	4.5	1.15	5.4
C_6H_4-2-Me	H	21	2.0	0.45	3.7
C_6H_4-4-Me	H	9	0.8	0.23	0.7
C_6H_4-2-CF_3	H	5	0.2	0.09	0.2
C_6H_4-3-F	H	4	0.5	0.15	0.3
C_6H_4-3-Cl	H	2	0.2	0.11	0.2
C_6H_4-4-Br	H	13	1.1	0.37	1.9
C_6H_4-2-OH	H	6	0.5	0.14	0.5
C_6H_4-4-OH	H	14	1.2	0.39	1.5
C_6H_4-2-OCH_3	H	2	0.2	0.32	0.2
C_6H_4-3-OC_2H_5	H	3	0.2	0.18	0.2
		*4	0.2	0.12	0.2
		5	0.7	0.48	1.1
NR^2R^3 =					
		3	0.2	0.31	0.2
		3	0.4	0.22	0.3
$COCH_3$	H	2	0.3	0.14	0.1
$CONH_2$	H	1	0.1	0.10	0.1
$CONHCH_3$	H	8	0.7	0.59	0.7
$CONHC_3H_7$	H	8	1.0	0.39	1.0

TABLE V

Urinary Excretion (Mean of 2 Values) in a 6 hr. Period following I.V. Administration to Dogs of 1 mg/kg, Expressed for Vol. in ml/kg and for Electrolytes in mEq/kg.



13

X	R	Vol.	Na^+	K^+	Cl^-
Control (extreme values from 108 exp.)		0.3 - 4.2	0.05 - 0.45	0.06 - 0.30	0.06 - 0.28
O	$NHCH_2CH{=}CH_2$	3	0.5	0.31	0.3
O	$NHCH_2C{\equiv}CH$	5	0.5	0.15	0.4
O	$NHCH_2CH{=}CHCH_3$	7	0.9	0.43	0.9
O	$NHCH_2CH_2CH_2CH_3$	33	4.5	1.15	5.4
O	$NH(CH_2)_4CH_3$	4	0.5	0.20	0.4
O	$NHCH_2C_6H_5$	25	3.0	1.3	3.8
O	$NHCH_2$ 	12	1.4	0.52	0.9
O	$NHCH_2$ 	28	3.2	0.83	2.2
S	$NHCH_2C_6H_5$	20	2.4	0.68	2.4
NH	$NHCH_2C_6H_5$	11	1.3	0.46	1.0
CH_2	$NHCH_2CH_2CH_2CH_3$	7	1.0	0.33	0.7
CH_2	$NHCH_2C_6H_5$	19	2.8	0.64	2.0
CH_2	$OCH_2CH_2CH_2CH_3$	14	1.9	0.61	1.4
CH_2	$OCH_2C_6H_5$	16	2.1	0.40	2.0

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staff of the Department of Pharmacology and to the Huntingdon Research Centre, Huntingdon, England, for the diuretic screening.

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3

4-(3-Sulfamoylphenyl)thiazolidin-4-ols

A Novel Class of Sulfonamide Compounds with Salidiuretic Activity

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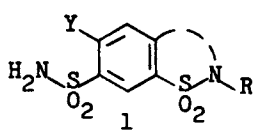
Along with other developments, the history of pharmaceutical research in our laboratories shows a close connection with the history of diuretics. A few years after the discovery in 1919 that antibiotic mercury compounds display diuretic effects (1, 2), investigators at Hoechst developed Salyrgan as the reference compound of the "Mercurials" (3). About 30 years ago, these organometallic drugs were displaced by sulfonamide derivatives which produced long acting diuresis (4, 5, 6). In 1959, researchers at Hoechst discovered the first potent short acting high-ceiling diuretic with a benzenesulfonamide structure, furosemide (7). Another interesting development in the field of high-ceiling diuretics was the synthesis of the acylphenoxyacetic acid derivative, ethacrynic acid (8). Another significant contribution to the high-ceiling diuretics which emanated from research in our laboratories was the development of piretanide (Hoe 118) during the last few years (9).

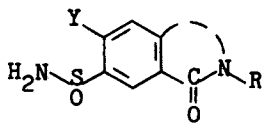
The present report will show the importance we attach to the long acting salidiuretics. When we first began our research into long acting diuretics, only the sulfonamide derivatives were known. At that time all commercial diuretics with a thiadiazide-like salidiuretic action could be derived from two general formulas, i.e., either from 3-sulfamoylsulfonamides (Structure 1) or from 3-sulfamoylbenzamides (Structure 2). Chlorthalidone (Compound 3) was an exception to this generalization (Scheme I).

Scheme I



1

3-Sulfamoyl-sulfonamides



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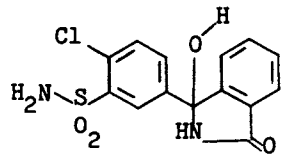
3-Sulfamoylbenzamides



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Chlorthalidone

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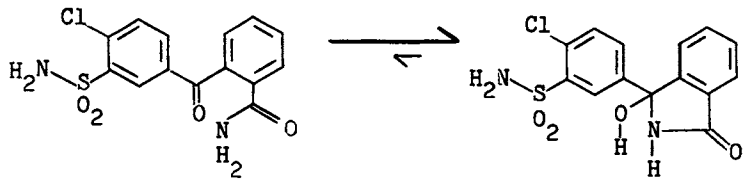
As very little was known about the structure-activity relationships of chlorthalidone (10), we were interested in elucidating the structural features which are important for the salidiuretic activity. That is, what is the significance of the fused aryl moiety, the carbamoyl moiety, the five membered ring, and the N-C-OH moiety with respect to the salidiuretic activity?

Compounds 4a and 4b (Scheme II) show chlorthalidone and its open chain tautomeric form (10).

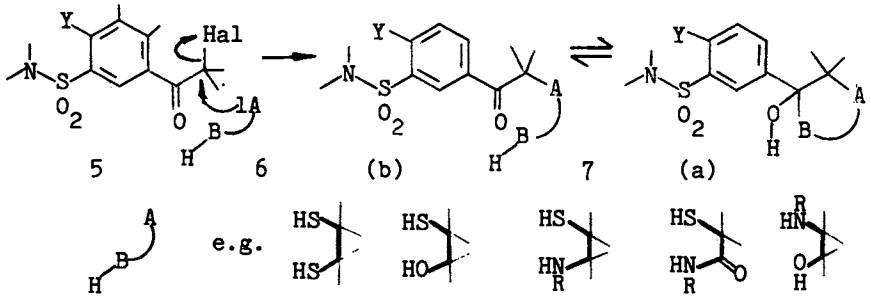
Scheme II



(a) (b)

To answer our first question as to the importance of the fused aryl moiety, we decided to prepare dearyl compounds (Scheme III) from α-haloacetophenones (Structure 5) and bifunctional molecules (Structure 6), where A represents the more nucleophilic portion of this molecule. The B-H portion of Structure 7b is then free to cyclize with the ketone carbonyl group giving ring form (Structure 7a) which would exist in tautomeric equilibrium with the open chain form. As indicated at the bottom, many such bifunctional molecules (Structure 6) were investigated and many of the resultant compounds display salidiuretic effects.

Scheme III



Thioureas (Structure 9) were used as the bifunctional molecules reacting as expected (11, 12) with the α-haloacetophenone (Structure 8) to give isothiouronium salts (Structure 10b) (Scheme IV). These compounds undergo cyclotautomerization to give thiazolidinol salts (Structure 10a). Treatment of these salts with weak bases such as triethylamine or sodium bicarbonate affords the free bases (Structure 11).

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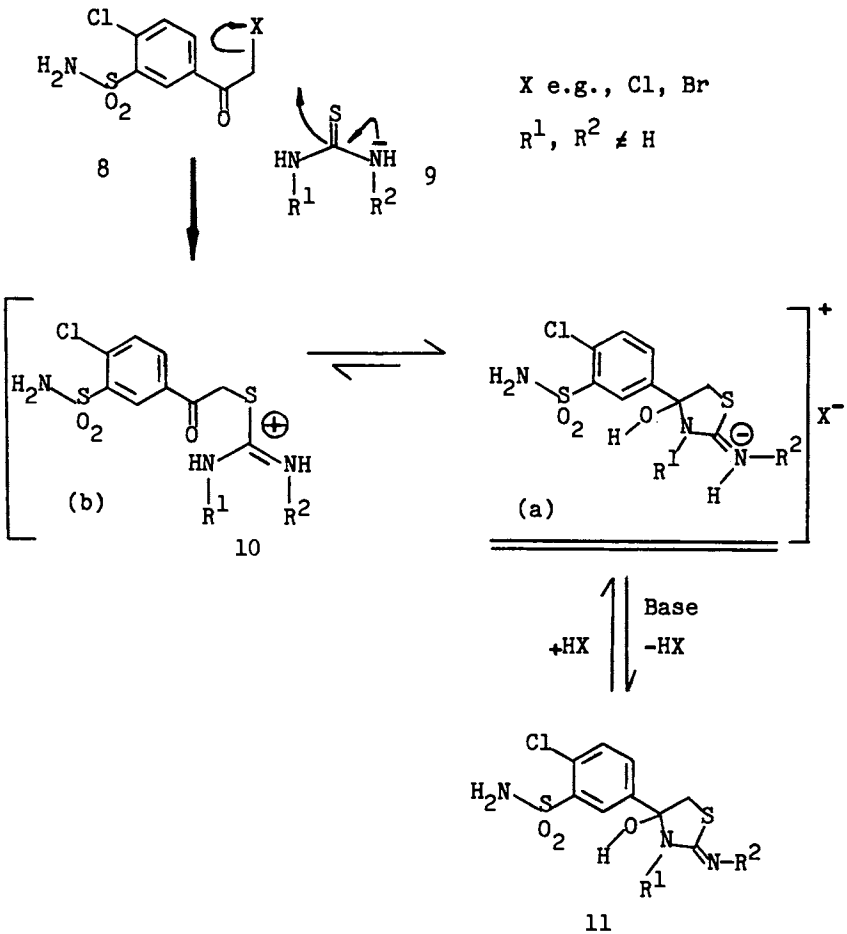
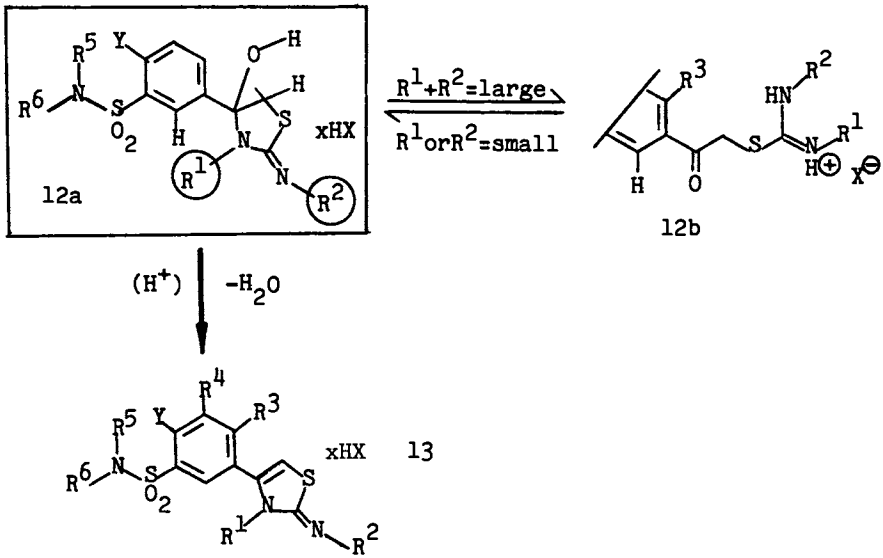
Scheme IV



These thiazolidinylbenzenesulfonamides (Structures 10 and 11) were found to be potent salidiuretic agents and extensive modifications were performed to elucidate further structure-activity relationships. The following comments with respect to structure-activity relationships pertain to studies with rats.

Structure 12a of Scheme V shows where modifications may be made.

Scheme V



As mentioned above, there is a tautomeric equilibrium between the thiazolidine and the open chain isothiourea forms (Structures 12a and 12b). This equilibrium could be proved in related structural systems by IR and NMR spectroscopic studies (11). The equilibrium is easily observed in the IR spectrum by examining the intensity of the carbonyl band near 1680 cm^{-1}, which is caused only by the open chain form. When both R^1 and R^2 are rather bulky substituents, such as cyclohexyl, cyclooctyl and cyclododecyl, there may be a carbonyl absorption at 1680 cm^{-1} suggesting a greater contribution of the open chain form. In the case where R^1 and R^2 are cyclododecyl, there is a marked reduction in salidiuretic activity. This reduced activity appears to be due more to the overall increased bulk of the molecule than just to its existing in the open chain form (Structure 12b). Optimal salidiuretic activity is associated with compounds where R^1 is a small substituent, such as methyl or ethyl, and R^2 is methyl, ethyl or even a bulkier substituent, such as cyclohexyl, cyclooctyl, benzyl, etc. Dehydration of the thiazolidines to thiazolines (Structure 13) is readily achieved by boiling with acetic acid. These compounds show reduced salidiuretic activity when compared to the corresponding thiazolidines (Structure 12).

Replacement of one or both sulfamoyl hydrogen atoms produces a decrease or a loss of salidiuretic activity in most of the known sulfamoyl diuretics. We were quite surprised to observe almost no decrease in salidiuretic activity when one of the sulfamoyl hydrogen atoms in formula 10 or 11 was replaced with alkyl groups, for example, with methyl to n-hexyl groups.

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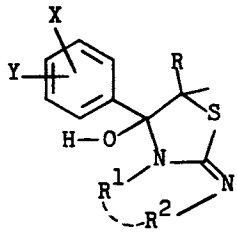
Furthermore, almost no decrease in activity was observed by similar replacement of one hydrogen atom (R^5 or R^6) of formula 12 with phenyl, carbamoyl, acetyl or aralkyl groups, such as benzyl or a substituted benzyl group.

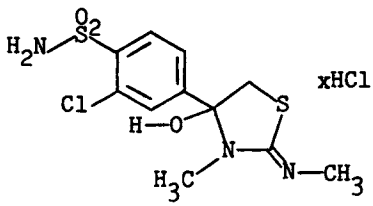
Good salidiuretic activity but a slightly enhanced toxicity was observed when both hydrogen atoms of the sulfamoyl group were replaced, for example, with methyls or with methyl and benzyl groups.

At this point we also tried to answer two other questions with respect to structure-activity relationships. First, is the sulfamoyl group necessary for salidiuretic activity? A literature survey revealed compounds of general structure 14 where X or Y are not a sulfamoyl group were reported by Manning and Houlihan (12).



14

A salidiuretic activity is associated with these compounds; however, compounds 12a containing a sulfamoyl group are much more potent as salidiuretic agents. The second question was whether or not the chlorine atom and sulfamoyl group could be interchanged. The isomer 15 of tizolemide (Compound 16, Hoe 740) was synthesized and was found to be almost devoid of salidiuretic activity.



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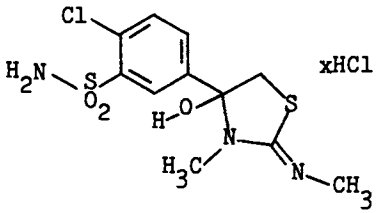
Optimal activity appears to be associated with the unsubstituted sulfamoyl group, so tizolemide was selected for further investigations.



16
Tizolemide
(Hoe 740)

The hydrochloride salt is an odorless, colorless, crystalline, substance which is moderately soluble in water. Most other sulfonamide diuretics have an acidic or neutral function at the position meta to the sulfamoyl group. In contrast, tizolemide bears a basic substituent at this position.

Tizolemide was compared with hydrochlorothiazide and chlorthalidone in various pharmacological assays. On the following figures the abbreviations Hoe 740, HCT and Ch-on appear to indicate these agents.

ION EXCRETION IN RATS

Figure 1 shows the dose-response curves in rats after oral administration of tizolemide, hydrochlorothiazide and chlorthalidone. Only sodium excretion is shown, since excretion of chloride and water follow in a parallel manner. The curves are generally flat over a dose range of 0.25 to 32 mg/kg which is typical for thiazide-like diuretics. However, at higher dosages, Hoe 740 exerts an additional effect on the excretion of sodium, chloride and water. Micropuncture studies in rats revealed that the most intensive inhibition of sodium-reabsorption by Hoe 740 is located in the distal tubule. A slight activity was also observed in the loop of Henle which might account for the high-ceiling behavior of Hoe 740.

ION EXCRETION IN DOGS

Figure 2 shows the dose-response curves with respect to the sodium and chloride excretion in dogs after oral administration of tizolemide, hydrochlorothiazide and chlorthalidone. In contrast to the previous results with rats, these agents are much more potent in dogs. The curves are nearly identical. There was no significant difference between these agents in dogs over the dose range investigated.

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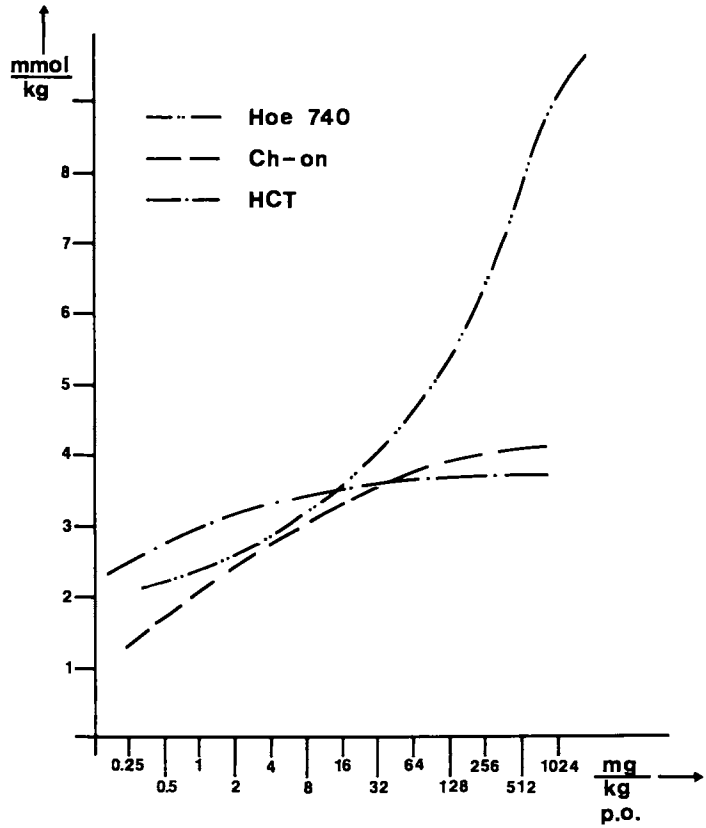

Figure 1. Sodium excretion, rat, 5 hr

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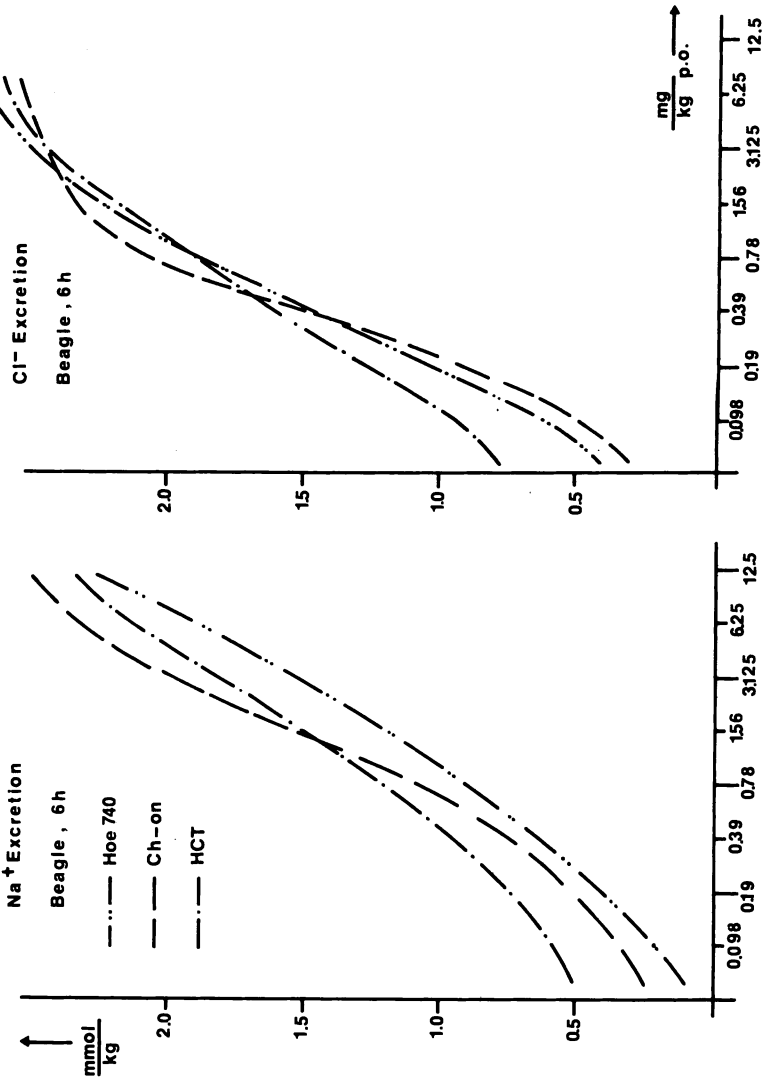

Figure 2. Sodium and chloride excretion, dog, 6 hr

THE ION RATIO

It is thought that a good diuretic agent should cause little or no enhanced excretion of potassium. It has been suggested that there is in many cases a close connection between the bicarbonate excretion and the excretion of potassium after treatment with diuretics (13, 14). Figure 3 shows the ratio of $Cl^-/Na^+ + K^+$ in dogs for Hoe 740, hydrochlorothiazide and chlorthalidone, produced in the previous experiment (Figure 2) over the saluretic active dose range. In the theoretical case where no other ions are excreted, this ratio would be equal to 1. If bicarbonate is also excreted, the ratio is less than 1. Figure 3 shows a higher $Cl^-/Na^+ + K^+$ ratio for Hoe 740 than for hydrochlorothiazide and chlorthalidone. This observation suggests that Hoe 740 would induce a lower excretion of potassium than chlorthalidone.

SODIUM AND POTASSIUM EXCRETION

Figure 4 shows the correlation between sodium and potassium excretion in dogs after i.v. administration of 12.5 mg/kg/h of chlorthalidone and Hoe 740. In comparison to the control group, there was a significant increase in sodium excretion after administration of both compounds. Chlorthalidone also causes a significant increase in potassium excretion, while potassium excretion after Hoe 740 is not significantly increased in comparison to the control group. This observation is presumably consistent with the higher $Cl^-/Na^+ + K^+$ ratio mentioned above for Hoe 740.

ANTIHYPERTENSIVE EFFECTS

Figure 5 shows the antihypertensive effects in rats treated with glycyrrhetinic acid after a daily administration of 50 mg/kg (p.o.) of Hoe 740, hydrochlorothiazide and chlorthalidone.

The glycyrrhetinic rat is a modified DOCA rat, as developed by Prof. Dr. Lindner (Hoechst AG) by use of glycyrrhetinic acid in place of 11-desoxycorticosteron-21-acetate. The high blood pressure is produced after a control period by treatment with Tyrode-solution as the drinking water and glycyrrhetinic acid (10 mg/kg daily). When a constant hypertension was achieved, the salidiuretic agent was administered while treatment with glycyrrhetinic acid was continued. The blood pressure was significantly decreased after Hoe 740, as well as, after treatment with the other two agents. When salidiuretic treatment was withdrawn, the blood pressure increased again but the previous hypertensive level was not achieved.

SERUM URIC ACID CONCENTRATIONS

Subchronic studies of the effects of Hoe 740 on serum uric acid concentrations have also been performed. Serum uric acid

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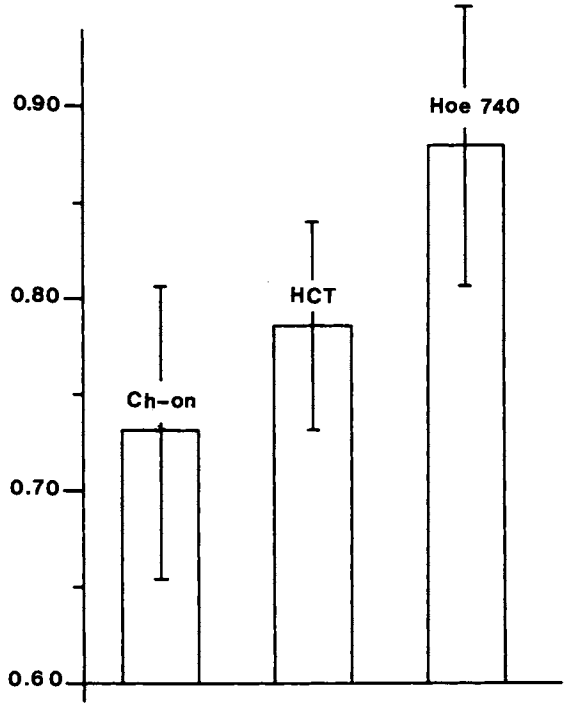

Figure 3. $Cl^-/Na^+ + K^+$, dog

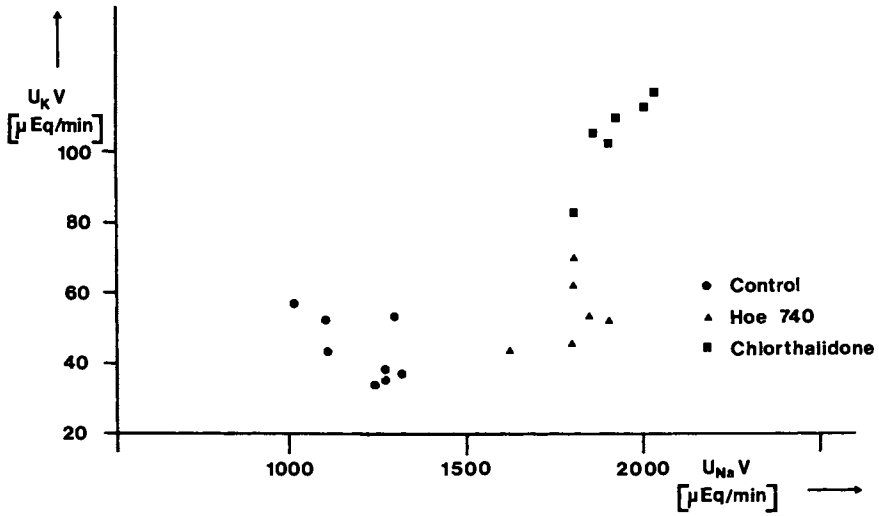

Figure 4. Correlation between Na^+ and K^+ excretion, dog

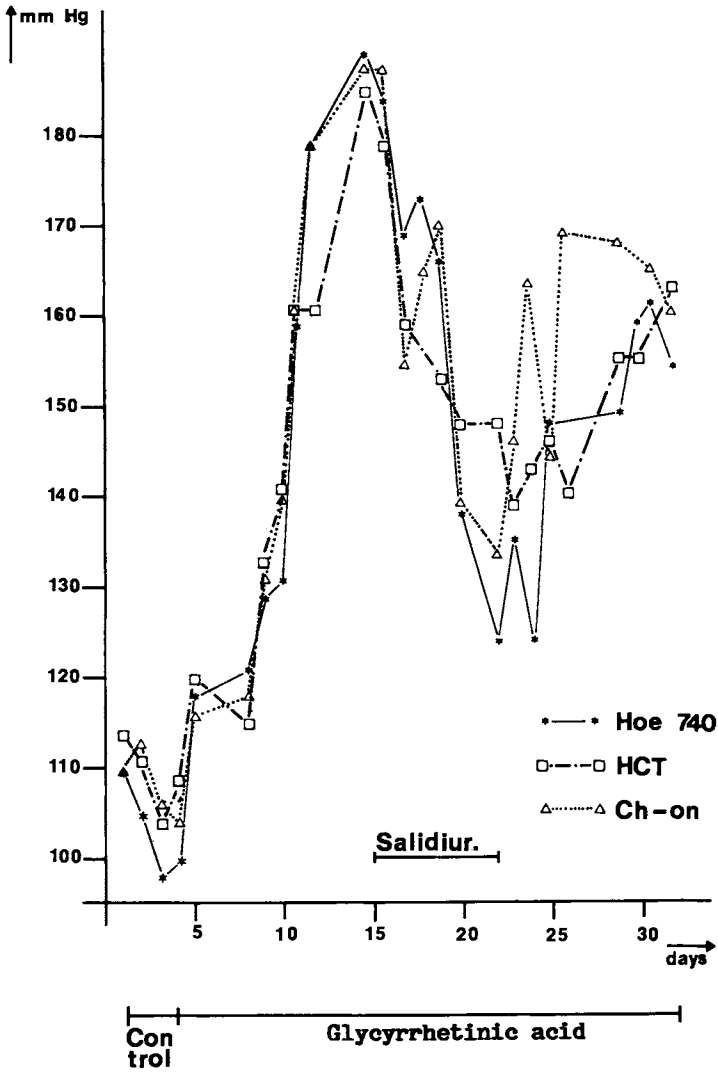

Figure 5. Antihypertensive effect in glycyrrhetinic rat

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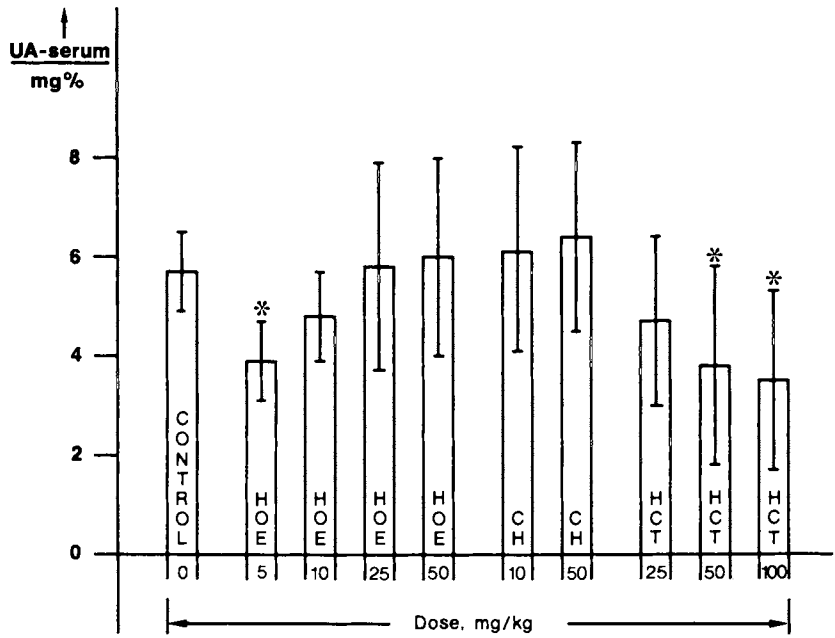

Figure 6. Effect of Hoe 740 on UA-serum concentration in comparison with chlorthalidone and hydrochlorothiazide in oxonic rat

concentrations have also been performed. Serum uric acid levels in dogs during 30 days treatment with 2.5, 8 and 25 mg/kg of Hoe 740 were not influenced; however, these levels were decreased in normal rats during the same length of treatment after oral administration of Hoe 740 at 4, 40 and 400 mg/kg.

The effect of Hoe 740 on serum uric acid was also studied in the oxonic rat (15, 16). Most mammals with the exception of some primates and humans convert uric acid into allantoin by means of the enzyme uricase. Allantoin is readily excreted in the urine because of its water solubility and its poor reabsorption in the kidney. Treatment of rats with the potassium salt of oxonic acid inhibits uricase and results in elevated serum uric acid levels. These hyperuricemic rats are treated once a day for three days with the test substance as well as oxonic acid. At the end of the test period, the animals are sacrificed and serum levels of uric acid are determined. Figure 6 shows the results with the oxonic rat. In this test, chlorthalidone produced at most a very slight increase in serum uric acid levels. Hydrochlorothiazide in higher doses produced a significant decrease in serum uric acid levels. In contrast, at low doses, Hoe 740 displayed an antihyperuricemic activity, whereas at doses higher than 10 mg/kg, no significant difference from control animals was observed. Hoe 740 is being investigated at the present time to elucidate the possible mechanism of this dose-related behavior.

Of special importance is that decreased serum uric acid levels after 10 mg/kg of Hoe 740 (p.o.) could be demonstrated also in Cebus monkeys (cebus albifrons) whose metabolism of uric acid is similar to man. The decrease of serum uric acid concentration was significant ($p<0.05$) after Hoe 740 in comparison to the control group.

Acknowledgments - We wish to thank Dr. Cragoe for the opportunity to present our work with these thiazolidines. We also wish to thank Dr. Lochelt, Dr. Schutz and Prof. Kramer for their subchronic studies in rats and dogs. Furthermore, I wish to thank my co-workers, Miss Ansuhn, Mrs. Fischer and Mr. Ahlborn. I wish to thank Dr. Lawrence Martin for his assistance in the English translation.

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4

Sulfonamide Diuretics

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More than twenty years have passed since the discovery of the thiazide diuretics. Since then many new developments in this field have occurred. The discovery of the thiazide diuretics in 1957-58 was the beginning of a new era in the treatment of edema and hypertension. Prior to the introduction of the thiazide diuretics, one had to rely on the mercurial diuretics and later, on the carbonic anhydrase inhibitors with their well known drawbacks. The carbonic anhydrase inhibitors acted mainly in the proximal tubule leading to increased urinary excretion of Na^+, K^+, and HCO_3^- and, as a consequence, to metabolic acidosis. At the time the carbonic anhydrase inhibitors were being developed, it was, however, the firm conviction of Drs. Beyer (1) and Baer of Merck Sharp and Dohme that a sulfonamide diuretic could be found that was saluretic, i.e., that increased urinary Na^+ and Cl^- excretion in equivalent quantities and therefore would not produce metabolic acidosis, if it worked at the appropriate site along the nephron. This working hypothesis eventually led to chlorothiazide.

It is now known that, depending on the site of action in the nephron, different diuretic profiles can be obtained, ranging from the carbonic anhydrase inhibitors to the thiazides and high-ceiling diuretics as is shown in Figure 1 (2, 3). Chemical structures, representing different types of diuretics have varied to a considerable extent.

The structures of these various classes of diuretics are exemplified by the carbonic anhydrase inhibitors, acetazolamide (4) and dichlorphenamide (5), the thiazide saluretic, chlorothiazide (6), the high-ceiling diuretics, furosemide (7), ethacrynic acid (8) and bumetanide (9), the uricosuric diuretic, tienilic acid (10), and the more recent compounds, muzolimine (11) and MK-447 (12), the last two representing compounds without a sulfonamide or carboxy group (Figure 2).

Some of the work that was carried out in our laboratories in the area of sulfonamide diuretics will be presented. The sulfonamide diuretics currently in use are effective and safe;

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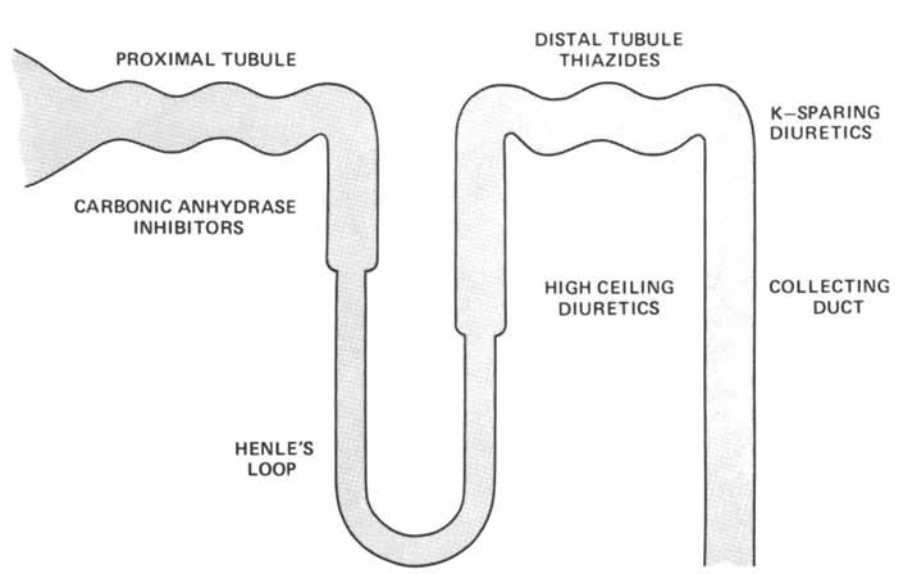

Figure 1. Representation of sites of action of diuretics in the nephron

TABLE I

Diuretic Screen

Reference Compounds	Unanesthetized Dog
Hydrochlorothiazide 5.0 mg/kg p.o.	1300 µEq Na/kg/210 min.
Furosemide 5./0 mg/kg p.o.	5200 µEq Na/kg/210 min.
Bumetanide 0.3 mg/kg p.o.	5700 µEq Na/kg/210 min.

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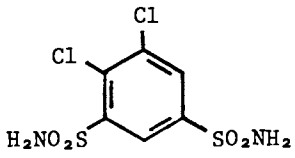
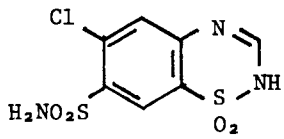


dichlorphenamide



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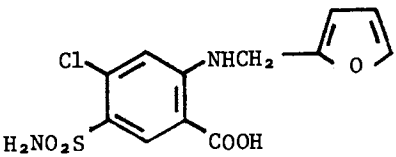
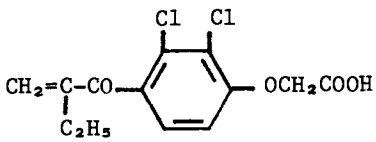
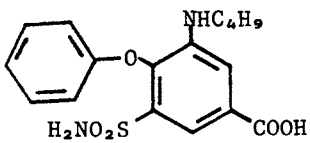


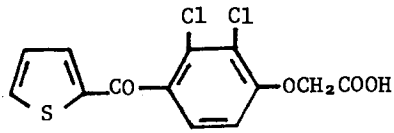
furosemide



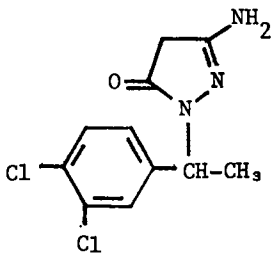
ethacrynic acid



bumetanide



tienilic acid



muzolimine

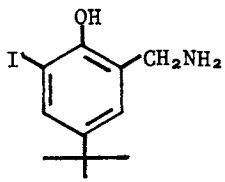


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Figure 2. Various classes of diuretics

there are, however, three undesirable side effects associated with the chronic administration of these compounds: (1) potassium depletion, (2) elevation of serum uric acid and (3) hyperglycemia. In patients with a normal fasting blood sugar, it would appear that the risk of precipitating diabetes is small. In patients who already have diabetes, the chance of disturbing the control of blood sugar is substantial. We hoped that our work would provide compounds either with less effect on potassium excretion or on blood glucose levels.

The compounds prepared were tested in rats and dogs; however, only results in dogs are reported as an indication of the relative potency of these compounds. Table I shows the values obtained with three standard sulfonamide diuretics in our diuretic screening using unanesthetized dogs. The values shown in Figures 3 to 7 also represent μEq Na/kg/210 min.

In our first approach we decided to study bi- and tricyclic compounds derived from intermediates which had yielded highly active diuretics.

Initially, we studied compounds derived from the 2-hydrazino-5-sulfamoylbenzoic acid (Compound 1) previously described by Sturm and co-workers (7) (Figure 3). The hydrazone (Compound 2) derived from phenylacetaldehyde, was only slightly active. Hydrazines such as compound 3 obtained by catalytic reduction of the corresponding hydrazone were moderately active at 5 mg/kg in the dog. On refluxing in dilute HCl, the hydrazine (Compound 3) cyclized to the indazolone (Compound 5), which was almost inactive. Fischer indole ring closure of the hydrazone (Compound 2) in acetic acid yielded the indole (Compound 4) which exhibited diuretic activity at 50 mg/kg. All compounds were administered orally.

Reaction of the 2-hydrazino-5-sulfamoylbenzoic acid (Compound 1) with cyclohexanone (Figure 3) followed by cyclization yielded the tetrahydrocarbazole (Compound 6) which was subsequently reduced catalytically in trifluoroacetic acid with platinum to the hexahydrocarbazole (Compound 7). Both compounds 6 and 7 had moderate diuretic activity at 100 mg and 50 mg/kg in the dog.

Another approach started with 2,4-dichloro-3-nitro-5-sulfamoylbenzoic acid (Compound 8, Figure 4). Treatment of compound 8 with sodium hydroxide yielded the salicylic acid derivative (Compound 9) (13). Reduction of the nitro group in compound 9 followed by fusion with butyric anhydride or benzoic anhydride gave the benzoxazoles (Compounds 10a and 10b, respectively). Only the 2-phenyl derivative (Compound 10b) showed appreciable activity at 20 mg/kg in the dog.

Similarly, the benzimidazole (Compound 12, Figure 4) was prepared. It was inactive. Starting with the 3-amino-4-anilino-5-sulfamoylbenzoic acid (Compounds 13a (9) and 13b), two other benzimidazoles (Compounds 14a and 14b, Figure 4) were prepared, but were also inactive as diuretics.

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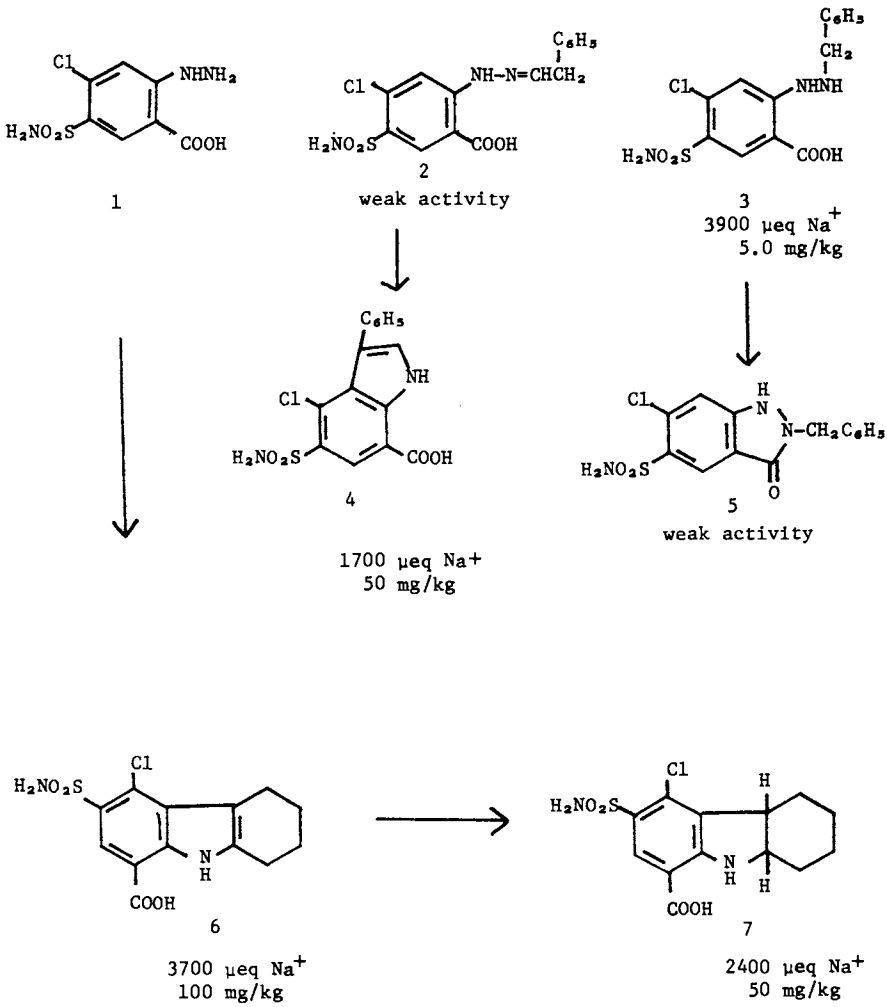


Figure 3. Compounds derived from the 2-hydrazino-5-sulfamoylbenzoic acid

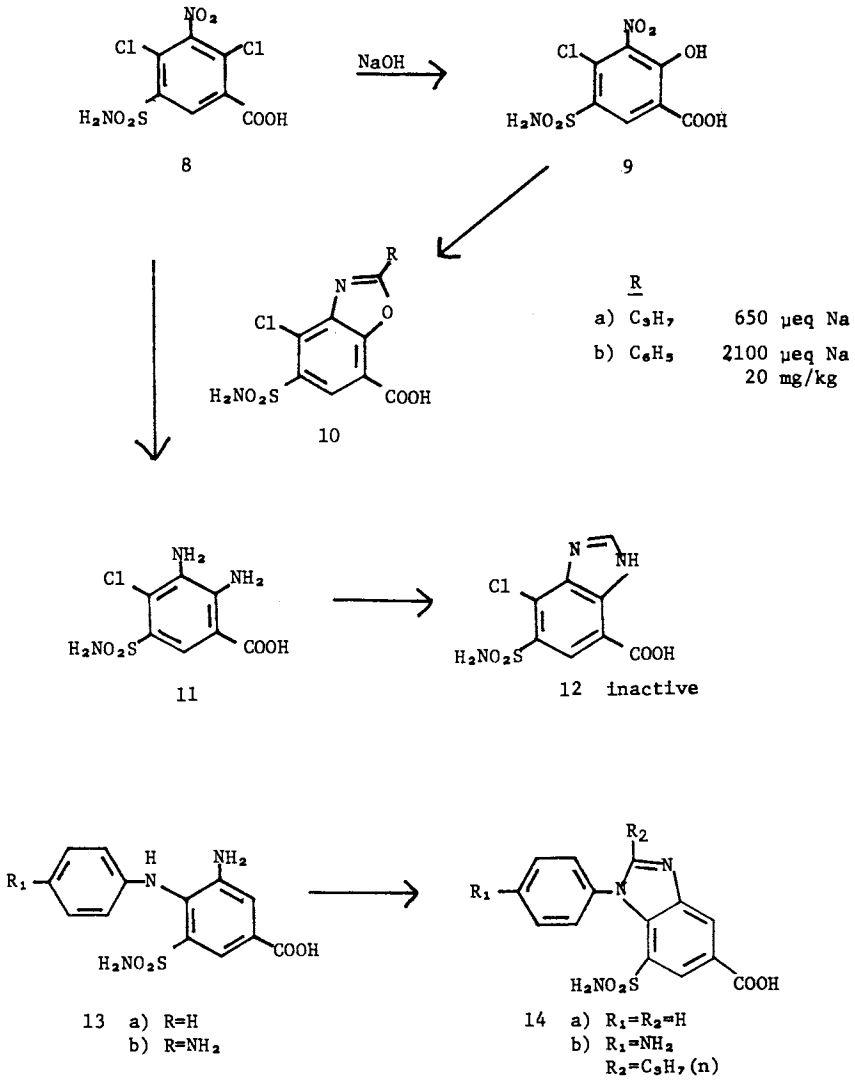


Figure 4. Benzoxazole and benzimidazole derivatives

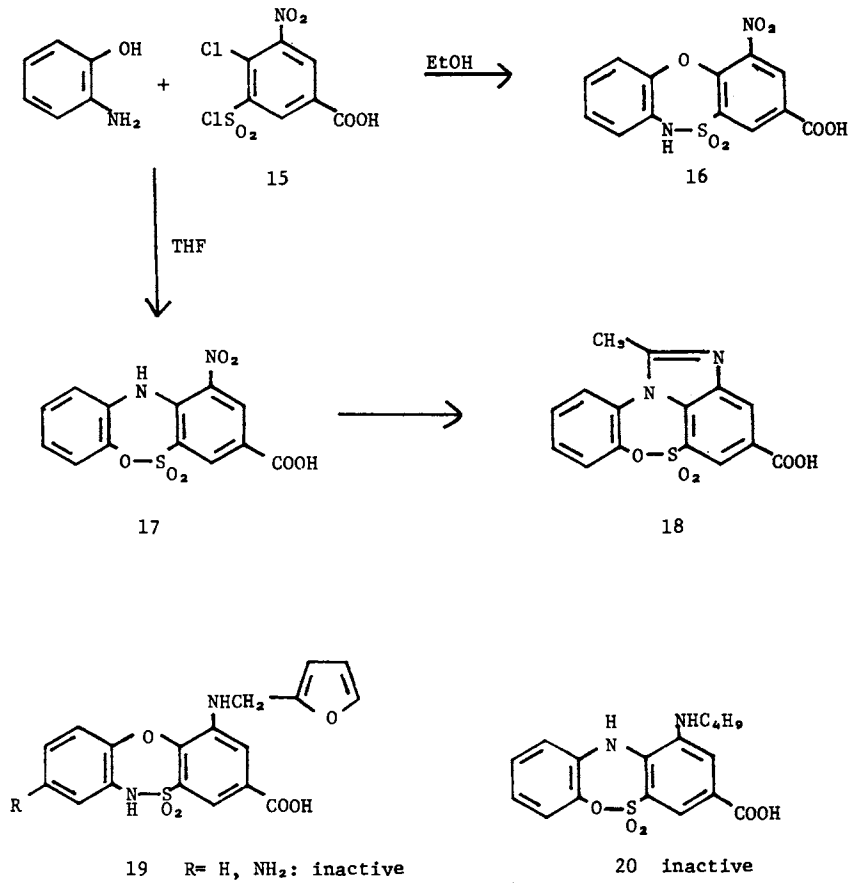


Figure 5. Tricyclic derivatives

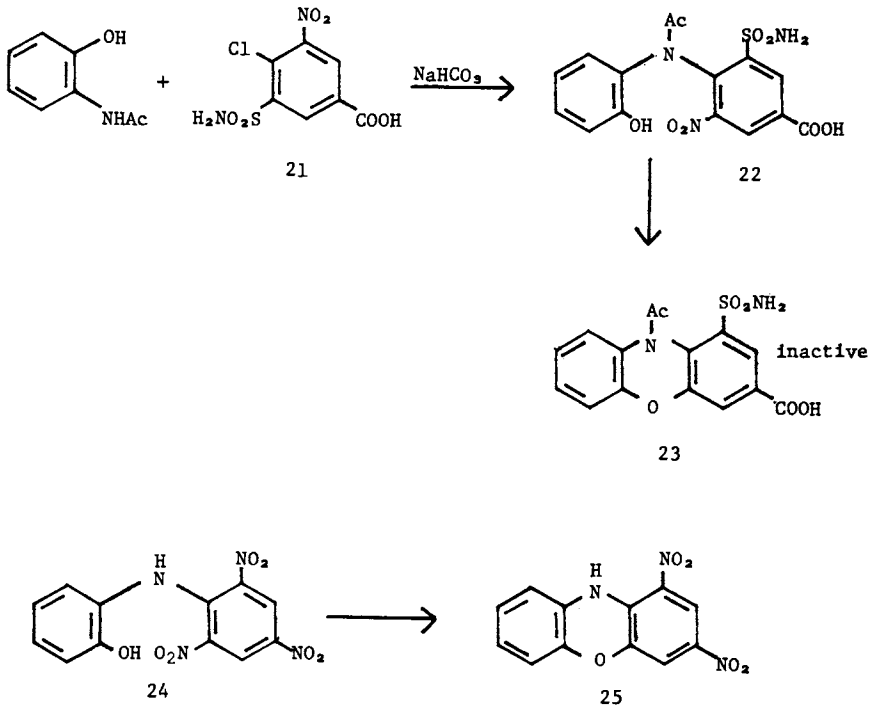


Figure 6. Phenoxazines and phenothiazines

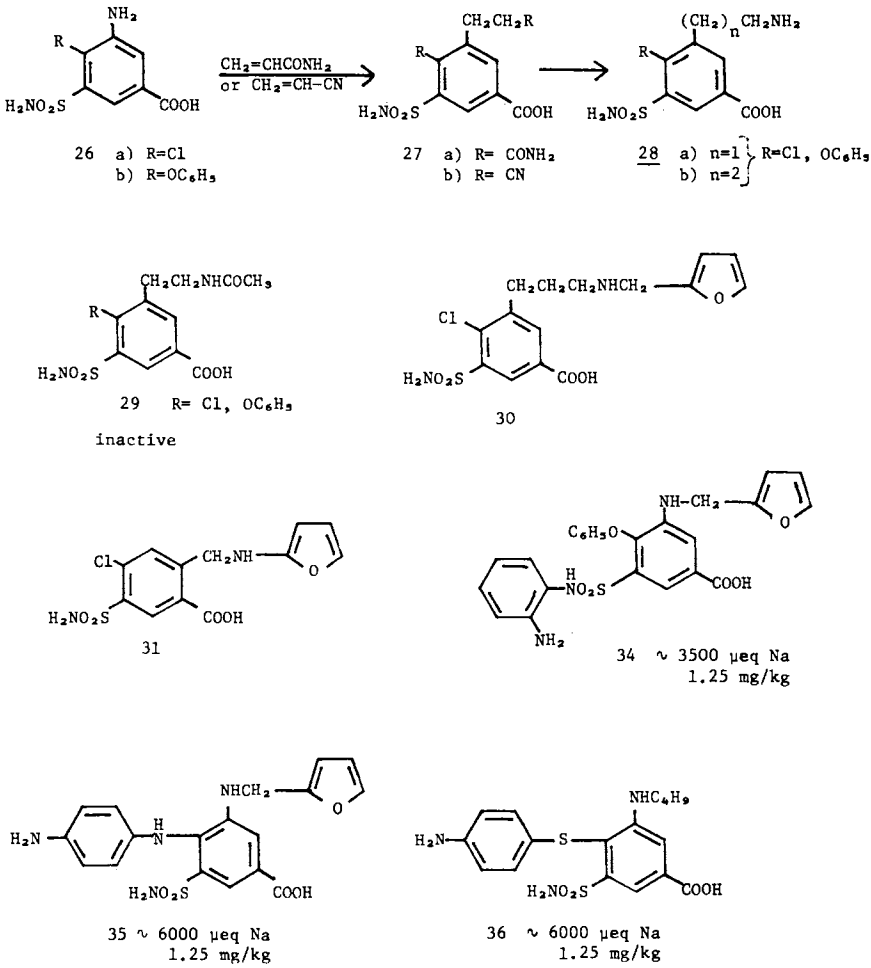


Figure 7. Monocyclic aromatic sulfonamide derivatives

Tricyclic derivatives, as shown in Figure 5, were also investigated. The reaction of o-aminophenol with 4-chloro-5-chlorosulfonyl-3-nitro-benzoic acid (Compound 15) (14) was of interest. Depending on the solvent, ethanol or THF, sulfonylation occurred on oxygen or nitrogen. Ring closure of the sulfonamide intermediate in aqueous $NaHCO_3$ yielded compound 16, whereas the sulfonyloxy intermediate ring closed directly to yield the isomeric tricyclic compound 17 (Figure 5).

The structure of compound 17 was confirmed by reduction of the nitro group to the amine and ring closure to the imidazole derivative (Compound 18) by refluxing in acetic anhydride.

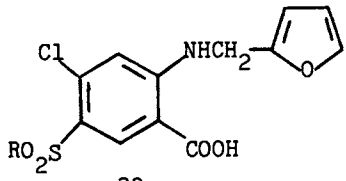
Several derivatives of these two tricyclic compounds were prepared, for example, compounds 19 and 20, both were inactive as diuretics (Figure 5).

Reaction of o-acetaminophenol in aqueous $NaHCO_3$ solution with the sulfamoylbenzoic acid (Compound 21) (14) gave the N-acetyldiphenylamine derivative (Compound 22) in low yield (Figure 6). Whether this compound arises by direct reaction of the highly reactive chlorosulfamoylbenzoic acid (Compound 21) with the acetamino group of the phenol or is formed via a Smiles rearrangement of an intermediate diphenyl ether has not been explored. The main reaction product was the phenoxazine (Compound 23). Similar ring closure reactions via a Smiles rearrangement to phenoxazines and phenothiazines, e.g., compound 24 to compound 25, have been reviewed by Truce, et al (15). The phenoxazine (Compound 23) as well as the deacetylated derivative had no diuretic activity.

Certain monocyclic aromatic sulfonamide derivatives were also studied. Starting with the 3-amino-5-sulfamoylbenzoic acid (Compound 26, Figure 7) (9, 14), the amino group was replaced by a propionamide or propionitrile group under conditions of a Meerwein reaction (Compound 16) using acrylamide or acrylonitrile, respectively, to yield compounds 27a and 27b. A Hoffmann degradation of compound 27a gave the aminoethyl derivative (Compound 28a). Reduction of the nitrile (Compound 27b) yielded the aminopropyl derivative (Compound 28b, Figure 7). The N-acetyl derivative (Compound 29) was inactive. The effect of inserting a three carbon chain in the 3-position of the sulfamoylbenzoic acid moiety of the bumetanide-type diuretics was studied. Compound 30, which is related to bumetanide, was only weakly active, as was compound 31, which differs from furosemide only by a CH_2 group (Figure 7). This may be related to the increased basicity of the benzylic amine function. Feit and co-workers have shown that the aromatic amino group in bumetanide can be replaced by an O- or S- ether linkage without a pronounced change in diuretic potency; however, in the anthranilic acid (furosemide) series, the structural requirements are more stringent.

It is generally accepted that substitution on the sulfonamide nitrogen of sulfonamide diuretics lowers the diuretic

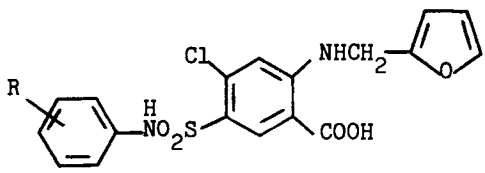
TABLE II



32

	R	Dose mg/kg p.o.	μEq/kg/4 hr Na^+	K^+	Ref.
a:	NH_2	6.25	5000	935	(Furosemide)
b:	$NHCH_3$	5	slight activity		
c:	$NHCH_2C_6H_5$	5	1000		
d:	NHC_6H_5	6.25	2200	400	(18)
	Control		370	340	

TABLE III



33

	R	Dose mg/kg p.o.	μEq/kg/4 hr Na^+	K^+	Ref.
a:	p-Cl	5	very slight activity		
b:	p-NH_2	5	5000	800	(18)
c:	p-NH-CH_3	5	slight activity		
d:	p-$N(CH_3)_2$	5	inactive		
e:	o-NH_2	2.5	5500	900	(18)
	Control		370	340	

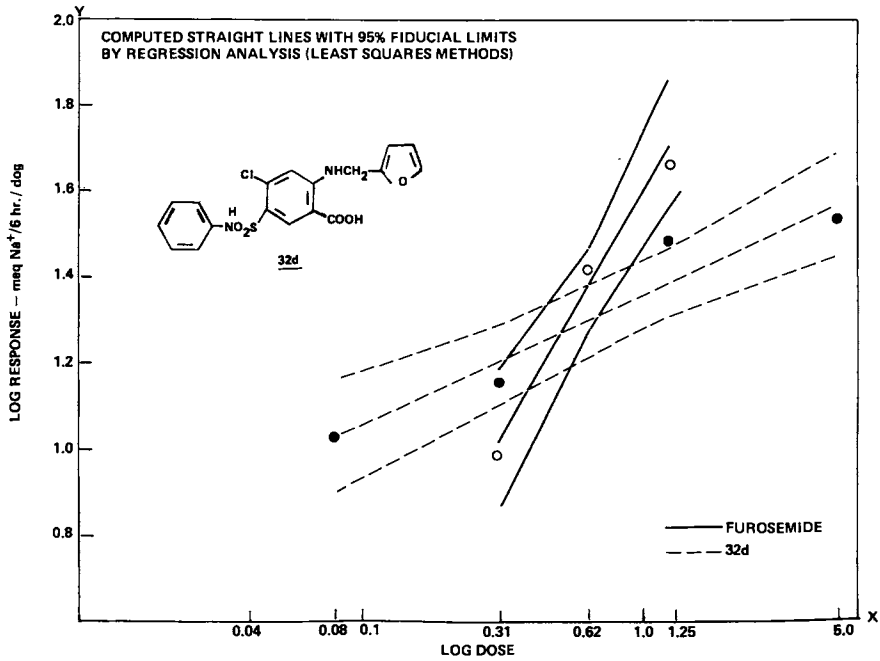


Figure 8. Comparative dose response curves—furosemide vs. compound 32d—Na⁺ excretion in dogs

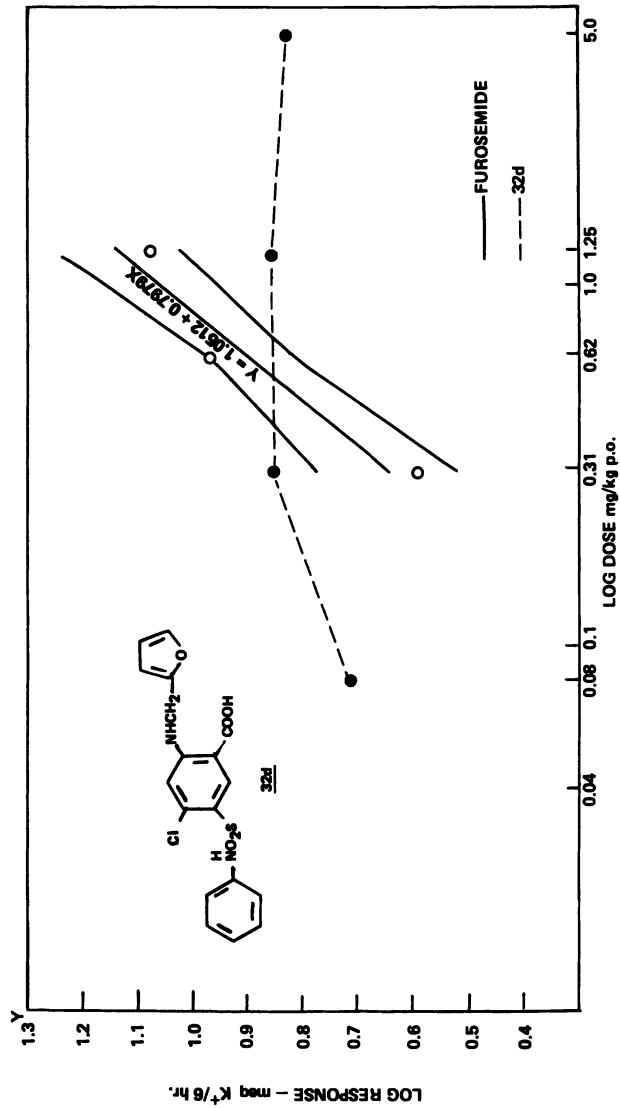


Figure 9. Comparative dose response curves—furosemide vs. compound 32d—K^+ excretion in dogs

activity. Since we were not primarily looking for enhanced potency but were interested in trying to modify some of the other properties such as potassium depletion and effects on blood glucose levels, we investigated this area.

Compound 32a (Table II) is furosemide, methylation of the sulfamoyl nitrogen as in compound 32b drastically reduced the diuretic activity, the N-benzyl derivative (Compound 32c) retained a modest degree of activity. Surprisingly, the N-phenyl derivative (Compound 32d) was quite natriuretic and appeared to have very little effect on potassium excretion as shown in Table II.

This observation was further explored by introducing a substituent on the phenyl ring attached to the sulfamoyl group of compound 32a. The p-chlorophenyl derivative (Compound 33a, Table III) was only weakly active; however, the p-aminophenyl derivative (Compound 33b) proved to be a very active diuretic. Methylation of the amino group as in compounds 33c and 33d greatly reduced the activity; the 4-dimethylaminophenylsulfonamide (Compound 33d) was actually inactive at the 5 mg/kg dose (Table III).

The o-aminophenylsulfonamide derivative (Compound 33e) was more active on a weight basis than the p-aminophenyl derivative (Compound 33b). Unfortunately, potassium excretion was also elevated.

The finding that a para- or ortho-aminophenyl group can enhance activity was also applied to the 3-amino-5-sulfamoylbenzoic acid series (Figure 7). Compound 34 had moderate diuretic activity at 1.25 mg/kg p.o. in the dog. Both compounds 35 and 36 were active diuretics (19, 20). Compound 36 was of somewhat greater interest and was active in a dose related sense over a range of 0.02 mg to 1 mg/kg. Thus, a number of compounds of potential interest had been found and considered worthy of further study.

Compound 32d (Table II) was of interest because it appeared to have very little effect on potassium excretion. It was an active diuretic in dogs and reached a natriuretic ceiling at a dose of approximately 6 mg/kg. Osmolar clearance increased two-to three-fold, while reabsorption of solute free water remained positive in the dog. Figure 8 shows the dose response curve for sodium excretion in dogs. At higher dosages, sodium excretion leveled off for compound 32d, whereas it continued upward with furosemide. Figure 9 shows the effect on potassium excretion, which was practically unaffected by higher doses. Clinical trials in normal volunteers, however, failed to produce any diuretic effects.

Compound 33b was also studied in greater detail. The diuretic effects in the dog resembled those obtained with furosemide, except that at doses above 10 mg/kg, the excretion of Na^+, Cl^-, K^+ and water decreased somewhat (Figure 10). During peak diuresis, the urine was almost isotonic. Renal plasma flow

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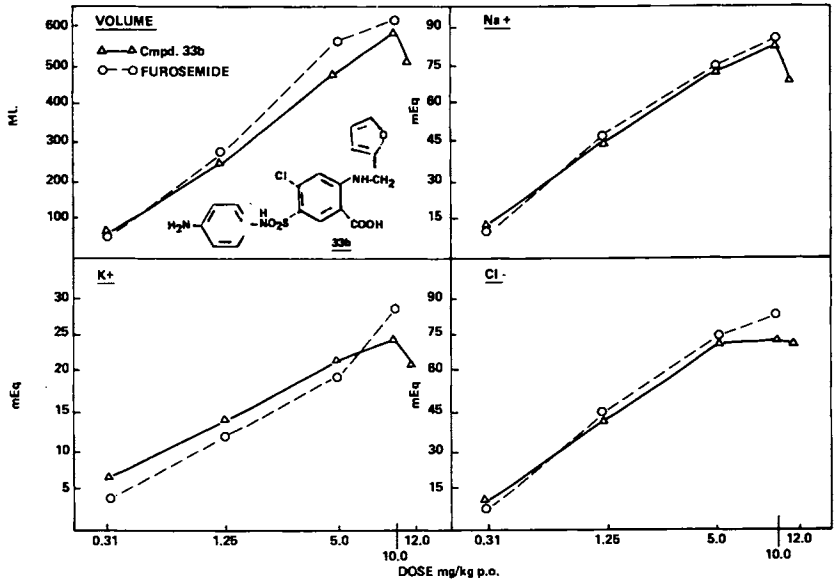

Figure 10. Diuretic effects of compound 33b and furosemide in dogs—excretion per dog per 6 hr

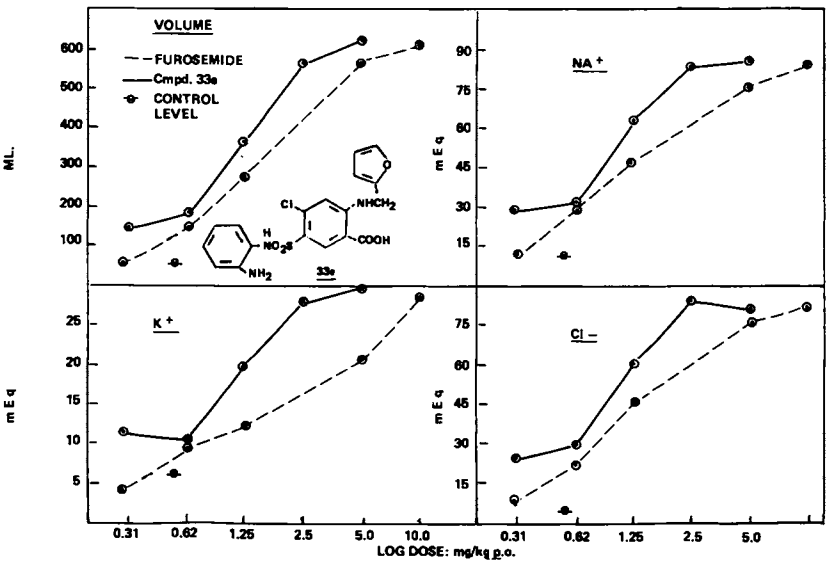

Figure 11. Diuretic effects of compound 33e and furosemide in dogs—excretion per dog per 6 hr

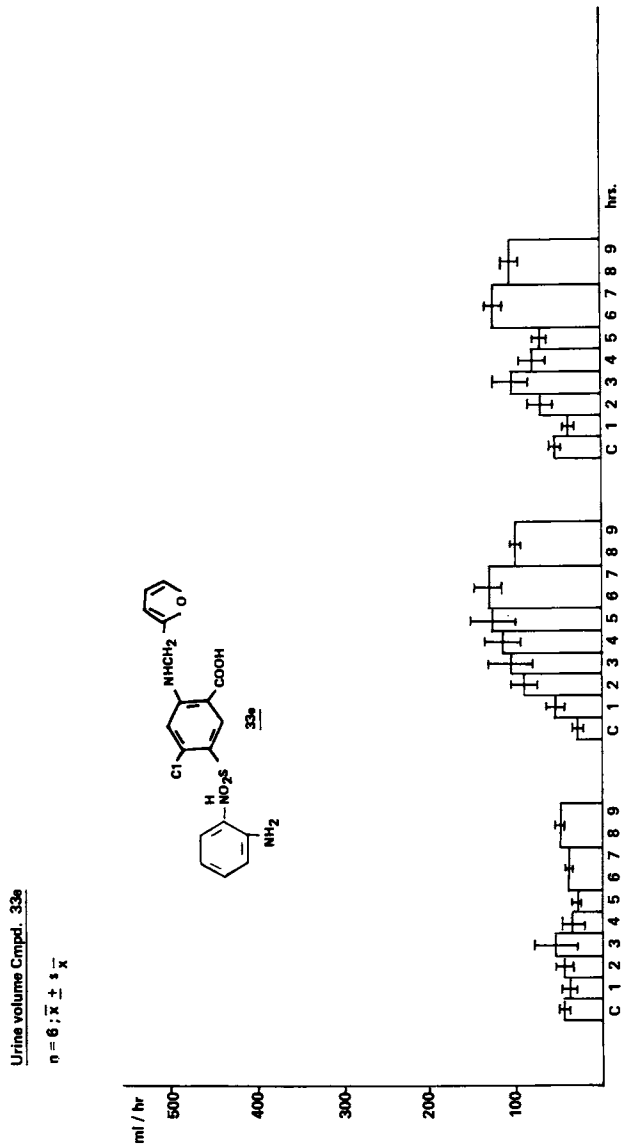

Figure 12. Diuretic response in normal volunteers

and glomerular filtration rate were only slightly affected.

In normal volunteers, doses of 40 to 120 mg produced a rapid onset of diuresis which persisted for 5 to 6 hours, similar to that of furosemide. The natriuretic effect reached a ceiling at the 80 to 120 mg dose and was about equal to 40 mg of furosemide.

Compound 33e was a somewhat more potent diuretic in dogs than furosemide. The onset of action was prompt, reaching a peak after 90 minutes and subsiding after 5 to 6 hours. The dose response curves for urine volume, Na^+, K^+ and Cl^- are shown in Figure 11.

In normal volunteers, contrary to the results obtained in animal experiments, compound 33e was not a high-ceiling diuretic. An activity plateau was reached at 80-120 mg, two to three times the threshold dosage (Figure 12). Its natriuretic effect at this level was less than that induced by 100 mg of hydrochlorothiazide. Interestingly, this compound did not produce a peak level of excretion, but rather a more constant effect persisting over five hours. This type of diuretic response may be desirable in the treatment of hypertension.

Unfortunately, none of the compounds described were potassium-sparing nor did they have appreciably less effect on glucose tolerance; however, no uricosuric effect was observed. This brief overview of some of our work on sulfonamide diuretics illustrates one of the problems we encounter in medicinal chemistry; namely, that encouraging biological data in animals do not always translate into equally favorable clinical results.

Acknowledgments - We wish to thank Dr. O. Büch, Dr. P. R. Hedwall and Dr. J. Kraetz, Basle, and Dr. M. J. Antonaccio, Dr. W. E. Barrett and Mr. R. Rutledge, Summit, for the pharmacological data and Dr. P. R. Imhof, Basle, for the clinical data.

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5

Diuretic and Uricosuric Properties of Tienilic Acid (Ticrynafen) in Mice, Rats, and Anesthetized Beagle Dogs

Antihypertensive Activity in SH Rats and Structure–Activity Relationship

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In the last twenty years, two series of compounds have been discovered which exhibit marked diuretic and saluretic activity. These are (a) the phenoxyacetic acids, the most prominent member being ethacrynic acid (1,2, Figure 1) and (b) the sulfamoylbenzoic acids, the most potent being furosemide (3) and bumetanide (4,5). The latter compound differs from furosemide in several respects, the most obvious being that the 5-chloro substituent is replaced by phenoxy.

These very potent natriuretic agents are sufficiently effective to satisfy the physician for use in the treatment of hypertension and cardiac diseases; however, they possess unwanted side effects which include potassium depletion, a diabetogenic propensity (6,7,8,9) and hyperuricemia (10-23). To solve the problem of potassium depletion, a search for new diuretic agents led to the discovery of compounds which block the tubular sodium-potassium exchange either directly, i.e., triamterene (24) and amiloride (25), or by blocking the action of aldosterone, i.e., spironolactone.

In regard to diuretic-induced hyperuricemia, the discovery in 1967 of tienilic acid was a major contribution to diuretic therapy. This compound, [2,3-dichloro-4-(2-thienylcarbonyl)phenoxy]-acetic acid, is an aryloxyacetic acid analog of ethacrynic acid which exhibits diuretic and uricosuric activity in mice, rats and dogs (28), as well as in man (29,30,31). More recently, another aryloxyacetic acid, an indanone (32,33,34,35), has been reported to possess potent diuretic and uricosuric activity in chimpanzees and in man.

The purpose of the studies which we will describe was to evaluate the diuretic and uricosuric effects of tienilic acid in mice, rats and dogs and the antihypertensive properties in SH rats preliminary to clinical trials.

METHODS

Diuretic and Uricosuric Activity in Mice (36) - Initial screening

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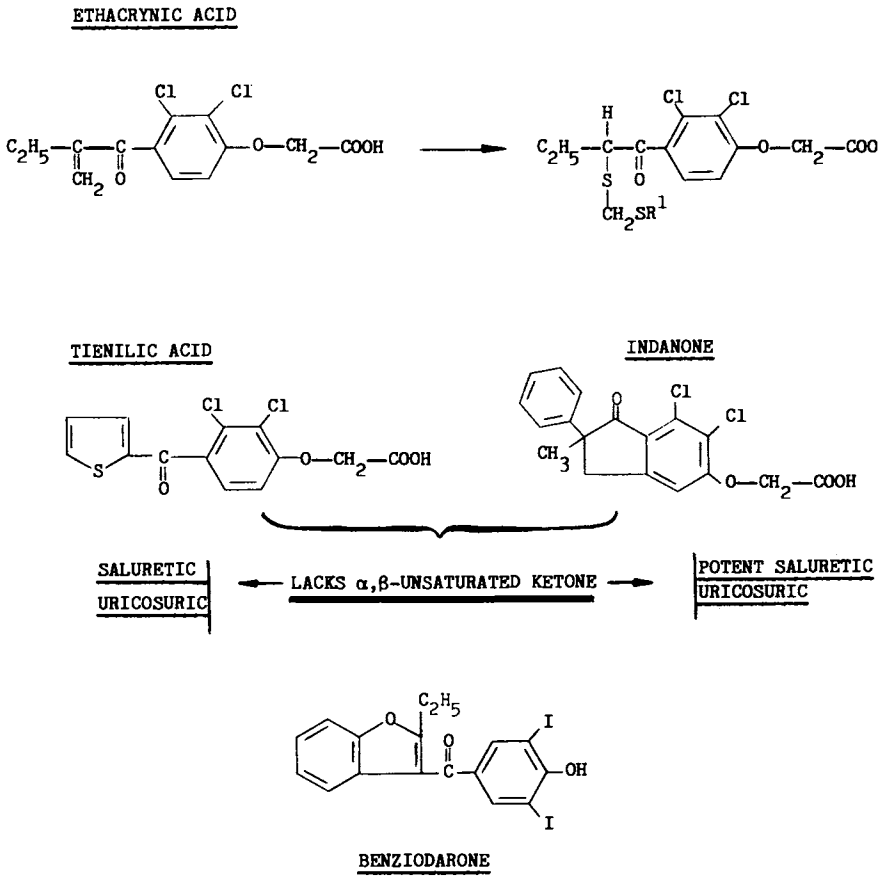

Figure 1. Compound structures

of diuretic agents were performed using groups of 6 male Swiss mice, weighing 22 ± 1 g, randomly selected and distributed in pairs into metabolism cages for 2 or 4 hours. Food and water were withheld for 2 hours prior to the experiments, urine was collected at 2 and 4 hours and the urine volumes and electrolytes measured. All mice were administered 1 ml of 0.9% saline orally and either simultaneously treated or not (control group) with diuretic agents. Test compounds (tienilic acid, ethacrynic acid and furosemide) were dissolved or suspended in the same 0.9% saline and administered by gavage. Four groups received tienilic acid orally at doses of 20, 50, 100 and 200 mg/kg and were compared to furosemide at 20 mg/kg, ethacrynic acid at 20 mg/kg and benziodarone (a potent non-diuretic uricosuric agent) at 100 mg/kg by the same route. For the general screening of diuretic and uricosuric drugs, such as tienilic acid and its congeners, the compounds were administered at doses of 100 mg/kg orally in comparison to furosemide or ethacrynic acid at 20 mg/kg.

Na^+ and K^+ analyses were determined using the flame photometer (Eppendorf), chlorides by microanalysis (37) and uric acid by the enzymatic spectrophotometric technique (38) or by a colorimetric method (39). Statistical analysis was made by application of Student's t test with simultaneous calculation of means (M) and standard deviation of the mean ($S/\sqrt{n}$).

Tienilic Acid-Benziodarone and Furosemide-Benziodarone Relationships in Mice - Groups of 6 mice weighing 22 ± 1 g were administered simultaneously either 100 mg/kg or 200 mg/kg of tienilic acid, or 200 mg/kg of benziodarone, or 20 mg/kg of furosemide or a combination of the same doses of tienilic acid + benziodarone or furosemide + benziodarone. After dosing, all animals were distributed by pairs into metabolism cages for 2 hours. Urine samples were collected for the determination of electrolyte and uric acid excretion using the same analytical methods as previously described. The statistical significance was based on a comparison of tienilic acid + benziodarone or furosemide + benziodarone versus tienilic acid alone, furosemide alone or benziodarone alone (Student's t test).

Diuretic Activity in Rats - Groups of 3 animals weighing 400 ± 5 g, deprived of food and water overnight, were given 20 ml/kg of water by intubation on the day preceding the experiment. After dosing, the animals were placed in metabolism cages for the collection of a 5 hour sample of urine. Diuretic drugs were administered as in the previous experiment in a volume of 20 ml/kg of 0.9% saline at the beginning of the experiment, while a control group was administered only the saline solution. Statistical significance refers to a comparison between treated and control rats (Student's t test). Dose-response relationships are illustrated in Figure 5 using the regression line procedure.

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Phenol-Red Retention Test in Rats (40,41) - Groups of 5 randomized male rats, weighing 180 ± 20 g, were given the test compounds orally 30 minutes before the intravenous injection of 75 mg/kg of phenol-red as a 1.5% solution in 0.9% sodium chloride. Heparinized blood samples were taken from the retro-orbital plexus at 15, 30, 45 and 60 minutes; color was developed by the addition of 0.05 ml of 0.1 M sodium hydroxide and read at 540 mμ on the spectrocolorimeter (Eppendorf). Results were calculated as percentage of variation in relation to the control value. Statistical significance was calculated by comparison with treated and control rats (Student's t test).

Antihypertensive Activity in Spontaneous Hypertensive Rats (Wistar-Okamoto Strain) - The studies were performed in Wistar hypertensive male rats which were approximately 25 weeks old and randomly placed in groups of six animals. The systolic blood pressure was determined by the tail cuff technique (Physiograph Narco System) before treatment and at 2, 5 and 24 hours after each daily administration of the drug or the vehicle, in acute (1 day) and in subacute experiments (8 days). In chronic studies, the blood pressure was measured indirectly before and 1.5, 3.5, 6, 12 and 18 months after the beginning of the experiment. In the last study, tienilic acid was mixed with the daily food at a level calculated to provide an oral intake of 100 and 200 mg/kg.

In the acute oral experiments, the rats (N = 6) were given 100, 200 and 400 mg/kg of tienilic acid; in the subacute experiments, the daily oral dose was 200 mg/kg. Statistical analysis was determined by Tukey's multiple comparisons procedure which permits the statistical classification of means between themselves or compared to control data. In Figure 17, statistical significance is illustrated by the addition of a broken line to the main solid line. Each point represents a mean value, and the vertical bars indicate the standard error of the mean.

In the chronic study, statistical significance was calculated by Student's t paired test.

In all the experiments, the blood pressure was measured indirectly in a control group of SH rats under the same conditions. In addition, in the chronic study, the treated SH rats were compared to control Wistar normotensive rats.

Triglyceride-Lowering Effect in Obese Rats (Fatty Strain) - Measurement of the triglyceride-lowering effect was carried out in groups of 5 to 7 obese male rats (Fatty strain) and compared to normal heterozygous rats as a first control group and to normal Wistar rats as a second control group.

Obese rats were randomly distributed into two groups of 5 to 7 animals which were given either 200 mg/kg of tienilic acid as an oral daily dose for 7 days or the vehicle alone. The other control groups (heterozygous rats and normal Wistar rats) were given only the fluid vehicle. Blood samples were taken at the end of the

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experiment from the retro-orbital plexus in order to determine plasma glucose, total lipids, cholesterol and triglyceride levels after daily treatment with tienilic acid. Statistical significance was calculated in comparison with treated and control rats (Student's t test).

Diuretic, Uricosuric and Clearance Studies in Anesthetized Beagle Dogs under Hydropenic Conditions - The diuretic, uricosuric and clearance studies were performed in beagle dogs weighing 10 to 13 kg which had been fasted overnight and then anesthetized by i.v. injection of mebubarbital, 30 mg/kg. During the experiment, the dogs were intravenously infused at a rate of 2 ml/minute with 0.6% saline, 0.08% PAH, 0.4% creatinine and mebubarbital, 5 mg/kg/hour using a constant rate infusion pump. After mesial laparotomy and catheterization of the ureters, each study started with a control period of two hours. Blood and urine samples were collected at 15 minute intervals. Then, the lysine salt of tienilic acid was injected intravenously at 5, 10 and 20 mg/kg. A group of 4 to 6 beagle dogs was used for each dose and for the control (placebo) evaluation. Sodium and potassium determinations were made using the Eppendorf Flame Photometer, chloride by the colorimetric micromethod (37) and urate by the enzymatic procedure (38). Other measurements and calculations which were made are as follows: PAH (42), creatinine (43), urea (44), pH, blood pressure, C_{osm} (osmolar clearance) after cryoscopic determination (using the freezing point depression), $T^C H_2O$ (free water reabsorption) and $T^C H_2O = C_{osm} - V$ (ml/min). The $C_{osm} - T^C H_2O$ relationship was studied using $T^C H_2O$ corrected for osmolar clearance and creatinine clearance as previously described (45): $\frac{T^C H_2O / C_{osm}}{C_{creat.}}$. All the clearance studies were determined using standard procedures. Statistical significance was calculated as previously described by the application of Tukey's test (Section 5). Data are illustrated in various figures as the mean values and the standard error of the mean (vertical bars) or as percentage of control values.

RESULTS

Diuretic Activity in Mice - As shown in Figure 2, the oral administration of tienilic acid (20, 50, 100, 200 mg/kg p.o.) gave a significant increase in urine volume and electrolyte excretion. However, in comparison with furosemide or ethacrynic acid, tienilic acid is about 1/5 to 1/10 as potent. Nevertheless, contrary to other diuretic and natriuretic agents, tienilic acid increases urate excretion.

The uricosuric activity of tienilic acid in mice is dose-related, as shown in Figure 3, in comparison with that of furosemide and ethacrynic acid in effective diuretic doses. In the same experiment, benziodarone, which possesses significant uricosuric

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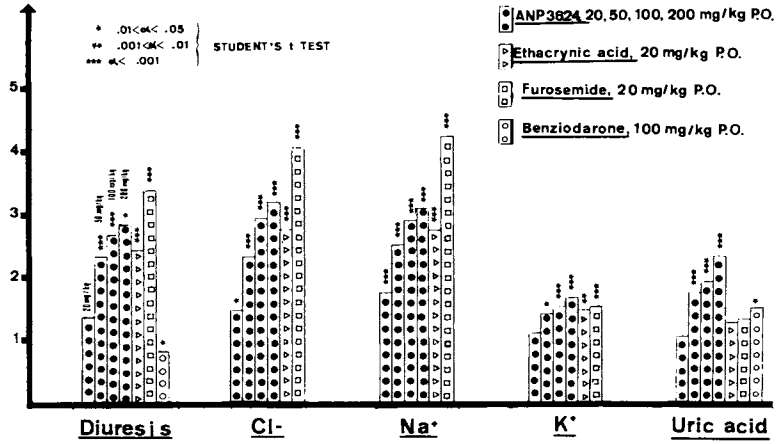

Figure 2. ANP 3624 (tienilic acid–ticrynafen)—diuretic and uricosuric activity in the mouse

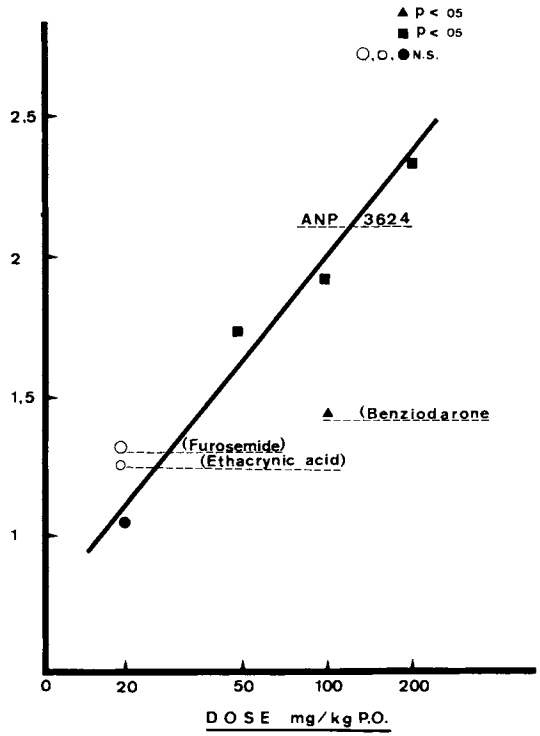

Figure 3. ANP 3624 (tienilic acid–ticrynafen)—uricosuric activity in the mouse

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properties in humans (46,67), has increased the urate excretion in mice without greatly altering renal function. However, in the benziodarone-tienilic acid interrelationship studies, it was demonstrated that benziodarone inhibits the diuretic effect of tienilic acid, but their uricosuric effects were synergistic (Figure 4). Conversely, the benziodarone-furosemide combination does not modify these parameters.

As seen in Table I, tienilic acid is the most active member of this series, and only three compounds possess both diuretic and uricosuric activities, i.e., ANP 3624 (tienilic acid), ANP 4316 (the 3-thienyl analogue of tienilic acid) and ANP 3860 (the 2-benzofuranyl analogue of tienilic acid). This latter compound, which produced an increase in urate elimination along with a weak natriuretic effect, possesses some of the structural features of the 2,3-dichlorophenoxyacetic acids and some of those of benziodarone, which is a benzofuran. From these studies, it is obvious that tienilic acid has a specific effect on the urate transport. This activity of the drug, initially observed in mice, has been confirmed in rats and dogs.

Oral Diuretic Activity of Tienilic Acid in the Rat - As seen in Figure 5 and according to many authors, ethacrynic acid was ineffective at a dose as high as 300 mg/kg (Γ = .29 NS); tienilic acid was weakly diuretic in doses up to 400 mg/kg [Γ = .96 ($P < .05$)] and furosemide exhibited marked diuretic activity at a dose of 50 mg/kg.

Uricosuric Activity of Tienilic Acid in the Rat as Measured by the Phenol-Red Test - In view of the difficulties in administering uric acid to small animals, the indirect method of Scarborough and McKinney (41) for screening uricosuric agents in rats was used. This indirect method does not measure the excretion of uric acid, but rather the phenol-red retention induced by all uricosuric substances.

In this test, as illustrated in Figure 6, tienilic acid and benziodarone exhibited similar inhibition of phenol-red elimination, while furosemide and ethacrynic acid were ineffective. It should be noted that in the same experiment probenecid and sulfinpyrazone reduced the phenol-red elimination and that acetylsalicylic acid strongly inhibited the activity of tienilic acid and benziodarone (unpublished data).

Diuretic Activity in Anesthetized Beagle Dogs - Diuretic and Natriuretic Activity - The acute effect of tienilic acid on urine and electrolyte excretion in anesthetized beagle dogs is illustrated in Figures 7-11. Intravenous administration of 5, 10, 20 mg/kg of tienilic acid caused a statistically significant dose-related excretion of urine (Figure 7) with an increase of renal sodium (Figure 8), potassium (Figure 9) and chloride excretion (Figure 10). At every dose, the sum of Na^+ and K^+ excretion was

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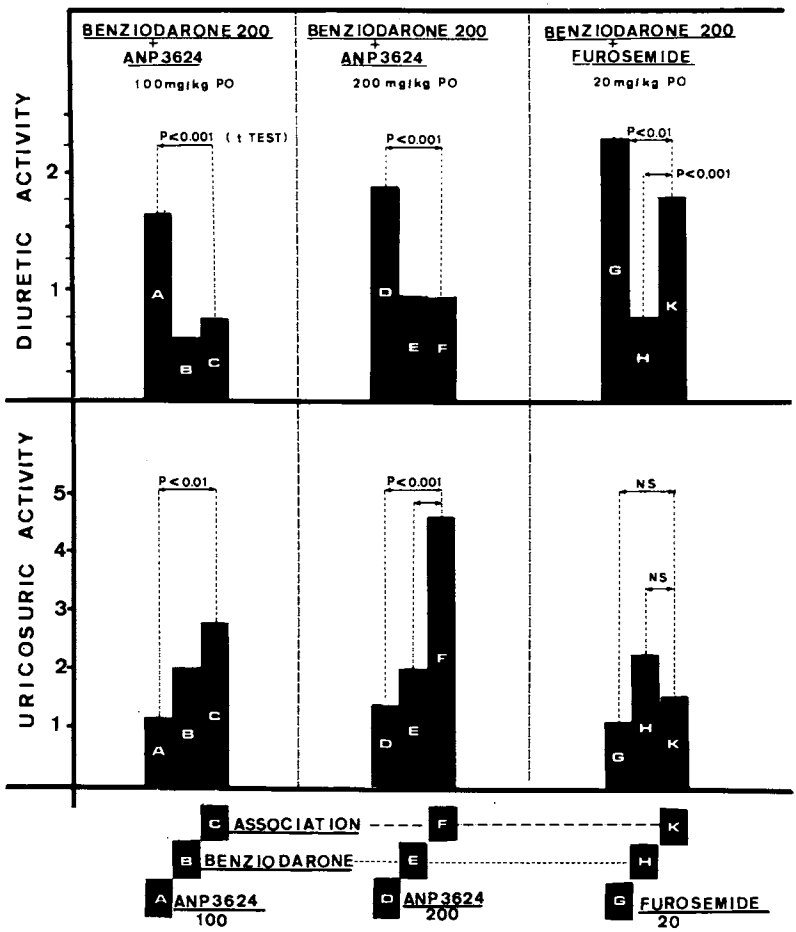
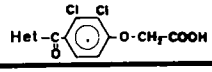


Figure 4. Antagonism benziodarone–ANP 3624 (diuresis) with synergistic increase of the uricosuric effect (mice N =6)

Table I



		DIURETIC ACTIVITY (MICE)				
ANP	HET.	H_2O	Na^+	K^+	$\frac{Na^+}{K^+}$	urates
3624**		2.7*	2.9*	1.5	1.9*	1.9*
3649		1.2	1.8	1.1	1.7	1
4357		.8	.7	.6	1.2	.9
3598*		1.8*	5.4*	1.5*	3.5*	1.1
4384		1.1	1.4	.8	1.7	1.1
4316**		2.7*	2.2*	1.9*	1.2	1.6*
4381*		1.6*	2	.1	1.8*	.9
4303		1.2	1.2	.9	1.3	1.1
4294		1.3	1.3	1.2	1.1	1.3
3860*		1.3	1.5*	1.7*	.9	1.6*
A*	ETACRYNIC ACID	2.4*	2.8*	1.5	1.9*	1.3
B*	FUROSEMIDE	3*	4.3*	1.5	2.8*	1.4

•, *, ★, $P < 0.05$ STUDENT's t TEST

A, B 20 mg/kg PO

ANP3624... 100 mg/kg PO

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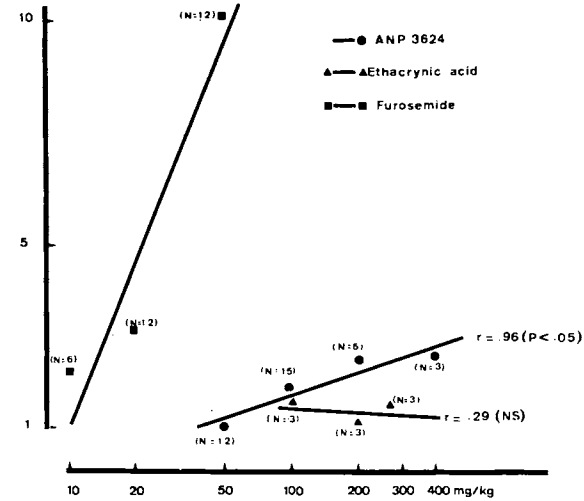

Figure 5. ANP 3624 (tienilic acid–ticrynafen)—diuretic activity in the normal rat

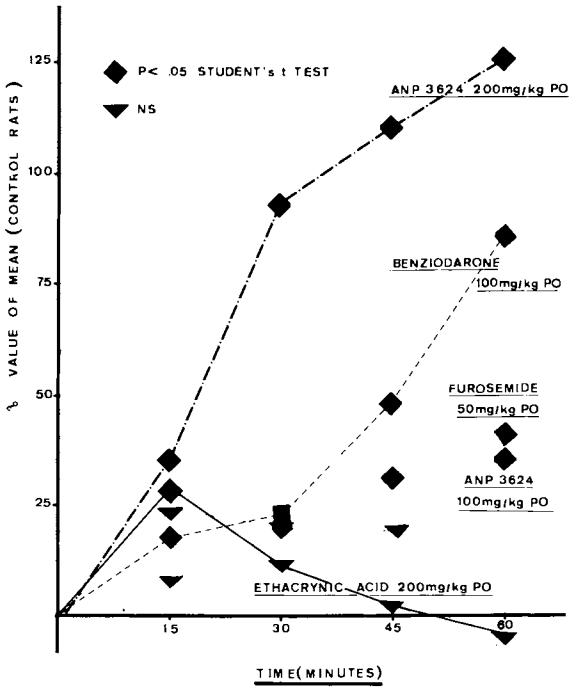

Figure 6. ANP 3624 (tienilic acid–ticrynafen)—retention of phenol red in the rat (N = 5)

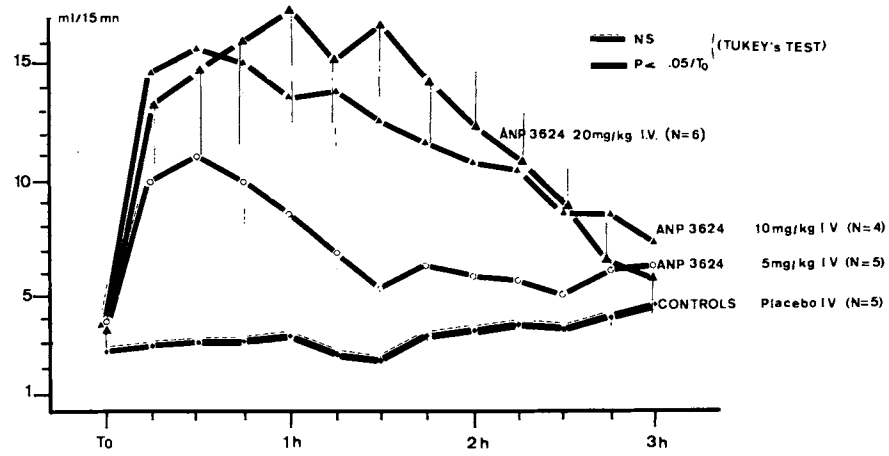

Figure 7. ANP 3624 (tienilic acid–ticrynafen)—diuretic activity in the anesthetized beagle dog

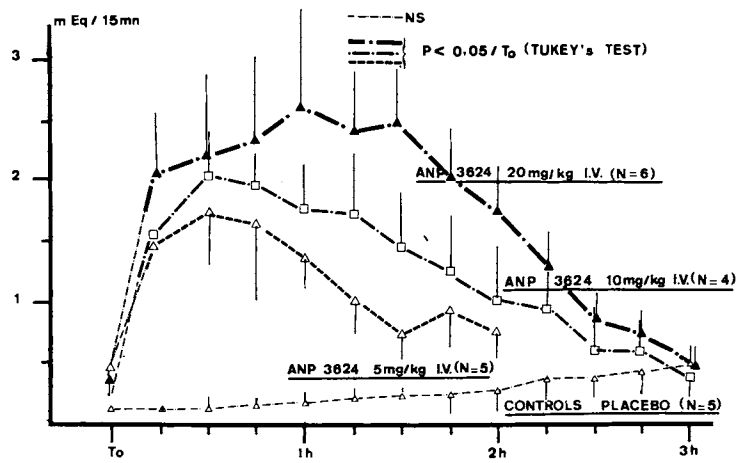

Figure 8. ANP 3624 (tienilic acid–ticrynafen)—diuretic activity in the anesthetized beagle dog (Na^+)

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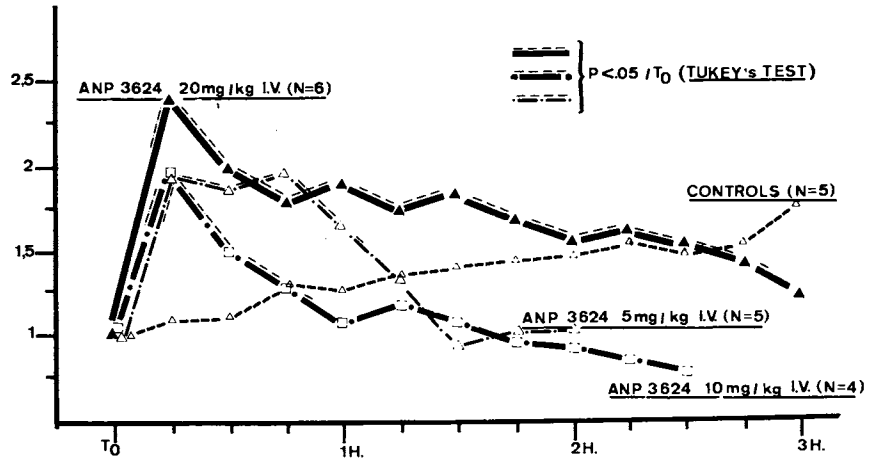

Figure 9. ANP 3624 (tienilic acid–ticrynafen)—diuretic activity in the anesthetized beagle dog (K^+)

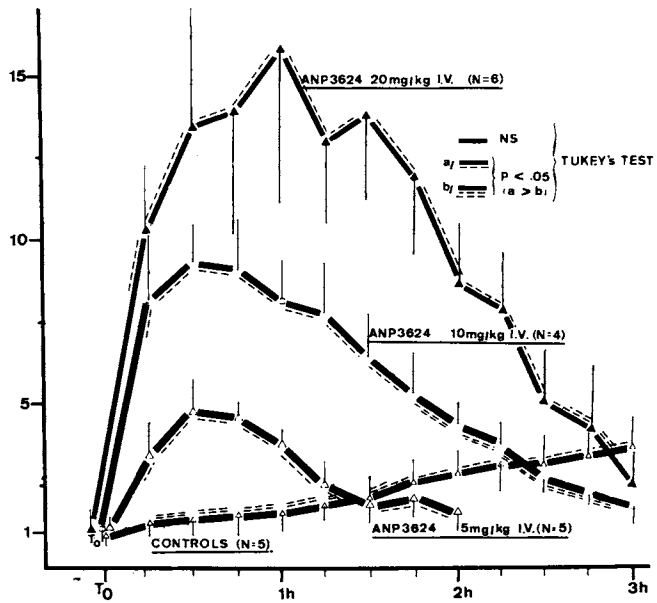

Figure 10. ANP 3624 (tienilic acid–ticrynafen)—diuretic activity in the anesthetized beagle dog (Cl^-)

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about equal to that of chloride excretion and the urinary pH remained unchanged. In these experiments, the total duration of urine and electrolyte excretion was approximately 3 hours. After intravenous administration, the maximal diuretic and natriuretic response occurred within 30 minutes after drug injection.

In all of the experiments the rise of Na^+ excretion was accompanied by a rise in K^+ excretion, but the Na^+/K^+ ratio was generally increased as with each of the diuretic substances (Figure 11).

Uricosuric Activity - The effects of tienilic acid on the urate excretion in anesthetized beagle dogs are seen in Figures 12-14. Intravenous administration of 10 and 20 mg/kg of tienilic acid caused a statistically significant urate excretion. The time of maximum increase in urine output is about the same as that for Na^+ excretion and the greatest uricosuric activity was observed 15 minutes after treatment, but a statistically significant response lasted for 3 hours (Tukey's test). In this experiment, 5 mg/kg of tienilic acid did not induce uricosuria. During the same period, no statistically significant changes in creatinine clearance were noted (Figures 13, 14).

Clearance Studies - As shown in Figures 13-15, tienilic acid at i.v. doses of 5, 10 and 20 mg/kg affected renal clearances, particularly osmolar clearance and free water reabsorption when exogenous creatinine excretion showed no statistically significant change.

In the hydropenic state, a slight rise of urine volume was accompanied by marked increases in total solute clearance. This effect, which is shown in Figure 15 (20 mg/kg i.v. of tienilic acid), caused an increase in free water reabsorption. But, interestingly, when these data were corrected for osmolar clearance and glomerular filtration rate, as previously suggested (45, 47-52), tienilic acid proved to decrease the negative free water clearance (Figure 16), i.e., it decreases free water reabsorption. In these experiments, after 20 mg/kg i.v. of tienilic acid (N = 6), C_{osm} increased from 0.60 ± 0.11 to 1.79 ± 0.26 ml per minute after 30 minutes when there was a progressive rise in T^CH_2O from 0.40 ± 0.07 to 0.80 ± 0.11. Using data factored by C_{osm} and glomerular filtration rate (Figure 16), T^CH_2O decreased from 3.527 ± 1.028 to 1.410 ± 0.366 after 90 minutes.

In the same experiment, 10 mg/kg of tienilic acid provoked a similar decrease in corrected tubular water reabsorption, i.e., dropping from 3.103 ± 1.258 to 0.918 ± 0.268 after 60 minutes. These disturbances in renal concentrating mechanism, which are summarized in Figure 16 and statistically analyzed by Tukey's test, were still significantly different from control values 3 hours after the diuretic was given; thus, the administration of 5 mg/kg of tienilic acid led to a significant change in the free water reabsorption.

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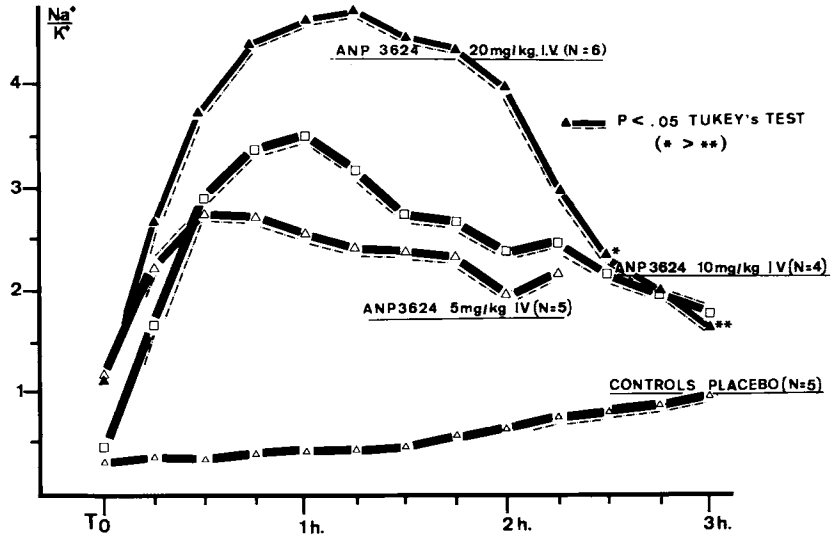

Figure 11. ANP 3624 (tienilic acid–ticrynafen)—diuretic activity in the anesthetized beagle dog

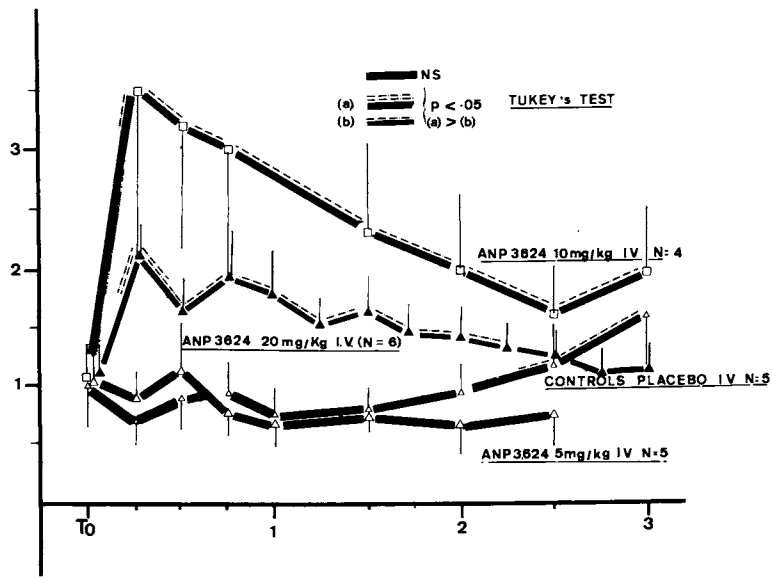

Figure 12. ANP 3624 (tienilic acid–ticrynafen)—uricosuric activity in the anesthetized beagle dog

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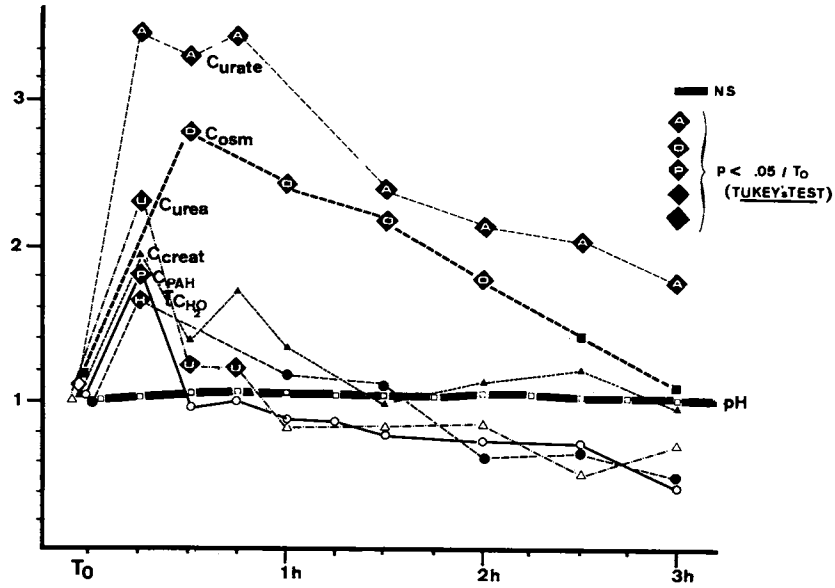

Figure 13. ANP 3624 (tienilic acid–ticrynafen)—10 mg/kg iv clearance studies in the anesthetized beagle dog (N = 3–5)

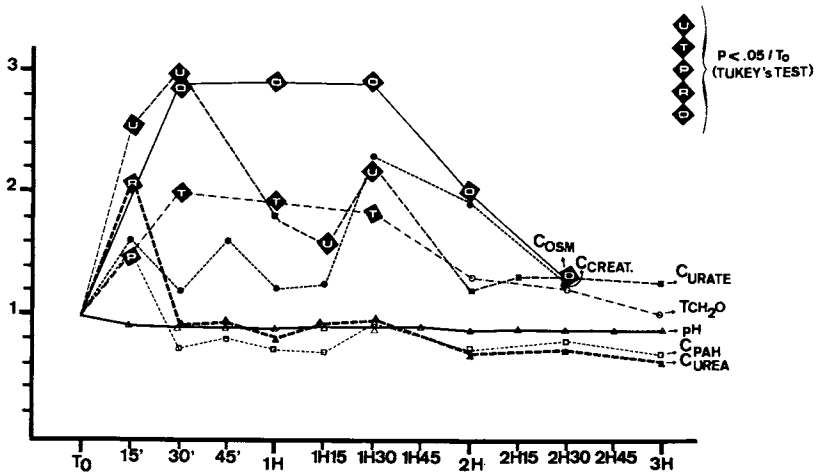

Figure 14. ANP 3624 (tienilic acid–ticrynafen)—20 mg/kg iv clearance studies in the anesthetized beagle dog (N = 6)

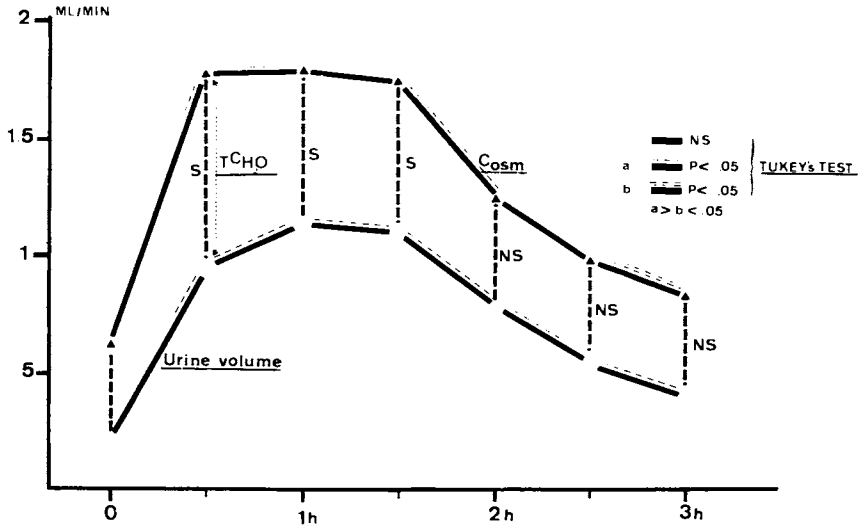

Figure 15. ANP 3624 (tienilic acid–ticrynafen)—20 mg/kg iv (N = 6) = diuretic activity in the anesthetized beagle dog

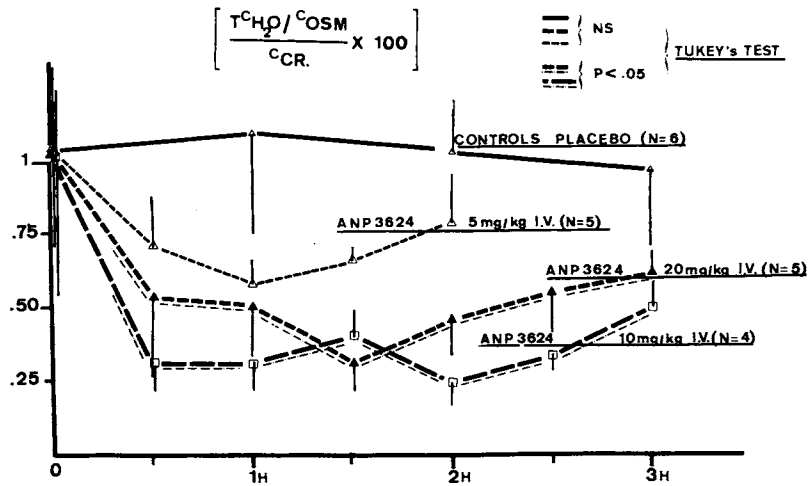

Figure 16. ANP 3624 (tienilic acid–ticrynafen)—diuretic activity in the anesthetized beagle dog

During the same period, minor variations in PAH, creatinine and urea clearances were induced by 10 and 20 mg/kg of tienilic acid as demonstrated in Figures 13 and 14. Notably, the clearance of PAH increased from 71.38 ± 11.22 to 100.04 ± 29.99 and from 66.4 ± 13.03 to 121.27 ± 39.16 at 5 mg/kg and 20 mg/kg of tienilic acid, respectively. Under the same experimental conditions, the glomerular filtration rate slightly increased, i.e., the filtration fraction had a value between 0.35 and 0.50, or remained unchanged. The time required for the maximum change in PAH clearance was usually about 15-30 minutes after administration of tienilic acid.

Antihypertensive Activity of Tienilic Acid in the SH Rat - The combined diuretic, natriuretic and uricosuric properties of tienilic acid led to a study of its potential antihypertensive activity in SH rats using acute, subacute and chronic experiments in which the classic cuff technique was employed.

Acute Antihypertensive Activity (Figure 17) - The systolic blood pressure of SH rats was significantly (Tukey's test) decreased after 2, 5 and 24 hours following administration of 200 and 400 mg/kg of tienilic acid by the oral route. The placebo did not produce any change in blood pressure, and tienilic acid at 100 mg/kg significantly decreased the blood pressure only after 2 and 5 hours following administration of the drug.

Subacute Antihypertensive Activity - Figure 18 shows that daily administration of 200 mg/kg of tienilic acid for 5 days produced a sustained antihypertensive response in SH rats at each time interval during the experiment. Three days after withdrawal of the drug, the blood pressure returned to control values.

Chronic Antihypertensive Activity (Figure 19) - Tienilic acid was administered orally to SH rats in daily doses of 100 and 200 mg/kg (mixed with food) for 6 months. For 100 mg/kg, N = 27; 200 mg/kg, N = 29; control normal rats, (N.R.) N = 15 and control SH rats (SHR) N = 25. The systolic blood pressure was measured at the beginning and then after 1.5, 3.5 and 6 months. As illustrated in Figure 19, tienilic acid (200 mg/kg) decreased the systolic blood pressure ($P < 0.05$ - Tukey's test) in SH rats after 3.5 and 6 months. Neither control nor tienilic acid at 100 mg/kg significantly affected the blood pressure measured indirectly in SH rats.

Interestingly, after 20 months, the systolic blood pressure was not significantly altered by either 100 or 200 mg/kg of tienilic acid. However, the mortality of the SH rats was statistically less in the animals receiving 200 mg/kg of tienilic acid, $T_{27}^{21} = 408$; $0.01 < \alpha < 0.05$ [Wilcoxon's non-parametric procedure (53)]. The respective numbers of surviving animals were 12/15 (normotensive Wistar rats), 4/21 (control SH rats), 16/27

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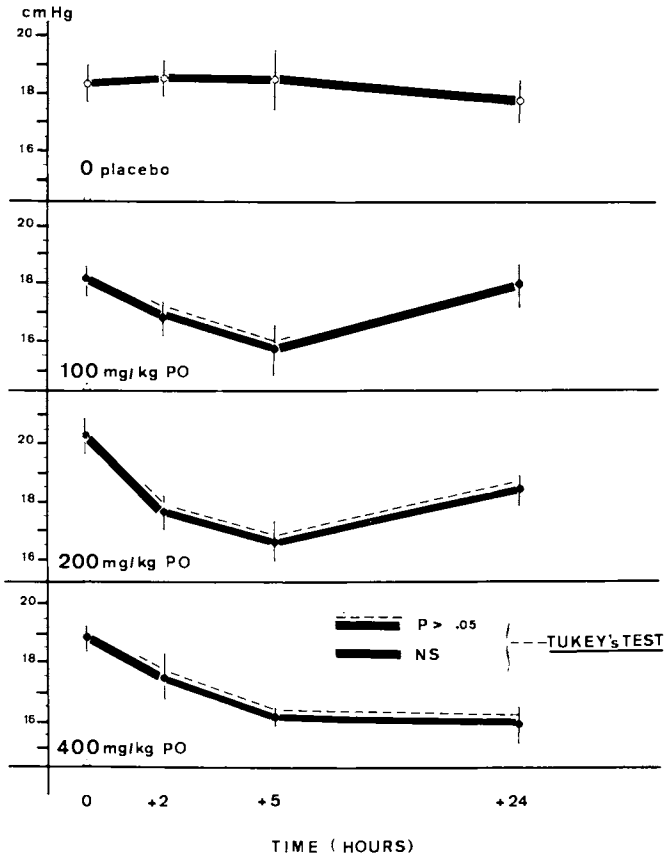

Figure 17. ANP 3624 (tienilic acid–ticrynafen)—acute antihypertensive activity in the SH rat (N = 6)

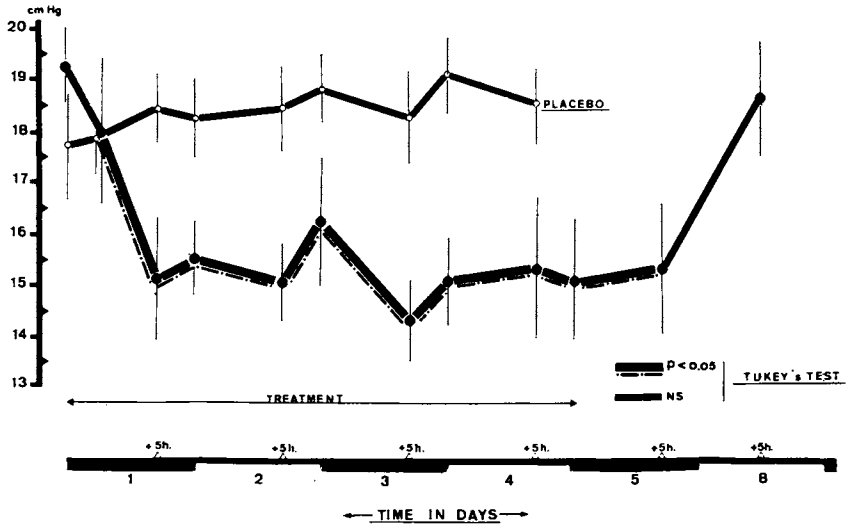

Figure 18. ANP 3624 (tienilic acid–ticrynafen)—200 mg/kg/day po—antihypertensive activity in the SH rat (N = 6)

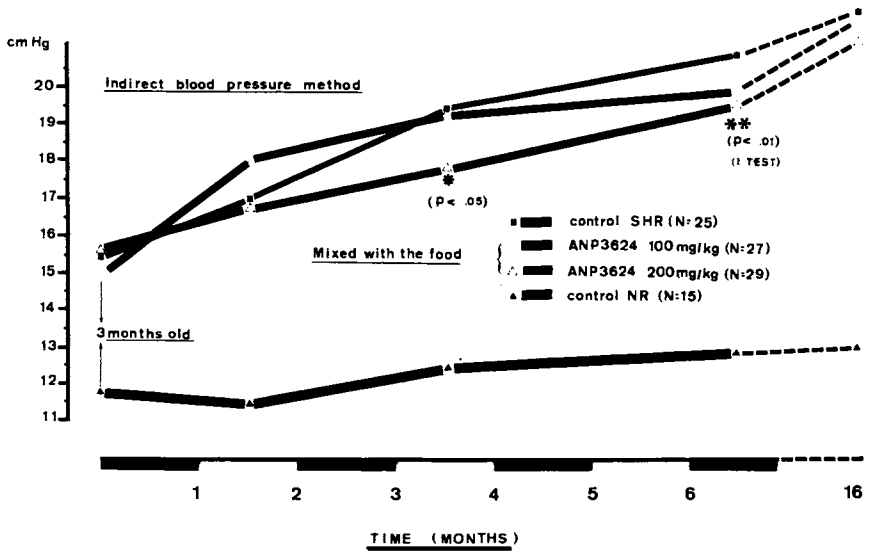

Figure 19. ANP 3624 (tienilic acid–ticrynafen)—antihypertensive activity. Chronic study in the SH rat (♂).

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(tienilic acid at 200 mg/kg) and 5/26 (tienilic acid at 100 mg/kg).

Blood Triglyceride-Lowering Effect in the Obese Rats (Fatty Strain) - In previous experiments, Maass (74) found that tienilic acid decreases the blood levels of triglycerides in normal rats. A similar activity has been found again in obese rats (Fatty Strain), but not in normal rats (Figure 20). However, total lipids, blood cholesterol and glucose levels remained unchanged in both animal models.

DISCUSSION

This paper describes the diuretic, natriuretic and uricosuric studies carried out over a period of years (28) in a variety of animals, including mice, rats and dogs. Recent pharmacological and clinical reports have confirmed the original observations (54-63) of Masbernard (29) who first reported the human pharmacological studies on this drug.

As shown in mice, rats and anesthetized beagle dogs, tienilic acid (ANP 3624), in contrast to furosemide, ethacrynic acid, the indanone and bumetanide, is a mild saluretic-diuretic agent at relatively high doses. But, in all the species studied in these experiments, tienilic acid exhibited the potent uricosuric activity required to decrease hyperuricemia, which is a cardiovascular risk factor.

In mice, the diuretic, uricosuric and natriuretic activities of tienilic acid were found to be dose-dependent (Figure 3); in normal rats, tienilic acid, in contradistinction to ethacrynic acid, provoked a dose-related diuretic and natriuretic response. In the same animal, this diuretic agent decreased phenol-red elimination, which has been regarded as an indirect measurement of a competitive antagonist of urate excretion in dogs, occurring mainly in the proximal tubule (64,65,66). This potential uricosuric activity as measured in the rat by antagonism of phenol-red elimination has been demonstrated by Lemieux (62) in a recent report which reveals that tienilic acid provoked a uricosuric effect in man, mouse, rat and dog (all species where urate reabsorption predominates). Indeed, in anesthetized beagle dogs, under hydropenic conditions, tienilic acid exhibits both diuretic and uricosuric properties over a wide dose range.

The diuretic activity was distinguished by a marked increase in water and Na^+ elimination with significant K^+ and Cl^- excretion. But, it was of interest to note that clearance studies showed a frank rise in both urate and osmolar clearances. This renders tienilic acid unique among the known diuretic agents, with the exception of another aryloxyacetic acid derivative, i.e., the indanone which also exhibited potent diuretic and uricosuric activities in chimpanzees and human subjects (32,33,34,35). As demonstrated in previous reports by other authors (54,60),

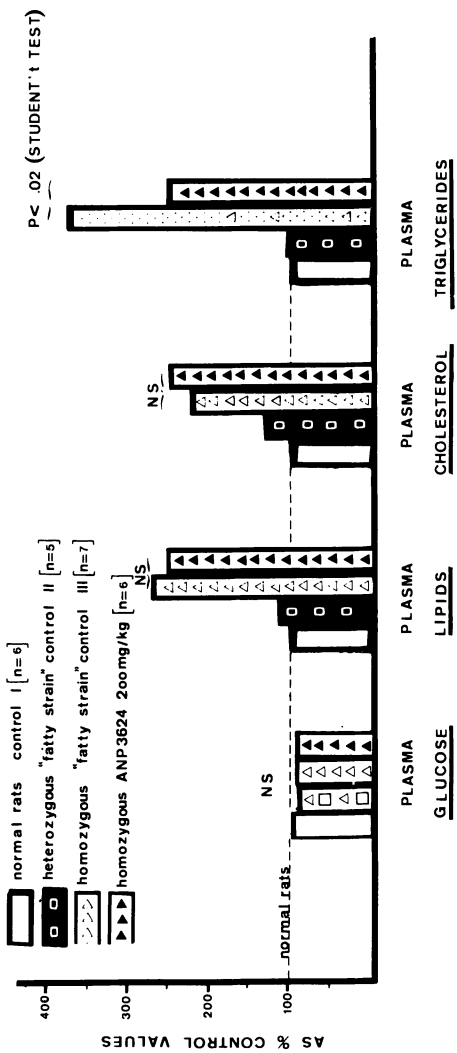

Figure 20. ANP 3624 (tienilic acid–ticrynafen)—200 mg/kg daily po (5 days)—study on the blood triglycerides lowering effect in the obese rat (fatty strain)

tienilic acid affects reabsorption and secretion of urate in dogs mainly in the proximal tubule. Other studies concerning the site of action of several non-diuretic uricosuric agents suggested the same conclusions (65,66,67,68). Inhibition of phenol-red excretion in rats by uricosuric agents and by diuretic-uricosuric substances indicates that they specifically depress urate reabsorption at the proximal site (64,65,66).

On the other hand, as summarized in Figure 15, tienilic acid exhibited a greater increase in osmolar clearance than in water excretion. Consequently, the calculated $T^{C}H_2O$ was significantly raised, as reported by Stote et al (54) and to a lesser degree by Maass et al (59). In conjunction with the previous data on $^{C}H_2O$, tienilic acid produced alterations in the cortical diluting segment of the distal nephron, unlike the other loop diuretics. However, when the reabsorption of solute free-water ($T^{C}H_2O$) was factored by osmolar clearance (^{C}osm) and glomerular filtration rate ($^{C}creat$) as previously reported (45, 47-52), free water reabsorption was surprisingly decreased by the administration of tienilic acid (Figures 15 and 16). A similar reversal was obtained when $T^{C}H_2O$ was corrected for osmolar clearance alone, attenuating the possible prominent part played by the creatinine clearance rate on the genesis of this phenomenon.

In the first absolute calculation of $T^{C}H_2O$ as it obtained from ^{C}osm, the action of tienilic acid was qualitatively similar to that of chlorothiazide. Using the corrected $T^{C}H_2O$, the tienilic acid results were approximately similar to those of ethacrynic acid and furosemide, while they were opposite to those of the thiazides. Considering these results in the light of the properties of tienilic acid in a hydrated state, these corrected calculations of $T^{C}H_2O$ do not exclude the possibility that tienilic acid could alter an additional site in the renal tubule. In an attempt to support this assumption, it is interesting to note that the calculation of both corrected $^{C}H_2O$ and $T^{C}H_2O$ from data reported in papers from several investigators, no change in the normal increase of hydrochlorothiazide activity on solute-free water reabsorption (69) was seen. On the other hand, the corrected calculations reversed the rather mild effect of tienilic acid on $T^{C}H_2O$ similar to that seen in the present study (54) and restored the $^{C}H_2O$ to the expected values for furosemide (70,71) and chlormerodrine (72) which, displayed paradoxically, altered absolute $^{C}H_2O$ values. More exactly, the corrected calculations of $^{C}H_2O$ and $T^{C}H_2O$ have normalized the paradoxical effects observed for furosemide or chlormerodrine, while the same corrections reversed the significance of the effects of tienilic acid in regard to solute-free water reabsorption. For example, in the experiment of Stote et al (54), tienilic acid very moderately increased the $T^{C}H_2O$ before the correction, and significantly decreased the $T^{C}H_2O$ value using the same calculation, while $^{C}H_2O$ in water diuresis was not modified when an analogous calculation was made with respect to urinary flow and inulin clearance. In the same way, in the report

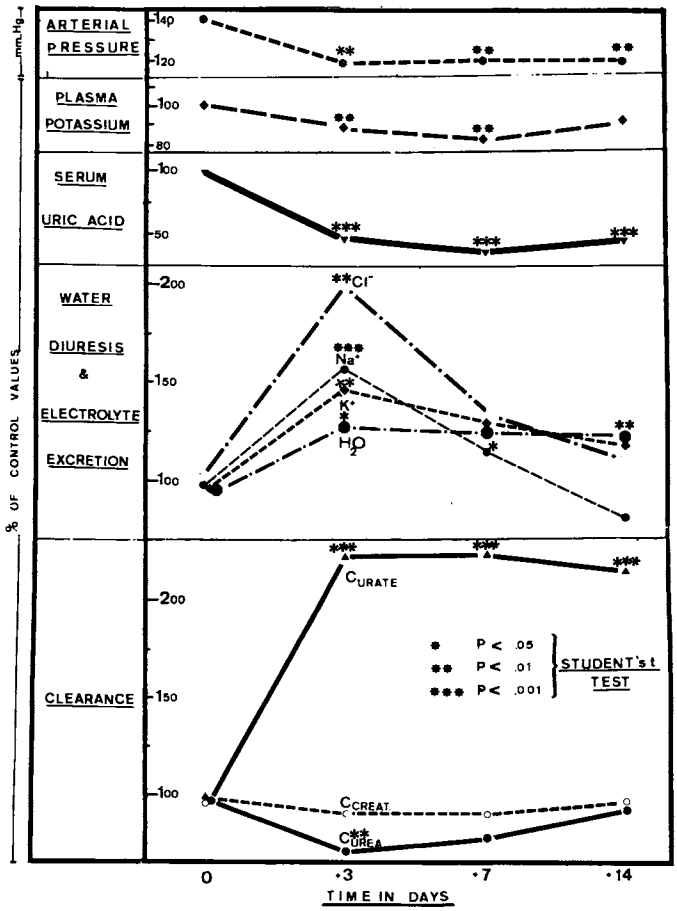

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Figure 21. Tienilic acid (250–750 mg daily)—antihypertensive and uricosuric effect in essential hypertensive patients (N = 12) (64)

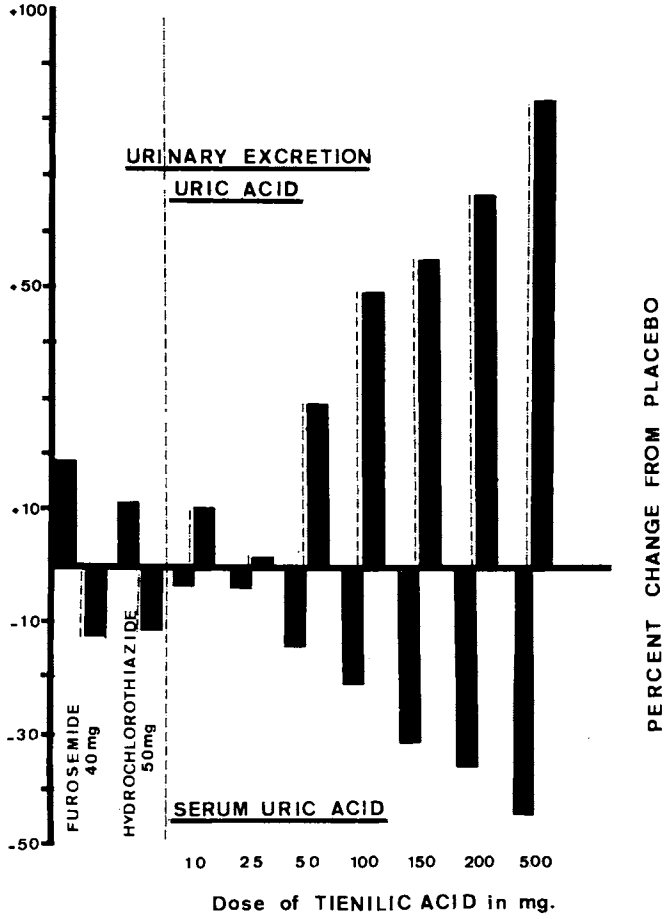

Figure 22. Tienilic acid—effect on uric acid in man—serum values and urinary excretion (54)

of Suki et al (70), using furosemide at 2 mg/kg i.v. paradoxically increased the calculated C_{H_2O} in dogs but markedly decreased the experimental value. However, in the report of Azer et al (71), which describes the proximal tubular rejection of sodium by furosemide, chlormerodrine, 8 mg/kg, did not change the $T^C_{H_2O}$ because of paradoxical data generated by his three experiments; but after correction, chlormerodrine depressed significantly solute-free water reabsorption in comparison to the control value.

In conclusion, it is suggested that these data are consistent with the idea that under some conditions of urinary excretion, tienilic acid possesses an additional site of action. On the other hand, it is of interest to observe that there is a good parallelism between the animal data and the clinical studies, as shown in Figures 21 and 22, which confirm the diuretic, uricosuric and antihypertensive properties of tienilic acid in man. It should be noted that the hypouricemia seen in man has not been observed in small rodents and dogs.

Finally, the combined diuretic, uricosuric, antihypertensive and triglyceride-lowering properties of tienilic acid observed in rats suggest that the control of these factors as risks in cardiac diseases may explain the increase of survival time of SH rats after chronic administration of tienilic acid.

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Ticrynafen: An Antihypertensive, Diuretic, Uricosuric Agent

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It is apparent that from a perusal of the articles in this publication that it is no longer sufficient to synthesize chemical structures which are modifications of existing diuretics in anticipation of obtaining a compound with similar diuretic activity. Examples of diuretics with markedly different chemical structures such as: mersalyl (1), ethacrynic acid (2), furosemide (3) and bumetanide (4), all of which act at the same site in the renal tubule have been known for some time. However, we also now know of two dichlorophenoxyacetic acid diuretics which act at different sites within the kidney tubule.

Ticrynafen is /2,3-dichloro-4-(2-thienylcarbonyl)phenoxy7-acetic acid. It was registered with the World Health Organization as tienilic acid; the USAN approved name, however, is ticrynafen. On the basis of its dichlorophenoxyacetic acid structure, it appears to be related to ethacrynic acid, /2,3-dichloro-4-(2-methylenebutyryl)phenoxy7acetic acid, but is chemically and pharmacologically quite different. For example, ethacrynic acid reacts avidly *in vitro* with the sulfhydryl amino acid, cysteine, while ticrynafen is quite inert. Since Burg (5) and others (6) have shown that it is the ethacrynic acid-cysteine adduct which is the active diuretic (at least *in vitro*), we were led to the conclusion that the two compounds would differ pharmacologically just as they differed chemically.

A pharmacologic difference was strongly suggested by our early observations on the effect of ticrynafen on diuretic and natriuretic activity in the mongrel dog. Ticrynafen, administered to the hydrated dog at doses of 12.5, 25 and 50 mg/kg orally, was compared, in an incomplete latin block design, with ethacrynic acid at oral doses of 3.1, 6.25 and 12.5 mg/kg. Urine was collected from these dogs for 5 hours and then for an additional 19 hours, for a total of 24 hours. Compared for diuretic and natriuretic effectiveness at 5 hours, ticrynafen was 1/26th and 1/23rd as active, respectively, as ethacrynic acid. However, after 24 hours collection, ticrynafen was 1/11th as active a diuretic and 1/14th as active a natriuretic agent as ethacrynic

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acid. These data demonstrated that not only did the two compounds differ in duration of action, with ticrynafen being an effective diuretic and natriuretic over a longer time span than ethacrynic acid, but also suggested that the natriuretic potency of ticrynafen differed from ethacrynic acid.

Since the response to a diuretic is principally determined by the locus in the nephron where the drug exerts its inhibitory effect on sodium reabsorption (7), we attempted to establish the site of renal action of ticrynafen. There are four major sites of diuretic activity in the renal tubule: a proximal site; two sites, medullary and cortical, in the thick ascending limb of the loop of Henle; and a far distal so-called sodium-potassium exchange site. At one time or another, practically every site in the tubule has been claimed as the active site for every diuretic (8). But, in recent years, it has been accepted that the pattern of electrolyte excretion, along with the determination of osmolal clearance in hydrated and dehydrated animals or man, will characterize the site of diuretic activity of a compound (7).

Adult female mongrel dogs were hydrated by giving an oral water load of 500 ml tap water thirty minutes prior to initiation of standard renal clearance procedure. Dogs were infused intravenously at 3 ml/minute throughout the course of the experiment with a 4% mannitol-phosphate buffer solution, pH 7.4 (9). Glomerular filtration rate was determined by the clearance of creatinine and effective renal plasma flow by the clearance of p-aminohippurate. Ticrynafen, administered intravenously to four hydrated mongrel dogs, caused a moderate natriuresis, kaliuresis and chloruresis with only a nominal increase in urine volume. The small change in urine flow rate above control (Table I) suggested that ticrynafen had little or no proximal activity. In this, ticrynafen differed from hydrochlorothiazide, furosemide and acetazolamide. All of these compounds increased sodium excretion which resulted in a positive osmolal clearance above controls (n = 6). However, only acetazolamide caused a positive free water clearance. These data suggested that ticrynafen was active in the thick ascending limb of the loop of Henle.

TABLE I

DIURETIC EFFECTIVENESS IN HYDRATED DOGS

Drug	Diuresis* ml/min	Sodium* FE %	C_{osm}* ml/min	C_{H_2O}* ml/min
Acetazolamide	3.2	3.3	+ 1.4	+ 1.8
Furosemide	10.0	15.6	+14.2	- 4.2
Hydrochlorothiazide	1.8	5.3	+ 4.4	- 2.5
Ticrynafen (n = 4)	0.2	6.3	+ 3.8	- 3.6

* change from control

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Adult, female mongrel dogs and dalmation coach hounds were fasted overnight. Using a constant rate infusion pump, the dogs were infused intravenously at 0.5 ml/minute throughout the course of the experiment with a 4% mannitol-phosphate buffer pH 7.4. The same standard renal clearance procedures were followed except that urine collection intervals were 20 minutes rather than the 10 minute collection periods used in the hydrated dogs. In the hydropenic dogs; however, ticrynafen is readily distinguished from furosemide by its lack of a significant affect upon $T^{c}H_{2}O$, the solute free water of reabsorption. Ticrynafen, administered intravenously at 15 mg/kg to eight hydropenic dogs increased urine flow rate above that of the controls (Table II) in the protocol. Again, the natriuretic effectiveness of ticrynafen was equivalent to that of hydrochlorothiazide, and again it increased osmolal clearance. However, the modest change on free water of reabsorption ($T^{c}H_{2}O$) strongly suggested a site of activity in the cortical diluting segment of the distal tubule.

TABLE II

DIURETIC EFFECTIVENESS IN HYDROPENIC DOGS

Drug	Diuresis ml/min	Sodium FE %	C_{osm} ml/min	$T^{c}H_{2}O$ ml/min
Acetazolamide	1.8	3.4	+ 2.5	+ 0.7
Furosemide	4.0	5.9	+ 3.6	- 0.4
Hydrochlorothiazide	0.9	3.0	+ 1.7	+ 0.8
Ticrynafen (n = 8)	1.5	3.2	+ 1.6	+ 0.1

Similar data have been obtained in the hydrated and dehydrated normal male volunteer (10). Ticrynafen at 500 or 1000 mg had no effect upon urine flow rate in the hydrated volunteers (Table III), suggesting a lack of proximal activity. Ticrynafen, given orally to hydrated subjects, increased sodium excretion and osmolal clearance but decreased free water clearance.

TABLE III

DIURETIC EFFECTIVENESS IN HYDRATED NORMAL VOLUNTEERS

Dose	Diuresis ml/min	Sodium FE %	C_{osm} ml/min	$C_{H_{2}O}$ ml/min
Control	16.0	1.3	3.4	12.8
500 mg (n = 3)	16.1	4.3	7.1	8.9
Control	15.0	1.0	3.3	11.8
1000 mg (n = 2)	14.7	5.7	7.7	6.9

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In hydropenic volunteers, ticrynafen, at 500 or 1000 mg orally, also increased sodium excretion and osmolal clearance. Unlike free water clearance however, tubular reabsorption of solute free water rose and was either equal to or exceeded that of the controls (Table IV). This observation, along with the observation in both hydrated and hydropenic normal volunteers, that only 4 to 6% of the filtered sodium load was excreted, clearly place the site of diuretic activity of ticrynafen in the cortical diluting segment of the distal tubule (10).

TABLE IV

DIURETIC EFFECTIVENESS IN HYDROPENIC NORMAL VOLUNTEERS

Dose	Diuresis ml/min	Sodium FE %	C_{osm} ml/min	T^CH_2O ml/min
Control	0.9	0.9	3.0	2.1
500 mg (n = 3)	3.3	4.6	6.4	3.1
Control	0.9	1.1	3.1	2.2
1000 mg (n = 2)	4.7	6.1	6.9	2.2

The congruence of results of activity of ticrynafen in hydrated and hydropenic man and dog upon free water clearance and the solute free water of reabsorption delineate its site of action (Table V). Proximal, loop and distal diuretics administered to hydrated dogs increased sodium excretion and osmolal clearance. Based on the physiologic principles outlined by Goldberg et al. (11), free water clearance (C^H_2O) was increased only by acetazolamide, a proximally active diuretic (12) and was decreased by loop and distally acting diuretics. But in hydropenic animals, the distally active compounds, hydrochlorothiazide and ticrynafen caused a slight increase or no change in T^CH_2O, clearly a different response than that obtained with furosemide (13), a "loop-agent".

TABLE V

SUMMARY OF THE EFFECTS OF DIURETICS UPON RENAL CONCENTRATING AND DILUTING MECHANISMS

Site of Action	Experimental Procedure: Hydropenic $T^C_{H_2O}$	Experimental Procedure: Hydrated C_{H_2O}
Proximal Tubule Acetazolamide	↑	↑
Loop of Henle Furosemide	↓	↓
Distal Tubule Ticrynafen	±	↓

To determine the pattern of electrolyte excretion in the dog, ticrynafen was administered intravenously, in single acute doses, to the phosphate-mannitol infused dog. At intravenous doses of 0.05 to 15 mg/kg, the compound enhanced urine volume and caused excretion of sodium, potassium and chloride. The maximum natriuretic response occurred promptly within the first 10 minutes after completion of the injection, and as the dose was increased there was a greater duration of natriuresis (Table VI).

TABLE VI

ELECTROLYTE EXCRETION FOLLOWING ADMINISTRATION OF TICRYNAFEN TO THE PHOSPHATE-MANNITOL INFUSED DOG

I. V. Dose mg/kg	No of Observations	Maximal FE Na %	Total Cumulative Excretion mEq During First Hour: Na	K	Cl
Placebo	10	2.09	5.29	1.49	2.64
0.05	3	2.93	9.39	1.36	6.33
0.5	6	3.91	7.02	1.13	4.00
2.5	1	7.40	12.96	2.80	11.61
5.0	4	10.05	19.31	2.09	15.46
15.0	3	11.60	33.85	4.58	31.80

When compared statistically with placebo, ticrynafen caused a significant natriuretic response at 0.5 mg/kg I.V. which increased in a linear fashion with dose, reaching a maximum effect with excretion of about 12% of the filtered sodium load at 15 mg/kg. The excretion of chloride parallels that of sodium but is somewhat reduced due to the presence of phosphate in the infusion-medium, suggesting that ticrynafen is a saluretic

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agent. A significant increase in potassium excretion occurred at a dose of 2.5 mg/kg, five times the minimum effective natriuretic dose. This observation makes it unlikely that ticrynafen has an inhibitory affect upon the sodium/potassium exchange site.

During maximal natriuresis in man, following administration of 500 mg ticrynafen, average sodium excretion increased 463 μEq/min., while potassium excretion increased only 16 μEq/min. Clearance ratio of calcium to inulin increased by approximately 1% and clearance ratio of magnesium 2.6%. Bicarbonate and phosphorus excretions were not significantly changed, and urinary pH consistently decreased. The acute administration of ticrynafen did not result in any significant change in GFR as determined by inulin clearance. This electrolyte pattern again supports a conclusion of a lack of activity of ticrynafen in the proximal tubule, while the similarity of electrolyte response to "thiazide-like" diuretics is consistent with an action in the cortical diluting segment of the distal tubule.

As a result of these observations, we have concluded that hydrochlorothiazide and ticrynafen, two compounds with markedly differing chemical structure, produce a similar pattern of electrolyte response due to their activity at the same site in the kidney tubule.

While diuretics of markedly differing chemical structure have not been recognized previously to be active at a cortical diluting site, compounds of widely differing structure - ethacrynic acid, bumetanide, furosemide and mersalyl - have been recognized to be active at a medullary diluting site on the thick ascending limb of Henle's loop. Thus, specific structure-function relationships do not appear to exist among diuretics except insofar as they are organic acids. But no one, I believe, would claim that all organic acids are diuretics, and conversely two diuretics, triamterene and amiloride, active at the far distal sodium/potassium exchange site clearly cannot be classified as organic acids.

However, molecular structures determining participation in the organic acid tubular transport system are clearly pertinent. The diuretic effect of mersalyl (14) and ethacrynic acid (15) appears to be due to inhibition by these drugs in the tubular lumen of active chloride transport in the thick ascending limb of Henle's loop. Similar data are available for furosemide *in vitro* (16), and in man (17); the diuretic response is determined by the amount of furosemide that reaches the renal tubule rather than the drug level in plasma (18). Similarly, in the dog, it has been found that those compounds which, administered in sufficient dose, inhibit renal transport of ticrynafen, also reduce ticrynafen natriuresis (19).

To add to the complexities facing the medicinal chemist synthesizing a new diuretic, is the observation that ticrynafen has a significant uricosuric activity. That an organic acid should have uricosuric activity is not totally unexpected. Any

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list of uricosuric agents, from acetoheximide to zoxazolamine will easily include 30 compounds of widely differing chemical structure, the majority of which are organic acids. However, weak organic bases such as zoxazolamine are also uricosuric. What is unexpected is that orally active diuretics which should increase uric acid excretion, generally cause a significant uric acid retention, possibly due to their contraction of extracellular fluid volume (20) and/or inhibition of urate secretion.

In the phosphate-mannitol infused mongrel dog, ticrynafen at doses of 0.5 to 15 mg/kg intravenously, is natriuretic and uricosuric (Table VII). Glomerular filtration rate was determined by the clearance of ^{3}H-inulin, and p-aminohippurate was omitted from the infusion solution. The dogs were not urate loaded. Under these conditions, ticrynafen was found to be natriuretic and uricosuric, roughly equivalent in uricosuric potency to probenecid. These data are in agreement with the original reports of uricosuria induced in the mongrel dog, reported by Beyer et al. (21) and by Sougin-Mibashan and Horwitz (22).

TABLE VII

DIURETIC AND URICOSURIC ACTIVITY IN THE MONGREL DOG

Compound	Sodium* FE %	C_{Ur}* ml/min	Urate* FE %
Probenecid 10 mg/kg I.V.	0	+ 16.2	+ 31
Hydrochlorothiazide 0.5 mg/kg + 0.5 mg/kg/hr	+ 5.3	- 2.3	- 1
Ticrynafen			
1.5 mg/kg + 1.5 mg/kg/hr	+ 2.1	+ 8.7	+ 18
4 mg/kg I.V.	+ 4.2	+ 7.5	+ 17
10 mg/kg I.V.	+ 4.7	+ 9.8	+ 25
15 mg/kg I.V.	+ 8.5	+12.6	+ 22

* change from control

C_{Ur} = clearance of urate

In man, ticrynafen is markedly uricosuric (Table VIII), the fractional excretion of urate rising to a maximum in one volunteer of 75% of the filtered load. As a consequence of the uricosuria, serum urate levels and, consequently, the filtered load of urate to the kidney is sharply reduced. In contrast, the ability of the orally active diuretics, especially the thiazides and furosemide, to retain uric acid and increase the filtered urate load is well recognized (23).

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TABLE VIII

URICOSURIC ACTIVITY OF TICRYNAFEN IN HYDRATED NORMAL MALE VOLUNTEERS

Volunteer		Dose mg	$U_{Ur} V$ µg/min	$C_{Ur}/C_{In} \times 100$
S-82	C	--	634	8.8
	E	500	2558	38.6
S-83	C	--	600	12.3
	E	500	2680	63.3
S-84	C	--	475	7.5
	E	500	2836	75.4
S-127	C	--	680	7.7
	E	1000	3173	58.8

Although researchers in the field of urate transport do not agree on the renal tubular site of urate reabsorption, a proximal site of urate transport is considered possible. Sulfinpyrazone, probenecid and aspirin are secreted by an organic acid secretory carrier located in the proximal tubule (24). Diamond and Meisel (25) have demonstrated that probenecid secretion is required for its uricosuric effect, and they have proposed that competition for binding at a urate-reabsorptive site on the tubular luminal surface may account for the effect of probenecid (26).

In the mongrel dog, competition with ticrynafen secretion reduced the uricosuric activity of ticrynafen as it reduced the natriuretic activity of the drug. Thus, increasingly a case may be made for the proximal transport of diuretics to act from the tubular lumen (19). It is a challenge to the medicinal chemist to fit his newly synthesized molecules to this renal transport system.

In summary, the data obtained in this study of ticrynafen clearly indicate that modification of chemical structures to obtain new diuretics requires the extensive participation of the physiologist.

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2-Aminomethylphenols: A New Class of Saluretic Agents

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A continuing search for new renal agents in our laboratories by screening carefully selected compounds for diuretic and saluretic activity in rats and dogs led to the discovery of 2-aminomethyl-3,4,6-trichlorophenol (Ia). The unusual structural features, attractive electrolyte excretion profile and saluretic potency of compound Ia relative to those of known diuretics (see Table I for an activity comparison) provided impetus for an extensive synthetic program. The data obtained facilitated the delineation of the structure-activity relationships for a variety of 2-aminomethylphenols and culminated in the development of 2-aminomethyl-4-(1,1-dimethylethyl)-6-iodophenol hydrochloride (MK-447). This compound was found to be a potent, high-ceiling salidiuretic agent with adjunctive antihypertensive and anti-inflammatory properties, and it is currently undergoing clinical evaluation. This report is a preliminary account (1) of the highlights of our research on this new class of saluretic agents.

TABLE I. Relative Saluretic Activities[a]

Compound	Species (Admin. Route) Rat (p.o.)	Dog (i.v.)
Ia	3	4
Hydrochlorothiazide	2	2
Furosemide	3	5
Ethacrynic Acid	0	5

[a]Presented as scores; assay protocols are described in the text, and scoring criteria are presented in Tables II and III.

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SALIDIURETIC ACTIVITY ASSAYS

The structure-activity relationships (vide infra) in this study were determined by evaluating each synthetic compound for salidiuretic activity in two animal species, the rat and the dog. A brief description of each assay protocol is given below along with the system used to score the biological results.

Oral Rat Assay - Female rats (Charles River, 150-170 g), housed in metabolism cages in groups of three rats per cage, were maintained overnight on a sugar diet with water ad libitum. At the time of the test, each animal was given the test compound orally either as a solution or suspension in 5 ml of water. Urine was collected over the 0-5 hr interval in graduated cylinders and subsequently analyzed for sodium, potassium and chloride content by standard methodology. The saluretic response was scored from 0 to 6 according to the natriuretic criteria indicated in Table II.

TABLE II. Scoring System for Oral Rat Assay

Score	μEq Na^+/cage, dose in mg/kg				
	1	5	16.5	50	81
0				0.3	0.3
±			0.4	0.7	0.9
1		0.3	0.5	1.2	1.5
2		0.4	0.6	1.8	2.4
3		0.5	1.4	2.4	2.9
4		1.4	2.7	3.3	
5	0.7	2.6	3.3	3.6	
6	2.1	3.6	4.2	3.9	

Intravenous Dog Assay - Conditioned female mongrel dogs, weighing approximately 20 kg in the postabsorptive state, were starved overnight and then given 500 ml of water orally 1 hr before induction of anesthesia with sodium pentobarbital (30 mg/kg, i.v.). After inducing anesthesia, each dog was prepared with an indwelling bladder catheter and primed with creatinine (4 g as a 10% solution in water) administered s.c. in multiple injection sites. To ensure uniform hydration and urine production, 1.5 ml/kg of an isoosmotic pH 7.4 phosphate buffer solution (20 mg phosphate/kg) was given i.v. as a priming injection prior to initiation of clearance studies and 3 ml/min of an isoosmotic pH 7.4 buffer containing 4% mannitol (6.9 mg phosphate/min) was infused during the experiment. At the start of

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timed clearances, the urinary bladder was emptied and replicate 15-min urine collections were made with venous blood samples being drawn at the midpoint of each period. Following this control phase, the test compound was administered i.v. stat (i.e., over a 5 min period) at 5 mg/kg. Urine was collected over replicate 15 min periods for 2 hr and subsequently assayed for electrolyte content by standard methodology. The average rate of natriuresis determined for the two highest consecutive 15 min collection periods was used to score the saluretic response from 0 to 5 on the basis of the sodium excretion rates indicated in Table III.

TABLE III. Scoring System for Intravenous Dog Assay

Score	μEq Na^+/min at 5 mg/kg I.V. Stat
0	0-99
±	active above 5 mg/kg
1	100-399
2	400-599
3	600-799
4	800-899
5	900

STRUCTURE-ACTIVITY RELATIONSHIPS

The initial phase of this investigation was directed toward determination of the biological consequences resulting from reorientation and structural modification of the hydroxyl (phenolic) and aminomethyl groups in compound Ia. As is shown in Table IV, orientation of these functional groups in either a meta (Compound Ib) or para (Compound Ic) relationship with concomitant positional interchange of the aminomethyl moiety with either the 3- or 4-chloro substituent resulted in ablation of activity. Hence, these results established the importance of maintaining an ortho relationship between the hydroxyl and aminomethyl groups.

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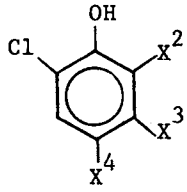
TABLE IV. Orientation Effects

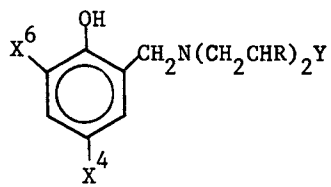


(I)

Compd.	X^2	X^3	X^4	Saluretic Score Rat	Dog
Ia	CH_2NH_2	Cl	Cl	3	4
b	Cl	CH_2NH_2	Cl	0	0
c	Cl	Cl	CH_2NH_2	0	0

The effects of N-substitution on saluretic activity were explored next and are tabulated for a representative series of structures in Table V. As the data indicate, alkylation of the amino group proved to be detrimental to activity as did acylation, with exception of N-trifluoroacetylation. In the latter case, the activity elicited upon oral administration of compound IIg in the rat most likely resulted from its *in vivo* hydrolysis to the active precursor, compound Ia. Indeed, the observed facile conversion of trifluoroacetamide IIg to amine Ia under mild hydrolytic conditions (e.g., 20°C, pH 8, 30 min) *in vitro* is consistent with this viewpoint.

Meadow, et al (2, 3) have reported some of the biological activities displayed by a series of tertiary amines of general formula III; compound IIIa was identified as the most active diuretic member of the series. In our laboratories, compound IIIa was found to be inactive in both the rat and dog diuretic assays under a variety of test conditions. This result is interesting in view of the activity, albeit weak, displayed by tertiary amine IIc.



IIIa, R=H; Y=O;
$X^4=CH_2CH=CH_2$;
$X^6=OCH_3$

Alteration of the aminomethyl group, e.g., by α-substitution (Compound IVa), homologation (Compound IVb) or simultaneous α-substitution and homologation (Compound IVc), led to substantial diminution in activity as demonstrated in Table VI. Furthermore,

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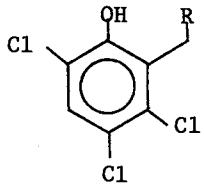
TABLE V

Effects of N-Substitution



(II)

Compd	R	Saluretic Score Rat	Dog
IIa	$NHCH_3$	1	
b	$N(CH_3)_2$	2	0
c	$N(CH_2CH_2)_2O$	±	1
d	$N(CH_3)CH_2$- 	1	1
e	NHCHO	0	0
f	$NHCOCH_2Cl$	1	0
g	$NHCOCF_3$	3	
h	$NHCOCH_2NH_2$	±	1

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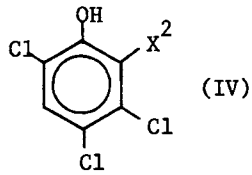
the corresponding salicylalcohol (Compound IVd), salicylaldehyde (Compound IVe) and salicylic acid (Compound IVf) analogs of compound Ia were devoid of demonstrable saluretic activity.

TABLE VI. Effects of Aminomethyl Group Alteration



(IV)

Compd.	X^2	Saluretic Score Rat	Dog
IVa	$CH(CH_3)NH_2$	±	
b	$CH_2CH_2NH_2$	1	1
c	$CH(OH)CH_2NH_2$	0	1
d	CH_2OH	0	0
e	CHO	0	0
f	CO_2H	0	0

To complete the preliminary SAR studies, the effects of hydroxyl group modification were determined; the results are presented in Table VII. As the data indicate, both O-alkylation (e.g., as in compounds Va-c) and replacement of OH by NH_2 resulted in structures with marginal saluretic activity. These results coupled with those presented previously (vide supra) indicated that: (a) the ortho orientation of the hydroxyl and aminomethyl groups (i.e., as in salicylamine) should be maintained, and that (b) these two functional groups must remain unsubstituted. Accordingly, the effects of nuclear substitution, both singularly and multiply, were investigated in a systematic manner as an approach to improving saluretic activity.

Data for a representative series of monosubstituted salicylamines (Compound VI) bearing substituents in the 4-position are shown in Table VIII. Although salicylamine itself (Compound IVa) is devoid of demonstrable salidiuretic activity, introduction of a chloro group in the 4-position (Compound VIb) imparts weak saluretic properties which are maintained upon replacement of the chloro group by lower alkyl groups up to and including a three carbon straight chain, with or without α-branching. However, introduction of the n-butyl group in the 4-position is not tolerated, since compound VIf did not exhibit demonstrable activity in either the rat or the dog assay.

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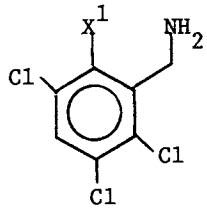
TABLE VII

Effects of Phenol Group Modification



(V)

Compd	X^1	Saluretic Score	
		Rat	Dog
Va	OCH_3	±	0
b	$OCH_2CO_2C_2H_5$	1	0
c	OCH_2CO_2H	±	
d	NH_2	±	0

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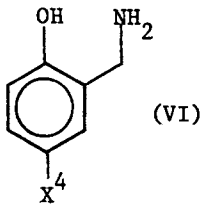
TABLE VIII. Effects of Nuclear Monosubstitution



(VI)

Compd.	X^4	Saluretic Score	
		Rat	Dog
VIa	H	0	0
b	Cl	1	1
c	C_2H_5	1	1
d	$C(CH_3)_3$	1	
e	$CH(CH_3)C_2H_5$	1	0
f	$(CH_2)_3CH_3$	0	0

Appropriate nuclear disubstitution led to marked enhancement of saluretic activity as indicated by the results tabulated in Table IX. The dichloro derivatives (Compounds VIIa-c) illustrate the importance of the 4,6-disubstitution pattern, i.e., VIIb is more active than the other positional isomers. This observation, coupled with the information gained from the monosubstituted series, prompted preparation of compounds VIId-i. Replacement of the 4-chloro group in compound VIIb with lower alkyl groups led to markedly improved activity which peaked in potency with the introduction of a 4-(1,1-dimethylethyl) moiety, i.e., compound VIIi. Interestingly, the 6-chloro, 6-bromo and 6-iodo derivatives (i.e., compounds VIIi, VIIk and VIIl, respectively) elicit marked salidiuretic responses, whereas, the 6-fluoro analog (Compound VIIj) is considerably less active. As will be discussed subsequently, the pronounced antihypertensive properties of MK-447 (Compound VIIl) served to distinguish it from a subseries of nearly equipotent saluretic agents which emerged during the course of this investigation. Finally, it should be noted that compound VIIm, the primary amine analog of compound IIIa (vide supra), displayed good activity in both rats and dogs.

The influences of nuclear trisubstitution on saluretic activity are presented in Table X for a group of compounds of the general structure VIII. It is noteworthy that shifting the 3-chloro substituent in compound Ia to the 5-position (i.e., to afford compound VIIIa) resulted in slightly improved activity in the dog; whereas, movement of the 6-chloro group in Ia to the

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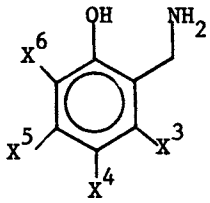
TABLE IX

Effects of Nuclear Disubstitution



(VII)

Compd	X^3	X^4	X^5	X^6	Saluretic Score: Rat	Saluretic Score: Dog
VIIa	Cl	Cl	H	H	1	1
b	H	Cl	H	Cl	2	±
c	Cl	H	H	Cl	0	1
d	H	CH_3	H	Cl	2	1
e	H	C_2H_5	H	Cl	4	5
f	H	$(CH_2)_2CH_3$	H	Cl	4	5
g	H	$CH_2CH(CH_3)_2$	H	Cl	3	2
h	H	$CH(CH_3)C_2H_5$	H	Cl	5	4
i	H	$C(CH_3)_3$	H	Cl	6	5
j	H	$C(CH_3)_3$	H	F	4	5
k	H	$C(CH_3)_3$	H	Br	6	5
l[a]	H	$C(CH_3)_3$	H	I	6	5
m	H	$CH_2CH=CH_2$	H	OCH_3	3	2

[a]Compound VIIl = MK-447

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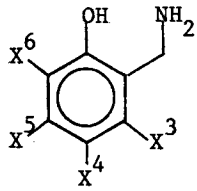
5-position (i.e., to form compound VIIIb) diminished activity in the dog while marginally enhancing activity in the rat. Furthermore, introduction of electron donating substituents (e.g., methyl and methoxy groups) in the 6-position, as in compounds VIIIf and VIIIg, proved to reduce activity in both test species.

TABLE X. Effects of Nuclear Trisubstitution



(VIII)

Compd.	X^3	X^4	X^5	X^6	Saluretic Score Rat	Dog
Ia	Cl	Cl	H	Cl	3	4
VIIIa	H	Cl	Cl	Cl	4	4
b	Cl	Cl	Cl	H	4	2
c	F	Cl	H	Cl	4	4
d	Cl	Cl	H	CF_3	4	2
e	CH_3	CH_3	H	Cl	3	2
f	CH_3	CH_3	H	CH_3	2	1
g	Cl	Cl	H	OCH_3	1	1
h	OH	Cl	H	Cl	1	0

Finally, the data recorded in Table XI illustrate the effects of nuclear tetrasubstitution on activity. These data reveal several interesting SAR trends. First, although replacement of the 3- and 5-chloro substituents of tetrachloro derivative IXa with methyl groups resulted in greatly enhanced activity in the dog, most surprisingly, the interchange of chloro and methyl substituents (i.e., conversion of Compound IXb to compound IXc) totally abolished activity. These results indicate that both steric and electronic effects contribute substantially in determining the saluretic efficacy of these structures. Reinforcement for the importance of these effects is provided by activity comparisons of structures IXe with IXf and IXg with IXh. Diminution of activity accompanies replacement

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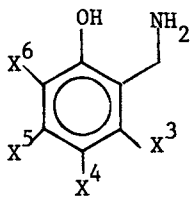
of a chloro substituent with a methyl group (electronic effect) in the first instance and reflects the rather stringent steric requirements for substituents in the 3-position in the second instance. Secondly, the results tabulated for compounds IXb, IXi and IXk suggest that, whereas the methyl groups in the 3- and 5-positions can be replaced with methoxy groups with maintenance of activity, their replacement with ethoxy moieties substantially reduces activity. The latter result is in accord with the steric restraints discussed above for substituents in the 3-position. Furthermore, it should be noted that compounds IXg and IXi display saluretic activities which are essentially of the same magnitude as those of the 4-(1,1-dimethylethyl)-6-halosalicylamines (i.e., VIIi, VIIk and VIIl) cited earlier.

TABLE XI. Effects of Nuclear Tetrasubstitution



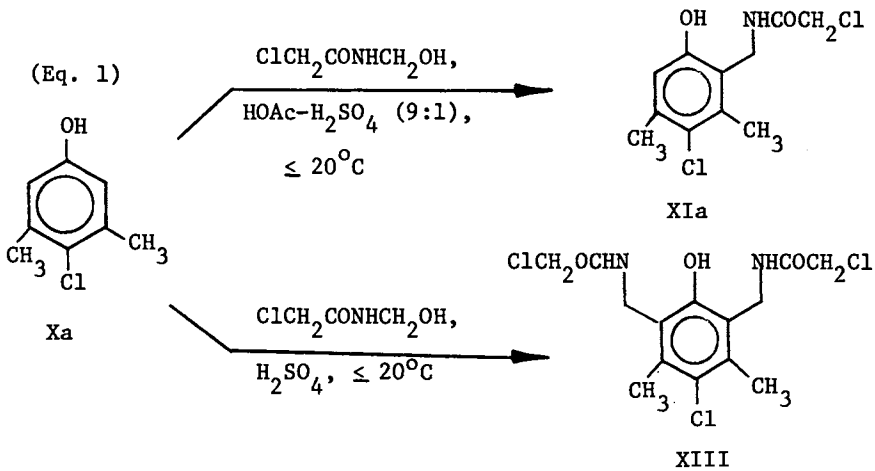
(IX)

Compd.	X^3	X^4	X^5	X^6	Saluretic Score Rat	Dog
IXa	Cl	Cl	Cl	Cl	4	1
b	CH_3	Cl	CH_3	Cl	4	5
c	Cl	CH_3	Cl	CH_3	0	0
d	CH_3	Br	CH_3	Br	4	5
e	CH_3	CH_3	Cl	Cl	5	2
f	CH_3	CH_3	CH_3	CH_3	3	1
g	CH_3	Cl	C_2H_5	Cl	5	5
h	C_2H_5	Cl	CH_3	Cl	1	1
i	OCH_3	Cl	OCH_3	Cl	5	5
j	OCH_3	Cl	OCH_3	Br	5	3
k	OC_2H_5	Cl	OC_2H_5	Cl	3	0

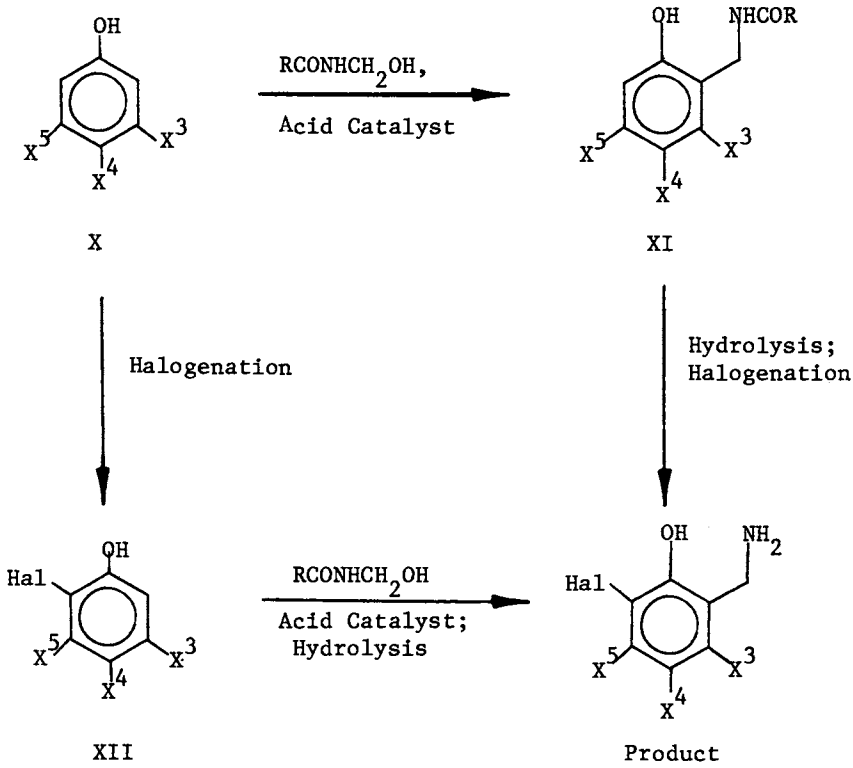
In summary, these SAR studies indicate that saluretic activity is elicited by 2-aminomethylphenols which are appropriately substituted with a hydrogen, methyl or methoxy group in the 3-position, a halo or lower alkyl (i.e., ≤ three carbon straight chain, preferably α-branched) moiety in the 4-position, a hydrogen or lower alkyl (or alkoxy) substituent in the 5-position and a iodo, bromo or chloro group in the 6-position, the optimal substituent combinations and patterns being determined by the cited SARs.

CHEMISTRY

The two general synthetic routes which were most frequently used to prepare the salicylamine derivatives studied during the course of this investigation are summarized in Scheme I. The first route involves acid-catalyzed nuclear amidoalkylation of an appropriately substituted phenol (Compound X) with an N-hydroxymethylamide, $RCONHCH_2OH$ (R=CH_3, CH_2Cl, CCl_3, CF_3 or C_6H_5), to give an N-acylsalicylamine of general formula XI. Subsequent hydrolytic N-deacylation of compound XI followed by halogenation affords the target product. The second route involves the same steps but in an altered order, i.e., halogenation of phenol X to give an o-halophenol of general structure XII followed by amidoalkylation and hydrolysis. In both sequences, the key amidomethylation step is accomplished readily via the Tscherniac-Einhorn reaction (4). The choice of the proper reaction sequence, as well as optimum conditions (i.e., $RCONHCH_2OH$, acid catalyst, reaction medium, etc.) for introducing the aminomethyl moiety, is governed primarily by the chemical nature and directing influences of the substituents X^3, X^4 and X^5. The importance of selecting the proper acid catalyst and reaction medium to control the amidoalkylation step is demonstrated in equation 1.



Scheme I


OH
X5
X3
X4
X
RCONHCH2OH,
Acid Catalyst
OH
NHCOR
X5
X3
X4
XI
Halogenation
Hydrolysis;
Halogenation
OH
Hal
X5
X3
X4
XII
RCONHCH2OH
Acid Catalyst;
Hydrolysis
OH
NH2
Hal
X5
X3
X4
Product

The desired amide (Compound XIa) is formed in good yield (60-70%) when HOAc-H_2SO_4 (9:1) is used, whereas, the use of H_2SO_4 alone leads to extensive diamidomethylation (diamide XIII), even when equimolar quantities of phenol Xa and N-hydroxymethylchloroacetamide are employed.

Application of these general synthetic routes to the elaboration of MK-447 (5) from 4-(1,1-dimethylethyl)phenol (Compound Xb) is shown in Figure 1. The reaction sequence, Xb ⟶ XIb ⟶ XIV ⟶ MK-447, is the preferred synthetic pathway for preparing large quantities of MK-447 and can be accomplished routinely in 50% overall yield. Since the 6-iodo substituent is introduced under very mild conditions (ICl, 0.5N HCl, 20°C) in the terminal step, this sequence is ideally suited for the synthesis of [^{131}I]-MK-447. The alternative pathway, Xb ⟶ XIIb (either directly or stepwise via chloromercurial XIIa) ⟶ MK-447, although quite useful, is less satisfactory than the former as discussed below. The intermediate o-iodophenol XIIb is somewhat deactivated to electrophilic substitution relative to phenol Xa and, therefore, XIIb requires more acidic conditions to facilitate the Tscherniac-Einhorn reaction. The stronger acid medium is conducive to deiodination and leads to reduced yields of MK447. Nevertheless, the alternative pathway proved to be the route of choice for elaborating MK-447 labeled with a 2-[^{14}C]-aminomethyl moiety. In this instance, conversion of o-iodophenol XIIb to [^{14}C]-MK-447 was achieved in 35% overall yield using N-(hydroxy-[^{14}C]-methyl)chloroacetamide which was conveniently generated *in situ* from [^{14}C]-paraformaldehyde and chloroacetamide.

MOLECULAR STRUCTURE, METABOLISM AND PHARMACOLOGY OF MK-447

Molecular Structure - As determined by potentiometric titration in water, MK-447 exhibits pKa_1 7.25 and pKa_2 10.75. These pKa values reflect the amphoteric properties of MK-447 and, when compared with those ($pKa_{1,2}$ 8.4, 10.5) displayed by 2-aminomethylphenol hydrochloride, they indicate that introduction of the 6-iodo substituent enhances the acidity of the phenolic hydroxyl group or, viewed from a different perspective, serves to reduce net molecular basicity, i.e., monodeprotonation occurs at a lower pH. When partitioned between 1-octanol and pH 7.4 buffer, MK-447 is localized essentially quantitatively (*ca.* 99%) in the lipid phase. The free base form of MK-447, readily liberated from MK-447 by neutralization with weak bases such as ammonia and sodium bicarbonate, is very soluble in non-polar organic solvents and relatively insoluble in aqueous media. The limited water solubility of MK-447 free base suggests that it has appreciable zwitterionic character in water. Indeed, the validity of this suggestion was confirmed by determining the effects of pH on λ_{max} for MK-447 in water. The bathochromic shift from 285 to 309 mμ which accompanies phenoxide formation (i.e., ArOH ⟶ ArO^-) was observed at pH 6. Hence, the observed solubility characteristics of MK-447 free base

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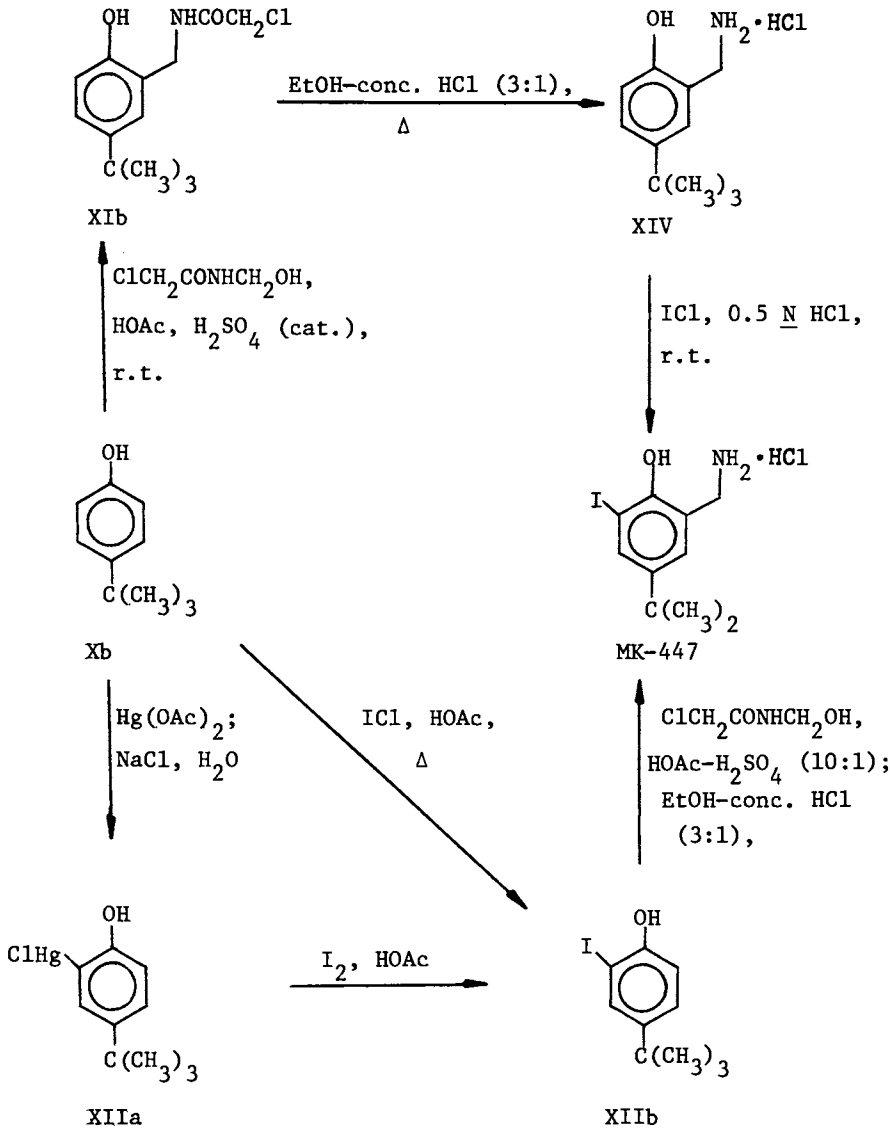
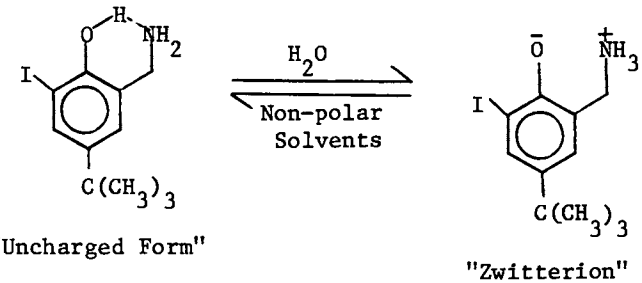


Figure 1. Synthetic routes to MK-447

reflect its unique ability to adjust molecular polarity in a solvent-dependent manner as illustrated in equation 2. This process should be quite facile since, a priori, minimal energy would be required to effect the depicted intramolecular proton transfer.

(Eq. 2)



"Uncharged Form" "Zwitterion"

Examination of the lowest energy ground state conformation of compound VIIi, calculated by the CNDO/2 formalism (6) and presented as an ORTEP projection (7) in Figure 2, affords additional insight into the physicochemical properties cited above. First, the plane formed by the Cl-C6-Cl-O-H_a atom array is coplanar with the aromatic ring and the phenolic hydrogen atom (H_a) is hydrogen bonded to the chloro substituent. This result is in accord with the recent demonstration of intramolecular hydrogen bonding in o-halophenols, including 2-iodophenols, by Kollman et al (8). Furthermore, although the aminomethyl group is situated substantially out of the plane of the ring ($\theta = 45^\circ$), a second intramolecular hydrogen bond exists between H_b of the amino group and the phenolic oxygen atom. The solid state structure of MK-447 free base, determined by the single crystal X-ray crystallographic technique, is in reasonable agreement with the calculated ground-state structure of compound VIIi. A comparison of these structures, shown by their superimposition (i.e., one over the other) in Figure 3, indicates that they differ in two respects: (a) compound VIIi contains a H_a...Cl, whereas, the $-OH_a$ group in MK-447 free base is not intramolecularly hydrogen bonded; instead, it is directed below and perpendicular to the plane of the aromatic ring and (b) the size of the dihedral angle θ (45° vs. 53°) is minimally different. The first structural difference is somewhat surprising and may well reflect the inherent difficulties associated with accurately locating a hydrogen atom proximate to an iodo substituent by X-ray crystallography. The minor difference in θ could either reflect the crystal packing forces in the solid state or stem from the well-known overestimation of attractive forces between non-bonded atoms by the CNDO/2 method (9). In any event, the substitution of iodo and aminomethyl groups vicinally to the hydroxyl moiety facilitates intramolecular hydrogen bonding which, coupled with the presence of a 4-(1,1-dimethylethyl) substituent, imparts substantial lipophilicity to MK-447 free base. Likewise, this nuclear substitution pattern is

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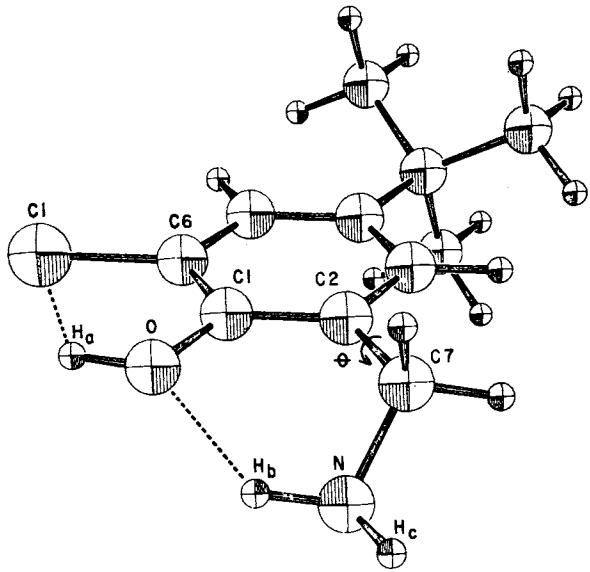

Figure 2. The lowest energy ground state conformation of compound VIIi, the 6-chloro analog of MK-447 free base. This conformation, presented as an ORTEP projection, was calculated by the CNDO/2 semiempirical technique.

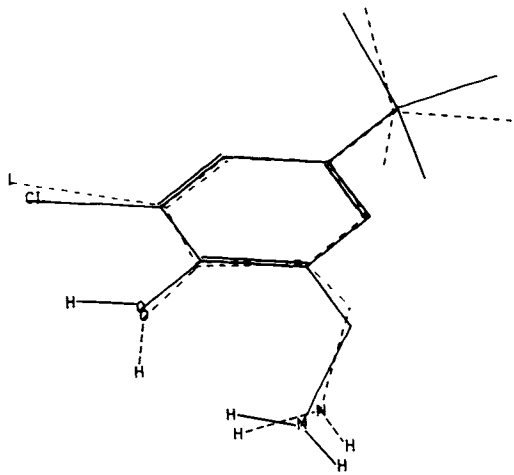

Figure 3. Comparison of the x-ray structure of MK-447 free base (dotted lines) with the CNDO/2-calculated lowest energy ground state conformation of 6-chloro analog VIIi (solid lines)

conducive to zwitterion formation in aqueous media and, thereby, severely limits the solubility of MK-447 free base, but not that of the parent hydrochloride, in water.

Metabolism - Studies (10) in rats, dogs and man indicate that orally administered [^{14}C]-MK-447 is rapidly absorbed, metabolized and excreted. Peak plasma drug levels were observed within 1 to 2 hr post drug administration in all three species; at this time, the parent drug accounted for ca. 15% of the total [^{14}C]-radioactivity in human plasma and ca. 5% of that in rat and dog plasma. The half-life of drug-related [^{14}C]radioactivity in the plasma was about 7.5 hr in man and dogs and 1 hr in rats. In each of these species, the principle route of drug elimination (primarily as metabolites XV and XVI, vide infra) was via the urine, whereas, the feces constituted the minor pathway for drug excretion.

As depicted in Scheme II, MK-447 is metabolized in rats and dogs almost exclusively to the corresponding O-sulfate ester (Compound XV). The major human metabolite of MK-447 has been isolated and tentatively assigned structure XVI, the N-glucuronide derivative of MK-447. The minor human metabolite corresponds to O-sulfate ester XV. The assignment of structure XV to the major rat and dog metabolite was confirmed by direct comparison of the isolated metabolite with an authentic sample of 2-aminomethyl-4-(1,1-dimethylethyl)-6-iodophenyl hydrogen sulfate which was synthesized by the O-sulfation process presented in Scheme II.

It should be noted that compound XV displays marked salidiuretic activity in both rats (saluretic score = 3) and dogs (saluretic score = 5). In view of the data presented in Table VII (vide supra) for O-substituted salicylamines Va-c, the observed activity after administration of O-sulfate ester XV suggests that O-desulfation occurs at the proper site in vivo, liberating MK-447 in agreement with the widely-accepted principle of microscopic reversibility, at least as it applies to enzymatic transformations. Hence, although ultimately eliminated via urinary and fecal excretion, metabolite XV may well serve in vivo as both a depot and a pro-drug form of MK-447. Finally, in spite of its structural similarity to thyroxine, MK-447 does not undergo detectable deiodination either in vitro or in vivo.

Pharmacology (11) - Orally administered in both normotensive and spontaneously hypertensive rats, MK-447 displayed marked saluretic and diuretic effects which were rapid in onset and relatively modest in duration, the major action having occurred within the first 5 hrs. A comparison of the salidiuretic activities of MK-447, furosemide and hydrochlorothiazide in normotensive rats is presented in Figures 4, 5 and 6. For those renal parameters measured, this comparison demonstrates that (a) the ceiling effects of MK-447 exceed those of furosemide and hydrochlorothiazide and (b) MK-447 is significantly more potent than furosemide. A precise comparison of the relative potencies of MK-447

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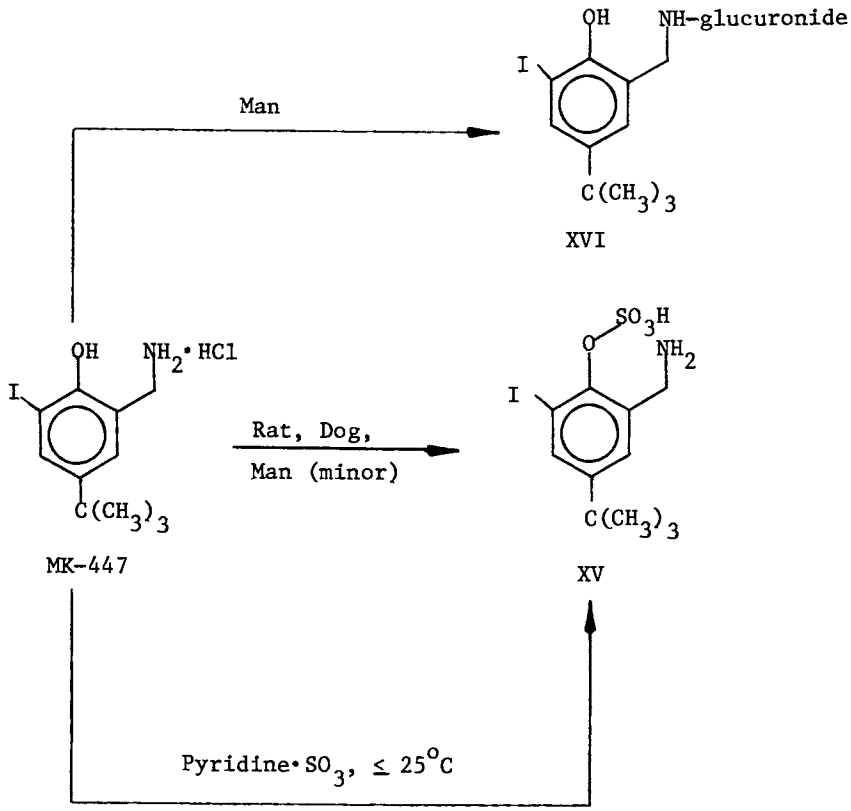
Scheme II

Metabolism of MK-447



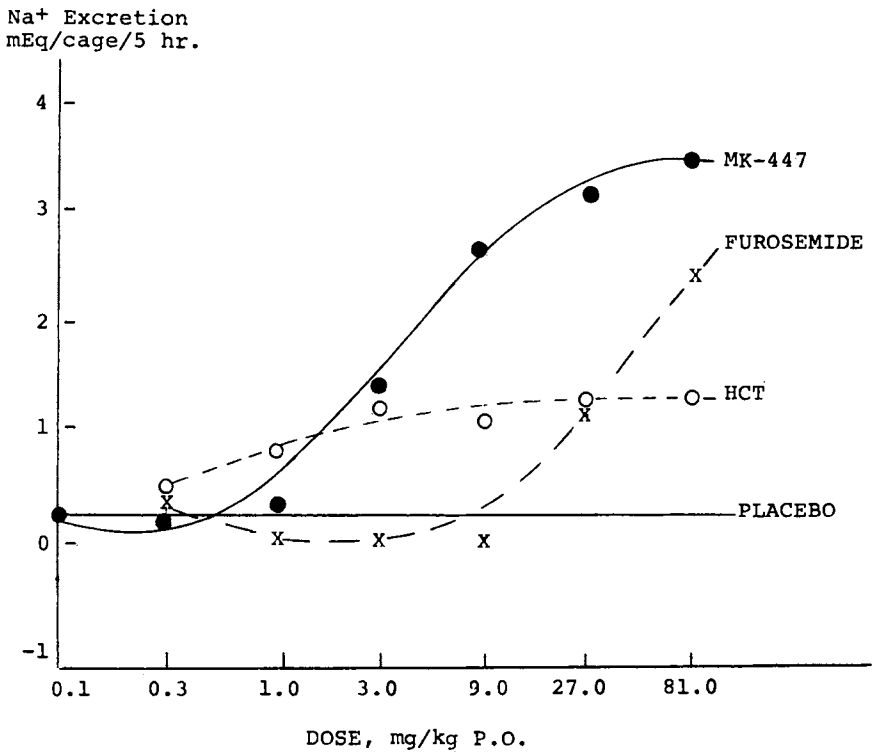


Figure 4. Dose–response regression lines for the natriuretic effects of orally-administered MK-447, furosemide, and hydrochlorothiazide in normotensive rats over a five-hr period. The data points are average values determined per cage for six to nine cages (three rats per cage of each drug. The placebo values for the same period were: Na^+, 0.28; K^+, 0.16; and Cl^-, 0.25 mEq/cage, and urine volume, 28 mL/cage.

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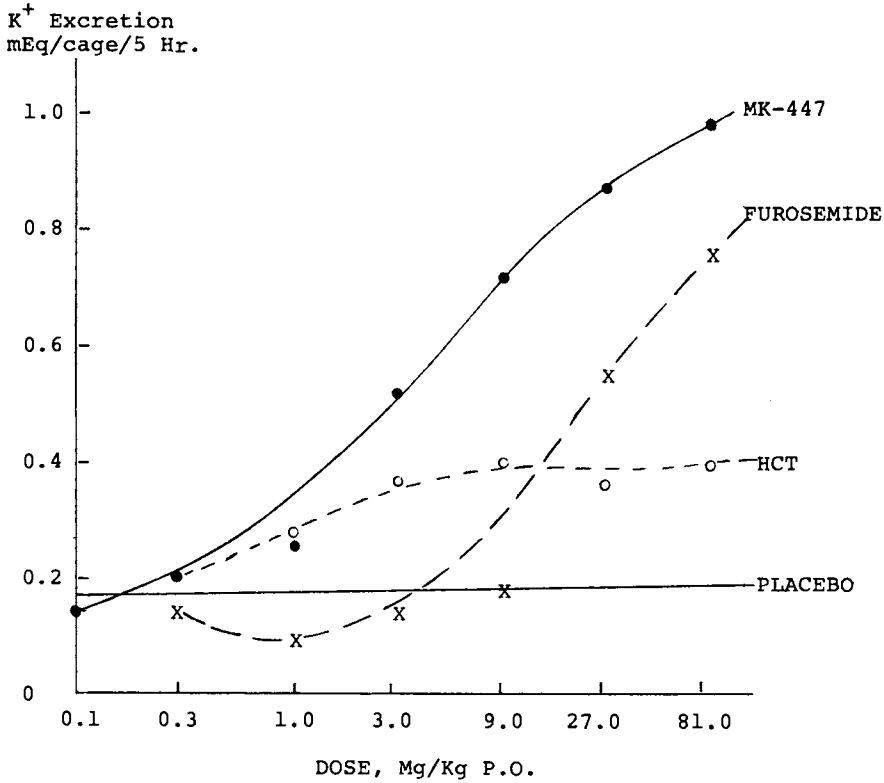

Figure 5. Dose–response regression lines for the kaliuretic effects of orally-administered MK-447, furosemide, and hydrochlorothiazide in normotensive rats over a five-hr period. The data points are average values determined per cage for six to nine cages (three rats per cage) at each dose of each drug. The placebo values for the same period were: Na^+, 0.28; K^+, 0.16; and Cl^-, 0.25 mEq/cage, and urine volume, 28 mL/cage.

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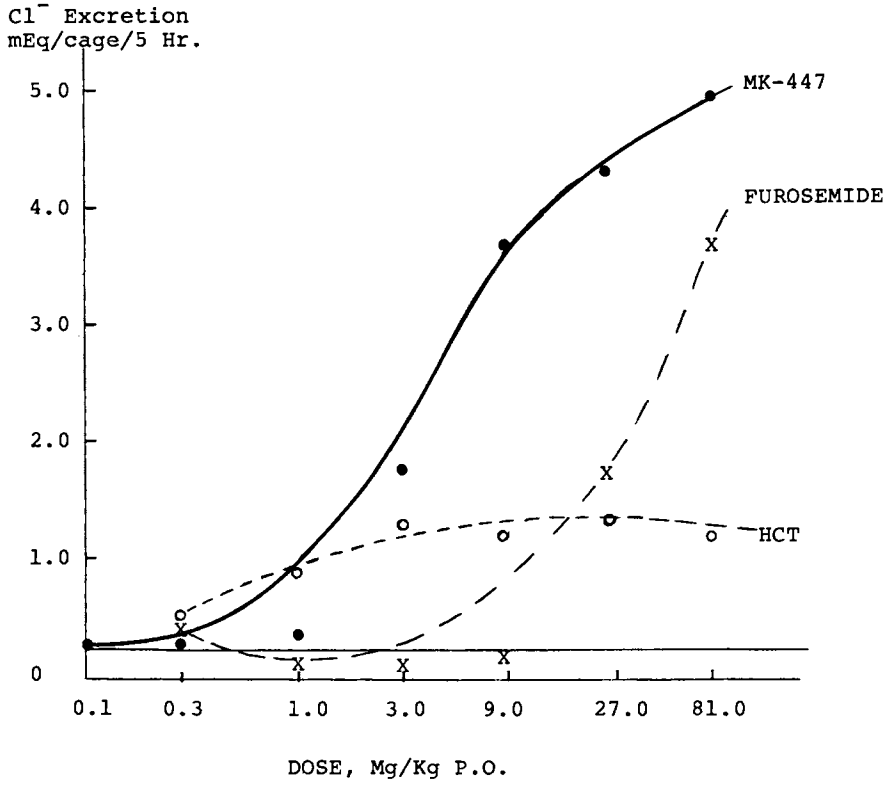

Figure 6. Dose–response regression lines for the chloruretic effects of orally-administered MK-447, furosemide, and hydrochlorothiazide in normotensive rats over a five-hr period. The data points are average values determined per cage for six to nine cages (three rats per cage) at each dose of each drug. The placebo values for the same period were: Na^+, 0.28; K^+, 0.16; and Cl^-, 0.25 mEq/cage, and urine volume, 28 mL/cage.

and hydrochlorothiazide is precluded by the lack of slope parallelism in their dose-response regression lines.

Evidence that the salidiuretic activity of MK-447 may relate to its ability to enhance kidney levels of PGE has been provided by Kuehl, et al. (12, 13) who showed that this drug has the ability to enhance the synthesis of PGEs in ram seminal vesicular microsomes and in incubating kidney slices. A typical experiment demonstrating the ability of MK-447 to facilitate the conversion of arachidonic acid to PGE_2 is shown in Figure 8. The mechanistic details for this action of MK-447 have been described (12, 14).

Support for the concept that the salidiuretic action of MK-447 relates to PG production is provided by the finding that treatment of rats with indomethacin (2 mg/kg p.o.) 1 hr prior to dosing with MK-447 affected both electrolyte excretion and urine volumes as shown in Table XII. This effect of indomethacin on the salidiuretic action of MK-447 was dose-dependent, since pretreatment with a lower dose (1 mg/kg p.o.) of indomethacin did not significantly affect the potency of MK-447. On the other hand, at a dose of 4 mg/kg p.o., indomethacin substantially reduced both the saluretic and diuretic effects of MK-447. Furthermore, indomethacin (4 mg/kg p.o.) alone marginally reduced rat urine volumes, which suggests that the observed effect of indomethacin pretreatment may be due only partially to a specific antagonism of the diuretic actions of MK-447.

The salidiuretic effects of MK-447, furosemide and hydrochlorothiazide given p.o. in unanesthetized dogs are compared in Table XIII. At each of the doses studied, MK-447 displayed saluretic and diuretic effects greater in magnitude than those of either furosemide or hydrochlorothiazide. As observed in rats, MK-447 elicited slightly more chloruresis than natriuresis in dogs. Kaliuresis was increased significantly by each of the three drugs. In anesthetized dogs, dose-related increases in electrolyte excretion and urinary volumes resulted from MK-447 given i.v. over the entire 0.1 to 25 mg/kg dose range. The Na^+/K^+ excretion ratio approached 14 at the highest dose (25 mg/kg i.v.).

When evaluated in spontaneously hypertensive (SH) rats, MK-447 (dose $\geq$ 0.312 mg/kg p.o.) exhibited antihypertensive activity. At doses of 1.25 and 5 mg/kg p.o., the antihypertensive effects of MK-447 were rapid in onset (within 1 hr), pronounced in potency and prolonged in duration (24 hr). In addition, the antihypertensive activity of MK-447 in SH rats was maintained upon repeated oral administration at 0.312 mg/kg as demonstrated in Table XIV. Under conditions where MK-447 (0.312 mg/kg p.o.) produced a pronounced antihypertensive response, furosemide at 20 mg/kg p.o. exhibited no hypotensive effect.

Evidence which suggests that the renal prostaglandins, in addition to their possible contribution to the salidiuretic

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TABLE XII

Antagonism of the Saluretic and Diuretic Effects of MK-447 by Indomethacin Pretreatment[a] in Normotensive Rats

Treatment	Dose (mg/kg p.o.)	Excretion Values[b], 0-5 Hr Period			
		Na^+ (mEq)	K^+ (mEq)	Cl^- (mEq)	Urine Vol (ml)
Placebo	0	0.28	0.16	0.25	28
MK-447	9	2.70	0.77	3.64	44
MK-447	27	3.19	0.88	4.35	47
MK-447	81	3.51	0.99	4.89	47
MK-447 + Indomethacin	9 + 2	2.52	0.85	3.59	42
MK-447 + Indomethacin	27 + 2	2.97	0.97	4.24	45
MK-447 + Indomethacin	81 + 2	3.25	1.02	4.70	43

[a]Pretreatment 1 hr prior to dosing with MK-447. [b]Average values per cage, 3 rats per cage.

TABLE XIII

Saluretic and Diuretic Effects of Orally Administered MK-447 (MK), Furosemide (F) and Hydrochlorothiazide (HCT) in Unanesthetized Dogs

Dose (mg/kg)	Excretion Values[a], 0–24 hr. Period											
	Na^+ (mEq)			K^+ (mEq)			Cl^- (mEq)			Urine Vol (ml)		
	MK	F	HCT	MK	F	HCT	MK	F	HCT	MK	F	HCT
0.2	10			6			12			260		
0.3	16	12	9	7	6	5	17	12	11	330	510	375
0.5			15			8			15			440
0.6	25			11			36			540		
1		22	19		8	8		25	26		660	800
1.3	38			19			46			570		
1.8	42			16			52			700		
2	50			17			73			890		
3		37			10			44			725	
5	59		24	18		10	77		31	960		590
10		57	26		18	12		69	31		1035	760

[a]Average values determined per dog for 3 to 47 dogs at each dose of each drug. The placebo values for the same period were: Na^+, 4.8; K^+, 6.3; and Cl^-, 6.3 mEq/dog and urine volume, 210 ml/dog.

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TABLE XIV

Antihypertensive Activity of MK-447 Administered Orally in SH Rats of the Wistar-Okamoto Strain

Group No. (No. of Rats)	Treatment	Day	Mean Arterial Pressure (mmHg ± SE)[a], Hr after Treatment			
			0	4	12	24
1 (17)	Saline 2 ml/kg p.o.	1	178±4	180±4	174±3	172±3
		2		173±4	171±3	171±3
		3		171±2	170±2	173±2
2 (5)	MK-447[b]	1	185±3	163±5	166±7	167±7
		2		157±7	161±6	156±5
		3		157±4	159±3	181±4

[a]Arterial pressure was recorded in unanesthetized male rats of 290 to 350 g body weight and 30 to 40 weeks age by a direct technique involving cannulation of the caudal artery. [b]MK-447 (0.312 mg/kg) was administered p.o. in water in volumes of 2 ml/kg daily for 3 days.

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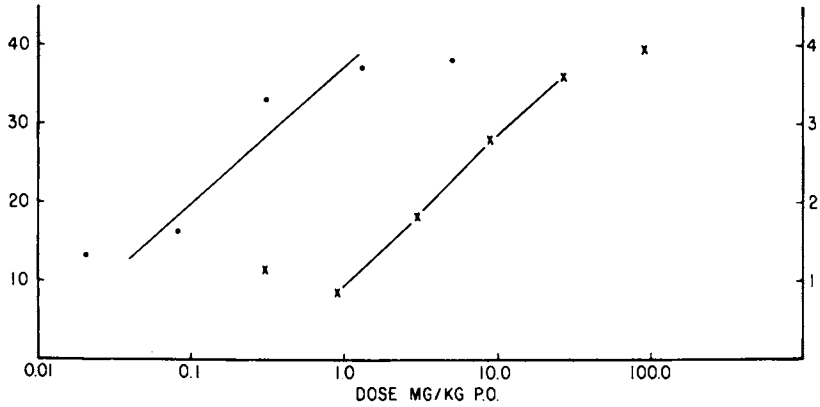


Figure 7. Dose–response regression lines for the effects of MK-447 on the mean arterial pressure and Na^+ excretion in spontaneously hypertensive rats. The data points for decreases in mean arterial pressure are average values per rat for four to six rats per dose; the data points for Na^+ excretion are average values per cage determined for nine cages (three rats per cage) at each dose.

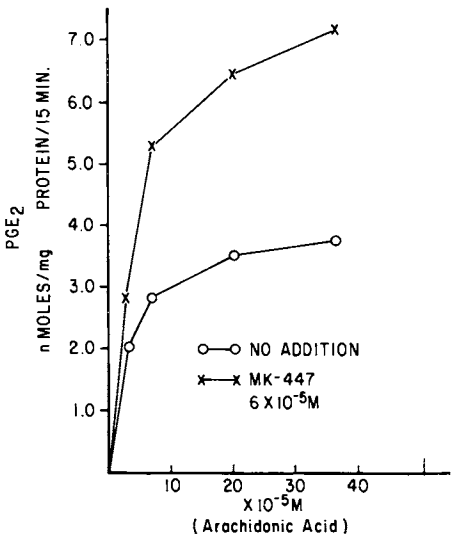


Figure 8. The stimulatory effects of MK-447 on the biosynthesis of PGE_2 in ram seminal vesicular microsomes as a function of substrate concentration

effects of MK-447 (vide supra), may also play a role in mediating the antihypertensive activity of MK-447 emerged from studies in SH rats. In this test species, the marked antihypertensive effects elicited by 0.078, 0.312 and 1.25 mg/kg p.o. doses of MK-447 were seriously attenuated by the oral coadministration of either indomethacin (1.25 mg/kg) or aspirin (20 mg/kg). Interestingly, MK-447 displayed no hypotensive activity in normotensive Wistar-Kyoto rats at doses up to 20 mg/kg p.o. Of even greater pharmacological interest was the observation that MK-447 exhibited antihypertensive activity in SH rats at subdiuretic doses, i.e., at doses ten-fold lower than those required for diuresis as shown by the dose-response regression lines in Figure 7.

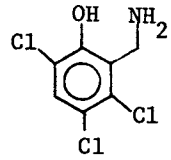
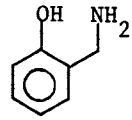
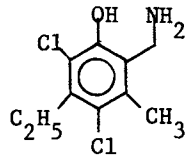
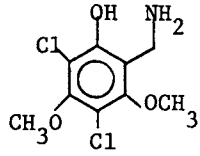
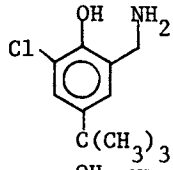
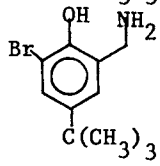
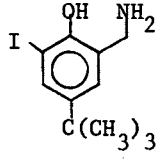
The results cited above indicate that, at least in SH rats, the antihypertensive activity of MK-447 is not solely dependent on its diuretic activity. Further support for this view emerges upon examination of the relative saluretic and antihypertensive activities recorded in Table XV for a series of salicylamine derivatives. Although compounds IXg, IXi, VIIi, VIIk and MK-447 display nearly equipotent saluretic activities in rats and dogs, their relative antihypertensive effects are markedly different. Hence, as noted earlier, the pronounced antihypertensive properties of MK-447 served to distinguish it from a subseries of potent salidiuretic agents which emerged from this study. It is interesting to note that the initial saluretic screening lead (Ia) is not antihypertensive in the SH rat, whereas, the parent structure, salicylamine (Compound VIa), has demonstrable, albeit weak, antihypertensive activity in the SH rat, but it is devoid of saluretic activity.

The third pharmacological attribute of MK-447, antiinflammatory activity, was demonstrated by its effect in reducing both carrageenan-induced foot edema in rats and croton-oil induced swelling in mouse ears (12, 13). This action was suggested to arise from the ability of MK-447 to scavenge an oxygen centered free-radical released in the conversion of PGG_2 to PGH_2. Evidence has been provided to show that this radical is an important inflammatory mediator (12, 13).

The initial clinical study (15) in normal volunteers has shown that MK-447 is a potent, high-ceiling diuretic in man as indicated by the natriuretic and kaliuretic data presented in Figure 9. In this study, MK-447 displayed marked dose-related saliuresis and diuresis with minimal kaliuresis. Recently, MK-447 was shown to elicit antihypertensive activity at diuretic doses in man (16). MK-447 is presently undergoing further clinical investigation. Whether the adjunctive antiinflammatory activity demonstrated for MK-447 in experimental animals will be observed in man is yet to be established.

Acknowledgments - We are indebted to Ms. S. J. deSolms, Mr. A. A. Deana and Mr. N. P. Gould for expert synthetic assistance, to Mr. E. L. Cresson for the UV studies, to Dr. L. S. Watson (deceased) and to Mr. H. F. Russo for the salidiuretic

TABLE XV. Relative Saluretic and Antihypertensive Activity

Compd.	Structure	Saluretic Score		Antihypertensive Score[a]	
		Rat	Dog	SH Rat	RH Dog
Ia		3	4	0	
VIa		0	0	1	
IXg		5	5	0	
IXi		5	5	1	1
VIIi		6	5	1	2
VIIk		6	5	2	2
MK-447		6	5	3	3

[a]Relative scores: outstanding activity = 3; moderate activity = 2; weak activity = 1; inactive = 0.

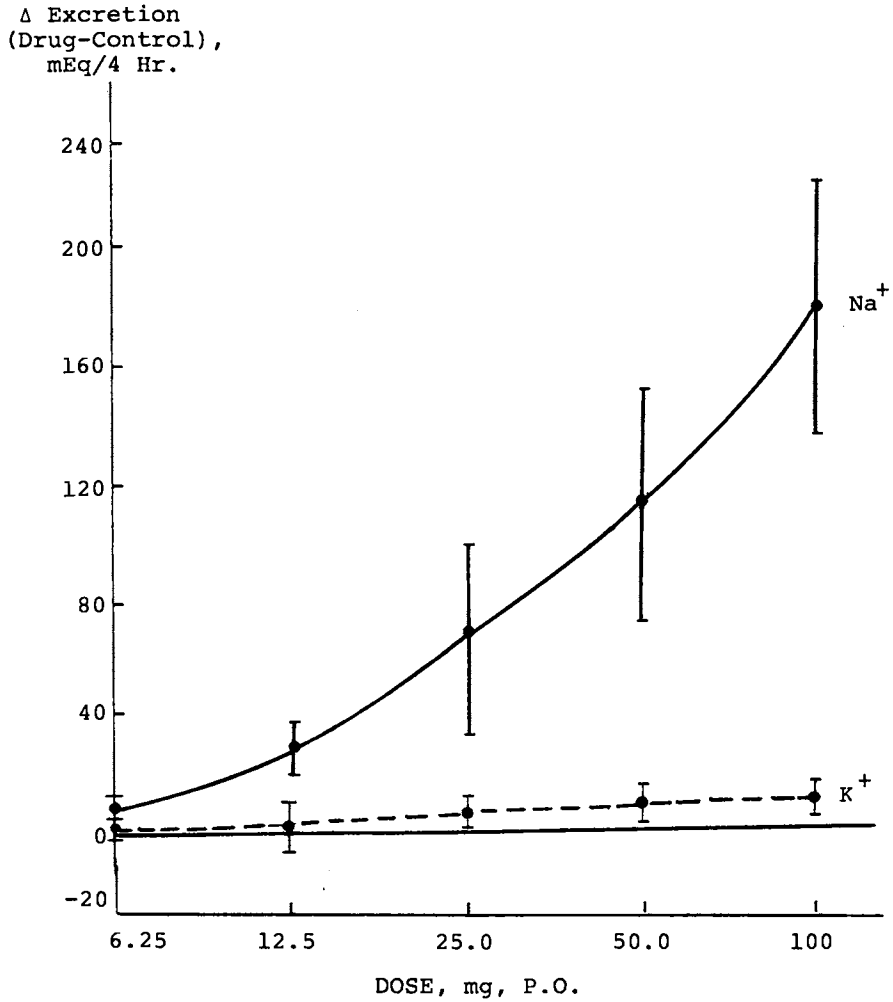


Figure 9. The natriuretic and kaliuretic effects of orally-administered MK-447 in normal human volunteers over a four-hr period. The data points are average values (Δ excretion = drug control) determined per volunteer in eight volunteers at each dose. Derived from the data of Affrime, M. B., et al. (15).

In Diuretic Agents; Cragoe, E.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

evaluations, to Dr. A. Scriabine and to Mr. C. T. Ludden for the antihypertensive studies and to Dr. C. G. Van Arman for the antiinflammatory results. Gratitude is expressed to Dr. J. L. Humes for providing Figure 8, to Dr. K. Hoogsteen, Dr. J. Springer and Mr. J. Hirschfield for the X-ray crystallographic analysis of MK-447 free base and to Dr. G. M. Smith for the CNDO/2 modeling studies. We wish to express our appreciation to Drs. D. J. Tocco and J. E. Baer and their colleagues for the metabolism studies, to Dr. R. O. Davies, Dr. J. M. Schrogie, Dr. K. E. Tempero and Dr. B. Lei for the clinical investigations and to Dr. J. M. Sprague (retired), Dr. C. A. Stone and Dr. R. F. Hirschmann for their guidance and encouragement throughout the course of this investigation.

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8

1-Aralkyl-2-pyrazolin-5-ones: A New Class of Highly Potent Diuretics with High Ceiling Activity

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The diuretics in use at the present time may be divided pharmacologically and clinically into compounds with either low-ceiling or high-ceiling activity.

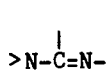
Compounds having a low-ceiling activity exhibit a leveling-off or plateau in their diuretic action. Beyond a certain point, the diuretic effect is not increased by an increase in dosage. Typical low ceiling diuretics are the thiazides. Also, such compounds as chlorthalidone and mefruside belong to this group. A further characteristic of this group is the protracted duration of activity which is particularly advantageous in long-term treatment (Figure 1).

In contrast to this, the dose/activity curves of high-ceiling diuretics run almost linearly over a wide range. Such diuretics have a reserve in capacity and can, therefore, also be used on thiazide-resistant patients. A major disadvantage is their short duration of activity which is coupled with rebound phenomena. Representative compounds having this type of activity are ethacrynic acid, furosemide and bumetanide.

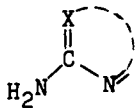
The medically most desirable combination of high-ceiling activity and protracted duration could not be realized until now. This was, therefore, the target of our own work in this area.

While it seemed improbable that progress could be made in this direction merely by working on known diuretics, we concentrated our attention on new classes of active substances.

Structural elements such as 1 and 2 which appear frequently in diuretic purines, triazines, pteridines, pyrazines and



1



2

pyrimidines, served as guidelines for selection.

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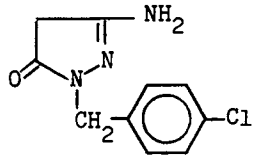
A number of new diuretics, such as furosemide, bumetanide and triflocin (1), have amphoteric properties, so we also looked closely at compounds having acidic and basic functions in the same molecule.

As a result of this extensive search, we discovered a new class of agents represented by compound 3 a few years ago which possessed moderate diuretic activity in rats and dogs.

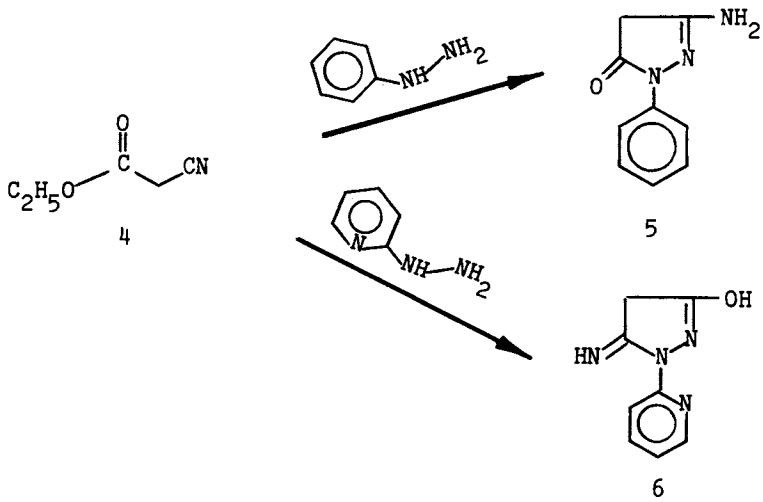


3

As may be seen, this is a 3-aminopyrazolin-5-one which is substituted in the 1-position by an aralkyl residue.

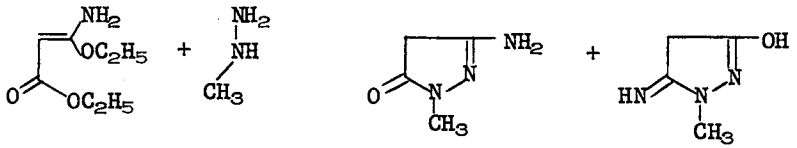
In contrast to the well-known and thoroughly investigated 3-amino-1-arylpyrazolin-5-ones, 3-amino-1-aralkylpyrazolin-5-ones had only been described in a few instances in the patent literature at the start of our work, and then, only as coupling components for magenta dyestuffs (2). The chemistry of this class will therefore be discussed first.

The preparation of 3-aminopyrazolin-5-ones (Formula 5) bearing aromatic substituents in the 1-position has been studied extensively by Weissberger and co-workers (3, 4, 5) and is carried out by reaction of arylhydrazines with cyanoacetate (Compound 4) in the presence of alkoxide. Certain heterocyclic hydrazine derivatives, such as 2-pyridylhydrazine, yield the isomeric 3-hydroxypyrazolin-5-imines (Formula 6) under these conditions.



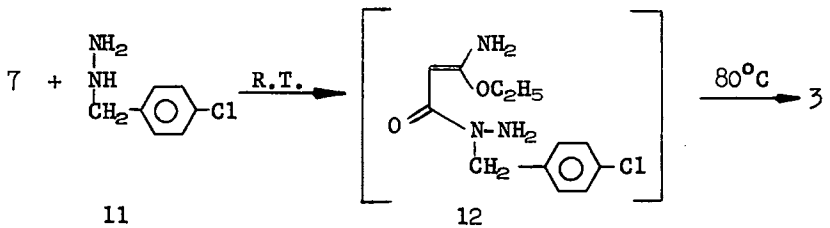
4 5 6

If β-amino-β-ethoxyacrylate (Compound 7) is employed instead of compound 4, then the 3-aminopyrazolin-5-one analogous to compound 5 is formed in both cases. In contrast to this behavior, aliphatic hydrazines, such as methylhydrazine (Compound 8), react even with compound 7 to give a mixture of 3-aminopyrazolin-5-one (Compound 9) and 3-hydroxypyrazolin-5-imine (Compound 10).

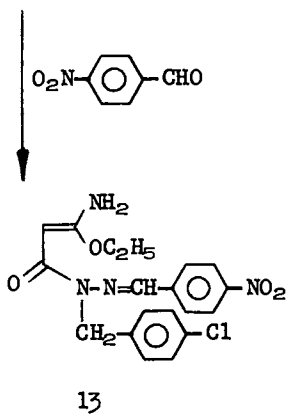


7 8 9 10

This result shows that both N-atoms of compound 8 are capable of reacting either with the ester function or with the imidate function of compound 7. One would expect a similar behavior from aralkylhydrazines. If one reacts 4-chlorobenzylhydrazine (Compound 11) with compound 7 under mild conditions for a short time and traps the primary product (Compound 12) with 4-nitrobenzaldehyde, then one isolates the hydrazone (Compound 13) in good yield.



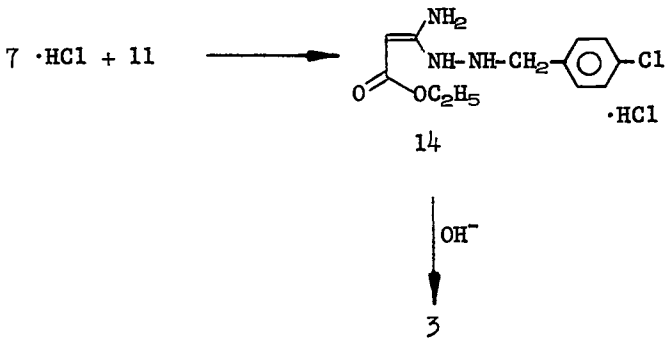
11 12



13

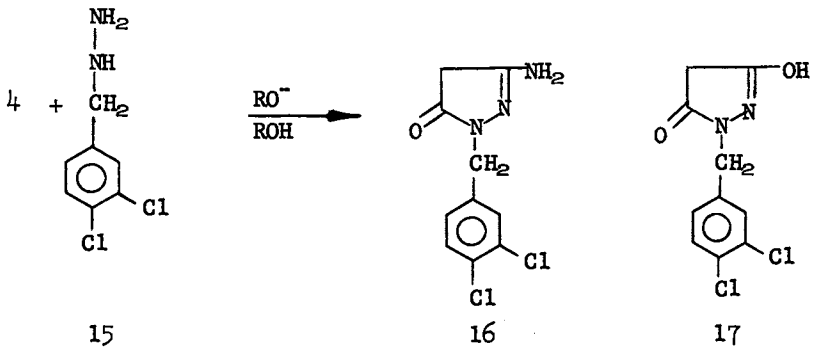
Under forcing conditions, compound 7 and compound 11 react smoothly to give compound 3 in good yield. This shows that the ester function reacts primarily and that the favored point of attack is the substituted N-atom of compound 11.

If the imidate function is activated by salt formation, then the amidrazone (Compound 14) is formed primarily, which does not react with aldehydes. In this case, the unsubstituted N-atom of compound 11 is the favored point of attack.

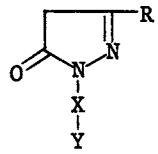


On addition of alkoxide, compound 14 is cyclized spontaneously to compound 3 so that the same product results from both reactions.

If aralkylhydrazines are reacted directly with compound 4 in the presence of alkoxide, then mixtures of the isomeric pyrazolines (Compounds 16 and 17) are formed, as shown in the reaction of 3,4-dichlorobenzylhydrazine (Compound 15).

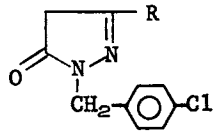


Since compound 3 is a polyfunctional species, it offers countless opportunities for molecular variation; therefore, a representative selection of analogs will be made for discussing the effect of structural changes on diuretic activity. The general formula 18 serves as a basis for this discussion.



18

In Table I, the qualitative relationship is presented between structure and activity for the pyrazolin-5-ones (Structure 19) as a function of the substitution in the 3-position.



19

The results show that alkylation or acylation of the amino group causes a drastic loss of activity. Replacement of amino by a hydroxy- or carbonyl group or groups derived from these moieties also leads to weak or inactive compounds. In this regard, it was all the more surprising that compound 26 (and similar pyrazolin-5-ones substituted by lower alkyl groups) possessed a noteworthy diuretic potency. A plausible explanation for these results is not possible at the present time.

The carbonyl group in the 5-position is of crucial importance for activity. Inter alia, this may be seen by the fact that 1-(4-chlorobenzyl)-3-hydroxypyrazolin-5-imine, isomeric with Compound 3, and the corresponding 3,4-dichlorobenzyl derivative (Compound 15) have no diuretic activity. Furthermore, 3-amino-1-(4-chlorobenzyl)pyrazolin-5-imine, prepared from 4-chlorobenzylhydrazine and malonitrile, is also inactive.

3-Aminopyrazolin-5-ones (Structure 32) possess amphoteric properties. It has been shown by NMR-studies that the equilibrium Compound 32 $\rightleftharpoons$ Compound 33 lies in favor of the enol form (Compound 33) in polar solvents.

In Diuretic Agents; Cragoe, E.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

TABLE I

The Qualitative Relationship between Structure and Activity for the Pyrazolin-5-ones (Structure 19) with Variations (R) in the 3-position.

No.	R	Activity
3	$-NH_2$	+
20	$-NHCH_3$	-
21	$-NHC_6H_5$	-
22	$-NH-COCH_3$	-
23	$-NH-CO-NHCH_3$	-
24	$-OH$	-
25	$-OC_2H_5$	-
26	$-CH_3$	+
27	$-C_6H_5$	-
28	$-COOC_2H_5$	-
29	$-COOH$	-
30	$-CONH_2$	-
31	$-CONHC(=NH)-NH_2$	-

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ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

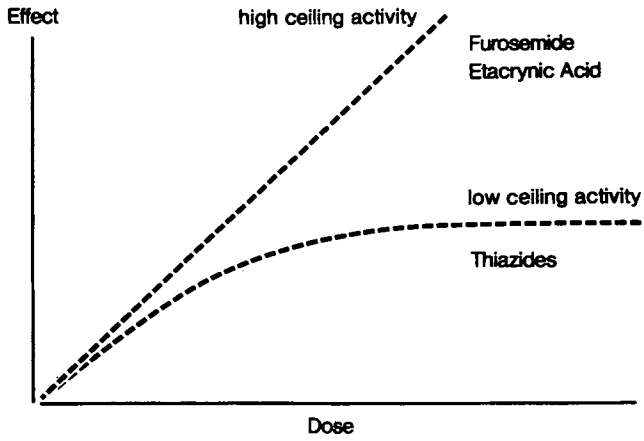


Figure 1. Dose–effect curves of diuretics with high-ceiling and low-ceiling activity

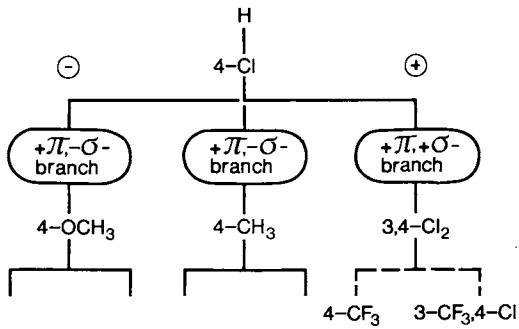
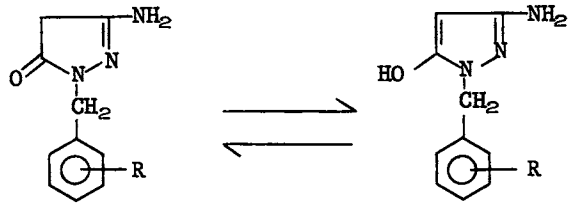


Figure 2. Topliss-operation scheme greatly simplified



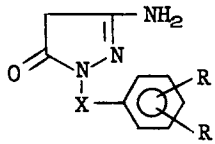
32 33

Our current data indicate that the capability of the compounds to undergo enolization seems to be of critical importance for activity. Inter alia, this also may be concluded from the fact that 3-aminopyrazolin-5-ones monoalkylated in the 4-position have diuretic activity, whereas, the analogs dialkylated in the 4-position are inactive. Replacement of the enolic hydroxyl group by a chlorine atom also leads to loss of activity.

The substitution pattern in the aromatic residue has been extensively investigated. In these studies, the operation scheme developed by Topliss (6) yielded good results. By the application of a somewhat simplified "Topliss tree" (Figure 2) to different subgroups of pyrazoles, we found that one always approached the area of optimal activity by following the $+\pi$, $+\delta$-branch as far as the 3,4-dichloro derivative.

Figure 3 shows the connection between the natural logarithm of the sodium excretion in the dog after oral administration of 3 mg/kg of each substance and the lipophilicity in the series of 3-amino-1-benzylpyrazolin-5-ones. The R_M value serves here as a measure of the lipophilicity (Figure 3). If one takes into account that these data were orientating values obtained on the intact animal, i.e., two dogs per substance, then the result, with a correlation coefficient of 0.63 and a random sample size of 24, may be regarded as satisfactory and serve as an aid for planning new syntheses. The results would be better if the pharmacological data could be refined by multiple repetition. This was only possible with a small number of compounds due to limited capacity.

We obtained very interesting results by variation of the bridge X between the heterocyclic and aryl moieties. A selection of compounds of general formula 34, which serves to illustrate this, is contained in Table II.



R=H, CH_3, Cl

34

TABLE II

The Average Activities of Compounds on Variation of the Bridge X between Heterocycle and Aryl Residue (Formula 34).

Subgroup	X	Activity *
I	-	-
II	$-CH_2-$	+
III	$-CH(CH_3)-$	+++
IV	$-CH_2-CH_2-$	+
V	$-CH_2-CH_2-O-$	++
VI	$-CH_2-CH=CH-$	++
VII	$-CH_2-CH_2-CH_2-$	-

*dog, 3 mg/kg p.o.;

-	<500 µEq	Na^+/Kg
+	500-1000	"
++	1000-2000	"
+++	>2000	"

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ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

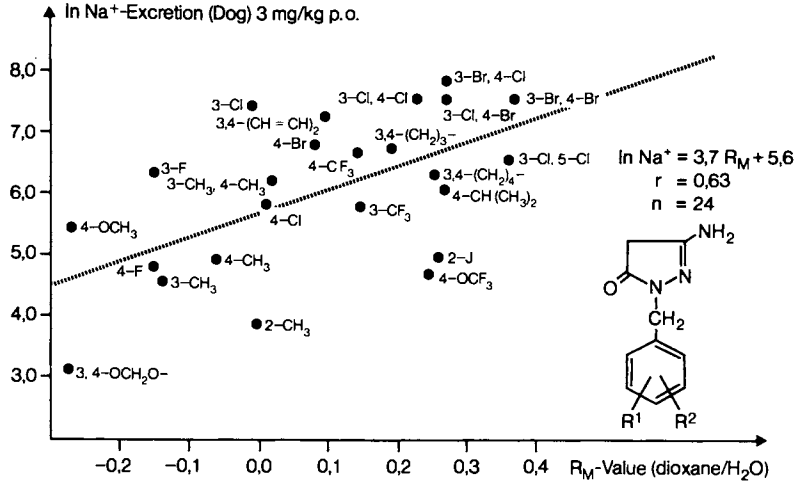


Figure 3. Quantitative structure activity relationship between sodium excretion in the dog and lipophilicity

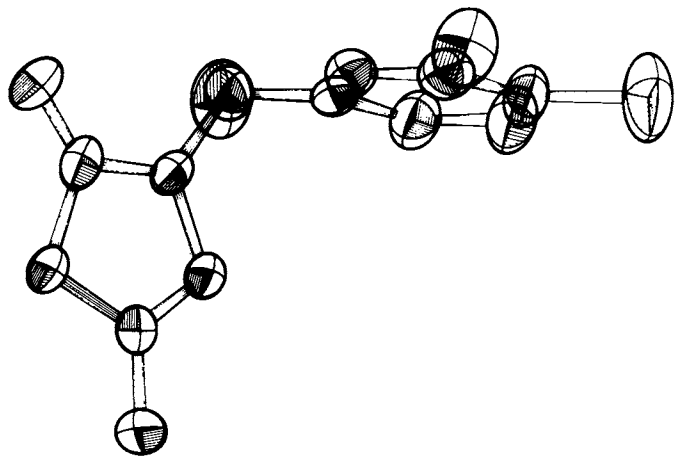
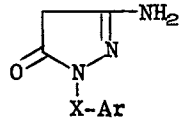


Figure 4. X-ray crystal structure of muzolimine

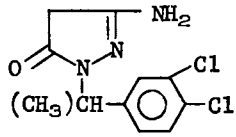
The results should, however, only indicate a trend in activity which was observed in the dog by variation of the bridge X. (Within the individual subgroups, the average was taken of the results obtained on compounds with different substitution patterns.)

An important guideline for continued work in this class of compounds was the observation that 3-amino-1-arylpyrazolin-5-ones (Subgroup I) were inactive; 3-amino-1-benzylpyrazolin-5-ones with a methyl branching in the methylene group (Subgroup III) were highly active. This is illustrated by the comparison of some unbranched 3-amino-1-benzylpyrazolin-5-ones with the corresponding branched derivatives (Table III, General Formula 35).



35

From the several hundred pyrazolin-5-ones synthesized in these laboratories, muzolimine (BAY g 2821, Compound 38, 3-amino-1-(3,4-dichloro-α-methylbenzyl)pyrazolin-5-one) was selected for clinical trials.



38

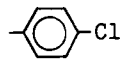
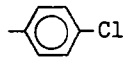
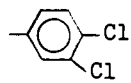
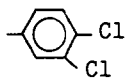
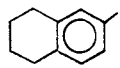
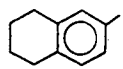
Figure 4 shows the X-ray crystal structure of muzolimine. In Table IV, some physicochemical data for muzolimine and furosemide are listed. These data show that BAY g 2821 is noticeably more lipophilic than furosemide, which is probably of great significance in explaining their different pharmacokinetics.

In Figure 5, the dose/activity curve in the dog is shown for different electrolytes. As can be seen, doses of muzolimine in the range 0.1 mg/kg to 3 mg/kg cause an almost linear increase in the excretion rate of chloride and sodium ions. The potassium excretion is only significantly increased by doses above 1 mg/kg. The bicarbonate excretion remains almost unaffected. Thus, muzolimine behaves similar to furosemide and must be added to the group of "high ceiling" diuretics.

An even clearer demarcation with respect to furosemide and the special activity profile of BAY g 2821 is seen when one studies the activity vs. time curve after a single dose of 1 mg/kg. When one measures the excretion volume and electrolyte

TABLE III

Comparison of the Activities of Some Unbranched 3-Amino-1-Benzylpyrazolin-5-Ones with the Activities of the Corresponding Branched Derivatives (Formula 35).

No.	-X-	-Ar-	Activity*
3	$-CH_2-$		+
36	$-CH(CH_3)-$		+++
37	$-CH_2-$		++++
38	$-CH(CH_3)-$		++++++ (muzolimine)
39	$-CH_2-$		+
40	$-CH(CH_3)-$		++++

*Increased sodium excretion; dog, 3 mg/kg p.o./1 hr.
+ = 500 $\pm$ 100 μEq Na^+/Kg

TABLE IV

Physicochemical Properties of Muzolimine in Comparison with Furosemide

	Furosemide	Muzolimine
pK_a	3.9	9.2
lg P (octanol/H_2O)	-.83	2.29
R_M (ethanol/H_2O)	-.62	-.84
R_M (dioxane/H_2O)	-.67	-.33

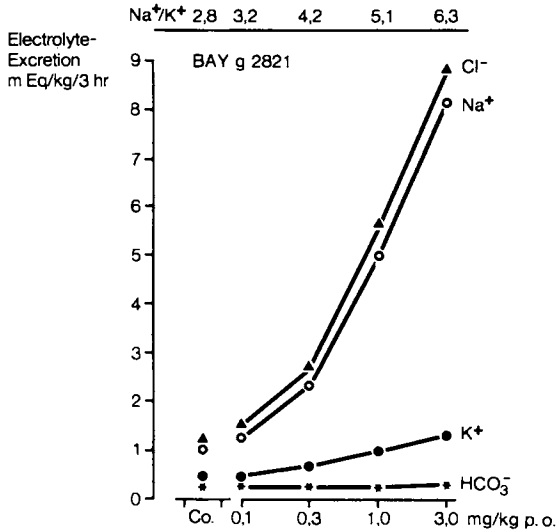


Figure 5. Dose–activity curves in the dog after application of muzolimine

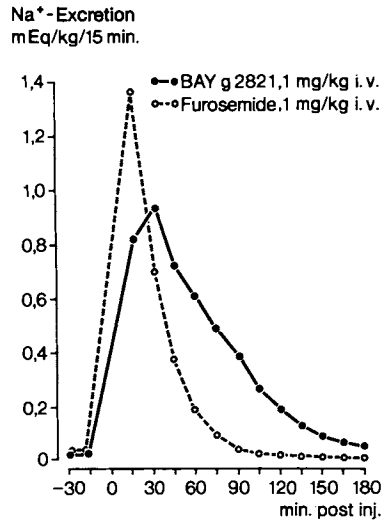


Figure 6. Time–response curves in the dog without substitution after a single dose of 1 mg/kg

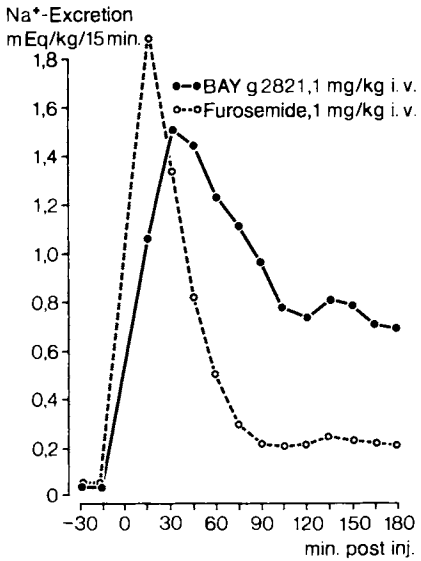


Figure 7. Time–response curves in the dog with substitution after a single dose of 1 mg/kg

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quantities at short intervals over 180 minutes, it is found that the action of furosemide is of rapid onset but soon drops away, whereas, with muzolimine, the initial increase in action is slower, but the duration is longer (Figure 6).

These differences become even more distinct when the salt and volume losses caused by the substance are replaced by an infusion carefully matched to the excretion rate. By this means, the counter-regulation effect in the healthy animal is nullified and the salt and water reserves present in the edematous patient are simulated. Under these conditions, one can see that the activity of BAY g 2821 is not only of distinctly longer duration, but is also higher than that of furosemide with respect to the total excretion (Figure 7).

For approximately two years, muzolimine has been under clinical test. A whole series of carefully controlled studies have been completed in the meantime and some have been published (7-13). In these studies, the activity of muzolimine, especially the novel combination of high-ceiling and long-acting activities on different forms of edema, particularly on cardiac and hepatogenic edemas, has been confirmed.

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9

Quincarbate: A Representative of a New Class of Diuretics with 1,4-Dioxino[2,3-g]quinolone Structure

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During an investigation of new quinolone derivatives (Figure 1), which were synthesized mainly for anticoccidiosis testing, it was found that some of these compounds possessed potent diuretic activity (1).

In this paper, we will discuss, in turn: (a) the structure-activity relationships (SAR) of the compounds of the series, (b) the physicochemical properties of some representative compounds, (c) the pharmacological profile and the metabolism of quincarbate, the compound selected for human pharmacological investigation and (d) the preliminary results of the evaluation of quincarbate in humans.

STRUCTURE-ACTIVITY RELATIONSHIPS

The diuretic activity was determined in saline loaded rats after oral administration of the compounds according to a modification of the method of Lipschitz (2). The volume of excreted urine as well as its electrolyte contents were measured over a 5-hour period. The activity was expressed as an ED_{200} value, i.e., the dose (mg/kg) which gave a 100% increase in the urinary volume over the control value.

Compound 1 (Figure 2) is a prototype structure which possesses the characteristics essential for diuretic activity. Variation of the ring structure, as well as, of the location of the substituents generally led to compounds with no oral activity at 50 mg/kg (Figure 3). Reorientation of the $EtOCH_2$-group to the vicinal carbon atom in the dioxino ring, as in compound 2, or the reorientation of the carboxyl group in the pyridone ring, as in compound 3, produced a considerable decrease in activity or to inactive compounds. Contraction (Compound 4), expansion (Compound 5), or opening (Compound 6), or reorientation (Compound 7) of the dioxino ring also produced inactive compounds. Likewise, the presence of methyl groups on the pyridone nucleus had a disastrous effect on the activity (Compounds 8 and 9).

Variation of R_3 revealed that a small alkoxyalkyl substituent

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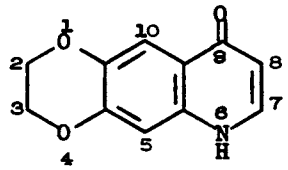


Figure 1. 1,4-Dioxino[2,3-g]-quinolone

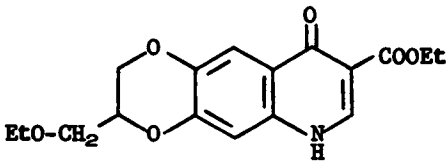


Figure 2. Compound 1, a prototype of the series

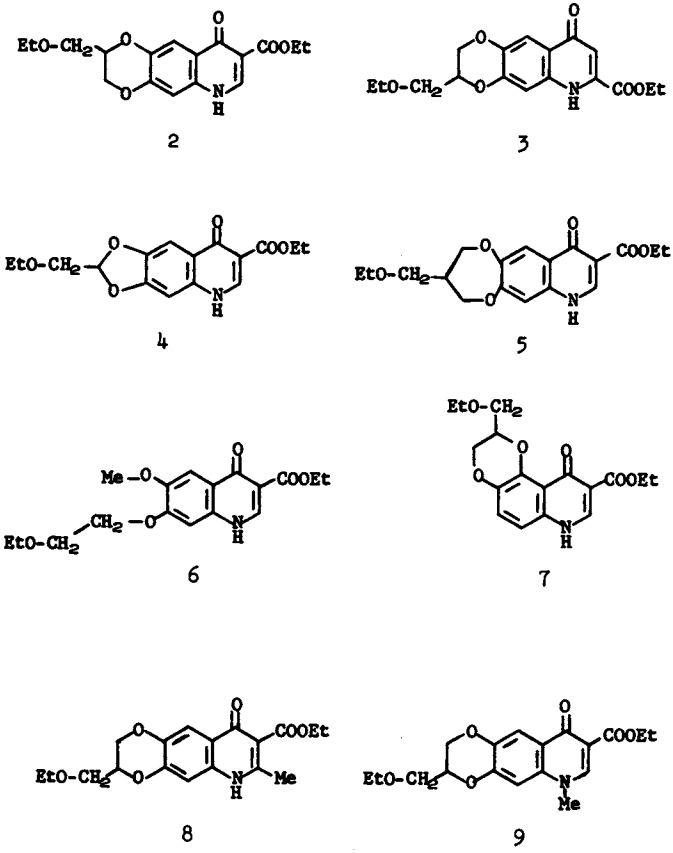
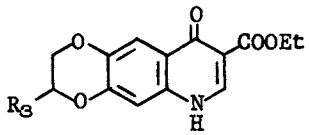


Figure 3. Inactive (a, b) analogs in which substituents or rings have been rearranged.

(a) Diureses estimated in saline-loaded rats; (b) inactive means: no activity at 50 mg/kg, orally.

was essential, since compounds 15, 16, 17, 18 and 19 were inactive, and it also showed that the oxymethylene bridge appeared optimal (Table I).

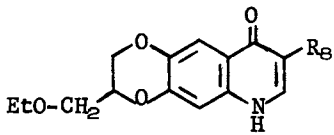
Table I. Effect of variation of R_3 in



no.	R_3	ED_{200}	no.	R_3	ED_{200} (mg/kg)
1	$EtO-CH_2$	3.8	15	H	>50 or inactive compounds
10	$HO-CH_2-$	10	16	Me-	
11	$MeO-CH_2-$	10	17	$Cl-CH_2-$	
12	$PrO-CH_2-$	15	18	MeO-CO-	
13	$MeO-CH_2-CH_2-O-CH_2-$	4	19	$n-C_8H_{17}-O-CH_2-$	
14	$EtO-CH_2-CH_2-$	~ 20			

The substituent R_8 (Table II) seemed to be less critical, since the carboxy or ethoxy carbonyl function can be replaced by an acetyl group (Compound 21) or even by an alkyl group (Compound 24). Whether the activities of these various compounds are due to metabolism has not been established.

Table II. Effect of variation of R_8 in



no.	R_8	ED_{200}	no.	R_8	ED_{200} (mg/kg)
1	-COOEt	3.8	25	$-CO-NH_2$	>50 or inactive compounds
20	-COOH	7	26	$-C\equiv N$	
21	-CO-Me	12	27	$-CH_2-NH_2$	
22	$-CH_2-OH$	4	28	-H	
23	-CHOH-Me	11	29	-Br	
24	-Et	10	30	$-NH_2$	
			31	$-NO_2$	

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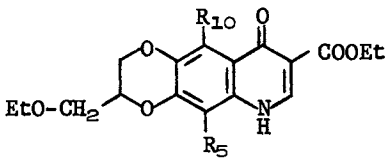
Substitution of the benzene nucleus produced marked change (Table III) in diuretic activity.

Table III. Effect of variation of R_5 and R_{10} in



no.	R_{10} (R_5=H)	ED_{200}	no.	R_{10}	R_5	ED_{200} (mg/kg)
1	H	3.8	38	NH_2	H	50
32	Me	4	39	OMe	H	>50 or inactive compounds
33	Cl	0.08	40	H	Cl	
34	Br	~0.4	41	Cl	Cl	
35	CF_3	~0.5				
36	NO_2	~3				
37	NHAc	13				

When R_{10} was a lipophilic, electronegative group, as in compounds 33, 34, 35 and 36, activity was increased, especially when R_{10}=Cl, as in compound 33. Compound 33 (whose recommended INN name is quincarbate) was the most active member of the series. Electron donating groups for R_{10}, e.g., NH_2 (Compound 38) and OMe (Compound 39), produced a considerable decrease in activity. It is remarkable that when the other hydrogen atom (R_5) was substituted by Cl, as in compounds 40 and 41, activity was totally lost.

Some variants of R_9 induce the pyridone ring I to assume its tautomeric form II (Figure 4). If R_9 is an oxygen atom, the pyridone configuration I dominated almost completely, as confirmed by IR data. This was also the case with compound 43 and probably with compound 42, but if R_9=OMe (Compound 44) or Cl (Compound 46), only tautomer II is possible. Since among the compounds which exist in one or the other tautomeric form, both active as well as inactive compounds are found (Table IV), the actual ring structure would appear not to be relevant.

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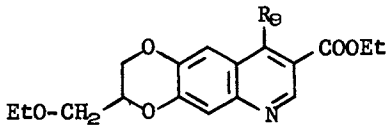
Table IV. Effect of variation of R_8 in



no.	R_8	ED_{200}	no.	R_8	ED_{200} (mg/kg)
1	OH	3.8	45	NHPr	> 50 or inactive compounds
42	SH	~13	46	Cl	
43	NH_2	~13			
44	OMe	30			

The SAR may be summarized by the statement that the tricyclic compound must be linear and unsubstituted at positions 2, 5, 6 and 7. Substitution at position 3 with a hydroxymethyl or alkoxymethyl group is necessary, while at position 8, a substituent such as a carboxyl, alkoxycarbonyl, hydroxyalkyl, acetyl, or alkyl group must be present. The oxygen atom normally present at position 9 may be replaced by certain groups, and substitution at position 10 by lipophilic electronegative groups increases the activity. A QSAR for substituents at position 10 confirmed these statements. In the calculations concerning R_8, three deviations from the regression were found, i.e., with compounds 28, 29 and 31.

PHYSICOCHEMICAL PROPERTIES

The compounds of this series possess an extremely low solubility in water (Table V). The partition coefficients indicated a rather lipophilic character, but the solubility in lipophilic solvents was generally very low, being far less than 0.1% in most solvents. The ester, quincarbate (Compound 33), exhibits good solubility (>10%) only in strongly protonating solvents, while the acids (Compounds 47 and 51) form soluble salts in aqueous alkali.

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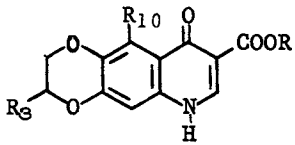
Table V. Comparison of solubility and partition coefficient with diuretic activity in rats

Structure				Solubility in water at pH 6 at 20°C (mg/L)	Partition coefficient octanol/ water at pH 7.5	Diuretic activity ED_{200} (mg/kg)
						
No.	R_3	R_{10}	R			
1	$EtOCH_2$	H	Et	30	> 50	3.8
33	$EtOCH_2$	Cl	Et	1	> 50	0.08
47	$EtOCH_2$	Cl	H	0.5	25	0.14
48	$EtOCH_2$	Cl	CH_2-CH_2OH	10		0.2
49	$EtOCH_2$	Cl	CH_2-CHOH-CH_2OH	20	4.5	0.5
50	$EtOCH_2$	Cl	CH_2-O-COMe	1.6		1.0
51	$HOCH_2$	Cl	H	1.5	1.7	~1
52	$HOCH_2$	Cl	Et	30	5.7	~0.1

Since the extremely low solubility (<30 mg/L) of the compounds might influence the bioavailability, some compounds were synthesized with more hydrophilic substituents (Table V, Compounds 48, 49, 51 and 52). However, no improvement in activity was accomplished.

It may be concluded that, in spite of the role played by the lipophilic character of certain groups (e.g., Cl at R_{10}), the lipophilicity of the whole molecule (partition coefficient) does not seem to be an important factor in the level of activity that is achieved (compare compounds 33 and 47 with 52, and compound 1 with 33). Also, an extremely low solubility does not seem to have a negative effect on the activity in the rat.

PHARMACOLOGICAL PROFILE

Quincarbate (Compound 33, Figure 5) was selected for development as a possible drug for human therapy. The various existing diuretics can be divided into two classes: "high ceiling" diuretics, of which furosemide is a well-known representative, and "low ceiling" diuretics, a class to which most diuretics of the thiazide type belong. The difference can be demonstrated most clearly in saline-loaded rats using the Lipschitz method (2). Using this procedure, quincarbate was shown to be a "high ceiling" diuretic with an efficacy comparable to that of furosemide

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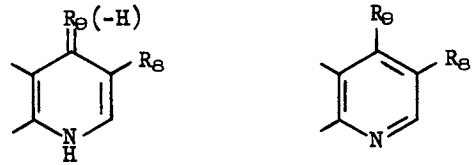

Figure 4. Tautomeric forms of the pyridine moiety

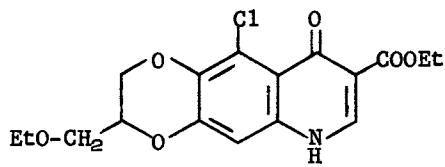

Figure 5. Structure of quincarbate

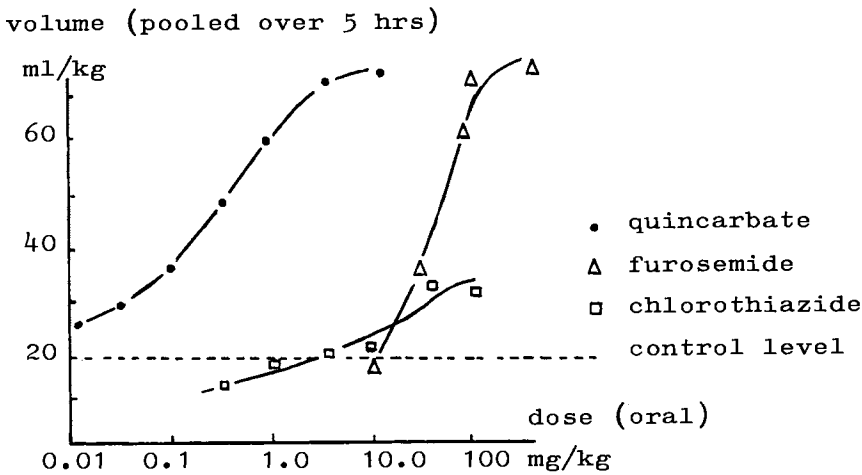

Figure 6. Dose–response relationships in male albino rats (strain Wistar-TNO, 160 ± 20 g), six animals per dose group

(Figure 6). Significant diuretic effects of quincarbate in rats are elicited at doses which are at least 200 times lower than the minimum dose required for furosemide and chlorothiazide. However, since the dose/response curves do not run parallel, a strict comparison of the potencies is not possible.

The electrolyte excretion caused by quincarbate, furosemide and chlorothiazide, respectively, parallels their diuretic response (Figure 7). Over the complete dose range, quincarbate gives a more favorable ratio of the Na^+/K^+ excretion when compared to furosemide, and it is even better when compared to chlorothiazide (Figure 8).

The dehydration of the animals caused by effective diuretics masks their effectiveness over longer time intervals. In order to prevent such an interference in the study of the duration of action, the rats were intermittently loaded with saline in a volume equal to the volume of urine excreted in the preceding period. From Figure 9, it is clear that quincarbate has a rapid onset of action, as does furosemide. However, the action continues over a period of seven hours, after which it gradually subsides, whereas furosemide has a much shorter duration of action.

When tested in rats not loaded with saline at a daily dose of 0.1 mg/kg for 14 days, the diuretic activity of quincarbate remained undiminished. No rebound effects were observed after discontinuing the administration of the drug. Also, in water-deprived rats, a diuretic effect was clearly seen.

Quincarbate shows a remarkable variation in its diuretic effectiveness in various species. In dogs, an ED_{200} value of 5 mg/kg orally was obtained. It is noteworthy that in this species the natriuretic response was more pronounced than the diuretic response (Figure 10). Surprisingly, in mice an antidiuretic effect was noted at low doses (1 mg/kg), whereas, at higher doses (46 and 100 mg/kg), some diuretic effect was apparent. In hamsters, quincarbate exhibited only marginal activity.

From both *in vitro* and *in vivo* studies, it can be concluded that quincarbate is not a carbonic anhydrase inhibitor. It exerted its diuretic effect even in drug-induced acidotic rats. The potency was unaffected in adrenalectomized rats; furthermore, no direct aldosterone antagonism was observed. In rats with artificially induced edema (partial hepatectomy), quincarbate proved to be highly effective.

Quincarbate was tested for antihypertensive activity in spontaneously hypertensive (SH) rats (3). The drug was administered orally, followed 20 hours later by a second dose; then 4 hours later, the blood pressure was measured by a cannula in the caudal artery. A slight activity was observed at 1 mg/kg, and a maximum effect was seen at 5 mg/kg. A prolonged action was indicated by noting that the original blood pressure was somewhat decreased even 20 hours after the first dose. With a dose of 5 mg/kg, the actual lowering (in mm Hg) was from 169.0 $\pm$ 9.6 to

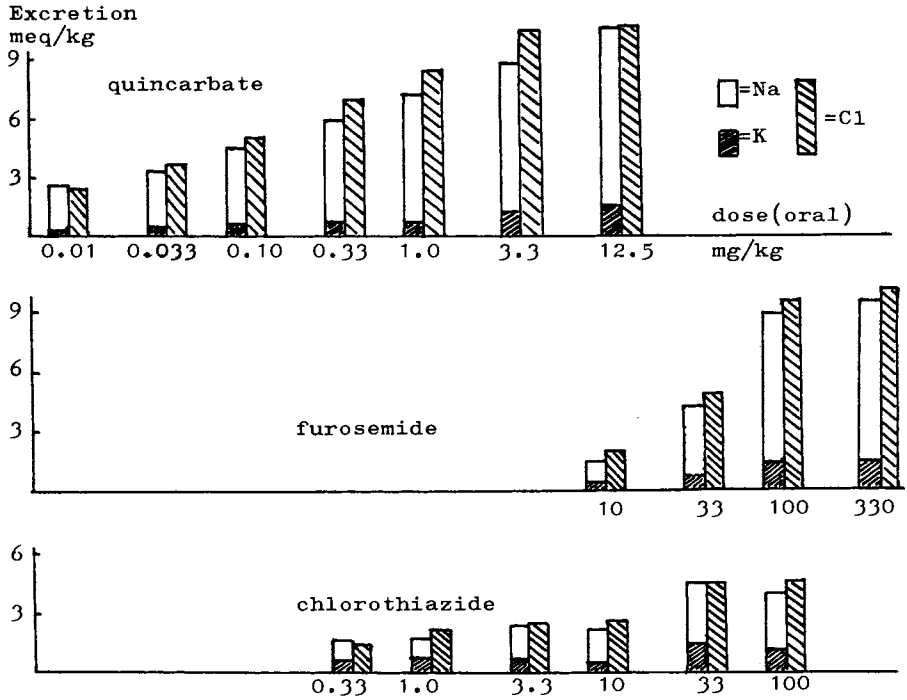

Figure 7. Dose–response of electrolyte excretion in saline-loaded rats. Six animals per dose group were used. Determinations of electrolytes were performed in pooled urine samples excreted in the five-hour period.

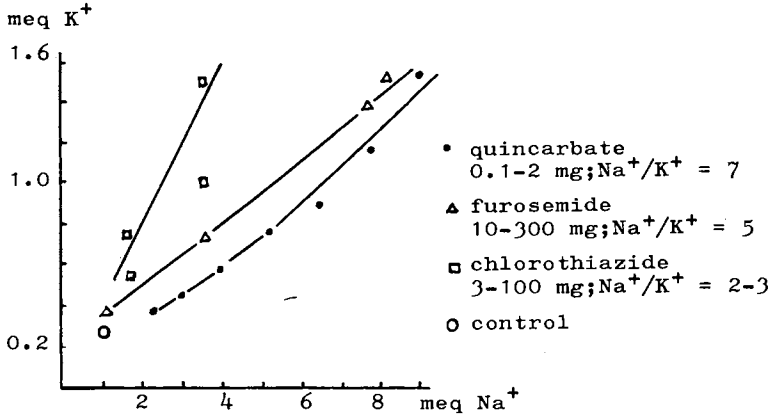

Figure 8. Ratios of Na+:K+ excretion in saline-loaded rats. (Further details in legend of Figure 7.)

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138.3 ± 7.3 at the fourth hour on the second day (3). In renal hypertensive rats, no conclusive effects were found with either quincarbate or furosemide. Following oral administration of quincarbate to rats at doses of up to 100 mg/kg, no toxic or neurotoxic phenomena were observed. Also, in chronic toxicity studies with rats and dogs, no serious side effects were observed at doses of 50 mg/kg. In rabbits and rats, quincarbate did not adversely affect pregnancy, nor did it show teratogenic potential.

METABOLISM

The metabolism of quincarbate was studied in rats, dogs, monkeys and humans by means of ^{14}C-labelled material. From a number of experiments in normal rats as well as in rats with cannulated or ligated bile duct, it can be deduced that the ratio of urinary to biliary excretion of metabolites of quincarbate is 2:1. The material that is excreted in the bile does not enter an enterohepatic circulation but is excreted in the feces.

Using the above-mentioned ratio, we calculated the amount of absorption on the basis of the urinary excretion of radioactive material. In all species, the degree of absorption was strongly dose dependent. In rats, the degree of absorption decreased from 45% at a dose of 1 mg/kg to 15% at the dose of 16 mg/kg. In dogs, the absorption percentage declined from 40% at the dose of 0.1 mg/kg to 2.25% at 50 mg/kg. In monkeys, at a dose of 1 mg/kg, the absorption was 10%. In humans, the degree of absorption declined from 60% at 0.015 mg/kg to 55% at 0.075 mg/kg and finally to 44% at 0.3 mg/kg. Hence, within the therapeutic range, about 50% of the dose will be absorbed. Urinary excretion in all species ended within 24 hours.

Plasma levels of radioactive material were extremely low at all times in rats, dogs and humans. Maximum levels tended to be reached within one to three hours. Neither unchanged quincarbate nor the corresponding acid, nor even conjugates, were found in the excretion products. In the urine of the rat and the monkey, seven metabolites could be identified. In dogs and man, only three of these were found. Of these three, the one which occurred in the major proportion by far proved to be compound 51 (Figure 11). Thus, both ether and ester hydrolysis must have occurred.

Since both the hydroxymethyl derivative of the acid, compound 51, as well as of the corresponding ester, compound 52, show high activity (Table V), these products may also play an important role in the observed diuretic effect.

Since one of the characteristics of quincarbate is its species dependent diuretic activity, a study was conducted to reveal whether or not this was a matter of metabolism. The diuretic activity of compound 52, the ester of the main metabolite, was studied in various species. In dogs, compound 52 showed an activity comparable to that of quincarbate, and, in mice, it was completely inactive at doses up to 10 mg/kg. Therefore,

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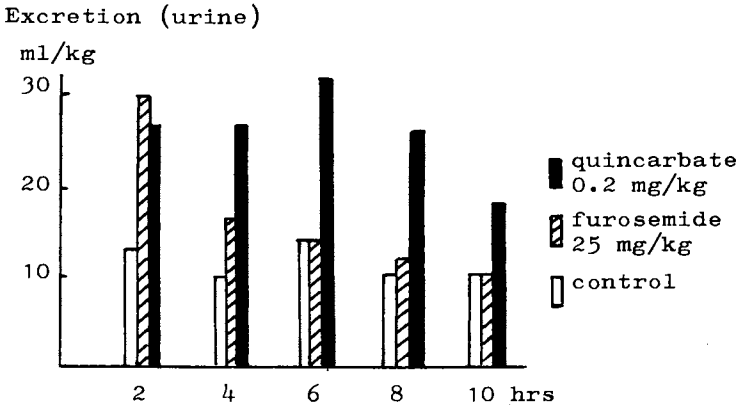

Figure 9. Duration of diuretic action in rats after single oral administration. Six animals per group were used. Rats were intermittently loaded with saline.

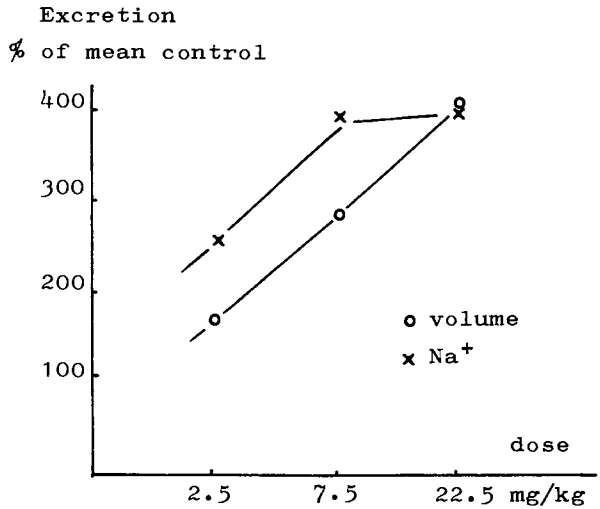

Figure 10. Urine and Na^+ excretion in beagle dogs after oral administration of quincarbate. Seven dogs per dose group were used. Excreted urine was pooled after seven hours.

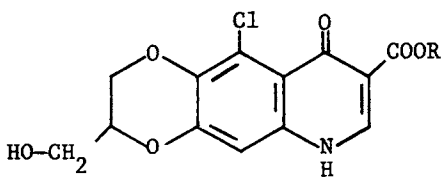

Figure 11. Main excretion product of quincarbate and its ester. 51: R = H. 52: R = Et.

it is unlikely that the species dependence is due to different degrees of formation of the active metabolites. It is possible that the degree of absorption of quincarbate in the various species determines, to a certain extent, the variation in diuretic potency.

HUMAN PHARMACOLOGY

The effect of quincarbate on the excretion of urine and of urinary electrolytes has been investigated in six small studies (4-8), including 34 healthy volunteers. In all these studies, quincarbate was given as a single oral dose, but several subjects ingested the drug in different dosages on subsequent test days. This resulted in a total of 84 single-dose administrations at different dose levels. The following preliminary conclusions may be drawn:

Potency. In the range of 5 to 40 mg, the dose/response curve of quincarbate is very flat. This might be partially explained by incomplete absorption at the higher doses in man. Quincarbate proved to be quite potent; thus, 10 to 20 mg given orally showed natriuretic effects comparable with those of 100 mg hydrochlorothiazide (4) (Table VI).

Table VI. Diuretic response in humans to quincarbate and hydrochlorothiazide (4) (double-blind study involving six healthy male volunteers between the ages of 22 and 32 years).

	Mean Cumulative Response					
	Diuresis (ml)		Natriuresis (meq)		Na^+/K^+ ratio	
	after 12 hrs	after 24 hrs	after 12 hrs	after 24 hrs	after 12 hrs	after 24 hrs
Placebo	695	1218	61	126	2.0	2.7
quincarbate						
5 mg	978	1598	145	239	3.4	4.0
10 mg	1162	1903	152	266	2.9	3.7
20 mg	1082	1998	170	309	3.4	4.1
40 mg	1315	2217	158	274	2.5	3.2
hydrochlorothiazide						
100 mg	1223	2110	183	310	4.2	4.9

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The oral activity of quincarbate was compared in four studies (4-6, 8) with that of furosemide. While 5 (or 20) mg of quincarbate proved to be more natriuretic than 20-25 mg of furosemide, 40 mg of furosemide sometimes showed a lesser and sometimes a greater effect than 20 mg of quincarbate. With 80 mg of furosemide, greater natriuresis and diuresis could be achieved than with quincarbate. Table VII gives the results of one of these studies.

Pattern of Activity. Quincarbate's diuretic activity starts within one hour after its oral ingestion and lasts for at least 8-9 hours, but (as can be inferred from Table VI) diuresis and natriuresis continue even during the 12-24 hour interval.

Table VII. Diuretic response in humans to quincarbate and furosemide (5) (double-blind placebo-controlled cross-over study involving four male and four female healthy volunteers between the ages of 22 and 61 years).

	Mean cumulative response after 8 hrs		
	Diuresis (ml)	Natriuresis (meq)	Na^+/K^+ ratio
Placebo	608	40.8	2.0
quincarbate			
5 mg	1232	137.3	4.6
10 mg	1262	143.9	3.5
20 mg	1204	142.4	3.9
furosemide			
20 mg	1214	117.2	3.3
40 mg	1772	180.8	4.2

While there is an increase in urinary K^+ excretion, it is less than the corresponding increase in urinary Na^+ excretion. This results in a favorable Na^+/K^+ ratio, comparable to that following ingestion of furosemide. Both the diuretic and natriuretic response are also well maintained in dehydrated subjects.

The main anion excreted concurrently with the increase in Na^+ and K^+ excretion is the chloride ion. There is no indication of any inhibition of tubular bicarbonate reabsorption. Neither the glomerular filtration rate (as measured by the rate of inulin clearance) nor the renal plasma flow (as measured by the p-aminohippuric acid clearance) are affected by quincarbate.

Side Effects and Tolerance. Up to the present time, quincarbate

has been tolerated extremely well; no clinical side effects of any importance have been observed. Occasionally, a slight decrease in serum Na^+ and/or K^+ was noted, as might be expected with any potent diuretic.

CINICAL STUDIES

Our clinical studies to assess quincarbate's efficacy and side effects are in their early stages. Only one (6) small double-blind cross-over study in hospitalized patients with peripheral cardiac edema has been completed. In this study, considerable improvement of clinical edema was apparent in all patients. In the cross-over study, an oral dose of 10 mg quincarbate was compared with 50 mg hydrochlorothiazide; the overall response to both drugs (improvement of edema, loss of weight, excretion of urine and Na^+, favorable Na^+/K^+ ratio, excretion of other electrolytes) was quite similar.

CONCLUSIONS

(1) Some dioxino[2,3-g]quinoline derivatives exhibit extremely potent diuretic activity with a high ceiling character in the rat.

(2) The structures of this group differ markedly from those of established diuretics.

(3) The structural variations permissible for diuretic activity are very limited.

(4) The most active compound, quincarbate (Figure 5), which at an oral dose of 0.08 mg/kg doubled the volume of the urine excreted by the rat, was studied extensively.

(5) Upon repeated administration of quincarbate to rats, no tolerance or rebound effects were noted.

(6) The diuretic activity of quincarbate was species dependent; it exhibited potent activity in rats and humans, moderate activity in dogs and monkeys and only marginal activity in mice and hamsters.

(7) The main metabolite of quincarbate (compound 51) also possessed diuretic activity.

(8) In chronic toxicity studies in rats and dogs, no notable toxic effects were observed at oral doses up to 50 mg/kg.

(9) In healthy humans, an oral dose of 5 mg of quincarbate showed a marked diuretic and natriuretic effect; and yet, even at the highest dose used (20 mg), no side effects of any importance were noted.

(10) In patients with peripheral cardiac edema, a considerable improvement of the edema was apparent with low doses (10 mg orally). However, further studies will be required before quincarbate can be assigned its place among future therapeutics.

Acknowledgments - We acknowledge the efforts of all our colleagues in the research laboratories of Philips-Duphar who have

involved in the studies which we have described. We mention especially K. S. Liem, M.D., who coordinated the studies in man, Dr. J. B. v.d. Schoot, who carried out the metabolic studies, and Mr. J. Tipker, who provided the valuable QSAR calculations.

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Etozolin: A Novel Diuretic

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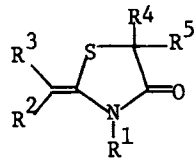
Introduction - Investigations to date seem to indicate that choleresis is, to some extent, part of the extrarenal effect of diuretic substances. However, more detailed studies, particularly with furosemide and ethacrynic acid, have proved that biliary osmolality is not affected hereby (1-4). The question as to whether choleretics, which do not influence the biliary electrolyte concentration, would make suitable models in the search for novel diuretic substances has never been investigated. We have recently described (5) some highly potent diuretic and well-tolerated compounds which were discovered in the course of investigating a new class of heterocycles (Structure 1), many of which possess choleretic properties.



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One of these compounds, ethyl Z-[3-methyl-4-oxo-5-(1-piperidinyl)-2-thiazolidinylidene]acetate (Compound 3, etozolin), has been introduced as a therapeutic agent on the German market under the trademark Elkapin®. Table I summarizes the current synthetic work in the field of 2-acylmethylene-4-thiazolidinones. Table II shows the substitution pattern of the compounds of type 1 which are active diuretics. As a consequence of these findings, six other series of heterocycles were synthesized and tested (Table III). None of the representatives of these heterocycles exhibited diuretic activity, even when the successful substitution pattern shown in Table II was employed (as far as this was feasible and led to stable compounds).

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TABLE I

Synthesized and Tested Derivatives of 1

Substituent	Groups	Notes
R^1	H, alkyl, aralkyl, CH_2X, $(CH_2)_2X$	X = functional groups such as -OR, $-NR_2$, acyl (R = lower alkyl)
R^2	H, alkyl, aryl, aralkyl, CH_2X, $(CH_2)_2X$; $NHCOCH_3$; CO_2R	only for $R^3 = CH_3$
R^3	CH_3 $-CO_2H$, $-CO_2R$, R-CO-, ArCO-, R-SO_2-, ArSO_2-, >NCO-, -HNCO-, R_2NCO-, -CN; pyridyl, benzimidazolyl, indolyl	only for $R^2 = CO_2R$ AR = aryl, heteroaryl
R^4	H, alkyl, aryl $R^4/R^5 = >CR^2$	
R^5	-N<, -N(-)-N<	aliph., arom., aliph.-arom., heteroaliph. and arom. amines and hydrazines

In Diuretic Agents; Cragoe, E.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1978.

TABLE II

Derivatives of 1 and Their Diuretic Activity Relative to Etozolin (3)

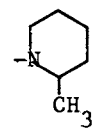
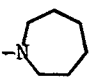
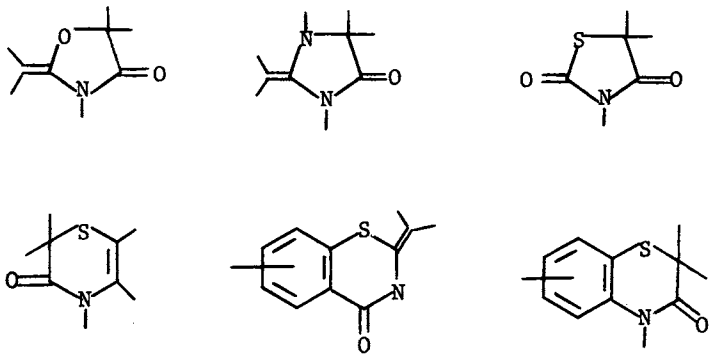
Compound No.	R^1	R^2	R^3	R^4	R^5	Diuretic Activity (Relative to 3)
2	H	H	$CO_2C_2H_5$		H	0.3
3	CH_3	H	$CO_2C_2H_5$		H	1.0
4	CH_3	H	CO_2CH_3		H	1.1
5	CH_3	H	CO_2H		H	1.3
6	CH_3	H	CO_2H		H	0.6
7	CH_3	H	$CO_2C_2H_5$		CH_3	0.1
8	CH_3	CH_3	$CO_2C_2H_5$		H	0.7
9	CH_3	$CO_2C_2H_5$	CH_3		H	0.6
10	CH_3	H	$CO_2C_2H_5$		H	0.6
11	CH_3	H	ϕSO_2		H	0.5
12	CH_3	H	CH_3SO_2		H	0.3

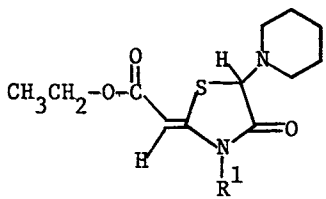
TABLE III

Schematic Representation of Heterocycles Structurally Related to 1



SAR of 2-Acylmethylene-4-Thiazolidinones - Changes in the substituent on the ring nitrogen (R^1) of compound 1 markedly affect diuretic activity. Even the change of R^1 = CH_3 to C_2H_5 leads to an abrupt loss of activity. Almost the same can be said of R^2 because activity is maintained only when R^2 = H or CH_3. Stereochemistry does not play an important role here since both the Z- and the E-isomer are active. Maximal activity is seen when R^3 is COOH or COOEt; the only other substituents allowed are methyl and phenylsulfonyl, and they produce less active compounds. Optimal activity is clearly achieved when R^4 is 1-piperidyl. Any enlargement or reduction of the ring size or any bridging or substitution reduces the activity and 2,6-disubstitution eliminates the activity. Introduction of a second substituent at C-5 (i.e., where R^5 is not H) does not lead to active compounds.

A particularly fascinating aspect is the homology of the most potent diuretic and choleretic substances, 3 and 13.



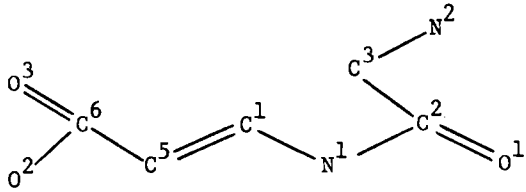
	R^1	
3	CH_3	etozolin
13	C_2H_5	piprozolin

However, as is illustrated below, compound 3 possesses only a very weak choleretic (side) effect and compound 13 has no diuretic effect. We investigated this phenomenon using spectroscopic, physical chemical and X-ray measurements. The spectroscopic and thermodynamic findings have been published (5), and some of the results of the X-ray studies will be discussed subsequently.

Comparison of 2-Acylmethylene-4-Thiazolidinones with Other Classes of Diuretics - As it will be discussed in detail below, the 2-acylmethylene-4-thiazolidinones represent a new class of high-ceiling diuretics to be added to the well known sulfamyl benzoic acids and acylphenoxyacetic acids. They all seem to have a similar effect on renal electrolyte transport. An examination of etozolin, furosemide and ethacrynic acid, each a representative of one of the three diuretic classes, reveals that they all have (a) the same main site of action (early distal tubular site) and (b) presumably, the same mechanism of action (inhibition of active chloride transport) (6-9).

At the present time, little is known about the mechanism of action of these diuretics; there are some indications that it involves inhibition of a renal adenyl cyclase (10). In order to develop working hypotheses on which to base an economical search

for new diuretics, it was of interest to investigate whether these chemically different substances had some common structural and/or electronic features. Such an approach seemed reasonable considering the molecular rigidity of these classes of compounds. Figure 1 shows on the left the three classes of high-ceiling diuretics: acylmethylene-4-thiazolidinones (14), sulfamoylbenzoic acids (15) and acylphenoxyacetic acids (16). The structural features thought to be important for activity are delineated in the figure (11). On the right, the electronic effects of these functional groups are indicated by the arrows; the length and direction of the arrows reflect their relative electron donor and acceptor properties. A striking feature is the general pattern that appears whose dominant characteristics are two groups with opposing electronic effects attached meta- or para- to each other and linked by a ring system suitable for the transmission of their electronic effects. The distances between comparable charge centers (compound 3, 5-N/O-C=O; furosemide, N_{ar}/S; ethacrynic acid, 1-O/=C-C=O) are in the range of 5.5-6.0 A. These distances were determined for compound 3, from X-ray data, see below; furosemide, calculated from the data of Price (12) and Dupont (13), ethacrynic acid data from Dreiding models. X-ray analysis of compound 3 (detailed data on comparative X-ray analysis of the acylmethylene-4-thiazolidinones will be published in a subsequent paper) furnishes interesting information (Figure 2). The heterocycle, including the structural fragment shown below, is virtually planar. The symmetry axis N/C-11 of the piperidine ring is approximately equatorial



to this plane, and the averaged plane of the piperidine chair is roughly vertical to it. The interatomic distances (Table IV) substantiate the inclusion of the ring sulfur S^1 in the electron distribution of the whole planar system. In accordance with its electronic situation and in contrast to the enone double bond of the ethacrynic acid (19), the exocyclic double bond is not nucleophilic; compound 3 does not react with sulfhydryl groups. This fact does not contradict our argumentation because the diuretic potency of the acylphenoxyacetic acids do not run parallel to the nucleophilic character of this bond (20).

Analysis of the X-ray data furnishing some explanations for the unusual SARs of the acylmethylene-4-thiazolidinones is as

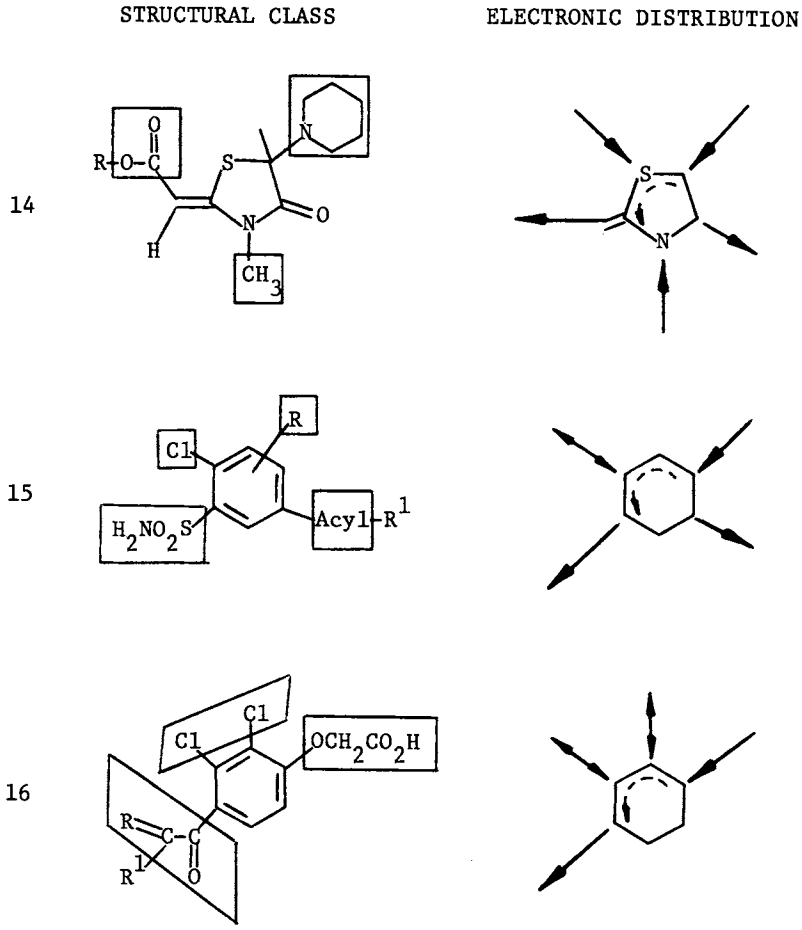


Figure 1. The three classes of high-ceiling diuretics with the moieties responsible for biological activity and their effects on electron distribution

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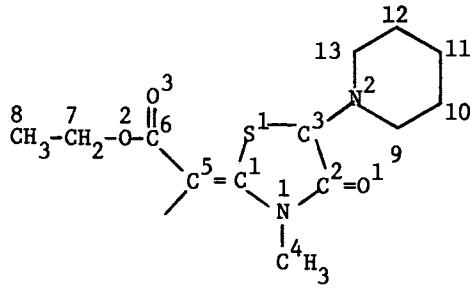

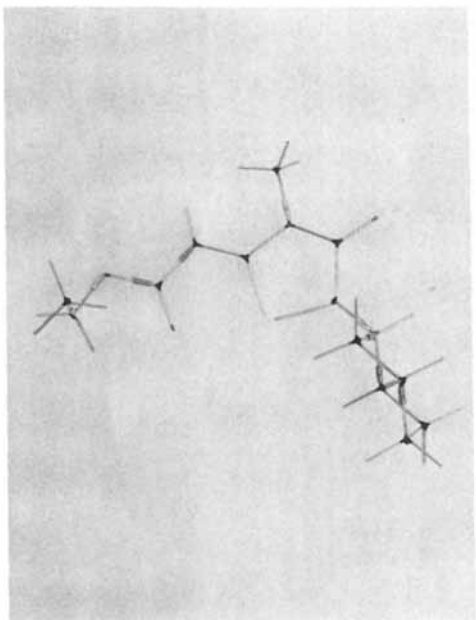

Figure 2. Dreiding Model of Compound 3 constructed on the basis of x-ray data. Viewed along the Z-axis (N_1: (0,0,0)). (The Dreiding model simulates planarity for the entire hetero ring not considering the deviation of S' by about 5°; thus, erroneously, 3-H is nearly eclipsed by C3-N2.)

TABLE IV

Characteristic Interatomic Distances and Bond Angles of Compound 3 and their Anticipated Values

Etozolin (3)	(Å)	Comparable Standard Values	(Å)	Ref.
N2-C3	1.43	1.47 (aliphatic)		14
C3-S1	1.89	1.82 (aliphatic)		14
S1-C1	1.74	1.82 (aliphatic) 1.72 (thiophene)		14 15
C1-C5	1.36	1.30 (acrylic acid)		16
C5-C6	1.41	1.47 (acrylic acid)		16
C6-O3	1.24	1.26 (acrylic acid)		16
C6-O2	1.34	1.28 (acrylic acid)		16
<C1-S1-C3	92°	91.9° (thiophene 100-105° (aliphatic) 105.5° (thiazolidine)		15 17 18

follows: 1) the exchange of the ring sulfur S^1 for other heteroatoms or a change in its oxidation state should affect or reduce its pseudo-aromatic state, 2) any increase in the size of the substituent on the ring nitrogen beyond CH_3 should alter the electron distribution in the molecule due to disturbance of planarity or of hyperconjugation; the same applies to the steric bulk and the nature of the acylmethylene group and 3) interaction between the piperidino nitrogen N^2 and the heterocyclic ring is dependent on the former's unhindered spatial flexibility. Other influences are probably attributable to the effects of the 5-amino group on transport (5).

PHARMACOLOGY

Acute Toxicity - The acute toxicity of etozolin was studied in male mice (NMRI) and rats (SIV 50). The calculation of the LD_{50} values was based on the probit analysis according to Weber (21). The results are shown in Table V.

TABLE V

Acute Toxicity of Etozolin in Mice and Rats Following i.g. and i.p. Administration

Species	Sex	Route of Administration	LD_{50} (mg/kg)	Confidence Limits p = 0.05 (mg/kg) lower	upper
Mouse	male	i.g.	8,670	7,310	10,270
Mouse	female	i.g.	9,360	7,290	12,030
Mouse	male	i.p.	1,210	1,093	1,340
Rat	male	i.g.	11,040	9,380	13,000
Rat	female	i.g.	10,250	9,260	11,350
Rat	male	i.p.	1,575	1,355	1,830

As the values shown in Table V indicate, the acute i.g. and i.p. toxicity of etozolin is extremely low. The LD_{50} values did not show any appreciable difference in male or female animals. The toxicity of the compound is slightly less in rats than in mice. The low p.o. toxicity of the drug was also confirmed in a pilot dose range-finding study in dogs. In this species, the first evidence of toxicity occurred at 1,600 mg/kg; at 3,200 mg/kg, the lower lethal dose range was reached.

Diuretic Activity - Clearance Tests in Dogs: These studies served to reveal the diuretic effect of etozolin as the hydrochloride salt in female dogs weighing between 10 and 29 kg (22).

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The doses administered i.v. ranged from 6 to 100 mg/kg. The animals were anesthetized by means of an i.v. injection of 30 mg/kg pentobarbital sodium (Nembutal®). In order to fill the extracellular space with a sufficient fluid, 25 ml/kg isotonic saline solution was infused i.v. within one hour followed by continuous i.v. administration of fluid at a rate of 2 ml/min to which enough creatinine and PAH were added to achieve and maintain constant plasma levels of about 20 mg % and 1.5 mg %, respectively. After a one-hour period of equilibration, the urine was collected during 10 to 20 min periods by means of a catheter. In the middle of each period, blood was withdrawn from the femoral artery. Na^+, K^+ and Cl^- as well as creatinine and PAH were measured in blood and urine. Furthermore, the urinary output and the urine pH were recorded. The first diuretic effects were noted at about 20 mg/kg and reached almost maximal values at 50 mg/kg. Table VI shows a typical result after i.v. administration of 50 mg/kg. The diuretic effect of etozolin reached its peak within 1 hour after i.v. injection. As in the other studies, there was a slight decrease in creatinine clearance, which is used as a measure of GFR in dogs. PAH clearance rates dropped to one-third or even less in each trial and, thus, approached the creatinine clearance values. This signified that the drug interfered with PAH secretion in the proximal tubule, so that PAH clearance could not be used to measure renal plasma flow following administration of this agent. The maximum tubular transport capacity for PAH (Tm_{PAH}) was determined in four separate studies. After i.v. injection of 50 mg/kg of etozolin, the mean Tm_{PAH} value fell from 7.28 to 1.76 mg/min. Both the urinary electrolyte excretion and total urinary output increased by multiples of their original values following administration of 50 mg/kg of etozolin and chloride excretion consistently exceeded that of sodium. The most reliable indicator of the potency of a diuretic, independent of body weight, is its percentage of inhibition of electrolyte reabsorption. In the example illustrated in Table VI, sodium reabsorption fell to 83% and chloride reabsorption dropped to 80%. Tubular potassium reabsorption also decreased, although no excretion levels were measured which were more than twice the baseline values. Urine pH was lower in all the tests than in the control periods, indicating an increase in hydrogen ion secretion.

The next studies were concerned with experimental changes in pH. Alkalosis was induced by i.v. infusion of an $NaHCO_3$ solution and acidosis by infusion of 0.1 N HCl. The action of etozolin was severely inhibited by metabolic alkalosis but was essentially unaffected by acidosis.

In a series of 18 tests involving 8 female dogs weighing 15 to 30 kg, a study was conducted to determine whether etozolin potentiates the effect of chlorothiazide and/or chlormerodrin, or conversely, whether peak chlorothiazide or chlormerodrin .

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TABLE VI

Effect of Etozolin (50 mg/kg) i.v. on Renal Function of a Female Dog of 17 kg

Time	Urine flow	GFR (C_{creat})	C_{PAH}	Urinary Excretion			Fractional Reabsorption			pH
				Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	
min	ml/min			micro Eq/min			%			Urine
-20-10	3.0	68	269	435	72	420	95.6	67.1	95.6	7.30
-10- 0	2.1	59	221	292	65	285	96.5	67.0	96.5	7.27
0	50 mg/kg Etozolin									
10-30	5.4	48	75	810	108	853	88.1	32.1	87.3	6.71
30-50	8.0	45	69	1088	136	1233	83.0	3.5	80.4	6.70
50-70	7.6	52	64	981	129	1102	86.9	20.4	85.0	6.82
70-90	6.5	40	57	793	117	929	86.5	7.1	83.0	6.87
90-110	5.8	46	55	684	105	794	89.6	26.6	87.7	6.73
110-130	4.7	42	52	554	94	653	89.3	36.5	89.2	6.67
130-150	4.7	36	54	550	89	625	89.5	23.9	87.4	6.59

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diuresis can be enhanced by this agent (23). Etozolin was found to have an additive effect on the maximum Na^+, Cl^- and K^+ excretion and urinary output produced by chlorothiazide and by chlormerodrin. Likewise, chlorothiazide and chlormerodrin enhanced the diuresis produced by etozolin. With the test methods used, it was not possible to establish whether these results were due to the three diuretics having different mechanisms of action or different sites of action in the nephron.

Micropuncture Studies in Rats - The object of performing diuretic studies in rats was to investigate the effect of etozolin in another species (22,24). First, dose-response curves were generated and then attempts were made to locate the site of action in the nephron using micropuncture techniques (25). Figure 3 shows the effect of i.g. administration of the drug in comparison to furosemide on the four-hour urinary output and Na^+, Cl^- and K^+ elimination, calculated as output per kg of body weight. Etozolin and furosemide were intubated orally as 1% tragacanth suspensions which were diluted with 0.2% saline solution. The controls received the same volume of tragacanth but without the diuretic. All the animals were then administered 40 ml/kg of a 0.2% saline solution i.g., so that the total volume of fluid intubated equaled 5% of the body weight. The test groups consisted of 8 to 12 animals which were kept in separate diuresis cages.

Both etozolin and furosemide had dose-related diuretic effects, furosemide being obviously the more potent of the two. Each compound produced a greater increase in chloride excretion than in sodium excretion. This tendency seemed to be somewhat more pronounced for etozolin than for furosemide.

Micropuncture Data - On the basis of the dose-response curves, an i.v. dose of 50 mg/kg of etozolin was selected for the micropuncture studies. Male anesthetized Sprague-Dawley rats were used. The animals were prepared for micropuncture in the usual way (25). Two to three late proximal and early distal punctures were made, and the drug was injected i.v. After an interval of 30 min, two to three more late proximal and early distal tubular segments were micropunctured. The tubular fluid was collected quantitatively and the inulin, sodium and potassium concentrations were determined. Table VII shows the micropuncture data for the late proximal nephron segments. Transit time was found to be extended after the administration of etozolin. None of the other parameters measured in the proximal convoluted tubule was significantly affected. Table VIII contains the micropuncture data of the early distal nephron segments.

Etozolin caused a pronounced increase in the flow rate of tubular fluid in the early distal tubular segments, a decrease in the TF/P-inulin ratio, an increase in sodium and potassium concentration and a rise in the TF/P-sodium and TF/P-potassium ratios. Fluid and electrolyte reabsorption in the loop of Henle

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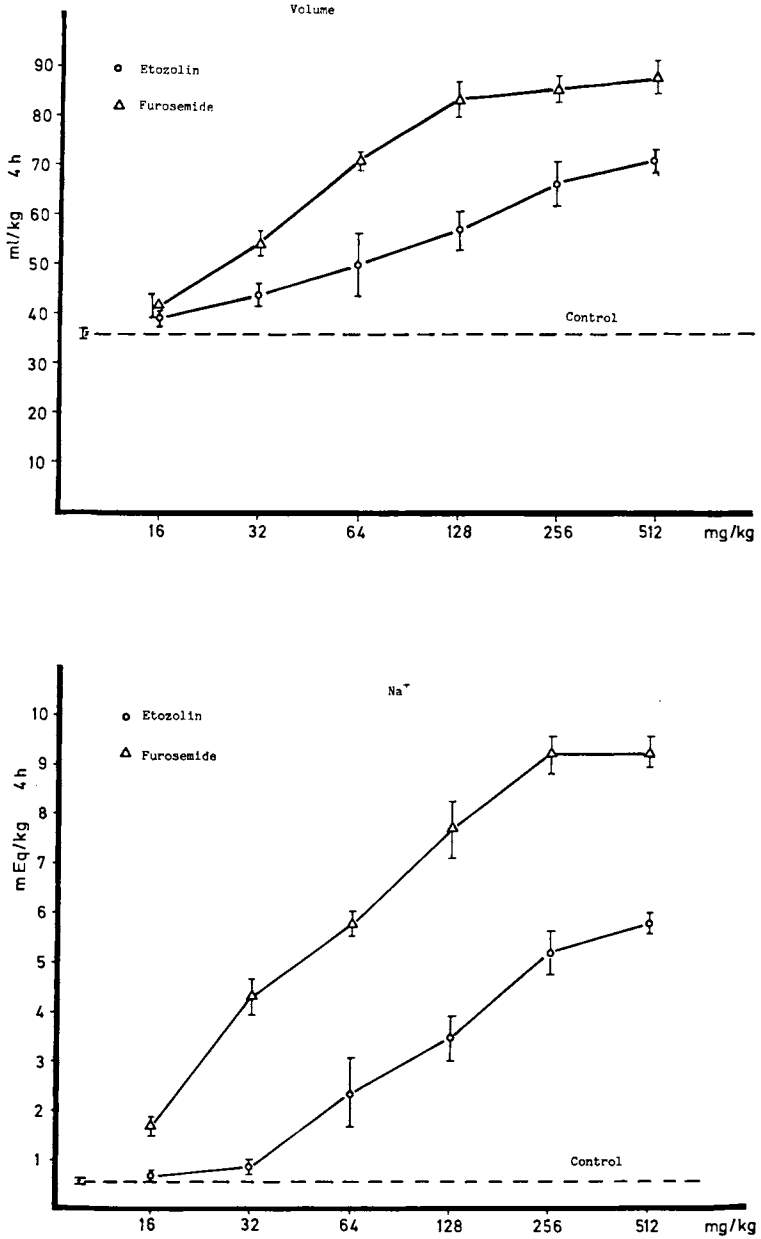

Figure 3. Comparison of the dose–response curves of etozolin and furosemide after i.g. administration to rats. Urinary output and Na^+ (top), Cl^- and K^+ (right) excretion over a four-hour period are shown ($\bar{x} \pm s_{\bar{x}}$, n = 8). The dotted lines represent the control values ($\bar{x} \pm s_{\bar{x}}$, n = 32).

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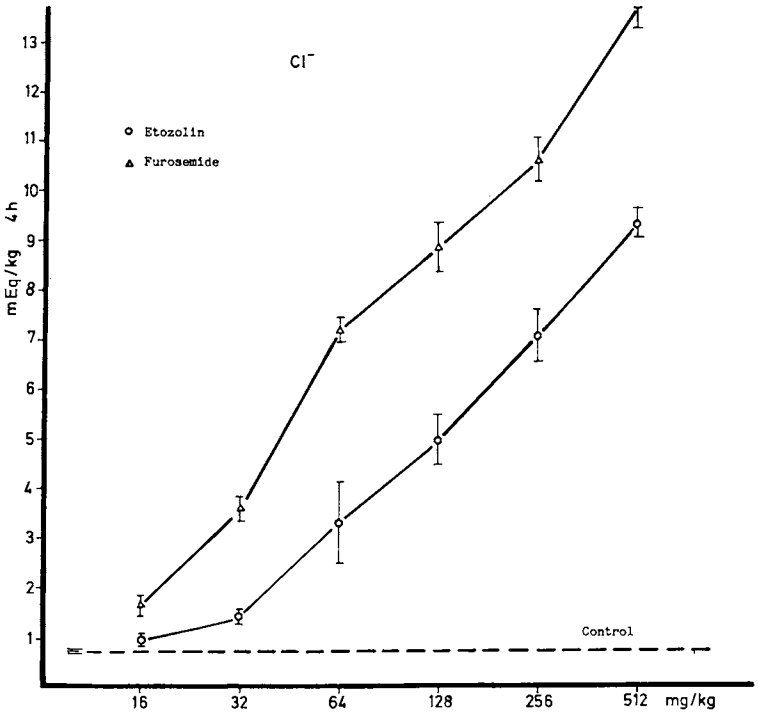

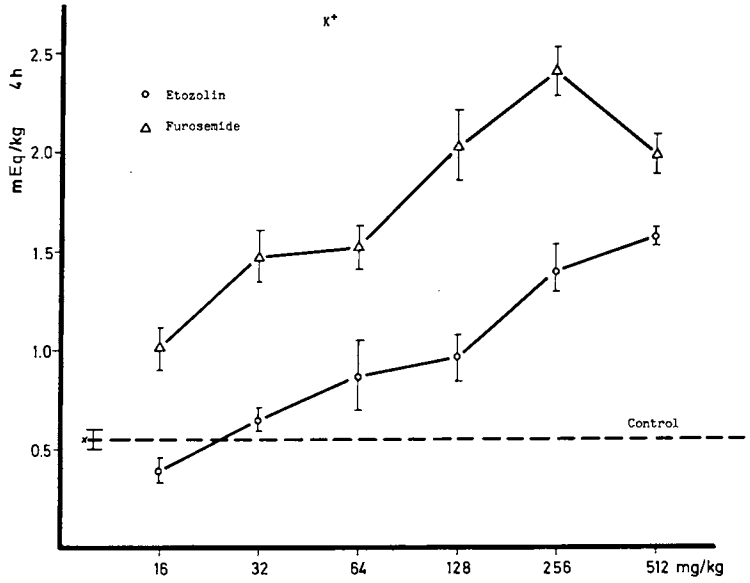

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TABLE VII

Summary of the micropuncture data obtained by measurement at the end of the proximal convoluted tubules of superficial nephrons. Transit time is the time from the diffuse coloration of the kidney surface after lissamine green injection until the dye columns converge into the terminal portions of the proximal convolution.

	Control	Etozolin 50 mg/kg i.v. stat; then, 50 mg/kg/h i.v.
Transit Time (sec)	7.53 ± 0.37 n = 6/12	10.8 ± 0.86* n = 6/11
Nephron Filtration Rate (nl/min)	27.9 ± 3.6 n = 7/11	22.3 ± 2.7 n = 6/10
Flow Rate (nl/min)	10.3 ± 0.7 n = 8/12	9.6 ± 0.9 n = 7/12
$\frac{TF}{P}$ inulin	2.68 ± 0.27 n = 7/12	2.49 ± 0.37 n = 6/10
$\frac{TF}{P}$ Na^+	1.02 ± 0.02 n = 7/12	0.99 ± 0.02 n = 8/11
$\frac{TF}{P}$ K^+	1.04 ± 0.05 n = 7/12	0.97 ± 0.04 n = 8/11

Mean ± S.E.M.; n = number of animals/number of measurements; $\frac{TF}{P}$ Inulin = tubular fluid to plasma inulin ratio; $\frac{TF}{P}$ Na^+ = tubular fluid to plasma sodium ratio; $\frac{TF}{P}$ K^+ = tubular fluid to plasma potassium ratio; nl = nanoliters; *p <0.01.

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TABLE VIII

Summary of the Micropuncture Data Obtained by Measurement at the Early Distal Tubular Site

	Control	Etozolin 50 mg/kg i.v., stat; then, 50 mg/kg/h i.v.
Nephron Filtration Rate (nl/min)	28.0 ± 2.9 n = 6/11	25.2 ± 4.4 n = 5/9
Flow Rate (nl/min)	6.0 ± 0.9 n = 6/11	8.9 ± 1.0* n = 5/9
$\frac{TF}{P}$ Inulin	4.98 ± 0.73 n = 6/11	2.72 ± 0.30* n = 5/9
Early Distal Sodium Concentration (mEq/L)	42.4 ± 3.5 n = 6/11	78.5 ± 8.5** n = 5/9
Early Distal Potassium Concentration (mEq/L)	1.12 ± 0.09 n = 6/11	2.52 ± 0.41** n = 5/9
$\frac{TF}{P}$ Na^+	0.274 ± 0.037 n = 6/11	0.543 ± 0.048** n = 5/9
$\frac{TF}{P}$ K^+	0.270 ± 0.031 n = 6/11	0.615 ± 0.074** n = 5/9

Mean ± S.E.M.; n = number of animals/number of measurements; $\frac{TF}{P}$ Inulin = tubular fluid to plasma inulin ratio; $\frac{TF}{P}$ Na^+ = tubular fluid to plasma sodium ratio; $\frac{TF}{P}$ K^+ = tubular fluid to plasma potassium ratio; *p <0.01; nl = nanoliters; **p <0.001.

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can be calculated from the late proximal and early distal data. Under the influence of the drug, fluid reabsorption in this nephron segment fell from 41.7 to 7.3%, sodium reabsorption from 82.9 to 48.7% and potassium reabsorption from 83.3 to 36%. It can be concluded from these findings that diuretic and natriuretic action of etozolin derives from the substance's inhibition of fluid and electrolyte reabsorption in the loop of Henle.

The major metabolite of etozolin, (Z)-[3-methyl-4-oxo-5-(1-piperidinyl)-2-thiazolidinylidene]acetic acid, was also subjected to detailed pharmacological investigation. It possesses potent diuretic properties and its site of action is also located in the ascending limb of Henle's loop. All the results suggest that this metabolite plays a major part in the biological activity observed for etozolin.

General Pharmacology - Extensive pharmacological screening did not reveal any results which were not attributable to the diuretic action of etozolin. The chemical relationship of this substance to known active choleretic compounds prompted us to undertake studies to compare etozolin and piprozolin (Compound 13) in rats. Piprozolin was also evaluated for diuretic activity in rats. Conscious animals weighing 160 to 200 g were employed. They were administered the test substance together with 5 ml/100 g body weight of a 0.2% saline solution and the urine was collected over a 4 hour period. Rats weighing 200 to 250 g, anesthetized with pentobarbital were used for the choleresis tests. The effluent bile was measured for 120 minutes with a drop counter. The results of these studies are presented in Figures 4 and 5.

Despite the close chemical relationship of the two compounds, etozolin showed only a slight choleretic action compared to piprozolin. While 100 mg/kg of piprozolin caused a rapid increase in bile secretion lasting over 90 minutes, only a slight, slow increase in bile secretion was observed after etozolin. Even 250 mg/kg of etozolin produced a marginal increase which subsided after 60 minutes, while 200 mg/kg of piprozolin had a stronger dose-related effect than did 100 mg/kg. While 50, 100 and 200 mg/kg of etozolin administered intragastrically caused a dose-related increase in urine elimination, piprozolin showed no significant effect at the same doses.

An important factor in long-term treatment with diuretics is their effect on glucose metabolism. Therefore, corresponding glucose tolerance tests were conducted in connection with the long-term experiments in rats and dogs (26). In rats, the dosages were 50, 250 and 2,000 mg/kg/day; the duration of the experiment was 18 months. Oral doses of 30, 120 and 480 mg/kg/-day were administered to dogs for 12 months. A glucose tolerance test was conducted in dogs after the 1st, 4th, 8th, 12th, 26th, 39th and 52nd week and in rats after the 26th and 78th week. Two g of glucose/kg of body weight were administered as a

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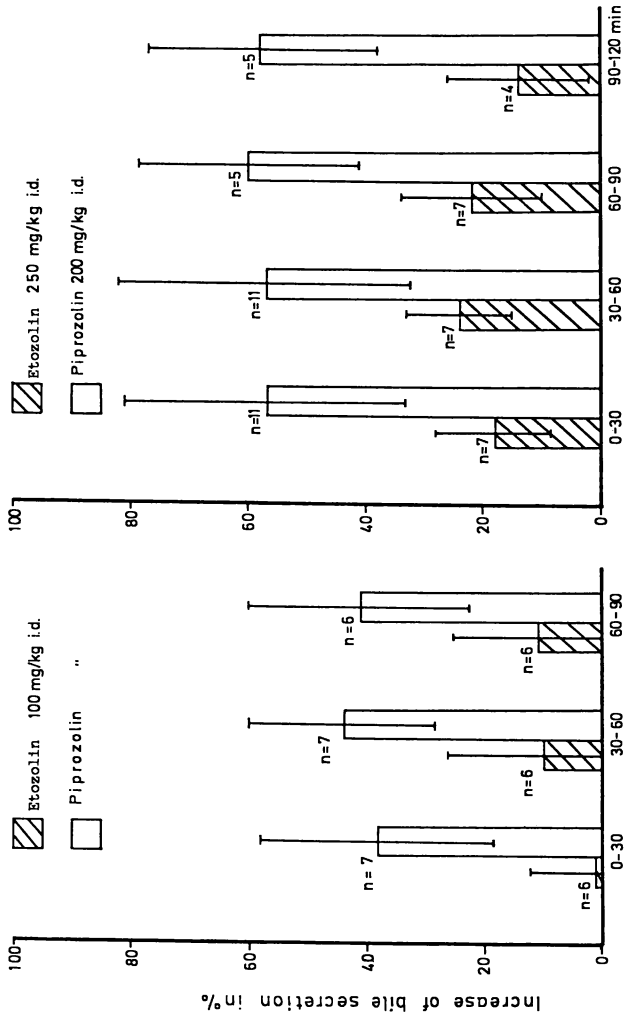

Figure 4. Effect of etozolin (100 and 250 mg/kg) and piprozolin (100 and 200 mg/kg) on bile secretion in anesthetized rats following i.d. administration

20% solution via a stomach tube. Following an 18 hour food abstinence period, serum glucose was measured before administration and then 1 hour and 4 hours after administration of the drug. There were no differences between the animals treated and the controls at any time during the experiment. Thus, the possibility of glucose intolerance in rats and dogs can be excluded even when high doses of etozolin are given over long periods of time.

Antihypertensive Effects in Rats - The antihypertensive properties of etozolin were tested in conscious rats with hypertension of different origin: (a) induced by desoxycorticosterone acetate (DOCA), (b) hereditary and (c) renovascular hypertension caused by experimental impairment of the renal circulation. Blood pressure was measured non-invasively at the base of the tail either via a condenser microphone or plethysmographically. Etozolin was administered intragastrically suspended in 1% tragacanth via a stomach tube once a day. The results of these tests are shown in Figures 6, 7 and 8. As we had expected, the most pronounced effects were observed in the DOCA-treated rats, since the impaired sodium balance was restored to its normal level and the volume of extracellular fluid was reduced as a result of the diuretic action of etozolin. The blood pressure was also significantly lowered in rats with renovascular hypertension and in animals with spontaneous hypertension. Since the three types of hypertension are caused by different mechanisms, there is reason to believe that the antihypertensive effect of etozolin derives not only from a reduction of the plasma volume, but also from peripheral mechanisms, possibly of the same kind as are postulated for the thiazides.

METABOLISM AND PHARMACOKINETICS

The fate of etozolin in the organism was investigated using ^{14}C-etozolin labeled in the 2-position of the thiazolidine ring (27). The absorption of ^{14}C-etozolin was $\geq$ 90% in man, $\geq$ 80% in rats and to a lesser degree in dogs. In all species, maximum ^{14}C blood levels were observed 2 to 3 hours after oral administration. After 24 hours, the concentration of radioactive material in the blood had dropped to approximately 8% of the maximum values in rats and dogs and to approximately 15% in man. In rats, the blood levels can be described by a one-compartment body model, the absorption half-life being approximately 0.6 hour, and the elimination half-life being approximately 6 hours. During absorption, etozolin underwent a distinct first-pass effect. In all species, the majority of the plasma radioactivity corresponded to metabolite I (see Figure 9), which also has diuretic activity. Unchanged drug appeared in plasma in considerably lower concentrations. In rats, more than 90% of plasma radioactivity could be ascribed to metabolite I. In man,

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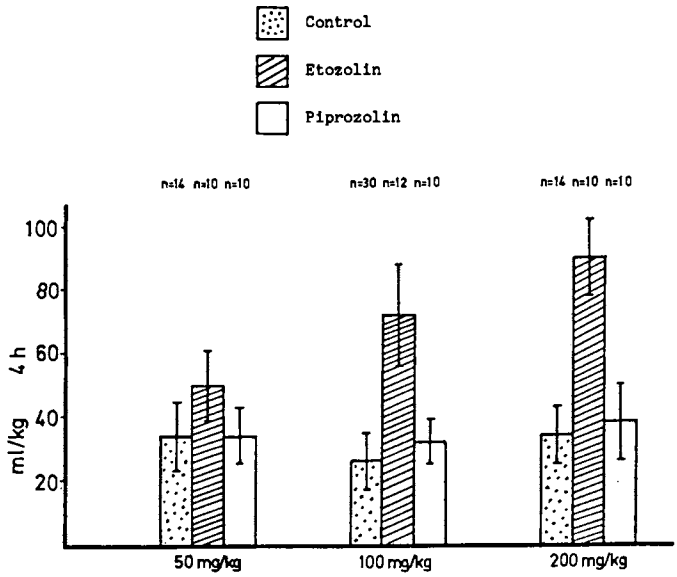

Figure 5. Effect of increasing doses of etozolin and piprozolin on urine excretion in conscious rats

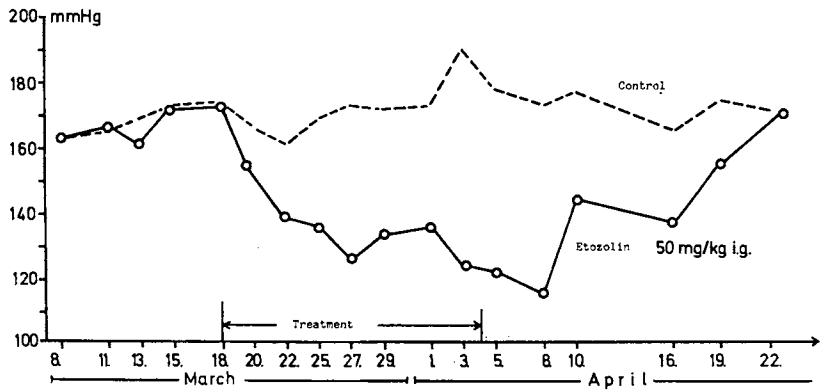

Figure 6. Effect of 50 mg/kg etozolin i.g. on DOCA-induced hypertension in rats: 19 drug treated animals; 10 control animals

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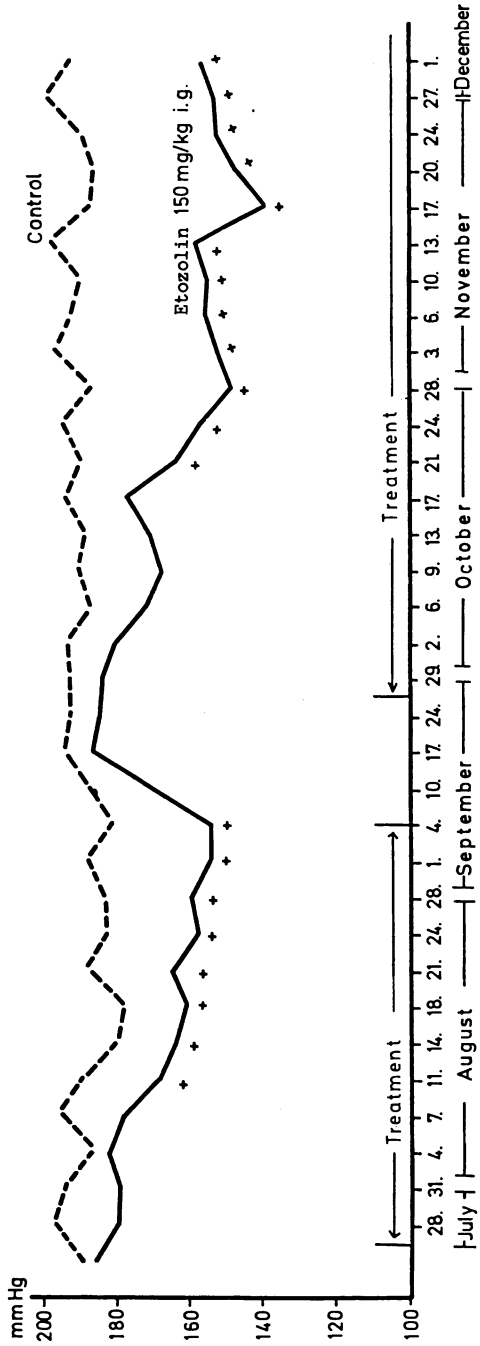

Figure 7. Effect of repeated i.g. treatment with 150 mg/kg of etozolin on the blood pressure of rats with spontaneous hypertension, $+ = p \geq 0.05$

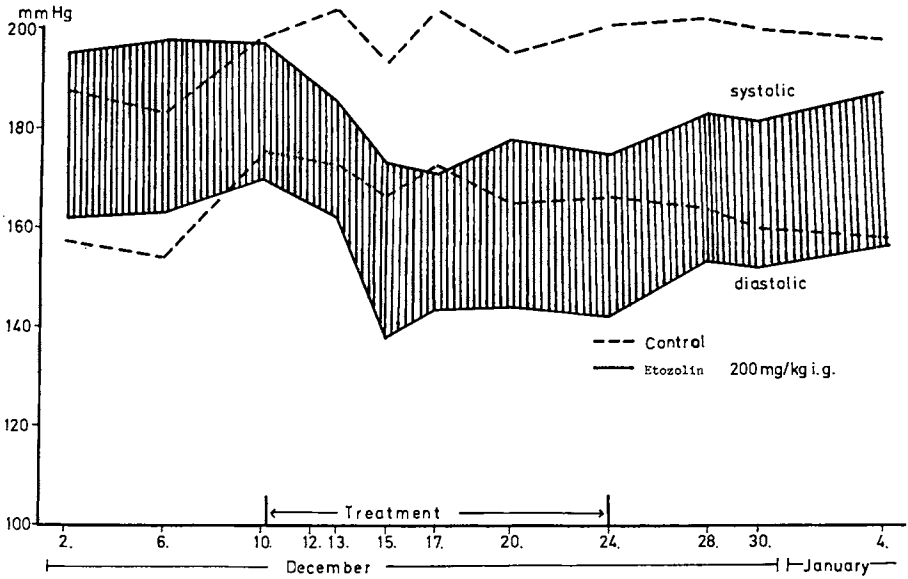

Figure 8. Effect of etozolin on systolic and diastolic blood pressure in rats with renovascular hypertension. Experimental and control groups consisted of 10 animals each.

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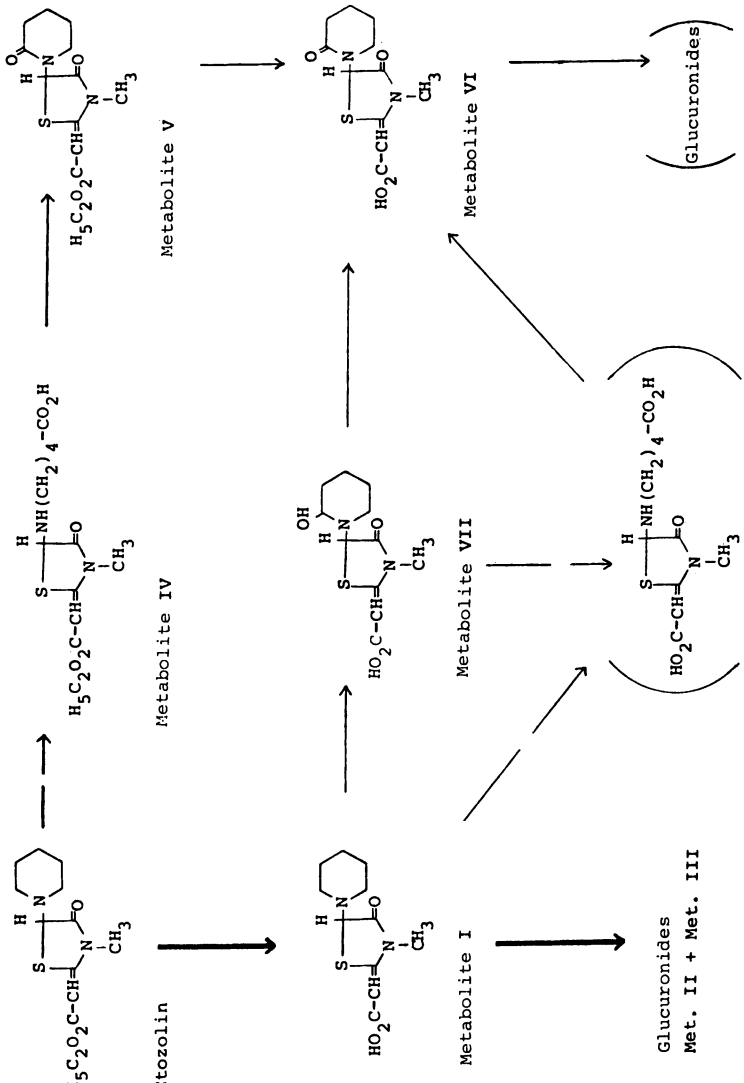

Figure 9. Proposed metabolic pathway of etozolin in rats, dogs, and man

approximately 80% corresponded to the sum of metabolite I and the unchanged drug during the 0 to 8 hour observation period. In all species, the main portion of the blood radioactivity was present in the plasma. In vitro investigation of human plasma revealed a low protein binding for etozolin and metabolite I (approximately 45% and 35%, respectively).

In rats, approximately 80% of the radioactivity was eliminated in the urine and the half-life was 6 hours; in man approximately 90% was eliminated in the urine and the half-life was 8.5 hours. The renal ^{14}C elimination in dogs ranged between 29% and 72%. In man, almost 100% of the radioactive dose was recovered in the excrement within four days. In rats, the highest ^{14}C concentrations were found in blood, kidneys and liver. In the other organs, the concentrations were generally much lower, the lowest being in the brain (27). Autoradiography of rats following repeated oral administration of 100 mg of ^{14}C-etozolin/kg/day from the 10-17 day of pregnancy revealed substantially lower radioactivity concentrations in the fetuses than in the mothers (28). The radioactivity was distributed uniformly throughout the fetal organs, the lowest concentrations occurring in the brain. The placenta would appear to act as a barrier, restricting the passage of radioactive compounds from the mother's circulation into the fetal tissue. The structures of the metabolites were elucidated in studies using rat, dog and human urine and rat bile obtained after oral administration of ^{14}C-etozolin (29). Seven metabolites were isolated. The metabolic pathway of etozolin is the same in rats, dogs and man, and is characterized in three steps (Figure 9): 1) enzymatic cleavage of the ester group, which leads to the main metabolite (metabolite I) in the plasma of all 3 species, 2) glucuronidation of the resulting metabolite I, leading to metabolites II and III, which are diastereoisomeric esters of the two enantiomeric forms of metabolite I with β-D-glucuronic acid and 3) oxidation of the piperidine moiety to metabolites IV-VII.

In all species, approximately 50-60% of the urinary radioactivity corresponded to the sum of the free metabolite I and the metabolite I glucuronides. The unconjugated form was more abundant in rats, while the metabolite I glucuronides predominated in man. Intermediate results were obtained using dogs. No unchanged substance was found in the urine of any of the species investigated. There was little variance in the excretion ratios and urinary metabolite profiles in rats following the oral intubation of different doses (3-100 mg/kg) of the drug, indicating that etozolin's metabolism is not dose related in this range.

A specific TLC analytical method was used to study the pharmacokinetics of the unchanged substance and its principal metabolite (metabolite I) following single and repeated administration of various doses by different routes (30). The pharmacokinetic behavior of etozolin and metabolite I could be

described by first-order process models in each study. Etozolin was metabolized fairly rapidly in each of the species investigated, only relatively low concentrations being found in plasma and none in urine. No deep compartment distribution (e.g., protein binding) was detected. Minor differences were observed in the excretion of the principal metabolite (half-life 4-8 hours), but the uniform distribution volume of approximately 1 L/kg indicates that the metabolite's apparent volume of distribution is identical in all three species.

Each species was administered a different dose range. The pharmacokinetic behavior of etozolin and its principal metabolite was not dose related in the ranges investigated (5-50 mg/kg i.v. in rats, 30-480 mg/kg i.g. in dogs, 400-800 mg orally in man). The plasma level time-curves for etozolin and metabolite I in man are shown in Figure 10.

Etozolin was investigated in long-term studies in man and dogs. The human study involved patients with impaired renal function. The pharmacokinetics of etozolin and metabolite I were unaffected by the size of the multiple doses given. It has been demonstrated that neither the parent substance nor metabolite I is likely to accumulate in the organism if the recommended therapeutic dosage intervals are observed.

CLINICAL STUDIES

Comparative Studies of the Diuretic Activity in Normal Volunteers - The diuretic effect of etozolin was first investigated in 8 healthy volunteers (31). A clear dose-dependent diuretic effect could be demonstrated. When compared to mefruside as a reference substance, both compounds showed a similar diuretic profile. The maximum effect occurred 2 to 4 hours after oral administration; the duration of the increased diuresis being about 10 hours. However, at the doses used, the total volume excreted was significantly higher with etozolin than with mefruside (Figure 11).

The diuretic profile of etozolin is different from that of furosemide, as shown by the total urinary output after equieffective doses. Furosemide, in contrast to etozolin and the thiazide diuretics, has a very rapid onset of diuresis, lasting about 2 hours with a rapid decrease in urine output followed by a distinct rebound phenomenon lasting over 12 hours (Figures 12, 13, 14).

Antihypertensive Activity - In a controlled trial in patients with essential hypertension, 200 mg of etozolin or half the diuretic dose showed an antihypertensive effect similar to that of a drug combination consisting of 150.0 mg of inositol nicotinate + 15 mg mefruside + 0.15 mg reserpine (Figure 15).

In another controlled study (32), lasting over 6 months, a pronounced antihypertensive effect could be observed (Figure 16).

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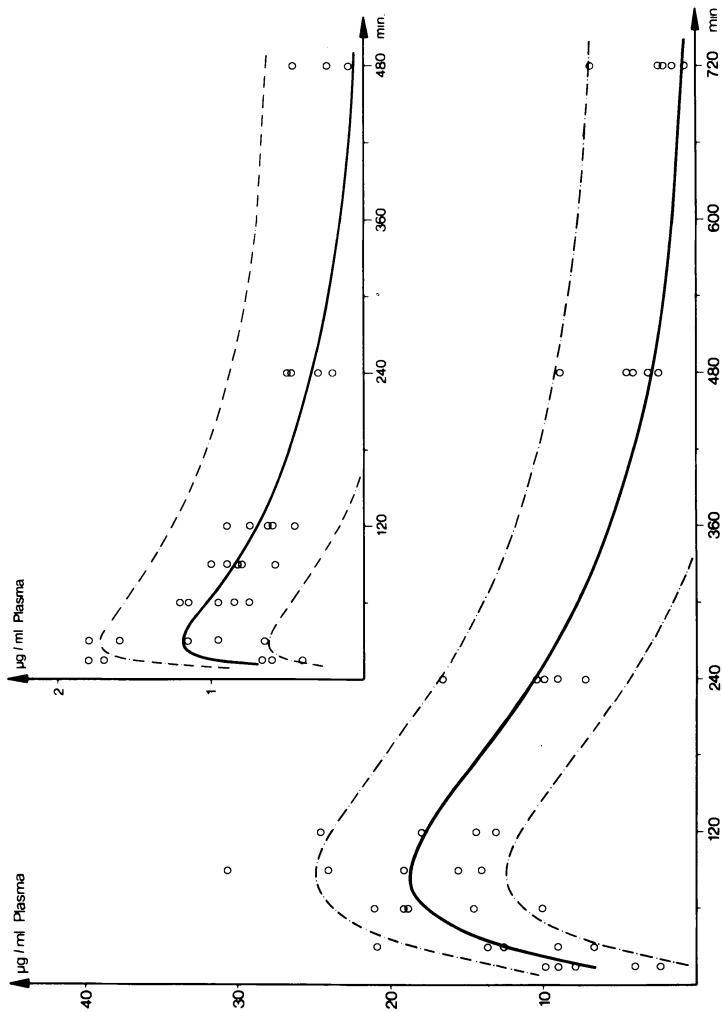

Figure 10. Plasma level time curves of the unchanged drug (top right) and metabolite I (bottom) after oral administration of 800 mg of etozolin to man. The 95% confidence interval of the function and the measured plasma level concentrations are also shown.

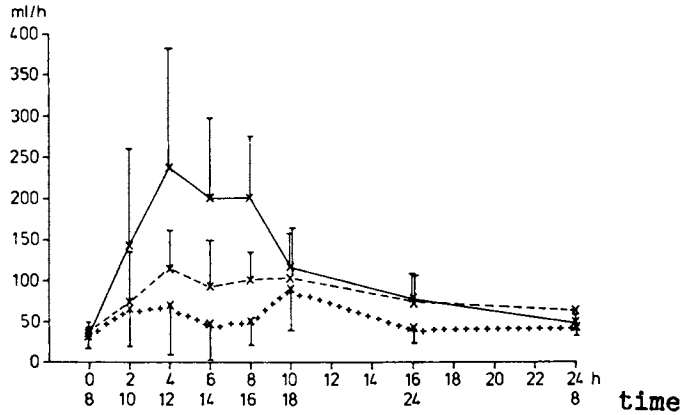

Figure 11. Mean value of urine volume. (——) Etozolin, 800 mg, n = 8; (– – –) mefruside, 50 mg, n = 8; (+ +) placebo, n = 8.

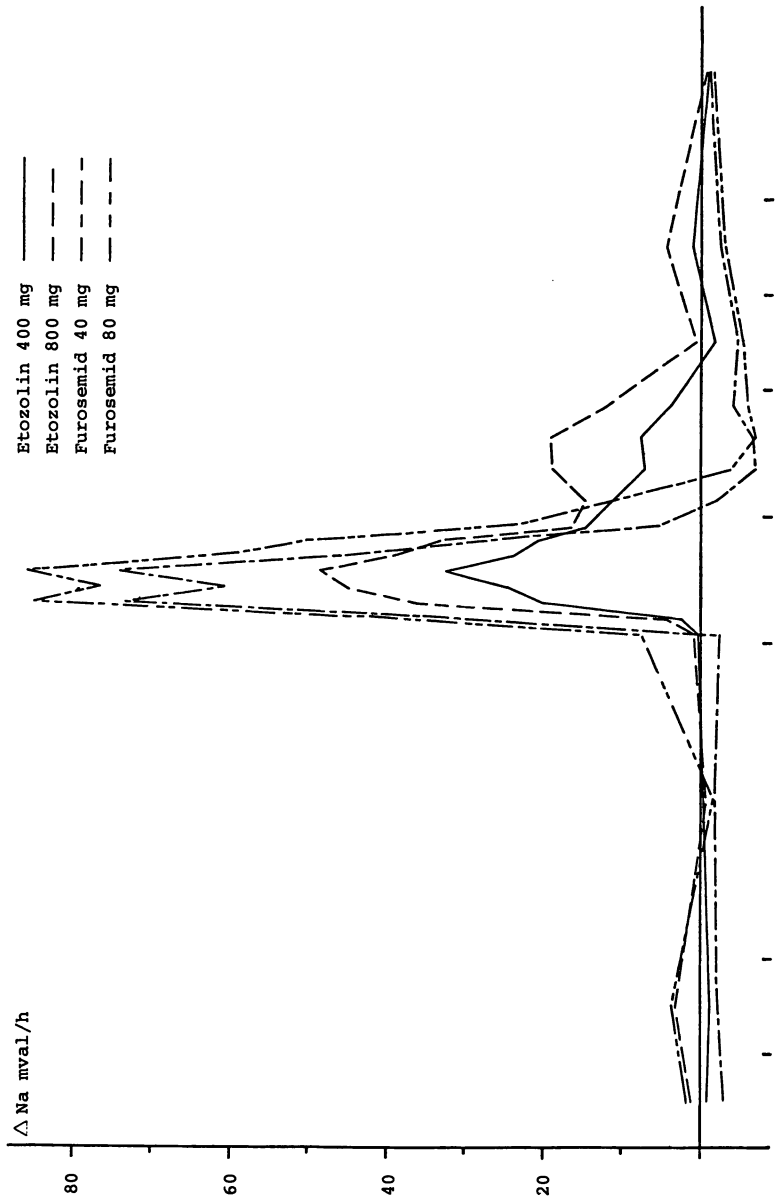

Figure 12. Dose and drug-dependent urinary excretion of sodium

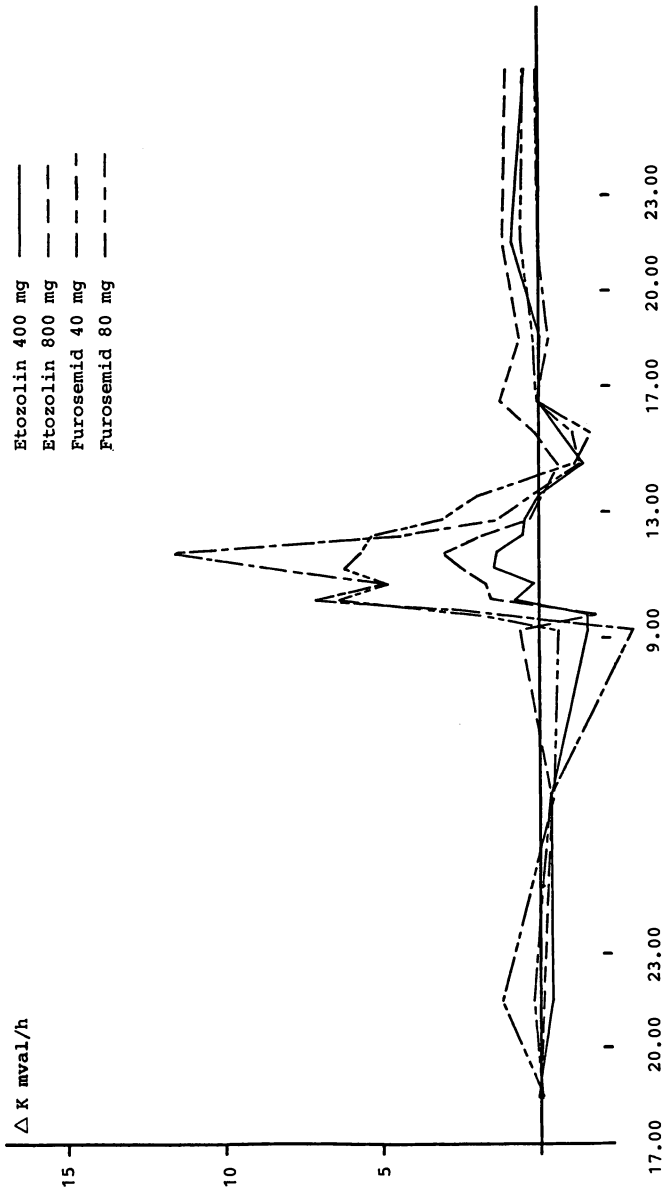

Figure 13. Dose and drug-dependent urinary excretion of potassium

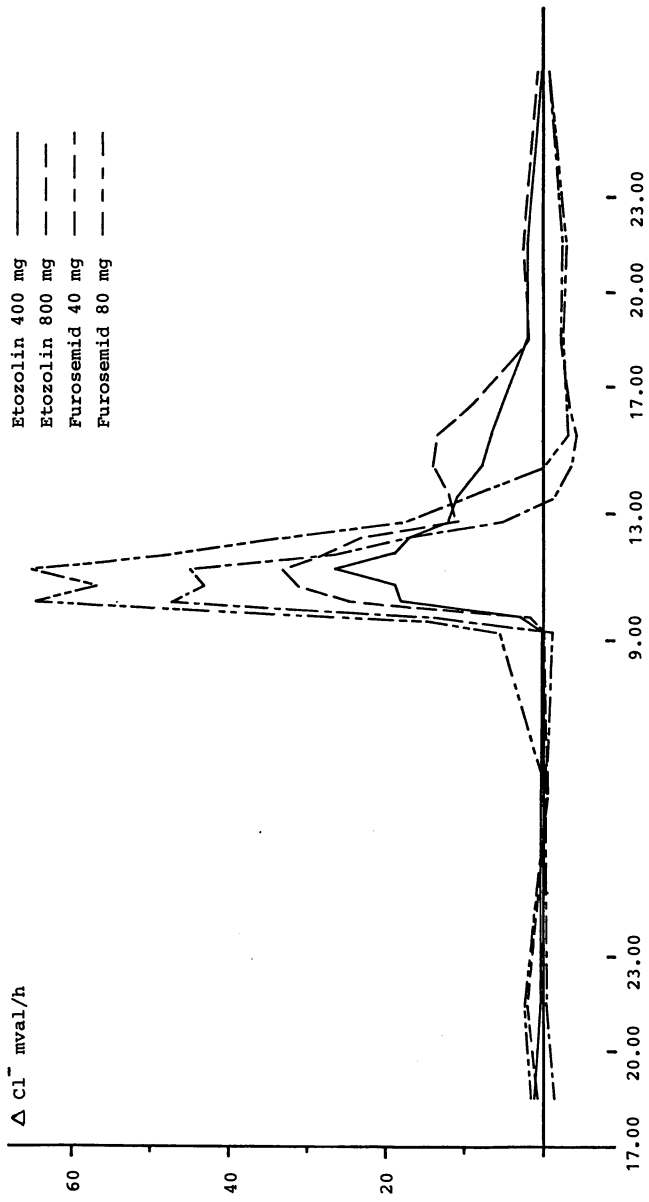

Figure 14. Dose and drug-dependent urinary excretion of chloride

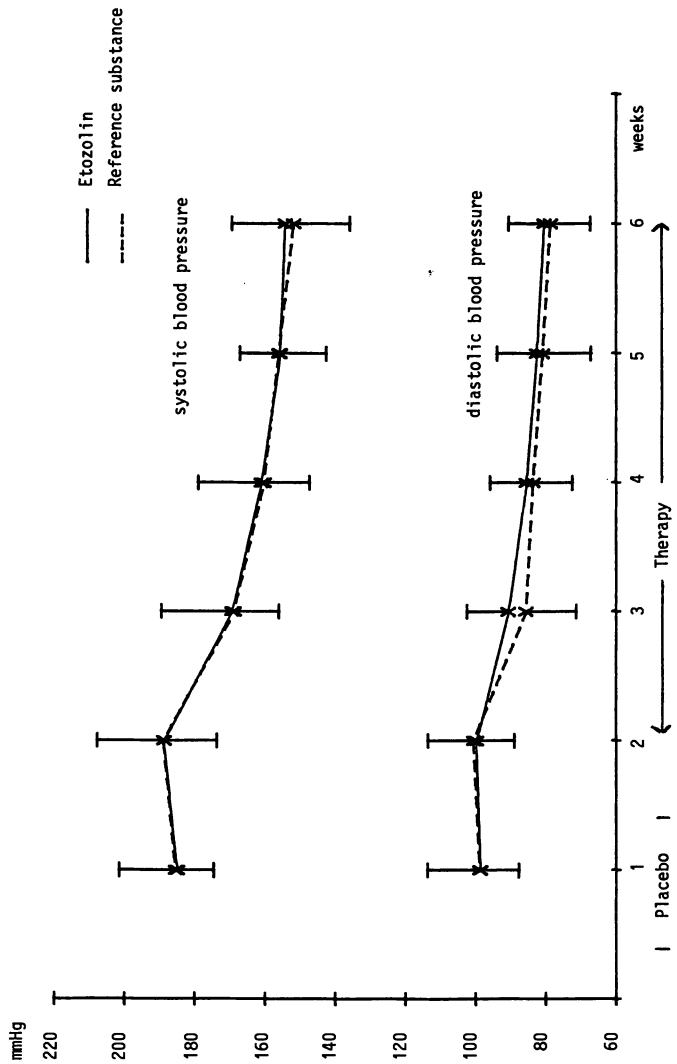

Figure 15. Curves of mean blood pressure values in a sitting position during six months therapy with etozolin and with a reference substance (combination)

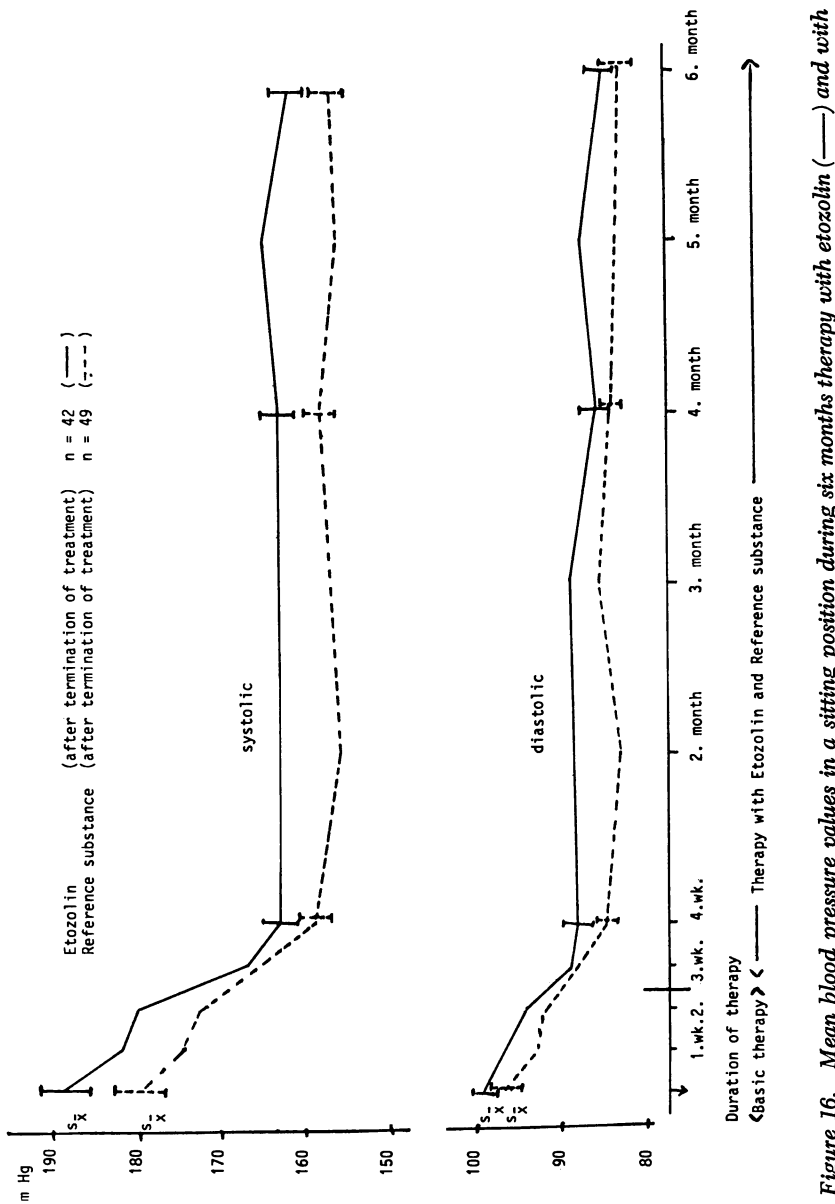

Figure 16. Mean blood pressure values in a sitting position during six months therapy with etozolin (——) and with a reference substance (combination) (- - -)

Side Effects - Out of 447 patients with edema of varying etiology, 29 (6.49%) reported side effects: nausea, 2.0%; vomiting, 1.6%; dizziness, 0.2%; headache, 0.7%; gastrointestinal complaints, 0.9%; skin rashes, 0.9%. Some patients complained of two or more side effects. It is yet to be established whether these side effects are attributable to etozolin alone since other drugs were administered concomitantly. The characteristic side effects of diuretic and/or hypotensive therapy, i.e., hyperuricemia and elevation of creatinine levels also occurred with etozolin.

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11

The Evolution of the (Aryloxy)acetic Acid Diuretics

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The advent of modern diuretic therapy occurred with the discovery of the diuretic properties of merbaphen (1, 2). In spite of the tremendous advances in the field, the mercurial diuretics, particularly the phenoxyacetic acids, merbaphen (Compound 1, Figure 1) and mersalyl (Compound 2, Figure 1) possess many pharmacodynamic attributes which are as good as, or superior to, the modern agents, including potent saluresis, proper urinary Na^+/Cl^- balance, an acceptable potassium excretion profile and uricosuric activity. In addition, much is known concerning their mechanism of action. Cafruny (3) and others have shown that the mercurials react with sulfhydryl-containing compounds both *in vitro* and *in vivo* and that such a reaction at the renal receptor is associated with the observed saluresis and diuresis. Also, the concentration of protein-bound sulfhydryl groups in renal cells is minimal when the concentration of mercury is maximal, which is also the time when diuresis is maximal.

The advantages of the mercurials were more than outweighed by their disadvantages which included tachyphylaxis, poor oral efficacy and toxicity. Using the mercurial phenoxyacetic acids as models, a search was initiated in our laboratories by J. M. Sprague and co-workers for a non-mercurial agent which would lack the drawbacks of the mercurials but would possess their diuretic properties.

Our approach to the solution of this problem was to search for a chemical moiety that would mimic the chemical behavior of the mercury atom in the diuretic mercurials, especially as it pertains to reactions with sulfhydryl groups. After a long investigation, the α,β-unsaturated ketones (3) were found to react by a Michael-type reaction with standard sulfhydryl-containing substances in a parallel fashion to mercurials (4) even under physiological conditions of pH and temperature as shown by the two equations in Figure 2.

Acryloylbenzoic acids of the type 3 were known at the time, but when synthesized proved to be inactive as diuretics. This

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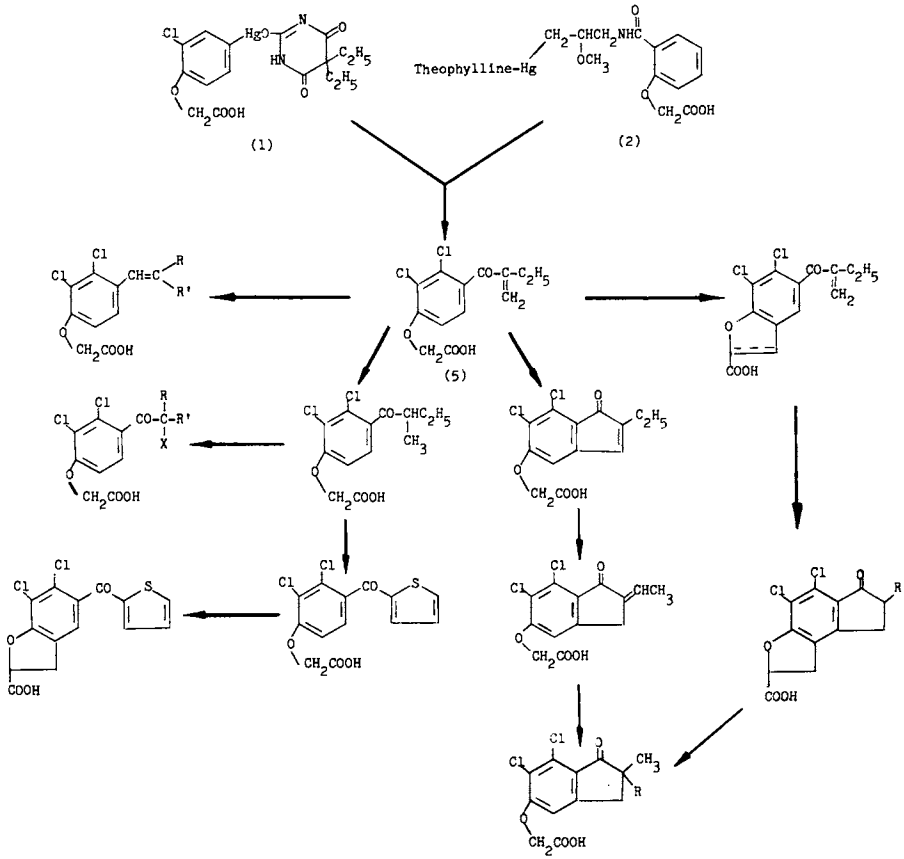
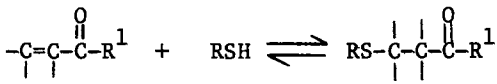


Figure 1. History of the phenoxyacetic acid diuretics

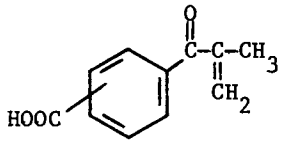


(3)

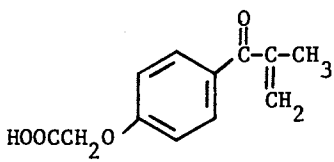
(4)

Figure 2. Reaction of α,β-unsaturated ketones and mercurials with mercaptans

was disappointing, but a knowledge of the critical structural requirements of the organic moiety for mercurials led us to prepare an organic portion of the α,β-unsaturated ketones more akin to that of merbaphen. This was achieved in compound 4 which, to our delight, had diuretic properties. The activity was very weak and of short duration, but it had the qualitative properties we were seeking. Thus, a lead had been established which, upon development, ultimately led to the discovery of ethacrynic acid (Compound 5, Figure 1) (4).



(3)



(4)

The information generated by the research in the ethacrynic acid series set off an explosion of ideas on the design of new diuretics both in our laboratories and in others. The history of how this family of diuretics evolved is illustrated graphically in Figure 1. The arrows portray the geneological relationship of each member of the family which will be discussed systematically in this paper. Interestingly, many of these structural types exhibit qualitatively and quantitatively different renal characteristics.

(Acryloylphenoxy)acetic Acids - After having established compound 4 as a lead, its development involved a systematic structural variation and correlation of biological activity with a series of physical properties, including pKa, H_2O/lipid distribution, protein binding, etc. In addition, correlation of some chemical properties was investigated. The compounds were initially screened intravenously in dogs where the sodium, potassium and chloride excretion and urine volumes were measured in comparison to controls. For convenience, the compounds were scored according to the criterion in Table I.

We first investigated the optimum location of the oxyacetic acid group in relation to the substituted acryloyl moiety of compound 4 (Table II). When these groups were ortho to each other, the compound was inactive. With the meta-orientation, activity was improved, but the para-orientation was optimal.

Next, attention was focused on the role of nuclear substituents (Table III). Only marginal activity was seen when the four nuclear substituents were hydrogen, and substituents in the 2-position made little contribution to activity. In the 3-position, although fluoro had little effect, the other halo, methyl and trifluoromethyl substituents bestowed a marked increase on the activity. The combined effect of two chloro

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TABLE I

Scoring System

Dog Assay[a]

5 mg/kg i.v. Stat Dose

Score	μEq. Na^+/Min. Excreted
0	0 - 99
±	Active Above 5 mg/kg
1	100 - 399
2	400 - 599
3	600 - 799
4	800 - 899
5	900 - 999
6	>1000

[a]Female animals were starved overnight, anesthetized with phenobarbital, creatinine primed, catheterized and infused with phosphate buffer at a rate of 3 ml/min. The drug was given i.v. at 5 mg/kg over a period of 5 min, and 15-min collections of urine were taken over a period of 2 h. The data recorded were the average of the two highest consecutive 15-min collections.

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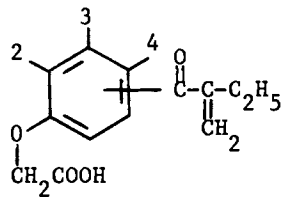
TABLE II

Effect of Location of the Acryloyl Moiety



Ring Position of $C_2H_5-\underset{\underset{CH_2}{\|}}{C}-\underset{\underset{O}{\|}}{C}-$	Cl	I.V. Dog Na^+ Scores
2	4	±
3	4	2
4	3	4

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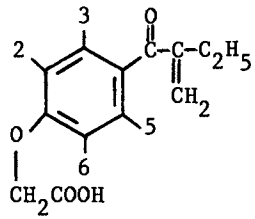
TABLE III

Effect of Nuclear Substituents



	Substituents				I.V. Dog Na+ Score	$T^a_{1/2}$
	2	3	5	6		
	H	H	H	H	±	>90
(MK-495)	Cl	H	H	H	±	27
	CH_3	H	H	H	0	>90
	H	F	H	H	±	27
	H	Cl	H	H	4	7
	H	Br	H	H	4	9
	H	I	H	H	4	9
	H	CF_3	H	H	3	14
	H	CH_3	H	H	3	48
	H	C_2H_5	H	H	±	43
(MK-595)	Cl	Cl	H	H	6	6
	Cl	H	Cl	H	±	6
	H	Cl	Cl	H	3	6
	Cl	H	H	Cl	±	22
(MK-695)	CH_3	CH_3	H	H	5	45
	CH_3	H	CH_3	H	0	58
	CH_3	H	H	CH_3	0	165
	CH_3	CH_3	CH_3	H	±	75
	CH_3	CH_3	H	CH_3	±	73
	CH_3	CH_3	CH_3	CH_3	±	60

[a]Time in minutes required for 50% reaction with a standard solution of mercaptoacetic acid.

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(MK-595, ethacrynic acid) or two methyl groups (MK-695) located in the 2- and 3-positions produced a dramatic increase in diuretic activity. Other orientations of two chloro or methyl groups were much less effective and each of the trimethyl and the tetramethyl analogs were virtually inactive.

As we had anticipated, the (acryloylphenoxy)acetic acids and the mercurial diuretics exhibited marked similarities in their reaction with sulfhydryl-containing compounds, both *in vitro* and *in vivo* (3, 4, 5). A measure of this property was recorded for each of our compounds as illustrated in the column headed $T_{\frac{1}{2}}$, which is actually the time in minutes required for each compound to give a 50% complete reaction with a standard concentration of mercaptoacetic acid at pH 7.4. These data indicate that the most active compounds react very rapidly with this model sulfhydryl reagent. Although, as one might expect, some compounds which react rather slowly have fairly good diuretic activity, and some that react very rapidly are inactive, e.g., the first compound, because they fail to meet other structural or chemical requirements. Compounds containing 5- and 6-substituents were markedly less active. This led us to believe that either the 2- and 3-positions (or the 5- and 6-positions) must remain unsubstituted to maintain activity. As will be seen later, this idea was dramatically disproved in two instances.

A few of the compounds that were prepared to evaluate the effect of substituents on the vinyl carbon atoms appear in Table IV. The first compound (where each R=H) reacts so rapidly and irreversibly with many nucleophiles (in contradistinction to the other compounds in this series) that it possesses very little activity. Each of the compounds bearing a single alpha (R^1) substituent, whether alkyl or cycloalkyl, exhibited potent diuretic activity and a rapid reaction with mercaptoacetic acid. The detrimental effect of an alkyl substituent on both the alpha (R^1) and the beta (R^2) carbon atom was even more pronounced when alkyl groups occupied both beta (R^2 and R^3) atoms. Hydroxy and methoxy groups on the beta (R^2) carbon atom were not tolerated.

Several compounds were studied in which the ether oxygen atom was replaced by other functionalities (Table V). Replacement of oxygen by sulfur provided compounds that were as potent as their oxygen isosteres; however, the corresponding sulfoxides possessed little activity. Replacement of oxygen by -NH-, $-N(CH_3)-$ or $-CH_2-$ drastically reduced or abolished activity. However, replacement of the oxygen atom by a bond provided the corresponding phenylacetic acids which possessed modest activity.

Representatives of the many groups which were evaluated as surrogates for the carboxymethyl moiety (R) are listed in Table VI. When a methylene proton is replaced by fluoro, full activity is maintained. However, substitution of one methylene proton by a methyl group is detrimental and substitution of both

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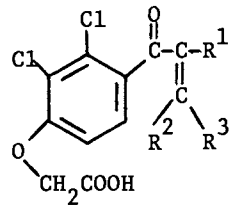
TABLE IV

Effect of Substituents on the Vinyl Carbon Atoms

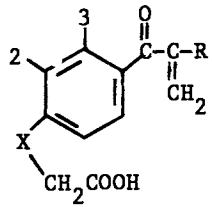


R^1	R^2	R^3	I.V. Dog Na^+ Score	$T_{1/2}$
-H	H	H	±	<1
$-CH_3$	H	H	5	1
$-C_2H_5$	H	H	6	6
$-CH_2(CH_3)_2$	H	H	5	5
	H	H	very active[a]	<94
	H	H	very active[a]	<55
$-CH_3$	CH_3	H	2	109
$-C_2H_5$	CH_3	H	5	>120
-H	CH_3	CH_3	1	109
$-C_2H_5$	OH	H	±	0
$-C_2H_5$	OCH_3	H	±	131

[a]Only tested by p.o. route

TABLE V

Effect of Varying the Atom that Bridges the Nucleus with the Acetic Acid Moiety



X	2	3	R	I.V. Dog Na^+ Score
-O-	H	Cl	CH_3	3
-O-	Cl	Cl	C_2H_5	6
-S-	H	Cl	CH_3	3
-S-	Cl	Cl	C_2H_5	6
-SO-	Cl	Cl	C_2H_5	±
-NH-	H	Cl	CH_3	±
$-NCH_3-$	H	Cl	CH_3	0
$-CH_2-$	H	Cl	CH_3	0
-	H	Cl	C_2H_5	2

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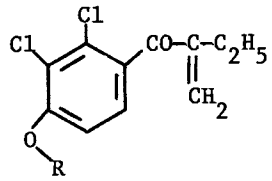
TABLE VI

Effect of Varying the Oxyacetic Acid Group



R	I.V. Dog Na^+ Score	$T_{1/2}$
$-CHFCOOH$	6	-
$-CH(CH_3)COOH$	2	2
$-C(CH_3)_2COOH$	±	<1
$-(CH_2)_3COOH$	1	<17
$-H$	±	<26
$-CH_2CONH_2$	±	6
$-CH_2COOC_2H_5$	very active[a]	12
$-CH_2SO_3^-Na^+$	6	<4
$-SO_2N(CH_3)_2$	active[a]	-

[a]Tested orally

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of them by methyl groups is disastrous, as is lengthening the chain by two methylene units. The phenol (R=H) and the acetamide ($R=CH_2CONH_2$) have little activity, but the ethyl ester is fully active. Replacement of carboxy by sulfonate ($R=CH_2SO_3Na$) gives a compound which is highly active i.v. but not p.o. Interestingly, the compound where $R=SO_2N(CH_3)_2$ exhibits oral activity.

The structure/activity (S/A) data on the (acryloylphenoxy)-acetic acids which we have presented indicate that both substitution of the 6-position of the nucleus and introduction of a methyl group on the carbon atom alpha to carboxy have detrimental effects on activity. However, a number of patents (e.g., 7, 8) have been assigned to Ciba-Geigy on benzofurans that can be viewed as 6 to α-cyclization products (Table VII) which violate both of these S/A indications. We prepared some examples of these structures, including the two shown in Table VII, and found them to be active in dogs, but the saturated forms were more active than the aromatic analogs (in which the double bond is present). It should be noted that these compounds have 6,7-dimethyl substituents and that the analogous (acryloylphenoxy)acidic acids are less active than the corresponding dichloro compounds (see Table III).

Many studies on the site and mode of action of ethacrynic acid have been reported. This compound belongs to the class of potent "loop" diuretics, which includes furosemide and bumetanide, since its principle action is in the distal portion of the loop of Henle (9-13). Like the mercurial diuretics (6), ethacrynic acid is excreted partially as the cysteine adduct (5) which has been shown to be active in man (14). The mercurials and ethacrynic acid also show similar effects on decreasing the protein-bound sulfhydryl groups in renal cells in dogs. No such effect was observed in rats, a species in which the drug is virtually inactive (15-18).

Ethacrynic acid and its analogs are readily cyclized to the corresponding indanones (I), shown in Table VIII, which can be brominated (II) and selectively dehydrobrominated to the corresponding indenones (III) or 2-alkylideneindanones (IV). Although Topliss (19) had found a deschloro analog to be inactive, compounds bearing chlorine atoms in the 6- and 7-positions were highly active, as seen with the two compounds listed on the second line of Table VIII which are the precise analogs of ethacrynic acid. This constitutes the second exception to the idea that the 5- and 6-positions must remain unsubstituted. Branching (i.e., where R and R^1 are CH_3) decreases activity in both series.

A hypothesis regarding the behavior of the (acryloylphenoxy)acetic acids (V) in body fluids is illustrated in Figure 3 which shows their reaction with a variety of sulfhydryl-containing compounds (VI), particularly cysteine and glutathione, to form adducts (VII). Upon reaching the nephron, a reaction occurs with the receptor sulfhydryl group (R^1SH, VIII)

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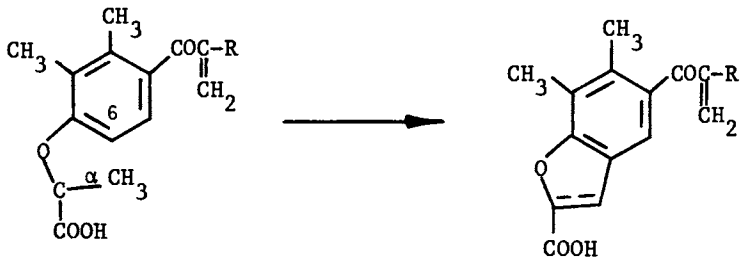
TABLE VII

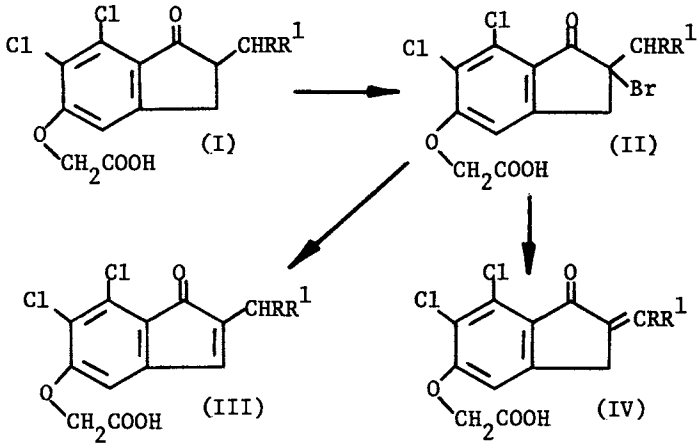
Benzofuran Analogs of (Acryloylphenoxy)acetic Acids



R	Double Bond Present	I.V. Dog Na^+ Score
$-CH(CH_3)_2$	NO	3
$-(CH_2)_2CH_3$	YES	1

TABLE VIII

Cyclic Analogs of (Acryloylphenoxy)acetic Acids



R	R^1	I.V. Dog Na^+ Scores: Indenyloxyacetic Acid (III)	I.V. Dog Na^+ Scores: 2-Alkylidene-indanyloxyacetic Acid (IV)
H	H	4	-
CH_3	H	4	4
CH_3	CH_3	3	2
$-(CH_2)-$		-	1
Ethacrynic Acid		6	-

to give a new adduct (IX). For each compound, the diuretic activity will depend, in part, on the equilibrium constants for reaction 1 vs. reaction 2 and the reaction rates. The most active compounds will be those whose equilibrium constants and reaction rates favor the receptor adducts (IX).

Confirmation of this concept was attempted by preparing some mercaptan adducts of ethacrynic acid and studying their diuretic effects in dogs (Table IX). It is seen that the methyl mercaptan adduct has only weak activity, but that the corresponding sulfoxide and sulfone are about as active as ethacrynic acid. From these selected examples, it will be seen that a range of activities are obtained, from that as great as ethacrynic acid, i.e., the cysteine and glutathione adducts, to complete inactivity, i.e., the mercaptoacetic and N-acetylcysteine adducts.

In the ethacrynic acid series, Free-Wilson and similar analysis were found to be of little value. However, a study by G. B. Smith and G. V. Downing proved to be quite enlightening. They prepared the contour map shown in Figure 4 which shows the correlation of each compound's distribution coefficient (between chloroform and pH 7.4 buffer) with its rate constant for reaction with mercaptoacetic acid. Each point shown on the map represents one compound with its i.v. dog diuretic score beneath the point. When either distribution coefficients or rate constants are considered separately, no correlation is evident; however, when both parameters are considered and lines drawn connecting points of equal diuretic activity, order emerges. The map defines regions or island of each activity which provided guidance in the design of compounds with maximal activity.

[4-(2-Haloacyl)phenoxy]acetic Acids - Another class of potent diuretics are those of the type shown in Table X. The most active members are those where R is alkyl or cycloalkyl, R^1=H and X=bromo. Comparing those where R=isopropyl, the compound in which X=Br is superior to those where X=Cl or I. These compounds react with mercaptans, but some of the most diuretic members react very slowly and the products of the reaction are different from those obtained with the acryloylphenoxyacetic acids, i.e., the X is replaced by H, while the mercaptan is oxidized to the disulfide.

(Vinylaryloxy)acetic Acids - About the time that the S/A relationships in the (acryloylphenoxy)acetic acid series seemed clear, compound 2 (Table XI) was prepared in which the carbonyl and vinyl groups were interchanged (20). The unexpected activity of this compound suggested the synthesis of the lower homolog (Compound 1) which was more active, and the introduction of a methyl group at R^1 which increased the activity (Compound 3). The introduction of a methyl at R^1 on compound 2 markedly decreased the activity (compound 4), but the cyclization to the

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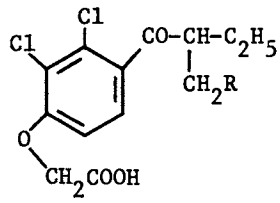
TABLE IX

Mercaptan Adducts of Ethacrynic Acid



R	I.V. Dog Na^+ Score	R	I.V. Dog Na^+ Score
$-SCH_3$	2	$-SCH_2COOH$	0
$-SOCH_3$	6	$-SCH_2CH(NHCOCH_3)COOH$	±
$-SO_2CH_3$	5	$-SCH_2CH(NH_2)COOH$	6
$-SCH_2-$	3	-SG (glutathione)	6
$-SC(CH_3)_3$	2	$-SCOCH_3$	4
$-S(CH_2)_4Cl$	1	$-S)_2$	2
-S-	±	$-SO_3^-Na^+$	0

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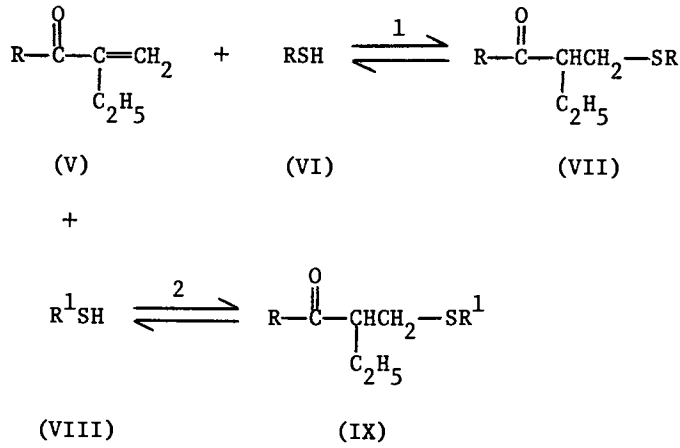


Figure 3. Reaction of (acryloylphenoxy)acetic acids with sulfhydryl compounds

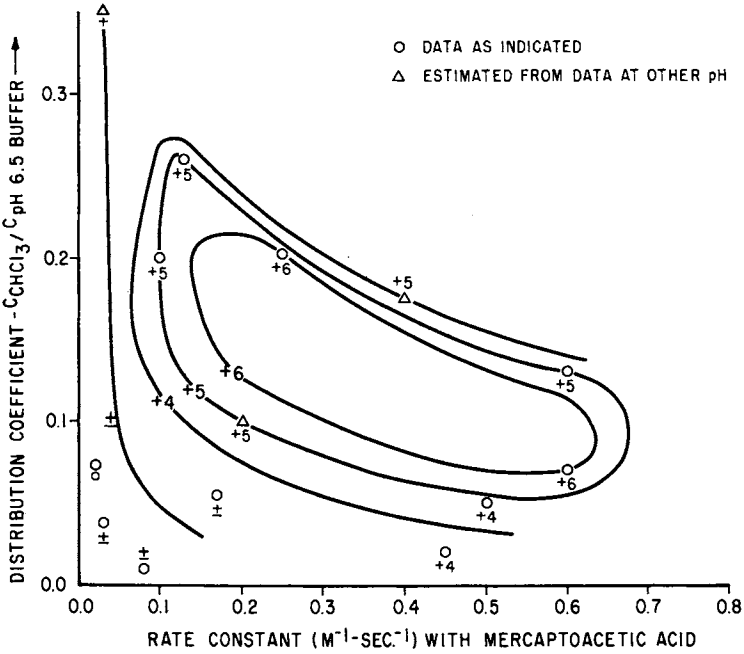
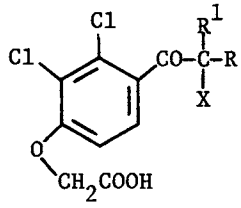


Figure 4. Activity contour map—chloroform distribution vs. rate constant

TABLE X

[4-(2-Haloacyl)phenoxy]acetic Acids



R	R^1	X	I.V. Dog Na^+ Score	$T_{1/2}$
$-C_2H_5$	H	Br	5	21
$-CH(CH_3)_2$	H	Cl	±	No R_x
$-CH(CH_3)_2$	H	Br	5	41
$-CH(CH_3)_2$	H	I	2	
	H	Br	6	>120
	H	Br	very active[a]	
$-C_2H_5$	CH_3	Br	+	21
$-C_2H_5$	C_2H_5	Br	±	41

[a]Active orally

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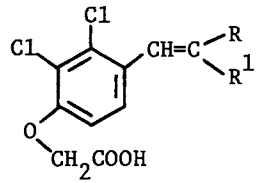
TABLE XI

(Vinylphenoxy)acetic Acids



Compound	R	R^1	I.V. Dog Na^+ Score	$T_{1/2}$
1	CH_3CO-	H-	5	56
2	C_2H_5CO-	H-	4	No Rx
3	CH_3CO-	CH_3-	6	
4	C_2H_5CO-	CH_3-	±	No Rx
5	$-COCH_2CH_2CH-$		6*	Very Slow
6	CH_3CO-	CH_3CO-	6*	2
7	CH_3CO	C_2H_5CO-	6*	2
8	C_2H_5CO-	C_2H_5CO-	4*	5
9	NO_2-	H-	1*	<1
10	NO_2-	CH_3-	6*	<1
11	NO_2-	C_2H_5-	6*	2
12	NO_2-	$CH_3(CH_2)_4-$	3*	8
13	CN-	H-	4*	No Rx
14	C_2H_5OOC-	C_2H_5OOC-	1	13
15	H_2NCO-	H_2NCO-	±	79
16	$-COCH(CH_2CH_2)_2N-$		3*	Very Slow

*Estimated from the response at a dose below 5 mg/kg

corresponding cyclopentanone (Compound 5) greatly increased activity.

Extending the series (21) to those where both R and R^1 are acyl groups greatly enhanced the activity with maximal potency appearing when both groups are acetyl (Compound 6). The compounds where R represents NO_2 (22) also were very active. Increased potency was observed when the second group (R^1) was methyl or ethyl (Compounds 10 and 11). Similar but weaker activity was observed in a number of other compounds (23) in which R and R^1 represent a variety of electron-withdrawing groups, i.e., cyano, ethoxycarbonyl, carbamoyl and sulfamoyl. The most active compounds, i.e., compounds 6-11, react the most avidly with mercaptoacetic acid.

In each series, the other structural requirements for activity paralleled that found for the (acryloylphenoxy)acetic acids. One notable difference was the high activity of mercaptan adducts which were inactive in the (acryloylphenoxy)-acetic acid series. Thus, compound 6 and its mercaptoacetic acid adduct were equipotent, while the corresponding adduct of ethacrynic acid was inactive.

Oral studies (Table XII) in dogs on representatives from each series indicate that compound 3 is four times as potent as furosemide, compound 6 is 70 times and compound 10 is 30 times as active.

We have discussed the three general types of phenoxyacetic acids shown in Figure 5. They each can react with sulfhydryl compounds, but each involves a different site on the molecule as shown by the arrows. Our current concept is that, although these compounds can react with receptor sulfhydryl groups and that such a reaction contributes to their activity, this reaction is not critical for saluresis. A series of (2,3-dichloro-4-saturated-acylphenoxy)acetic acids, which cannot react with sulfhydryl groups, were made and tested in dogs (Table XIII). Many of these exhibited appreciable saluresis. Particularly significant are dihydroethacrynic acid (Compound 1) and the water adduct of ethacrynic acid (Compound 4) which exhibited weak, but significant activity. Although these compounds exhibited only modest activity, this observation was of profound theoretical significance and set the stage for a major breakthrough in the (acylphenoxy)acetic acid family of diuretics. To obtain a complete picture, the compounds that were made for subsequent studies were evaluated orally in chimpanzees, rats and dogs according to the criterion shown in Tables XIV, XV and XVI.

Evaluation of "dihydroethacrynic acid" (Compound 1, Table XIII) in chimpanzees revealed that it had a weak but significant saluretic activity and that, in contrast to ethacrynic acid, it was uricosuric.

(1-Oxoindanyloxy)acetic Acids - Since 2-halo compounds, such as compound 1 in Table XVII, had been used to synthesize the

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TABLE XII

Oral Dog Activity of (Vinylphenoxy)acetic Acids

Cmpd. No. from Table XI	mg/kg p.o. Dose	Ave. 6 hr. mEq. Excretion[a] Na^+	K^+	Cl^-
3	2	16	3	21
6	0.4	34	6	36
10	0.4	23	6	29
11	1	24	5	26
16	5	12	4	17
Furosemide	0.4	8	3	11
	10	19	4	18
Placebo	-	2	1	2

[a]Oral tests were carried out on a colony of trained female mongrel dogs weighing 8–10 kg. All dogs received 100 ml of water the previous day and were fasted overnight. On the day of the test, 250 ml of water was administered orally, followed by 500 ml of water (orally) 1 hr later. One hour after the last oral priming dose of water, bladders were emptied by catheterization and the study was commenced by administration of compound or placebo. Compounds were given in gelatin capsules and the animals were maintained in metabolism cages for collection of spontaneously voided urine. Spontaneous urine was combined with bladder urine collected by catheterization at the end of 6 hr. Urine volumes were measured, and aliquots were analyzed for sodium, potassium and chloride content by standard methodology. Values are reported as geometric means.

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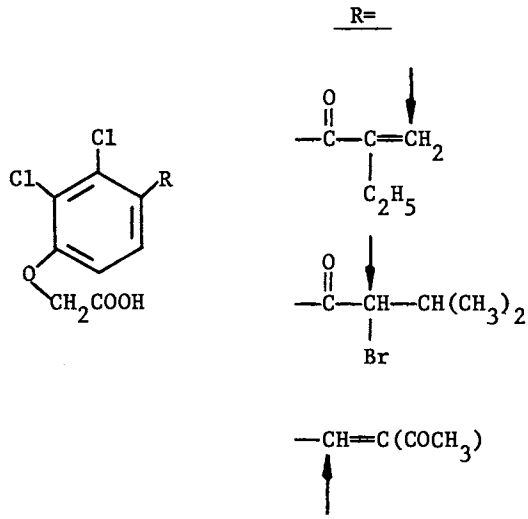
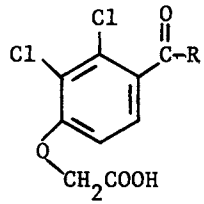


Figure 5. Site of reaction

TABLE XIII

(2,3-Dichloro-4-saturated-acylphenoxy)acetic Acids



Compd. No.	R	I.V. Dog Na^+ Score
1	$-CH(CH_3C_2H_5$	2*
2	$-CH_2C_2H_5$	1
3	$-C(C_2H_5)-OCH_2$	3
4	$-CH(CH_2OH)C_2H_5$	3
5	$-CH(CH_2CN)C_2H_5$	2
Ethacrynic Acid		6

*Estimated value from data at 1 mg/kg

TABLE XIV

Chimpanzee Data: Excretion following 0.5 mg/kg i.v. or 5 mg/kg p.o.[a]

μEq/Min Na^+	Score	$\Delta C_{Urate}/C_{Inulin}$
0-49	0	0-.05
50-99	±	.05-.09
100-199	1	.10-.19
200-299	2	.20-.29
300-399	3	.30-.39
400-499	4	.40-.49
500 and Above	5	.50 and Above

[a]Fasted, male chimpanzees weighing 21-77 kg were immobilized with phencyclidine (which was shown not to affect the results) (1.0-1.5 mg/kg i.m. plus 0.25 mg/kg i.v. as needed) and were prepared by catheterization for standard renal clearance studies using routine clinical asceptic procedures. Pyrogen-free inulin (i.v.) was used to measure glomeruluar filtration rate (GFR). Clearance of inulin, urate and the excretion rates of Na^+, K^+ and Cl^- were determined by standard Auto Analyzer techniques. (Inulin and urate in chimpanzee plasma are freely filterable.) Average control clearances were calculated from three 20-min consecutive periods. Drug-response values were derived as the average of eight 15-20 min clearance periods after oral administration of an aqueous solution of the compound through an indwelling nasal catheter. All data are reported as the difference between (average) treatment and control values obtained from single experiments.

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TABLE XV

5 Hr. p.o. Rat Scoring System[a]

Score	mEquivalents of Na^+ at the Given mg/kg Dose				
	0	3	9	27	81
0	.3	.3	.3	.3	.3
±	.3	.3	.3	.5	.9
1	.3	.3	.4	.7	1.5
2	.3	.3	.5	1.1	2.4
3	.3	.3	.9	1.9	2.9
4	.3	1.0	2.1	2.9	-
5	.3	1.7	2.9	3.4	-
6	.3	2.9	3.9	4.1	-

[a]Female rats (Charles River, 150-170 g) were maintained overnight on a sugar diet with water ad libitum. The test substance was dissolved in pure DMF and subsequently diluted with water (which contained 3 drops of Tween-80 per 100 ml) such that the final vehicle was 4% DMF. At the time of the test, animals were given the vehicle (as placebo) or test substance suspended in a final volume of 5.0 ml p.o. Rats were housed in groups of three in metabolism cages. Urine was collected for the 0-5 hr interval in graduated cylinders and was analyzed for sodium, potassium and chloride content. Animals that received placebo were run concurrently. Results are reported as milliequivalents per cage and are the geometric means of three cages per dose level. Standard methodology was used for determination of electrolyte levels.

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TABLE XVI

5 Hr. p.o. Dog Scoring System[a]

Score	mEquivalents of Na^+ at the Given mg/kg Dose					
	0	1	2	5	10	20
0	2	2	2	2	2	2
±	2	2	2	2	8	>8
1	2	2	2	8	15	25
2	2	2	8	15	25	35
3	2	8	15	25	35	45
4	2	15	25	35	45	55
5	2	25	35	45	55	65

[a]Oral tests were carried out on a colony of trained female mongrel dogs weighing 8-10 kg. All dogs received 100 ml of water the previous day and were fasted overnight. On the day of the test, 250 ml of water was administered orally, followed by 500 ml of water (orally) 1 hr later. One hour after the last oral priming dose of water, bladders were emptied by catheterization and the study was commenced by administration of compound or placebo. Compounds were given in gelatin capsules and the animals were maintained in metabolism cages for collection of spontaneously voided urine. Spontaneous urine was combined with bladder urine collected by catheterization at the end of 5 hr. Urine volumes were measured, and aliquots were analyzed for sodium, potassium and chloride content by standard methodology. Values are reported as geometric means.

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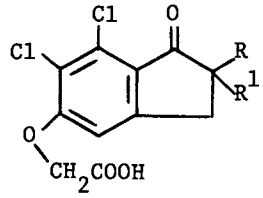
TABLE XVII

2-Substituted-Indanones



Compd No.	R	R^1	P.O. Na^+ Score Rat	Dog	P.O. Na^+ Chimp Score	Urate
1	$-C_2H_5$	-Cl	3	1	2	±
2	-H	-H	0	0		
3	$-CH_3$	-H	2	1		
4	-Et	-H	2	1	4	±
5	-Pr	-H	±	1	5	1
6	-i-Pr	-H	2	3	5	2
7	-Bu	-H	±	1	1	1
8	-sec-Bu	-H	2	±		
9	-t-Bu	-H	2	2	1	±
10	-Am	-H	1	1	1	±
11		-H	1	1	5	2
12		-H	1	1	2	3
13		-H	1		3	1
Ethacrynic Acid			0	6	5	0 (retains urate)

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(indenyloxy)acetic acids and the (2-alkylideneindanyloxy)acetic acids described in Table VIII, the possibility that it could function as a pro-drug was considered. However, its saluretic activity in rats and its uricosuric activity in chimpanzees revealed it to be qualitatively distinct from the mercurials and the unsaturated aryloxyacetic acids. It was assumed that the observed biological differences were due to the unchanged compound, even though metabolic studies indicated that some of the expected dehydrohalogenation had occurred. This led us to prepare analogs of compound 1 that were chemically more stable, such as compound 4, which is an annulated form of "dihydroethacrynic acid" (24-27). Its activity profile was very similar to that of compound 1.

The representative compounds of Table XVII exhibit a range of saluretic and uricosuric responses when R represents a variety of alkyl, cycloalkyl and aryl substituents. The 2-isopropyl analog (Compound 6) appeared to have the overall optimum activity. The effect of structural modification of the nuclear substituents and reorientation or variation of the oxyacetic acid moiety was about the same as had been observed in the (aryloxy)acetic acid series.

A most significant contribution to the activity of these (indanyloxy)acetic acids (27-28) occurred with the introduction of a second 2-substituent (Table XVIII). These compounds were generally considerably more active than the corresponding monosubstituted compounds. To quantitate these comparisons in rats, dose-response curves with high statistical significance were developed. The numbers in parenthesis in Table XVIII are the fold greater activity found for each 2-R, 2-CH_3 compound as compared to the monosubstituted analog (Compound 1). Optimal diuretic activity was seen with compounds 4 and 6 (MK-196), but the latter compound exhibited optimal dual (diuretic-uricosuric) activity. Increasing the size of R^1 to ethyl (Compounds 7 and 8) was detrimental to diuretic activity. Since uricosuric and diuretic activities do not run parallel, some compounds which are very weak diuretics, e.g., (2-ethyl-3-phenyl-6,7-dichloro-5-indanyloxy)acetic acid, exhibit potent uricosuric activity.

Substitution on the phenyl group of MK-196 had a marked effect on diuretic and uricosuric activity (Table XIX). Although there was considerable species variation, the p-fluoro derivative (Compound 4) showed the best overall activity. Replacement of the phenyl group of MK-196 by a 2-thienyl group gave a compound which was equipotent to MK-196. Replacement of the carboxy group of MK-196 by a number of groups, including 5-tetrazolyl, usually produced compounds with considerably decreased saluretic activity.

Numerous structural variations were made in positions 1, 2 and 3 of the indane ring (29). Reduction of the 1-oxo group of MK-196 to hydroxy provided two diasteriomers, both of which were

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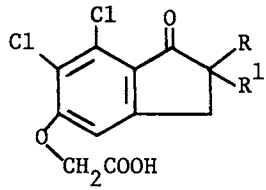
TABLE XVIII

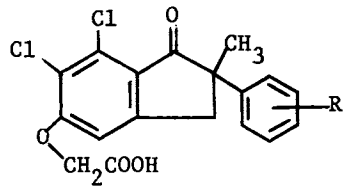
(2,2-Disubstituted-Indanyloxy)acetic Acids



Compd. No.	R	R^1	P.O. Na^+ Scores: Rat	P.O. Na^+ Scores: (FOLD x Compd. 1)	P.O. Na^+ Scores: Dog	P.O. Chimp Scores: Na^+	P.O. Chimp Scores: Urate
1	$-CH_3$	H-	2	(1)	1		
2	$-CH_3$	CH_3-	3	(4)	1	3	±
3	-Et	CH_3-	3	(15)	3	5	1
4	i-Pr	CH_3-	3	(53)	3	4	±
5		CH_3-	2	(5)	2	2	1
6		CH_3-		(50)	3	5	3
7		C_2H_5-	2			2	1
8		C_2H_5-	1		±	3	1
9	$-CH_2CH_2CH_2CH_2-$		2		1	3	1

TABLE XIX

Effect of Substituents on the Phenyl Group of MK-196



Compd. No.	R	P.O. Na^+ Scores Rat	Dog	P.O. Na^+ Chimp Scores	Urate
1	H	3	3	5	3
2	4-Br	±	1	±	±
3	4-Cl	±	1	3	2
4	4-F	2	3	3	4
5	4-OCH_3	2		4	±
6	4-OH	±	2	1	±
7	3-OH	1	2		
8	4-NH_2	1	1	2	±
9	4-NO_2	4	2	1	±
10	4-SO_2NH_2	1	5	5	±
11	4-$COCH_3$	±		5	±

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about as active as the parent (30). Alkyl or aryl groups in the 3-position usually decreased saluretic activity, and either did not affect or increased the uricosuric activity. A 3-oxo substituent generally provided compounds with activities only slightly below that of their parents (31).

The influence of the chiral center at the 2-position can be seen from the study involving the four enantiomeric pairs (Table XX). Although there was some variation among species, the enantiomeric effect was most pronounced with compounds 1 and 4.

Four of the (indanyloxy)acetic acids have been studied clinically: compounds 6 and 11 from Table XVII and compounds 5 (MK-473) and 8 (MK-196) from Table XVIII. The relative saluretic and uricosuric potencies in man were predictable from the animal data, i.e., MK-196>MK-473>compound 11>compound 6 (Table XVII).

MK-196 has received extensive pharmacological, biochemical and clinical evaluation (32-34). It produced a sustained antihypertensive effect in SH rats (32-33) at oral doses of 0.5-7.5 mg/kg. It was more potent than furosemide or hydrochlorothiazide when given daily for three days. It also was antihypertensive in renal hypertensive monkeys but not in renal hypertensive dogs.

MK-196 is well absorbed in mice, rats, dogs, monkeys, chimpanzees and humans (34-36). In rats and dogs, there was little metabolism of the drug, which is excreted mainly in the feces. In mice and monkeys, there was some metabolism and about equal elimination by the fecal and urinary routes. In chimpanzees, the major elimination is via the urine and, in man there is considerable metabolism, primarily to the 2-(p-hydroxyphenyl) derivative (Compound 6, Table XIX).

In chimpanzees (37-39), the urinary excretion of MK-196 is increased ten-fold when the urine is alkaline. The drug is secreted by the renal tubule and passively back-diffuses across the tubular epithelium by a pH-dependent process.

Since it is known that urate is reabsorbed and secreted in the proximal tubule, the studies in chimpanzees (38) suggested that the action of MK-196 on the clearance of urate is localized at this site. Micropuncture studies in rats (40) indicate that the drug affects electrolyte reabsorption in the loop of Henle and collecting duct, while concentration gradient studies in dogs (42) implicate sites in the proximal tubule and ascending loop of Henle.

Biochemical studies by Kuehl (43) and his colleagues indicate that MK-196 and other compounds in this series owe at least part of their activity to their effects on prostaglandin metabolism. MK-196 and its enantiomers (Table XXI) inhibit PGE-9-ketoreductase, the enzyme that converts PGE_2 to $PGF_{2\alpha}$. To a lesser extent, it inhibits PG-15-hydroxydehydrogenase, the enzyme which deactivates prostaglandins. MK-196 has a greater inhibitory effect on these enzymes than the classical diuretics as shown in Table XXI. The overall effect would be to increase the renal levels of PGE_2.

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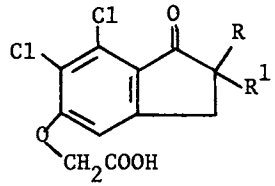
TABLE XX

Enantiomers of (Indanyloxy)acetic Acids



Compd. No.	R	R^1	Enantiomer	P.O. Na^+ Scores Rat	Dog	P.O. Chimp Scores Na^+	Urate
1		H	+	2	2	3	2
			-	1	1	2	1
2		CH_3	+	3	2	2	1
			-	2	1	2	2
3	$-CH(CH_3)_2$	CH_3	+	2	3	5	4
			-	3	5	4	1
4		CH_3	+	1	1	1	1
			-	3	2	6	2
Furosemide				3	5	5	0*
Hydrochlorothiazide				2	2	1	0*
Probenecid							1†

*Decreased Excretion

†Dose = 10 mg/kg

TABLE XXI

Effect of MK-196 on Prostaglandin Metabolism

Compd.	Enantiomer	ID_{50} μM	
		9-Ketoreductase[a]	15-Hydroxydehydrogenase[a]
MK-196	±	15	178
MK-196	+	145	159
MK-196	-	8.5	126
Mersalyl Acid		50	351
Ethacrynic Acid		307	709
Furosemide		499	2419
Hydrochlorothiazide		>2514	>2514

[a]Measured by determining the enzymatic conversion of ^{3}H-PGE_2 to ^{3}H-15-keto-PGE_2 in the presence of NADP. [b]Measured by the method of Oien et al (43).

In man, MK-196 (44-46) was found to be a well tolerated saluretic-diuretic. An oral dose of 10 mg elicited a total 24 hour saluresis equivalent to that of 40 mg of furosemide. Administration of furosemide caused a significant increase in plasma urate levels, whereas the continued use of MK-196 caused less increase in plasma urate levels. Daily oral administration of either 10 or 15 mg of MK-196 lowered the blood pressure of hypertensive patients as much as 50 mg of hydrochlorothiazide did. This drug is currently undergoing extensive clinical trials.

Indeno[5,4-b]furan-2-carboxylic Acids - The interesting activity exhibited by the benzofuran analogs of the (acryloylphenoxy)-acetic acids of the type seen in Table VII suggested the synthesis of some indeno[5,4-b]furan-2-carboxylic acid analogs of the active (indanyloxy)acetic acids. As seen in Table XXII, these compounds are potent diuretics (47, 48). In this series, uricosuric activity was exhibited only by those compounds where R^1 was phenyl. Since these compounds have two chiral centers, two diasteriomers are possible, and in one instance both diasteriomers (Compounds 3 and 4) were obtained and shown to have quite similar diuretic and uricosuric activities.

(Acylphenoxy)acetic Acids (Table XXIII) - The observation of saluretic and uricosuric activity in tienilic acid (Compound 1, Table XXIII) and some of its analogs (49) and in "dihydroethacrynic acid" (Compound 2, Table XXIII) (26) revealed that pharmacodynamic activity existed in several (2,3-dichloro-4-acylphenoxy)acetic acids. The fact that activity also was observed in compound 3 and a number of related structures indicated that the portion of the molecule represented by R could be alkyl, heterocyclic, aryl or aralkyl.

Since annulation of (acryloylphenoxy)acetic acids and (indanyloxy)acetic acids to the corresponding dihydrobenzofurans or dihydroindeno[5,4-b]furans either maintained or improved activity, an analogous annulation of representative (acylphenoxy)acetic acids was carried out (50) as recorded in Table XXIV. Studies involving compound 1 in rats, dogs and chimpanzees revealed it to be 40 to 100 times as potent a diuretic as tienilic acid with about equal activity as a uricosuric agent. Most interesting is the fact that compound 1 is a racemate which upon resolution affords two enantiomers in which there is a complete separation of pharmacodynamic properties. The minus-form possesses only uricosuric activity, while the plus-enantiomer possesses only diuretic properties. This permits the formulation of combinations of the enantiomers to obtain any desired ratio of diuretic to uricosuric activity. It is noteworthy that compounds in which R represents a variety of aryl, aralkyl, or heterocyclic groups retain the activity exhibited by

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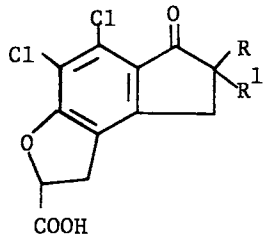
TABLE XXII

Indeno[5,4-b]Furan-2-Carboxylic Acids



Compd. No.	R	R1	P.O. Na^+ Scores Rat	Dog	P.O. Na^+ Chimp Scores Urate	
1	$-(CH_2)_4-$		5	4	5	0
2	CH_3		4	4	3	0
3 (α)	CH_3		3	5	5	5
4 (β)	CH_3		4	6	6	4

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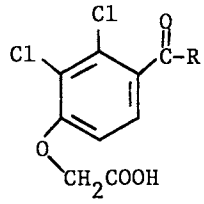
TABLE XXIII

(4-Acylphenoxy)acetic Acids



Compd. No.	R	P.O. Rat Scores	I.V. Dog Scores	1 mg/kg I.V. Chimp Na^+ Δ mEq/min	1 mg/kg I.V. Chimp $\frac{C_{Urate}}{C_{Inulin}}$
1		1	1	434	.376
2	$-CH(CH_3)C_2H_5$	±	1	400	.213
3	$-CH_2-$ 	±	1	363	.309

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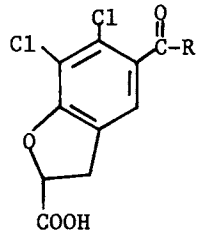
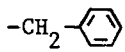
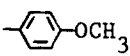
TABLE XXIV

4-Acyldihydrobenzofurans

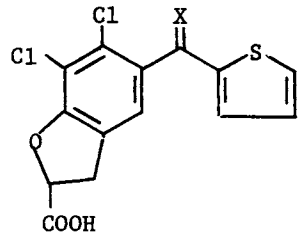


Compd. No.	Enantiomer	R	P.O. Biological Activity Scores			
			Diuretic			Uricosuric
			Rat	Dog	Chimp	Chimp
1	±		5	4	5	3
	+		0	0	0	5
	-		6	5	5	0
2	±		3	3*		
3	±		3	3*	5	1
4	±		3		4	2
5	±		3		5	3
6	±		3			
7	Tienilic Acid		1	±	3	4

*I.V. Scores

TABLE XXV

Reduction Products of 5-Acylbenzofuran-2-Carboxylic Acids



Compd. No.	X	P.O. Biological Activity Scores			
		Diuretic			Uricosuric
		Rat	Dog	Chimp	Chimp
1	O	4	4	4	3
2	H(OH)	1	4*	5*	1
3	H_2	±	4	2*	1

*I.V. Data

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TABLE XXVI

COLLABORATORS IN THE PHENOXYACETIC ACID RESEARCH

ORGANIC CHEMISTS

A. AUGENBLICK	N.P. GOULD	J.K. HORNER	C.M. ROBB
J.J. BALDWIN	C.N. HABECKER	S.F. KWONG	E.M. SCHULTZ
J.B. BICKING	W.J. HOLTZ	J.W. MASON	J.M. SPRAGUE
W.A. BOLHOFER	W.F. HOFFMAN	F.C. NOVELLO	G.E. STOKKER
A.A. DEANA			T.P. STROBAUGH

PHYSICAL AND ANALYTICAL CHEMISTS

B.H. ARISON	G.V. DOWNING	W.R. MCGAUGHRAN	G.B. SMITH
E.L. CRESSON	Y.C. LEE	W.C. RANDALL	K.B. STREETER

BIOCHEMISTS

D.E. DUGGAN	E.H. HAM	F.A. KUEHL	H.G. OIEN

PHYSICIANS

G.H. BESSELAAR	Z.E. DZIEWANOWSKA	J.J. SCHROGIE	K.F. TEMPERO
R.O. DAVIES			

BIOLOGISTS

J.E. BAER	C.H. DUNCAN	E.K. MAZACK	L.S. WATSON (Dec.)
K.H. BEYER	R.M. EVANS	J.E. MICHAELSON	T.I. WISHOUSKY
D.L. BOHN	G.M. FANELLI	H.F. RUSSO	A.G. ZACCHEI

ACKNOWLEDGMENTS

A. SCRIABINE	R.F. HIRSCHMANN	C.A. STONE

compound 1.

Of the many modifications of the structure of the benzofurans that have been made, one of the more interesting involves that of the carbonyl group. Reduction to the hydroxymethylene (one diasteriomer) or to methylene produced compounds which maintained good activity in dogs and chimpanzees but not in rats (see Table XXV).

In summary, the rational design of diuretics based upon either the structural features or mechanism of action of the mercurial diuretics led to the discovery of about a dozen new types of potent diuretics. One of these, ethacrynic acid, has become a clinically useful drug and several others show similar promise.

We wish to pay tribute to the chemists, biologists and physicians listed in Table XXVI who contributed to these studies which were conducted over a period of more than a decade.

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