ANNUAL REPORTS IN MEDICINAL CHEMISTRY, 1969

Sponsored by the Division of Medicinal Chemistry of the American Chemical Society

Editor-in-Chief: **CORNELIUS K. CAIN**

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McNEIL LABORATORIES, INC.
FORT WASHINGTON, PENNSYLVANIA

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LIST OF CONTRIBUTORS

PREFACE

This present annual volume is the fifth of a series. Traditionally, a fifth anniversary is a time for retrospection. Readers familiar with the series from its beginning may find it instructive to compare this volume with the first. Many changes will be apparent, and these changes reflect progress in Medicinal Chemistry - greater sophistication in theories, in synthesis, in the approach to new and old problems, in interpretation of subtle differences in biological effects, etc. Equal or greater progress is expected the next five years.

Thanks due the contributors to each volume - authors, section editors and many others - are more deserved each year. There is more to sift and condense, and the material is rarely, if ever, simpler. Their efforts are greatly appreciated.

Fort Washington, Pennsylvania
June, 1970

Cornelius K. Cain

AWARD ADDRESS

Excursions in Medicinal Chemistry - Renal Agents

James M. Sprague
Merck Sharp & Dohme Research Laboratories, West Point, Pa.

Third Award in Medicinal Chemistry, Twelfth National Medicinal Chemistry Symposium of the American Chemical Society, Seattle, Washington, June 22-25, 1970

Medicinal chemists are concerned with the discovery of new therapeutic agents, with the structural design of biologically-active substances and with a chemical interpretation of how these substances bring about their biological effects. Some results of our group in these areas of concern are presented here. Of the several areas that might be selected, the discussion is limited to specific topics in the renal field.

The rapid rate of discovery of highly effective drugs in all fields of therapy over the past twenty to thirty years has been the subject of much discussion. In the renal field, particularly diuretic agents, the notable developments have occurred mainly in the last fifteen years, starting with the first of the sulfonamide type drugs. Prior to this period, the organomercurial diuretics had been the only effective agents since their discovery in 1920. The progress in this area has closed the gap between the level of desirable therapy and the level of therapy attainable prior to these recent developments. Among the newly developed drugs, we have achieved many desirable attributes. Nevertheless, in spite of these advancements, there still exist gaps to be filled before we reach our goal of the elusive, totally satisfactory drug. So far, all of the highly effective drugs cause an undesirable excretion of potassium in the urine that can result in hypokalemia. They also decrease urinary uric acid excretion and increase blood uric acid concentration, a hyperuricemia that has led to gout in predisposed individuals. Many of these drugs can disturb carbohydrate metabolism leading to increased blood glucose levels. While these shortcomings do not occur in all patients and frequently can be overcome by judicious handling of the dose regime and by adjuvant medication, they can and do limit drug use.

These, then, are the major deficiencies to be filled by the design of new diuretics, and they constitute the immediate objectives of many medicinal chemists working in the area of renal drugs. The following discussion concerns some selected aspects of our own work that has been directed to the search for compounds to fill these deficiencies. This discussion will deal with three categories of compounds: (1) compounds designed to mimic the mercurials, (2) the sulfonamides, particularly the hydrothiazides and (3) a heterocyclic class of potassium-sparing diuretics. A complete or detailed analysis of structure-activity relations will not be presented; these either have been or will be published. Emphasis is placed on the inception of these compounds and on certain points related

to a chemical interpretation of how compounds of these classes may exert their biological activity.

Soon after the introduction of organomercurials as diuretics, the idea arose that their biological action resulted from the reaction with and consequent blockade of essential sulfhydryl groups. This was the prevailing concept in 1948-1951, when various of our attempts to design non-mercurial diuretics began. After extensive and sophisticated investigations that have been reported from many laboratories, this concept is still held today. In a recent review, Cafruny states that "the mechanism of action probably involves a firm attachment of mercury to a sulfhydryl group of a renal enzyme that helps to generate energy for sodium transport or to a sodium carrier".

Even today, after the development of many powerful and useful new drugs, the organomercurials are unique among these potent diuretics, in spite of their several disadvantages. The mercurials cause little or minimal potassium loss and, in fact, under certain conditions, may exhibit a potassium-sparing action. They are not known to disturb carbohydrate metabolism. They do not cause uric acid retention and certain of them have been shown to have a uricosuric action in man. Whether these desirable properties are a consequence of their structure and mode of action or result from the intermittent or spaced manner of administration is not clear. But these properties of the mercurials continue to be a challenge to the medicinal chemist and present objectives for the design of new structures.

From these considerations, we have developed in our laboratory several series of highly active compounds that were designed to react selectively with functionally-important sulfhydryl groups, or possibly other nucleophilic groups, that are essential for sodium transport. These compounds generally contain an activated double bond attached to a carboxylic acid structure of a type expected to assist transport into, or excretion by, the kidney. Figure 1 shows a general structure for these compounds,

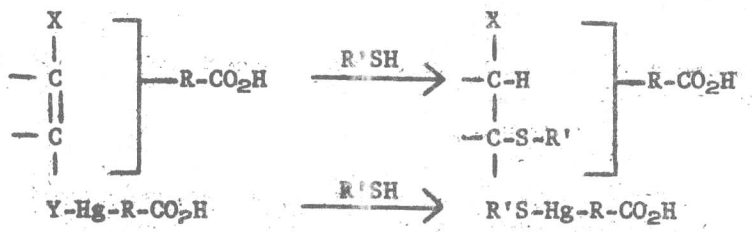


Figure 1

in comparison with a generalized structure for the analogous mercurials, and their reaction with compounds containing SH groups.

Figure 2 gives some of the types of activated ethylenic structures and carboxylic acids employed. All of these ethylene structures have

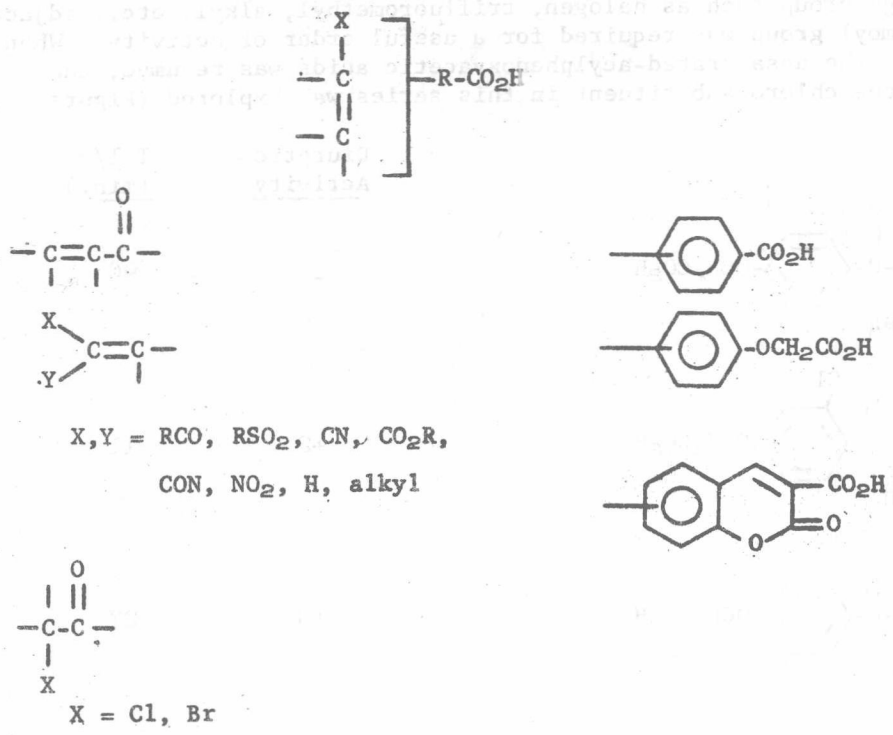


Figure 2

yielded compounds of high activity. The α-haloacyl group has also given active diuretics. Among the carboxylic acid structures, the phenoxyacetic acids are particularly active and have received most attention.

The α,β-unsaturated-acyl compounds (Figure 3) were the first studied,

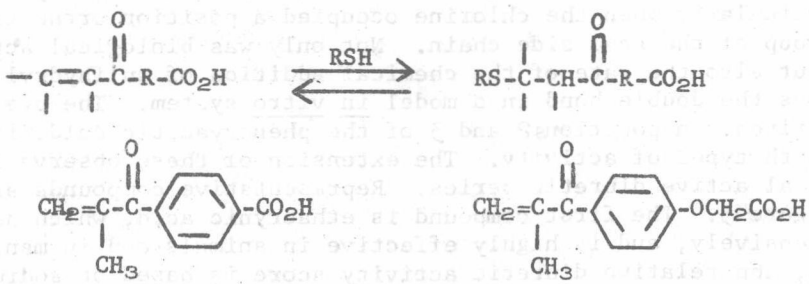


Figure 3

but the initial compounds showed only marginal activity in dogs. At this point in our program, the investigation of these structures was temporarily interrupted by the more promising results arising from the simultaneous study of the aromatic-disulfonamide series that led to the thiazides. In these sulfonamide series, it was clearly demonstrated that an

"activating" group such as halogen, trifluoromethyl, alkyl, etc., adjacent to a sulfamoyl group was required for a useful order of activity. When the work on the unsaturated-acylphenoxyacetic acids was resumed, the effect of the chloro substituent in this series was explored (Figure 4).

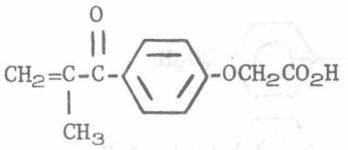
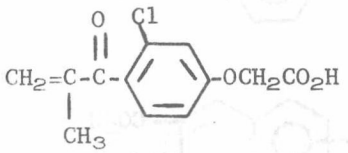
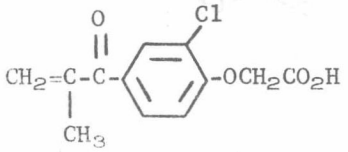
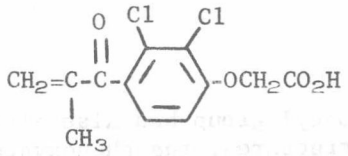
	Diuretic Activity	T 1/2 (min.)
	±	90
	+2	11
	+1	27
	+3	1

Figure 4

Here too, as in the sulfonamides, a tremendous boost in activity was observed, particularly when the chlorine occupied a position ortho to the carbonyl group of the acyl side chain. Not only was biological activity increased but also the rate of the chemical addition of sulfhydryl compounds across the double bond in a model in vitro system. The presence of two chlorines, in positions 2 and 3 of the phenoxyacetic acid, further increased both types of activity. The extension of these observations led to several active diuretic series. Representative compounds are given in Figure 5. The first compound is ethacrynic acid, which has been studied extensively, and is highly effective in animals and in man. In the figures, the relative diuretic activity score is based on sodium excretion in dogs following an intravenous administration of a standard dose and the chemical reactivity toward mercaptoacetic acid, as a model sulfhydryl compound, is recorded as the time in minutes for half reaction at pH 7.4 (T 1/2).

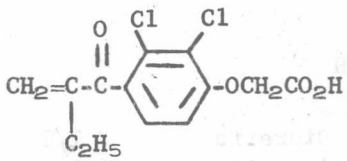
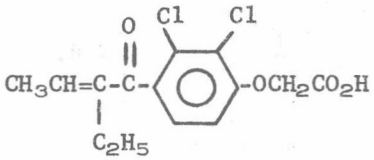
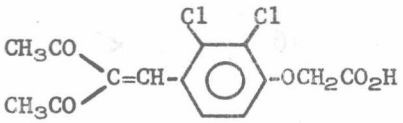
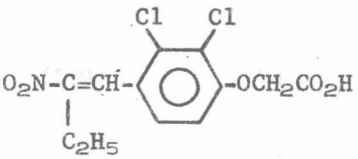
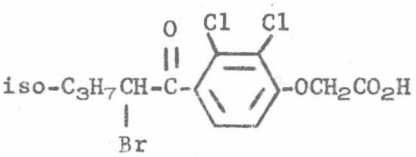
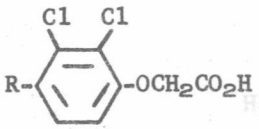
		Diuretic Activity	T 1/2 (min.)
(1)		+6	< 1
	Ethacrynic Acid		
(2)		+5	(210)
(3)		(+7)	2
(4)		+6	2
(5)		+5	(41)

Figure 5

The importance of the presence and position of unsaturation and of halogen in the acyl group is seen in Figure 6. Notable is the weak but real activity of the saturated compound (compound 1). The activity of this compound becomes unequivocal at a dose higher than the standard dose used here for comparison. The introduction of the double bond into the acyl group raises diuretic activity, particularly when it occupies the terminal position (compound 2, ethacrynic acid) which also gives the highest reactivity toward sulfhydryl in vitro. The α-bromo substituent (compound 4) also increased activity; however, not to the level of the unsaturated compounds. But when the bromo substituent is attached to a secondary carbon of the acyl group, as in compound 5, still higher activity results.



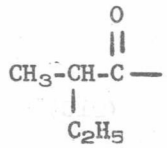
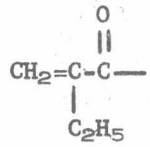
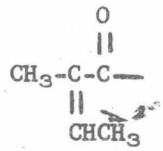
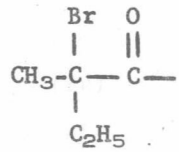
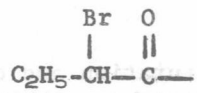
	R	Diuretic Activity	T 1/2 (min.)
(1)		±	N.R.
(2)		+6	< 1
(3)		+2	(97)
(4)		+	21
(5)		+5	9

Figure 6

Figure 7 shows the point of nucleophilic attack in the three series where a high level of diuretic activity is found. From these results, one may conclude that the phenoxyacetic acid structure, properly substituted, possesses inherent diuretic activity which is accentuated either by unsaturation or by an α-halogen substituent that is capable of attack by a nucleophilic group such as a sulfhydryl. When comparison of diuretic and natriuretic activity is made on the basis of the dose size for given response in dogs, the activated-vinylphenoxyacetic acids (Figure 7, type 1) proved to be most active, for example, the diacetylvinyl-dichlorophenoxyacetic acid (compound 3, Figure 5).

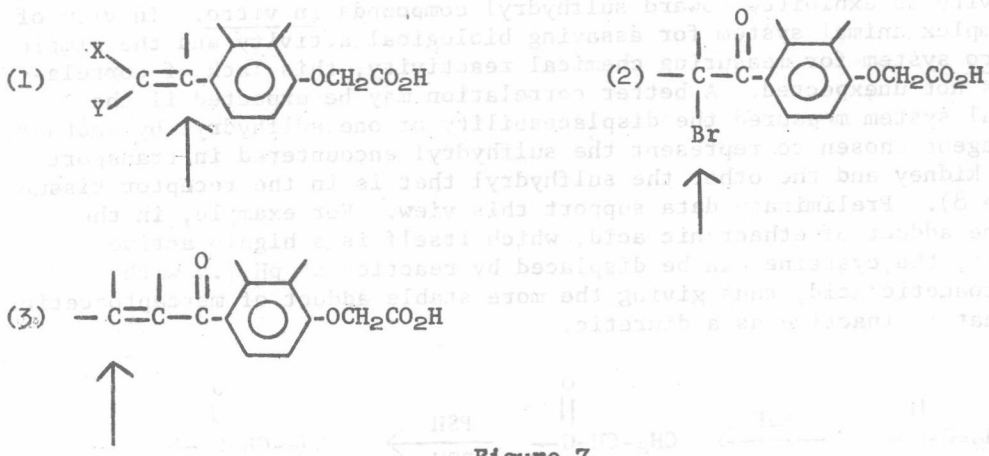


Figure 7

This summary of our results brings us to the question: What is the evidence that the diuretic activity of these mercury-free structures is related to the reaction with a sulfhydryl system or related nucleophilic system? The evidence is mostly circumstantial and must be drawn largely from the extensive studies with ethacrynic acid.

(1) As already noted, maximum diuretic activity is observed only with those structures that are capable of reaction with sulfhydryl.

(2) Ethacrynic acid decreases the protein bound sulfhydryl of the kidney in a manner similar to the mercurials.

(3) Ethacrynic acid reduces the binding of the mercury by kidney tissue.

(4) Ethacrynic acid is excreted in the urine, in large part, as the cysteine adduct in a manner similar to the excretion of mercurials as the cysteine conjugates.

(5) Only those sulfhydryl (or other nucleophilic) adducts of ethacrynic acid are diuretic if they also show in vitro a ready exchange with another sulfhydryl reagent.

From his studies with ethacrynic acid, Cafruny concludes that ethacrynic acid does react with protein-bound sulfhydryl of renal cells. He further states "that the data indicate that ethacrynic acid occupies the same "receptors" and may share the same mechanism of action as the mercurials" and that ethacrynic acid "probably blocks reabsorption of sodium in the same way as mercurials and in most respects is the "non-mercurial mercurial" diuretic it was designed to be".

As can be seen from the data in the figures, there is a lack of quantitative correlation between the biological activity and the chemical

reactivity as exhibited toward sulfhydryl compounds in vitro. In view of the complex animal system for assaying biological activity and the simple in vitro system for measuring chemical reactivity, this lack of correlation is not unexpected. A better correlation may be expected if the chemical system measured the displaceability of one sulfhydryl by another, one reagent chosen to represent the sulfhydryl encountered in transport to the kidney and the other the sulfhydryl that is in the receptor tissue (Figure 8). Preliminary data support this view. For example, in the cysteine adduct of ethacrynic acid, which itself is a highly active diuretic, the cysteine can be displaced by reaction at pH 7.4 with mercaptoacetic acid, thus giving the more stable adduct of mercaptoacetic acid that is inactive as a diuretic.

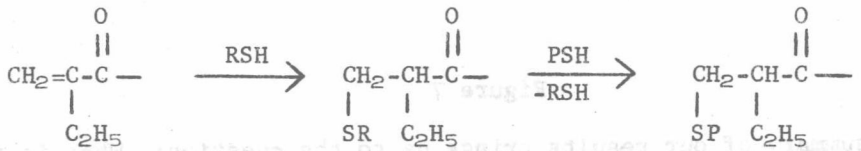


Figure 8

This approach to the design of structures that mimic the diuretic activity of the mercurials has led to the extremely potent compounds that lack certain of the disadvantages of the mercury-containing drugs and may share, in part, a common mechanism of action. However, other desirable attributes of the mercurials have not been reproduced. Ethacrynic acid, the only compound that has had extensive study both in animals and in man, causes potassium loss and uric acid retention and perhaps some disturbance of glucose metabolism although this latter effect appears to be minimal compared to that observed with many diuretics of the sulfonamide class.

To this point in the discussion, biological activity has referred to the response following a standard intravenous or oral dose. For comparison within the series, this is adequate. However, these compounds exhibit a quantitatively different dose response from that of the thiazides. The response to ethacrynic acid is less than for hydrochlorothiazide at the low doses but, at higher doses, the ethacrynic acid response exceeds that obtainable with any dose of chlorothiazide or of any of its relatives. The ethacrynic acid congeners all exhibit a similar dose response curve, showing the higher maximums than the thiazides, and differing within the series only by the position on the dose scale.

During the course of our work that is described here, a novel sulfonamide diuretic, furosemide, was developed in Germany. This compound (4-chloro-N-(2-furylmethyl)-5-sulfamoylanthranilic acid), a sulfonamide that has appreciable carbonic anhydrase inhibitory activity and shows no reaction toward sulfhydryl compounds, exhibits a dose response that is unique among all known sulfonamide diuretics. The response is similar to that of ethacrynic acid with similar high maximum of natriuresis.

However, it, too, causes potassium loss, uric acid retention and an alteration in glucose metabolism. How two compounds, ethacrynic acid and furosemide, of such diverse structures elicit similar biological responses lacks, so far, a chemical interpretation.

The sulfonamide diuretics all trace their origin to the observation that the inhibition of carbonic anhydrase in the kidney, first by sulfanilamide and, later, by the much more potent acetazolamide, leads to excretion of sodium but very little chloride, and large amounts of potassium and bicarbonate. From this beginning have arisen many sulfonamide diuretics of widely different structures that cause more chloride, less bicarbonate and less potassium excretion than the first carbonic anhydrase inhibitors. As this electrolyte excretion pattern improved with structural modifications, the inhibitory activity toward carbonic anhydrase, as measured in vitro, generally decreased markedly. Our investigation of aromatic-disulfonamides and the related thiazides illustrates this trend. A marked improvement in the electrolyte excretion pattern was noted with 5-chloro-2,4-disulfamoylaniline (CDSA)(Figure 9). Activity

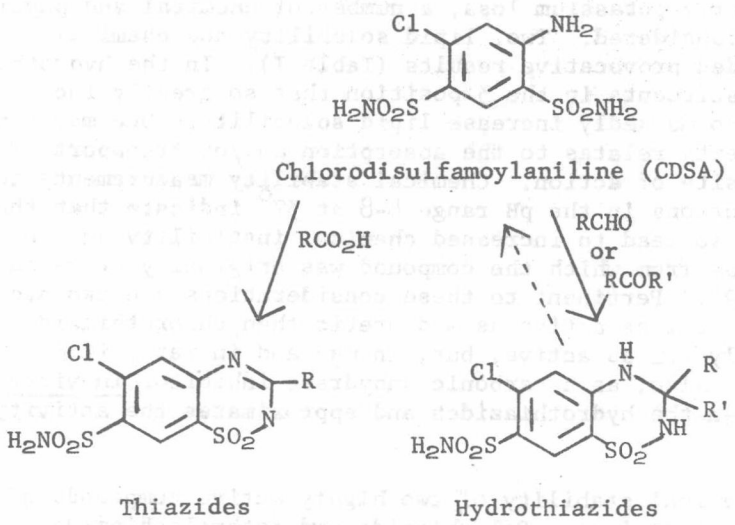


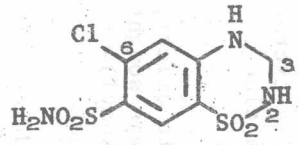
Figure 9

was further improved by cyclization to the thiazides and hydrothiazides. These are saluretic agents causing excretion of approximately equivalent amounts of sodium and chloride, little or no bicarbonate and substantially less potassium than the earlier sulfonamides. These sulfonamides all exhibited, in vitro, considerably weaker inhibition of carbonic anhydrase than acetazolamide; CDSA has approximately 1/60, chlorothiazide 1/40 and the hydrothiazides 1/200 - 1/600 the activity of acetazolamide. Thus, the hydrothiazides possess enzyme inhibitory activity that is only equal to, or even less than, sulfanilamide. Modification of the substituent in the 3-position of the hydrothiazide structure has produced

the greatest increase in diuretic activity. As measured in terms of the amount of compound that is required for a given response, the hydrothiazides range up to 2000 times more active than chlorothiazide. Within the hydrothiazide series, this increased diuretic activity is achieved without a marked change in the relative in vitro enzyme inhibition. These low enzyme inhibitory activities, together with the marked improvement in the electrolyte excretion pattern and the increased potency of the thiazides, generally, raise the question of the relevance of enzyme inhibition to the saluretic activity and to the potassium loss. Nevertheless, the inhibitory activity, although low, is real and all of the compounds contain the unsubstituted-sulfamoyl group (or a group metabolizable to such a group) that has been repeatedly shown to be responsible for enzyme inhibition. Furthermore, over the years, reports have appeared dealing with the action of the sulfonamide (including the thiazide) diuretics on many enzyme systems, but carbonic anhydrase remains the most sensitive to inhibition by these compounds.

In searching for a rationalization of these structural influences and for a chemical basis for the design of compounds to surmount the deficiencies, particularly potassium loss, a number of chemical and physical parameters has been considered. Two, lipid solubility and chemical stability, have yielded provocative results (Table I). In the hydrothiazide series, the substituents in the 3-position that so greatly increase diuretic activity also markedly increase lipid solubility. One may consider that this property relates to the absorption and/or transport of the compound to the site of action. Chemical stability measurements in aqueous buffered solutions in the pH range 4-8 at 37° indicate that these structural changes also lead to increased chemical instability and to hydrolysis to the CDSA from which the compound was originally prepared by cyclization (Figure 9). Pertinent to these considerations are two properties of CDSA. It is less active as a diuretic than chlorothiazide in the dog, possibly only 1/2 as active, but, in man and in rats, it is 2-3 times as active. Also, as a carbonic anhydrase inhibitor, in vitro, it is more active than the hydrothiazides and approximates the activity of chlorothiazide.

However, the chemical stability of two highly active compounds of this series presents exceptions. Polythiazide and methyclothiazide (compounds 4 and 5, Table I) having methyl substituents on the nitrogen in the 2-position exhibited little or no cleavage in vitro to the corresponding CDSA derivative. But, for one of these compounds, polythiazide, a major excretion product in the urine is reported to be the cleaved product, the methyl-CDSA derivative. For the other hydrothiazides, no reliable quantitative data are available on the nature of the form excreted in the urine although detectable cleavage is reported. These results suggest the intervention of in vivo processes and that metabolic cleavage of the benzothiadiazine ring may occur. The incubation of representative hydrothiazides with liver or kidney homogenates in vitro, however, did not show increased cleavage over that observed in the buffer chemical system.



3-Substituent	Relative Diuretic Activity Dog	Man	Lipid Partition[1]	Relative Carbonic Anhydrase Inhibition[2]	Hydrolysis % 24 hrs., 37° pH 4	pH 8
(1) H	3+	10	1.0	0.4	1	13
(2) H (6-CF_3)	3+	10	0.8	0.03	8	6
(3) $C_6H_5CH_2$-(6-CF_3)	4+	100-200	15	0.07	18	68
(4) CF_3CH_2S-CH_2-(2-CH_3)	5+	500	14	1.3	(<5)	0
(5) $ClCH_2$-(2-CH_3)	4+	200	26	1.6	(<1)	0
(6) Cl_2CH-	5+	100-200	4	0.3	45	5
(7) 5-norbornen-2-yl	4+	250	63	0.3	--	--
(8) cyclopentyl-CH_2	5+	1000-2000	19	0.6	37	92
(9) 4'-methyl-spirohexane	5+	100	7	0.7	85	78
Chlorothiazide	2+	1.0	0.3	5	< 1.0	< 1.0
CDSA	(+)	2-3	--	3.4	--	--

[1] octanol - pH 6.5 buffer

[2] sulfanilamide = 1.0

Table I