(C14H16N2O3S·HBr) C, H, N, Br.

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References and Notes

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to the presence of the benzo ring and the nature of the substituents on the benzo ring. Thus, when compounds exist as the open form in solution, they tend to cyclize under dehydration conditions to the thiazinone (for example, XIII), whereas when they are in the cyclic thiazoline form they undergo dehydration to either the thiazole ring (for example, XII, XIX) or the lactone (for example, XVI, XXII, XXV).

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(Vinylaryloxy)acetic Acids. A New Class of Diuretic Agents. 1. (Diacylvinylaryloxy)acetic Acids

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A series of (diacylvinylaryloxy)acetic acids was synthesized and tested in dogs for saluretic and diuretic activity. Several compounds exhibit a high order of activity, the most active being [2,3-dichloro-4-(2,2-diacetylvinyl)phenoxy]acetic acid (3). This compound is about three times as potent as [2,3-dichloro-4-(2-methylenebutyryl)phenoxy]acetic acid (ethacrynic acid) but is qualitatively similar in causing a prompt increase in the excretion of water and in the excretion of sodium and chloride ions in approximately equimolar amounts. Saturation of the double bond of 3 virtually abolishes activity lending support to the hypothesis that the saluresis induced by these compounds, like that of ethacrynic acid, is related at least in part to a chemical reaction with protein-bound sulfhydryl groups. Four mercaptan adducts of 3 were prepared; these probably function as prodrugs in producing saluresis. The adduct with mercaptoacetic acid is as active as 3 itself.

The high saluretic and diuretic activity of ethacrynic acid,^{1,2} [2,3-dichloro-4-(2-methylenebutyryl)phenoxy]acetic acid (I), apparently is associated with the presence of the double bond in conjugation with the carbonyl group. It has been proposed that this compound acts by the binding of renal sulfhydryl-containing enzymes by means of this electrophilic center.^{1,3} This paper is a report of the synthesis and renotropic properties of a series of (diacylvinylaryloxy)acetic acids of general structure II which incorporate a double bond activated toward nucleophilic attack by two carbonyl groups.



Chemistry. The (diacylvinylaryloxy)acetic acids presented in Table I were prepared by a process outlined below involving the piperidine or piperidine acetate catalyzed Knoevenagel condensation of ethyl (formylaryloxy)acetates (III) (Table II) with β -diketones followed by acid hydrolysis of the resulting ethyl (diacylvinylaryloxy)acetates (IV) (Table III).



In an alternate procedure, (2,3-dichloro-4-formylphenoxy)acetic acid, obtained by hydrolysis of the ethyl ester, was condensed under acidic conditions with the cyclic diketones 1,3-indandione and 4-cyclopentene-1,3-dione to yield products 13 and 14.



Geometrical isomers of acids 5 and 12 can exist since

Table I. (2,2-Diacylvinylaryloxy)acetic Acids

				C=	-CH-ArO-CHR(CO2H			
				$\mathbf{R}^2 \mathbf{CO}'$					
No.	-ArO-	R	R'	R ²	Mp, °C	Recrystn solvent	Yield, %	Formula	Analyses
1		н	CH ₃	CH3	172.5-174.5	EtOAc	52	$C_{14}H_{14}O_{5}$	С, Н
2	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	н	CH3	CH,	158-161	<i>i</i> - P rOH	6 8	C ₁₄ H ₁₃ ClO ₅	С, Н
3		н	CH3	CH ₃	184.5-186.5	EtOAc	70	$C_{14}H_{12}Cl_{2}O_{5}$	C, H, Cl
4		CH3	CH,	CH ₃	15 2- 154	Hexane- EtOAc	36	$C_{15}H_{14}Cl_2O_5$	С, Н
5^a		н	CH ₃	C_2H_5	185-186	i-PrOH	8	$C_{15}H_{14}Cl_2O_5$	С, Н
6		н	C_2H_s	C_2H_5	168-170	EtOAc	19	$C_{16}H_{16}Cl_2O_5$	С, Н
7		н	CH3	CH,	168.5-170.5	EtOAc	54	$C_{16}H_{18}O_{5}$	С, Н
8		Н	CH3	CH,	185.5-188.5	EtOAc	48	$\mathbf{C}_{_{18}}\mathbf{H}_{_{16}}\mathbf{O}_{_{5}}$	С, Н
9		Н	CH,	CH3	102-104	Hexane- EtOAc	48	$C_{14}H_{13}ClO_{5}$	С, Н
10	CI	Н	CH ₃	CH3	146.5-149	EtOAc	51	$C_{14}H_{13}ClO_{5}$	С, Н
11		н	CH3	CH,	113.5-115.5	Hexane- EtOAc	29	$C_{14}H_{12}Cl_2O_5$	C, H, Cl
12 ^b		Н	CH3	C ₆ H ₅	170-171	CH ₃ COC ₂ H ₅	80	C ₁₉ H ₁₄ Cl ₂ O ₅	C, H, Cl

^a ¹ H NMR (Me₂SO-d₆) δ 0.88 (3 H, t, CH₃CH₂), 2.40 (3 H, s, CH₃CO), 2.40 (2 H, q, CH₃CH₂), 4.90 (2 H, s, OCH₂), 7.15 (2 H, s, aryl H), 7.78 (1 H, s, vinyl H). ^b ¹ H NMR (Me₂SO-d₆) δ 2.50 (3 H, s, CH₃CO), 4.80 (2 H, s, OCH₂), 6.9-7.9 (7 H, m, aryl H), 8.06 (1 H, s, vinyl H).

these contain differing acyl groups. ¹H NMR spectra show that only one isomer has been isolated in each case and, further, serve in the assignment of structures to these isomers.

The methyl groups of [2,3-dichloro-4-(2,2-diacetyl-vinyl)phenoxy]acetic acid (3) give two singlets, at 2.40 and at 2.15 ppm (Me₂SO-d₆). According to assignments determined by Phillips and Currie,⁴ the singlet at 2.40 must be attributed to the methyl of the acetyl group cis to the ethylenic proton, and the singlet at 2.15 to the methyl in the acetyl group trans to this proton. The resonance of the methyl group of the isomer of [2,3-dichloro-4-(2-acetyl-2-propionylvinyl)phenoxy]acetic acid obtained, i.e., 5, mp 185–186°, occurs as a singlet at 2.40 ppm. It follows then that the acetyl group of 5 is cis to the ethylenic proton so that the structure of 5 is as represented below (Z configuration).

It is reasonable to assign, on steric grounds, the structure shown below to the obtained isomer, mp 170-171°, of [2,3-dichloro-4-(2-acetyl-2-benzoylvinyl)phenoxy]acetic acid (12) where benzoyl is cis to the ethylenic hydrogen. This assignment is supported by comparison of the chemical shift of the ethylenic proton of 12 (8.06 ppm) to that of the analogous proton in 3 (7.73 ppm) which is cis to an acetyl group. This downfield displacement of the ethylenic proton signal in 12 of over 0.3 ppm may be ascribed to the location of the proton cis to the benzoyl group (E configuration).



Compounds containing sulfhydryl groups add readily to the double bond of the (diacylvinylaryloxy)acetic acids at slightly basic pH. Four sulfhydryl adducts of 3 are presented in Table IV. Hydrogenation of the double bond

No	- 4 = 0-	P	OHC-A	$ArO-CHRCO_2C_2H_5$	Viold %	Formula	Analwasa
NO.	-AIO-		Mp, C	Recrystn solvent	rield, %	Formula	Analyses
20 ^a	~~~~~	Н	$41 - 42^{b}$	EtOH	76	$\mathbf{C}_{11}\mathbf{H}_{12}\mathbf{O}_{4}$	
21	CI ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Н	59-6 1.5	Cyclohexane-benzene	57	$C_{11}H_{11}ClO_4$	С, Н
22		Н	92-93	Cyclohexane	94	$\mathbf{C}_{11}\mathbf{H}_{10}\mathbf{Cl}_{2}\mathbf{O}_{4}$	C, H, Cl
23 ^c	CI O	CH ₃	67.5-68.5	Cyclohexane	61	$C_{12}H_{12}Cl_{2}O_{4}$	d
24	H3C CH3	Н	56-57	Cyclohexane	50	$C_{13}H_{16}O_{4}$	С, Н
25		н	99-100	EtOH	68	$C_{_{15}}H_{_{14}}O_{_{4}}$	е
26	CI - 0-	Н	58-60	BuCl	38	$\mathbf{C}_{11}\mathbf{H}_{11}\mathbf{ClO}_{4}$	С, Н
27	ci	Н	55-56	Cyclohexane-benzene	71	$\mathbf{C}_{11}\mathbf{H}_{11}\mathbf{ClO}_{4}$	С, Н
28	c ic i	н	52-53	EtOH-H ₂ O	85	$C_{11}H_{10}Cl_2O_4$	C, H, Cl

Table II. Ethvl (Formylaryloxy)acetates (III)

^a D. N. Robertson, J. Org. Chem., 21, 1190 (1956). ^b Lit. 42-42.5°. ^c Obtained from 2,3-dichloro-4-hydroxybenzaldehyde and ethyl 2-bromopropionate; reaction time 16 h. ^d C: calcd, 49.51; found, 49.97. H: calcd, 4.15; found, 4.57. Cl: calcd, 24.36; found, 23.78. ^e C: calcd, 69.75; found, 69.18. H: calcd, 5.46; found, 5.58.

of 3 gave [2,3-dichloro-4-(2-acetyl-3-oxobutyl)phenoxy]acetic acid (19), a compound incapable of functioning as a saluretic-diuretic agent by the proposed sulfhydrylbinding mechanism.



Saluretic-Diuretic Effects and Structure-Activity Relationships. Compounds 1-19 were tested in dogs for their saluretic and diuretic properties. Results obtained on iv administration are presented in Table V along with the reaction rates of the unsaturated compounds 1-12 with the sulfhydryl-containing reagent mercaptoacetic acid. Test results on oral administration of selected compounds are given in Table VI.

The (diacylvinylaryloxy)acetic acids and their mercaptan adducts are saluretic and diuretic agents; in many cases, the compounds are very potent. Their activity is qualitatively similar to that of I^2 in causing a prompt increase in the excretion of water and of sodium and chloride ions to an approximately equal degree. Potassium ion excretion is also increased but less markedly than sodium excretion. The most highly active compounds (2, 3, 5, 17) are 3-5 times as potent as ethacrynic acid, iv or po, and produce comparable peak effects of electrolyte and water excretion.

Some structure-activity relationships can be discerned by examining the effects of structural changes on the saluretic action of the highly active 3. Activity is highly dependent on the extent and nature of ring substitution: removal of the 2-chlorine atom (to give 2) reduces activity; removal of both chlorine atoms (1) nearly abolishes activity; replacement of the chlorine atoms of 3 by methyls (7) or a fused benzene ring (8) reduces activity to 1/4-1/2that of 3. The para relationship between the ether oxygen and vinyl group is essential, for the ortho and meta isomers exhibit only minimal activity (9-11). Replacement of one acetyl group by propionyl (5) may slightly enhance activity; replacement of one acetyl group by benzoyl (12) or both by propionyl (6) strongly reduces saluretic activity.

The activities of these compounds do not correlate with the data on the kinetics of their reaction with mercaptoacetic acid (Table V). It is likely that mercaptoacetic acid is a poor model for the functionally important renal sulfhydryl-bearing structures with which these agents may react in producing saluresis. The idea that sulfhydryl

Table III. Ethyl (2,2-Diacylvinylaryloxy) acetates

DIGO

			R.O	UU \				
				C = CH	I-ArO-CHRCC	$O_2C_2H_5$		
No.	-ArO-	R		CO R ²	Mp, °C	Recrystn solvent	Yield, %	Formula ^a
29		Н	CH ₃	CH,	85.5-86.5	Cyclohexane-benzene	63	$C_{16}H_{18}O_{5}$
30	~~~~	Н	CH3	CH3	55.5-57	Cyclohexane- <i>i</i> -PrOH	45	$C_{16}H_{17}ClO_5$
31		Н	CH3	CH3	123-125	Cyclohexane-EtOH	90	$C_{16}H_{16}Cl_2O_5$
32		CH,	CH3	CH,	110-112	Hexane-EtOAc	87	$C_{17}H_{18}Cl_{2}O_{5}$
33 ^b	CI → CI → CI →	Н	CH3	C_2H_5	Oil		с	
34 ^d		Н	C_2H_5	C_2H_s	Oil		с	
35	H3C CH3	Н	CH ₃	CH,	83-92 ^e		45	
	\square							
36	~~~~	н	CH ₃	CH,	118-120.5	EtOH	40	$C_{20}H_{20}O_5^{f}$
37	c	н	CH3	CH3	Oil		с	
38	c1	Н	CH ₃	CH3	82.5-84.5	Cyclohexane-benzene	77	$C_{16}H_{17}ClO_5$
39	ci	н	CH,	CH3	85-87	Hexane–EtOAc	70	$C_{16}H_{16}Cl_2O_5$
40 ^g	CIO	н	CH ₃	C_6H_5	98.5-100.5	EtOH	72	$C_{21}H_{18}Cl_2O_5$

^a All compounds with formula given were analyzed for C and H and values were within $\pm 0.4\%$ of calculated values. ^b Obtained by condensation of 22 with 2,4-hexanedione; reaction time 72 h. No determination was made of stereochemical composition. ^c The yield of crude oily product appeared to be quantitative. ^d Obtained by condensation of 22 with 3,5-heptanedione; reaction time 72 h. ^e The product which crystallized was used without purification or analysis. ^f C: calcd, 70.57; found, 70.12. H: calcd, 5.92; found, 6.12. ^g See Experimental Section. This ester was assigned the E configuration (see text).

Table IV. Sulfhydryl Adducts of 3

		CH ₃ CO CHCH- CH ₃ CO SR			
No.	R	Mp, $^{\circ}$ C	Recrystn solvent	Yield, %	Formula ^a
15 16 17	-CH ₃ -CH ₂ CH ₂ OH -CH ₂ CO ₂ H	115-117 100-103 164-166	Benzene Cyclohexane CH ₃ CN	62 39 36	$\begin{array}{c} C_{15}H_{16}Cl_{2}O_{5}S\\ C_{16}H_{18}Cl_{2}O_{6}S\\ C_{16}H_{18}Cl_{2}O_{7}S\end{array}$
18	CO2H	164-166	EtOAc	26	$\mathbf{C}_{21}\mathbf{H}_{18}\mathbf{Cl}_{2}\mathbf{O}_{7}\mathbf{S}$

^a All compounds were analyzed for C and H and values were within $\pm 0.4\%$ of calculated values.

binding is involved at least in part with the mechanism of action of these compounds is supported by the observation that saturation of the double bond of 3 to produce 19, which cannot add -SH, reduces saluretic activity to a very low level.

The four adducts (V) of 3 with mercaptans (15-18)

exhibit good saluretic and diuretic activity, the adduct with mercaptoacetic acid being as active as 3 itself when administered either iv or po. These adducts may function as prodrugs of 3 by an exchange reaction with receptor sulfhydryl-containing protein (R^2SH) as seen below, thus forming the same product (VI) with the receptor as when

Table V. Iv Activity in Dogs

		μe (cont	quiv/min excrete rol period/drug p	Urine vol, ^b	SH binding. ^c	
Compd	Dose, ^a mg/kg	Na ⁺	K⁺	Cl-	control/drug	$t_{1/2}$, min
1	10.0	60/336	67/106	16/254	1/4	<1
2	1	21/1318	104/232	4/1588	1/11	2
3	1	26/1615	28/104	26/1730	1/19	2
	0.1	36/984	14/87	14/1096	1/9	
4	1	41/847	27/71	3/696	2/8	<1
5	1	4/2025	7/13	2/1936	2/16	
6	1	23/676	12/68	14/815	2/7	5
7	1	38/673	5/58	3/711	1/6	17
8	1	12/393	45/104	5/580	1/4	8.5
9	25	9/42	30/40	2/41	1/1	4
10	10	29/94	12/20	3/26	2/2	3
11	25	68/244	41/50	22/139	2/3	< 2
12	10	14/583	25/105	6/683	1/6	3
13	10	82/118	61/44	8/29	2/2	
14	10	24/213	21/94	6/180	3/4	<1
15	1	32/571	18/78	3/565	2/6	
16	1	79/405	30/80	27/447	2/5	
17	1	79/1569	58/178	28/1628	1/4	
	0.1	46/1421	51/173	5/1991	2/13	
18	1	44/802	37/124	5/913	1/7	
19	10	4/198	6/35	4/241	1/3	
Ethacrynic acid	1	32/188	46/52	11/244	4/6	<1
-	10	48/2989	22/194	64/3324	1/22	

^a The compounds were administered as Na salts in H_2O . ^b The procedure is described in the Experimental Section. Control values are averages of data from two 15-min clearance periods prior to dosage. Response values are averages of data from two consecutive 15-min periods during which Na⁺ excretion was maximal; these periods usually occurred between 15 and 45 min after dosage. Changes in glomerular filtration rate during these experiments were minimal and did not show consistent trends. The data are from single representative experiments. ^c The time in minutes required for one-half of a standard amount of test compound to react with an excess of mercaptoacetic acid at pH 7.4 and 25° in solution in DMF-phosphate buffer. The procedure is analogous to that described by Duggan and Noll.¹⁰

Table VI. Oral Activity in Dogs^a

	Dose	No. of	me	quiv/ ccrete	Urine		
Compd	mg/kg	dogs	Na⁺	K⁺	Cl-	vol/6 h	
3	0.4	8	34	6	36	762	
	1.0	8	30	7	37	492	
7	5	3	34	6	38	480	
17	0.4	5	7	3	8	575	
	1.27	6	27	6	34	750	
Ethacrynic	1	10	21	5	26	560	
acid	3	8	37	7	42	571	
Placebo		35	2	1	2	180	

^a Procedure is described in the Experimental Section.

3 is used alone. Evidence has been presented⁹ that the analogous mercaptan adducts of ethacrynic acid function as prodrugs.



Experimental Section

Melting points were taken on a Thomas-Hoover capillary apparatus and are uncorrected. The ¹H NMR spectra were taken on a Varian T-60 instrument. Where analyses are indicated only by symbols of the elements, the analytical results for these elements are within 0.4% of the theoretical values.

The following hydroxybenzaldehydes and hydroxynaphthaldehydes were prepared according to the cited references: 2chloro-4-hydroxybenzaldehyde,⁵ 2-chloro-5-hydroxybenzaldehyde,⁶ 3,5-dichlorosalicylaldehyde,⁵ 2,3-dimethyl-4-hydroxybenzaldehyde,⁷ and 4-hydroxy-1-naphthaldehyde.⁸ 4-Hydroxybenzaldehyde and 5-chlorosalicylaldehyde are commercially available.

2,3-Dichloro-4-hydroxybenzaldehyde. Chloroform (208 g, 1.75 mol) was added dropwise to a stirred mixture of Ca(OH)₂ (280 g, 3.78 mol), Na₂CO₃ (320 g, 3.02 mol), 2,3-dichlorophenol (142.6 g, 0.875 mol), and H₂O (2 l.) at 60–70° during 2 h. The mixture was heated at 70° for 1 h, then cooled, and acidified with concentrated HCl. The product was extracted into EtOAc and dried over MgSO₄. Solvent evaporation left the product as a solid residue which was recystallized from toluene to yield 37.8 g (23%) of the aldehyde, mp 177–182°. Two recrystallizations from CH₃CN gave the analytical sample, mp 184–185°. Anal. (C7H₄Cl₂O₂) C, H, Cl.

Ethyl (Formylaryloxy)acetates. General Method. Table II. A mixture of the appropriate hydroxybenzaldehyde (0.5 mol), ethyl bromoacetate (133.6 g, 0.8 mol), K_2CO_3 (166 g, 1.2 mol), and DMF (375 ml) was stirred and heated at 55–60° for 2.5 h. The mixture was then poured into 500 ml of water. The product ester separated as an oil which slowly crystallized. It was collected and recrystallized.

Ethyl (2,2-Diacylvinylaryloxy)acetates. General Method for Compounds 29-39 in Table III. A mixture of the appropriate ethyl (formylaryloxy)acetate (0.15 mol), β -diketone (0.165 mol), piperidine (3 ml), and ethanol (200 ml) was warmed at 45° to obtain a clear solution (ca. 25 min) and then allowed to stand at room temperature for 24 h. When the product was a solid, the solution was chilled to precipitate the product was an oil, the solution was diluted with water; the oily product was taken up in Et₂O and used in the next step without purification.

Ethyl (E)-[2,3-Dichloro-4-(2-acetyl-2-benzoylvinyl)phenoxy]acetate (40). A solution of ethyl (2,3-dichloro-4formylphenoxy)acetate (5.5 g, 0.02 mol), 1-phenyl-1,3-butanedione (3.25 g, 0,02 mol), and piperidine acetate (100 mg) in toluene (50 ml) was heated at reflux under a constant water separator for 1.5 h until the theoretical amount (0.36 ml) of water was collected. Toluene was removed by vacuum distillation and the semicrystalline residue was recrystallized (see Table II). (2,2-Diacylvinylaryloxy)acetic Acids. General Method. Table I. A solution of the ethyl (2,2-diacylvinylaryloxy)acetate (0.15 mol) in HOAc (250 ml) and 5% aqueous HCl (100 ml) was heated at 100° for 40 min and then cooled and diluted with H₂O (350 ml). The solid product that separated was dissolved in 5% NaHCO₃ solution. The solution was filtered and acidified with concentrated HCl. The precipitated product was purified by recrystallization.

(2,3-Dichloro-4-formylphenoxy)acetic Acid. A solution of ethyl (2,3-dichloro-4-formylphenoxy)acetate (50 g, 0.151 mol) and NaHCO₃ (25.5 g, 0.3 mol) in water (900 ml) and ethanol (450 ml) was heated at 100° for 2 h and then cooled. The sodium salt of the product which precipitated was dissolved in boiling H₂O and the solution acidified with concentrated HCl. The precipitated product acid weighed 40 g (94%), mp 204–210°. Recrystallization from CH₃CN gave the analytical sample, mp 210–212°. Anal. (C₉H₆Cl₂O₄) C, H, Cl.

[2,3-Dichloro-4-(1,3-dioxo-2-indanylidenemethyl)phenoxy]acetic Acid (13). A solution of (2,3-dichloro-4-formylphenoxy)acetic acid (2.5 g, 0.01 mol), 1,3-indandione (1.5 g, 0.01 mol), and H₂SO₄ (0.2 ml) in acetic acid (50 ml) was heated at 65° for 3 h and then cooled. The product precipitated and was recrystallized from EtOH to yield 1.5 g (39%) of acid, mp 229-232°. Anal. (C₁₈H₁₀Cl₂O₅) C, H; C: calcd, 57.32; found, 57.75.

[2,3-Dichloro-4-(1,3-dioxocyclopent-4-en-2-ylidenemethyl)phenoxy]acetic Acid (14). A stirred suspension of (2,3-dichloro-4-formylphenoxy)acetic acid (1.0 g, 4 mmol) in BF₃·Et₂O (20 ml) was treated with a solution of 4-cyclopentene-1,3-dione (0.39 g, 4 mmol) in Et₂O (20 ml). The mixture was refluxed for 5 h. The yellow solid which was present was washed with Et₂O and dried: wt 0.60 g (46%); mp 227-229° dec. Two recrystallizations from EtOH gave the analytical sample: yellow crystals; mp 217-219° dec. Anal. (C₁₄H₈Cl₂O₅) C, H, Cl.

[2,3-Dichloro-4-(2-acetyl-3-oxobutyl)phenoxy]acetic Acid (19). Compound 3 (6.6 g, 0.02 mol) was dissolved in 50 ml of EtOH and hydrogenated at 25° and 1 atm over 1.0 g of a 10% Ru on charcoal catalyst. When 0.02 mol of H₂ had been absorbed, the catalyst was filtered off and EtOH evaporated. The residue was recrystallized twice from EtOAc to obtain 2.0 g (30%) of crystalline product, mp 156–158°. The ¹H NMR spectrum shows the keto/enol ratio to be approximately 4:1 (in Me₂SO-d₆ at room temperature): ¹H NMR (enol) δ 1.97 (6 H, s, CH₃), 3.64 (2 H, s, CH₂Ph); ¹H NMR (keto) δ 2.16 (6 H, s, CH₃), 3.10 (2 H, d, CH₂Ph), 4.26 (1 H, t, CHAc₂). Anal. (C₁₄H₁₄Cl₂O₅) C, H.

Sulfhydryl Adducts of 3. General Method. Table IV. Compound 3 (13.2 g, 0.04 mol) and the mercaptan (0.06 mol, except 0.04 mol in the case of 2-mercaptobenzoic acid) were dissolved in saturated NaHCO₃ solution at 25°. After 10 min, the solution was acidified to congo red with concentrated HCl. The product acid separated as an oil which slowly crystallized. It was collected by filtration and recrystallized to constant melting point.

Biological Procedures. Intravenous Diuretic Assay. Conditioned female mongrel dogs, weighing approximately 20 kg, in the postabsorptive state were given 500 ml of water orally 1 h before induction of anesthesia with sodium pentobarbital (30 mg/kg iv). After induction of anesthesia, each dog was prepared with an indwelling bladder catheter, and at this time creatinine (4 g as a 10% solution) was administered sc 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 of phosphate/kg) was given iv as a priming injection before commencing clearance studies, and 3.0 ml/min of an isoosmotic pH 7.4 phosphate buffer containing 4% mannitol (6.9 mg of phosphate/min) was infused during the experiment. At the start of 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 iv as a priming injection and replicate 15-min clearances were obtained for a period of 2 h. Electrolytes and creatinine were assayed by standard Auto-Analyzer methods.

Oral Diuretic Assay. 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 h 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 h. Urine volumes were measured, and aliquots were analyzed for sodium, potassium, and chloride content by standard methodology. Values are reported in Table VI as geometric means.

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