[(Aminomethyl)aryloxy]acetic Acid Esters. A New Class of High-Ceiling Diuretics. 3. Variation in the Bridge between the Aromatic Rings To Complete Mapping of the Receptor[1]

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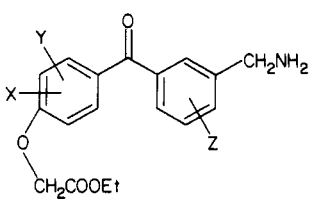
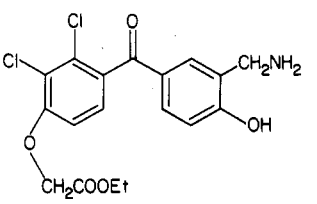
Continued structural evaluation of the [(aminomethyl)aryloxy]acetic ester diuretics has produced a series of compounds in which the functional group that bridges the two aromatic rings has been varied. Diuretic screening of these analogues in rats indicates that the keto group can be effectively replaced with an ether or thio ether function with a slight increase in potency, whereas the methylene and sulfoxide linking groups lead to diminished saluretic potency. Replacement with either $-SO_2-$, $-COCO-$, $-CH_2O-$, $-CONH-$ or direct bond results in a loss of activity. Although the series was designed according to QSAR criteria, the traditional linear free-energy properties of these compounds do not correlate with diuretic potency. However, conformational analysis of the series by potential energy calculations indicates that all active compounds have an accessible conformation that matches the bridge atom–carboxylate distance of the very potent dihydrobenzofuran analogue **56**. Conformational calculations of several compounds in which the aminomethyl group was varied suggests that the active conformation is probably a low-energy conformation. Consideration of rotation about the bridge could not distinguish between two possible orientations of the aminomethyl ring in the active conformation. However, there is a quantitative negative linear correlation between diuretic potency and the protrusion into space of the group that bridges the two aromatic rings.

Earlier papers[2] in this series have described a new class of [4-aroylphenoxy]acetic ethyl esters in which a 3-aminomethyl substituent on the aroyl function plays a pivotal role in modulating the diuretic/saluretic potency. These compounds, represented generically by structure **1**, were shown to possess a high-ceiling diuretic profile in rats, dogs, and monkeys. Molecular features that were found to enhance oral diuretic activity for this series include (1) a 3-(aminomethyl)-4-hydroxybenzoyl substituent attached to the phenoxyacetate ring, (2) chloro substituents at the 2- and 3-position of the oxyacetate aromatic ring, and (3) a prodrug moiety (such as an ester) that liberates the carboxylate of the oxyacetate side chain.



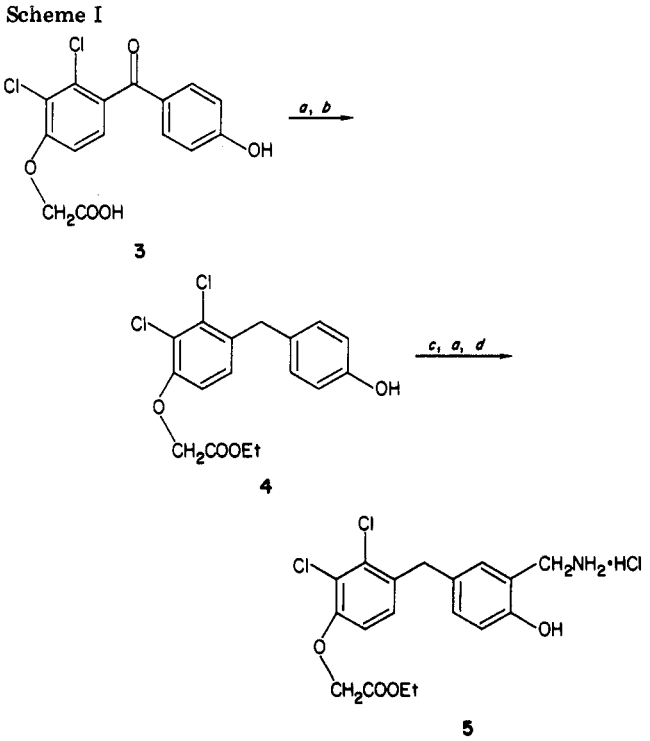
1



2

Structurally, the salicylamine-substituted phenoxyacetic ester **2** contains the essential pharmacophoric elements of two separate classes of diuretics: the (dichlorophenoxy)-acetic acids[3] and the 2-(aminomethyl)phenols[4] (e.g., MK-447). The present study was undertaken in an attempt to evaluate the importance of the functional group connecting these two pharmacophores. Among the (4-aroylphenoxy)acetate derivatives described earlier,[2] the substitution pattern indicated in **2** elicited optimal saluretic effects and was, therefore, chosen for demonstrating the effects of the variable linking group. Strictly speaking, our conclusions apply only to this substitution in the salicylamine portion of the molecule. Because one objective of these studies was to understand the relationship between physical and biological properties, we designed the set of compounds with this in mind. Specifically, we attempted to prepare analogues with a wide uncorrelated variation in electronic and hydrophobic properties. The standard deviation of π is 0.83 and that of σ is 0.37. While not

Scheme I



[a] EtOH, H_2SO_4. [b] $NaBH_4$, CF_3COOH. [c] $ClCH_2CONHCH_2OH$, HOAc, H_2SO_4. [d] HCl, EtOH, Δ.

(1) Portions of this work were presented in September 1982 at the 184th National Meeting of the American Chemical Society [see "Abstracts of Papers", 184th National Meeting of the American Chemical Society, Kansas City, MO, 1982; American Chemical Society: Washington, DC, 1982] and related work in February 1983 at the Drug Information Association Meeting, *Drug Inf. J.* **1984**, *18*, 95–113.

(2) (a) Lee, C. M.; Plattner, J. J.; Ours, C. W.; Horrom, B. W.; Smital, J. R.; Pernet, A. G.; Bunnell, P. R.; El Masry, S. E.; Dodge, P. W. *J. Med. Chem.*, in press. (b) Plattner, J. J.; Fung, A. K. L.; Smital, J. R.; Lee, C. M.; Crowley, S. R.; Pernet, A. G.; Bunnell, P. R.; Buckner, S. A.; Sennello, L. T. *J. Med. Chem.*, in press.

(3) Cragoe, E. J., Jr. In "Diuretics, Chemistry, Pharmacology and Medicine"; Cragoe, E. J., Jr., Ed.; Wiley-Interscience: New York, 1983; Chapter 4.

(4) Smith, R. L. In "Diuretics. Chemistry, Pharmacology and Medicine"; Cragoe, E. J., Jr. Ed.; Wiley-Interscience: New York, 1983; Chapter 5.

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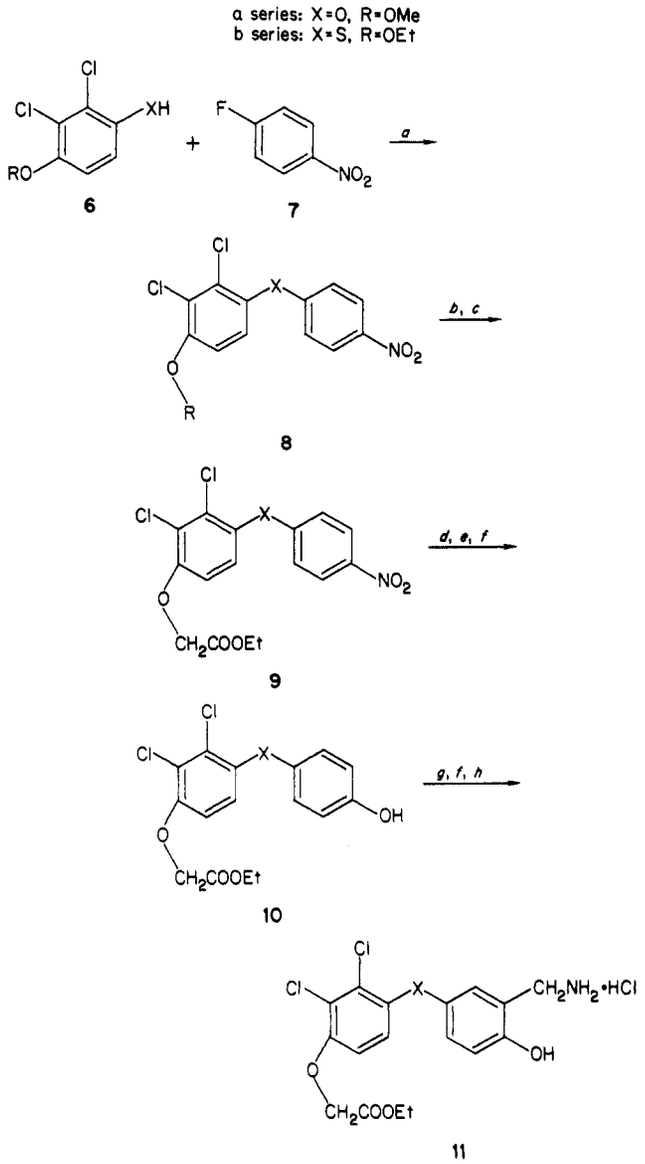
Scheme II



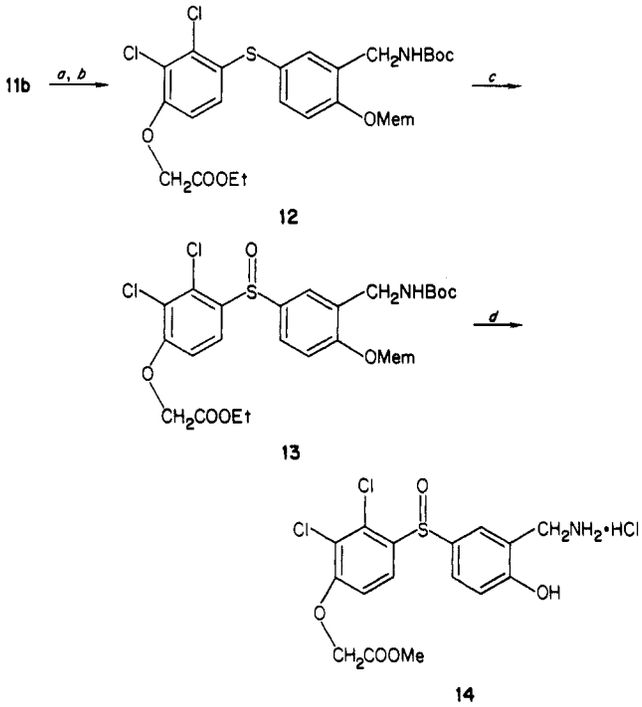
[a] NaH, DMF. [b] HBr, HOAc. [c] $BrCH_2COOEt$, K_2CO_3. [d] H_2/Pd-C. [e] $NaNO_2$, H_2SO_4, H_2O, Δ. [f] EtOH, H_2SO_4. [g] $ClCH_2CONHCH_2OH$, HOAc, H_2SO_4. [h] EtOH, HCl, Δ.

optimal, they are acceptable.[5] The R^2 between π and the σ is 0.51, again acceptable but not optimal.

The first paper in this series established the requirement for the basic nitrogen functionality and that the substituents on the nitrogen must be small. We interpreted this to mean that the binding site for the amino group of these compounds on the target biomolecule must be of limited size.[2a] In a similar manner our second study demonstrated the necessity for the carboxylate function and that there are spatial constraints at this position also.[2b] With these two sets of results in mind, we designed the compounds described in this paper to vary length of the bridge and hence the distance between the amino and carboxylate groups.

Chemistry. The wide range of functional character for each of the linking groups in the 4-substituted phenoxyacetates described required that we develop a unique synthetic pathway for each analogue (Schemes I-IX). The compounds prepared in this study are listed in Table I,

Scheme III



[a] Di-*tert*-butyl dicarbonate. [b] Mem-Cl, $(C_2H_5)_3N$. [c] Iodobenzene dichloride, pyridine, H_2O. [d] HCl, MeOH.

where cross-reference is also made to the appropriate reaction schemes.

The first key synthetic strategy for essentially all of the target compounds was to develop the appropriate methodology in order to generate the requisite bridge between the salicylamine and (dichlorophenoxy)acetate residues. This was accomplished by Friedel–Crafts chemistry for the diketone and sulfone compounds, by a nucleophilic displacement reaction for the ether-linked analogues (**11a**, **11b**, and **28**), and by a simple acylation for benzanilide **38**. The diphenylmethane congener **5** was obtained from a benzophenone precursor[2a] by reduction with $NaBH_4$ in trifluoroacetic acid[6] while sulfoxide **14** was generated by selective oxidation of an appropriately blocked thio ether precursor. The biphenyl analogue **52** was prepared from a substituted phenylacetaldehyde derivative by ring annelation with Nazarov's reagent[7] followed by aromatization with NBS. Entry into the rigid benzisoxazole nucleus **41** was achieved by a base-catalyzed cyclization of a ketoxime intermediate.

The second key transformation in this study involved the introduction of the aminomethyl substituent adjacent to the phenolic hydroxyl. For most of the compounds this was accomplished by means of the Tscherniac–Einhorn reaction[8] followed by hydrolysis of the amidomethyl adduct. Careful control of the stoichiometry and reaction conditions was necessary in order to obtain the desired mono addition product. In general, the phenols containing an electron-donating substituent at the para position required the use of an $HOAc–H_2SO_4$ reaction medium while the electron-deficient systems required concentrated H_2SO_4 as the reaction solvent. Utilization of the Tscherniac–Einhorn procedure for the methyleneoxy-

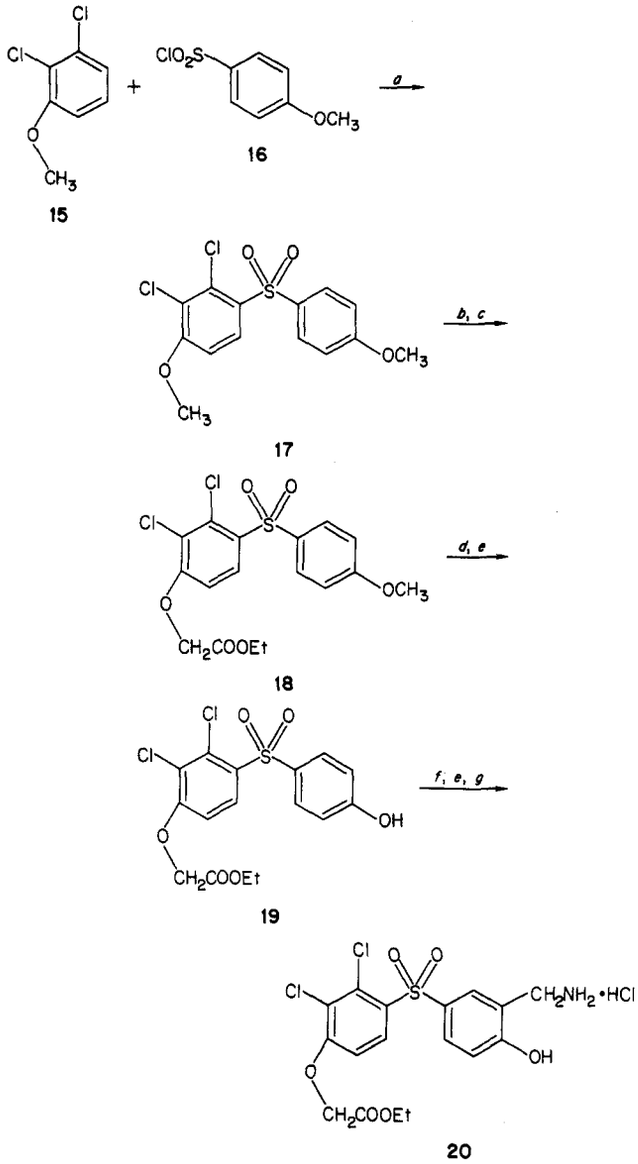
(5) Martin, Y. C.; Panas, H. N. *J. Med. Chem.* **1979**, *22*, 784.

(6) Gribble, G. W.; Leese, R. M. *Synthesis* **1977**, 172.

(7) Nazarov, I. N.; Zavyalov, S. I. *Zh. Obshch. Khim.* **1953**, *23*, 1703. Konst, W. M.; Witteveen, J. G.; Boelens, H. *Tetrahedron* **1976**, *32*, 1415.

(8) Zaugg, H. E.; Martin, W. B. *Org. React.* **1965**, *14*, 52.

Scheme IV



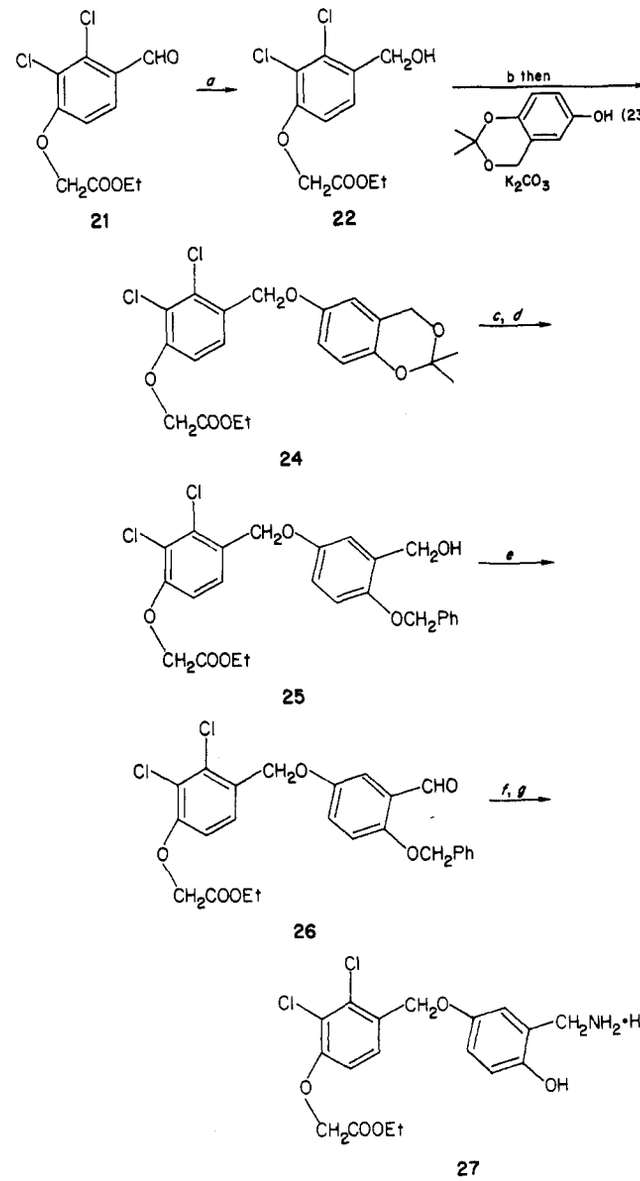
[a] $AlCl_3$, CH_2Cl_2, 10 °C. [b] $AlCl_3$, CH_2Cl_2, Δ. [c] $BrCH_2COOEt$, K_2CO_3. [d] HBr, HOAc, Δ. [e] EtOH, H_2SO_4. [f] $ClCH_2CONHCH_2OH$, H_2SO_4. [g] HCl, EtOH, Δ.

linked analogue **27** gave a complex mixture of products, and therefore an alternative pathway to the salicylamine was developed (Scheme V). Alcohol **25**, prepared as outlined in the reaction scheme, was oxidized to the protected salicylaldehyde **26** with PCC.[9] The corresponding oxime then gave **27** by catalytic hydrogenation over Pd–C. Attempted oxidation of **25** without the benzyl ether protecting group resulted in cleavage of the methyleneoxy bridge and the formation of a quinone-like material. Elaboration of the salicylamine function in an analogue **52** followed a similar course (Scheme IX), proceeding from a salicylaldehyde intermediate to final product by reduction of the corresponding oxime.

Scheme VI deserves a final comment. Attempted alkylation of phenol **30** with ethyl bromoacetate/K_2CO_3 gave a mixture of products due to competing alkylation at the carbon adjacent to the ketone. In order to reduce the acidity of the α-keto protons, the corresponding ketal was prepared. This compound, upon alkylation, gave exclusive

(9) Piancatelli, G.; Scettri, A.; M'Auria, M. *Synthesis* **1982**, 245.

Scheme V



[a] $NaBH_4$. [b] $SOCl_2$, $CHCl_3$. [c] HOAc, H_2O. [d] $PhCH_2Br$, K_2CO_3. [e] PCC. [f] $NH_2OH \cdot HCl$, pyridine. [g] H_2/Pd-C.

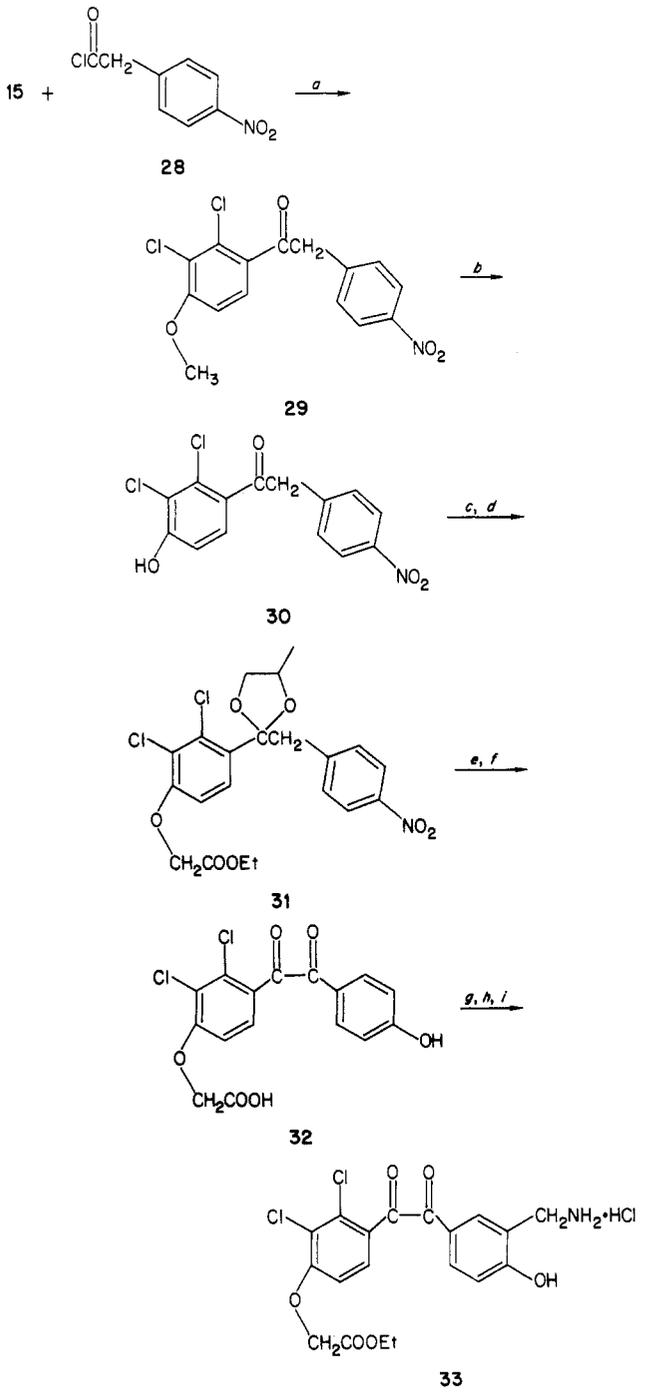
reaction at the phenol. Subsequently, a fortuitous oxidation occurred during the diazotization/hydrolysis procedure and led to the formation of benzil **32**. This unexpected oxidation most probably involved a nitrosation at carbon followed by hydrolysis of the resulting nitrite ester to give diketone **32**.

Results

A. Saluresis–Diuresis in Rats. Pharmacologic evaluation of the target compounds in this study is limited to rats, since previous work[2a] within this series showed a good correlation between activity in rats, dogs, and monkeys. Dose–response experiments measuring the urine volume, Na^+, K^+, and Cl^- concentration were used to evaluate the saluretic properties of the target compounds. The relative natriuretic potencies of these derivatives are represented as an ED_2 as described in Table I. Although only the Na^+ excretion was used to determine these values, the urine volume and Cl^- excretion generally paralleled that of the Na^+, and thus either of these parameters could also be used for relative potency comparison.

As can be seen from the data in Table I, the keto group can be effectively replaced with an ether or thioether

Scheme VI

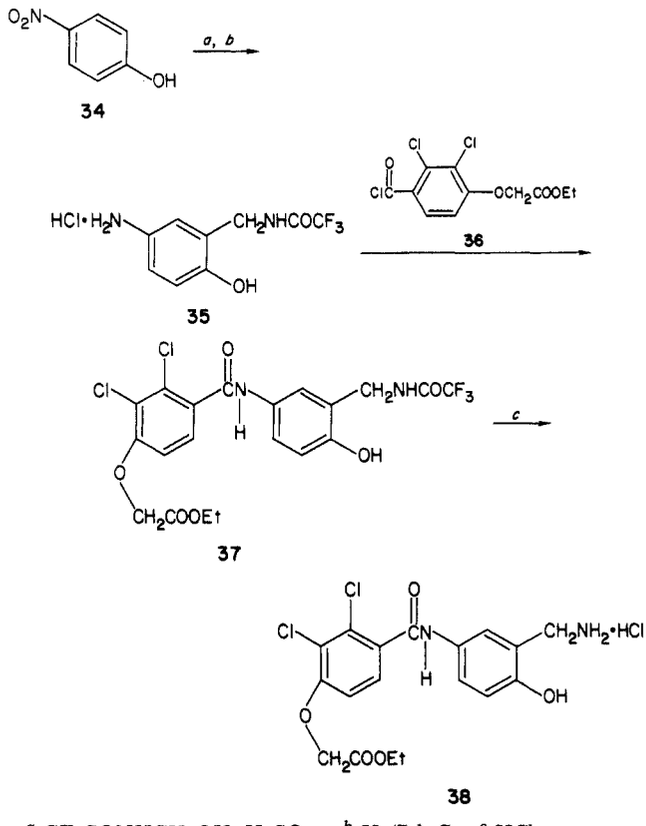


[a] $AlCl_3$, CH_2Cl_2. [b] HBr, HOAc. [c] $HOCH(CH_3)CH_2OH$, *p*-TsOH, Δ. [d] $BrCH_2COOEt$, K_2CO_3. [e] H_2/Pd-C, HCl. [f] $NaNO_2$, H_2O, H_2SO_4, Δ. [g] $ClCH_2CONHCH_2OH$, H_2SO_4. [h] EtOH, H_2SO_4. [i] HCl, EtOH, Δ.

function with a slight increase in potency, whereas the methylene and sulfoxide linking groups lead to diminished saluretic activity. Replacement with either $-SO_2-$, $-COCO-$, $-CH_2O-$, $-CONH-$, or direct bond, however, results in a loss of activity.

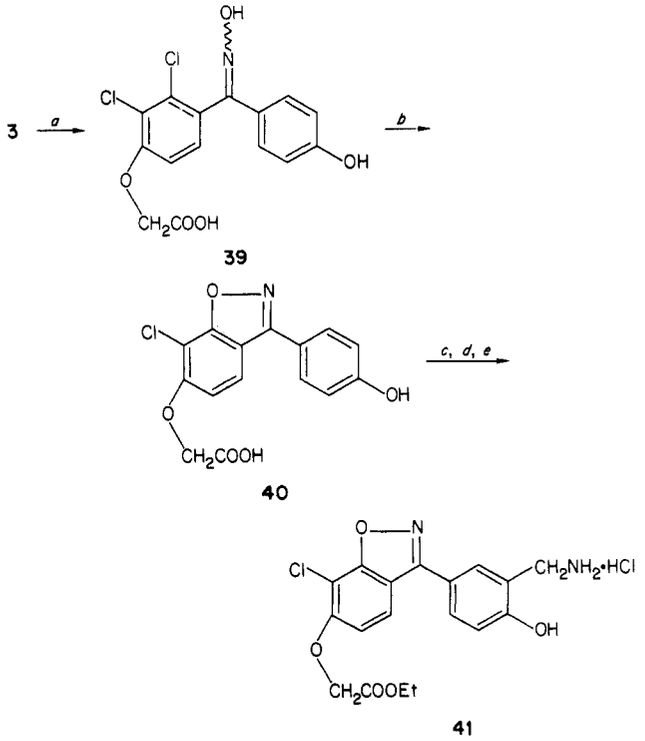
B. Physical Properties of the Compounds. Nonlinear regression analysis fit of the change in ultraviolet spectrum of compound **2** with pH shows that the compound has two pK_a values, 6.38 and 10.6. Since the largest increase in wavelength of maximum absorbance occurs with the lower pK_a value, it was concluded that this ionization involves loss of the proton from the phenol of the cationic form to produce the zwitterion. This proposed

Scheme VII



[a] $CF_3CONHCH_2OH$, H_2SO_4. [b] H_2/Pd-C. [c] HCl, EtOH, Δ.

Scheme VIII

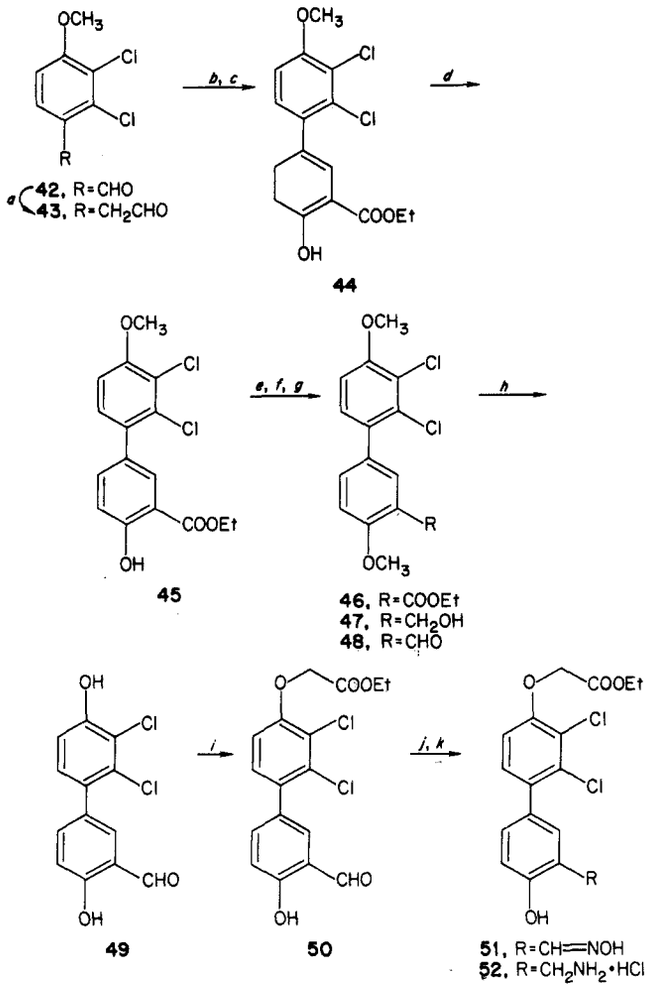


[a] NH_2OH·HCl, pyridine. [b] KOH, EtOH. [c] $ClCH_2CONHCH_2OH$, H_2SO_4. [d] EtOH, H_2SO_4. [e] HCl, EtOH, Δ.

ionization pattern is shown in Scheme X.

The pK_a values for the ionization of the phenol and pH 7.0 distribution coefficients of some of the compounds are listed in Table II. Also listed in Table II are literature

Scheme IX



[a] Darzen's reaction. [b] *N*-(Trimethylsilyl)morpholine, *p*-TsOH. [c] Nazarov's reagent. [d] NBS, benzoyl peroxide. [e] CH_3I, K_2CO_3. [f] B_2H_6. [g] PCC. [h] BBr_3. [i] $BrCH_2COOEt$, K_2CO_3. [j] $NH_2OH \cdot HCl$. [k] H_2-Pd/C.

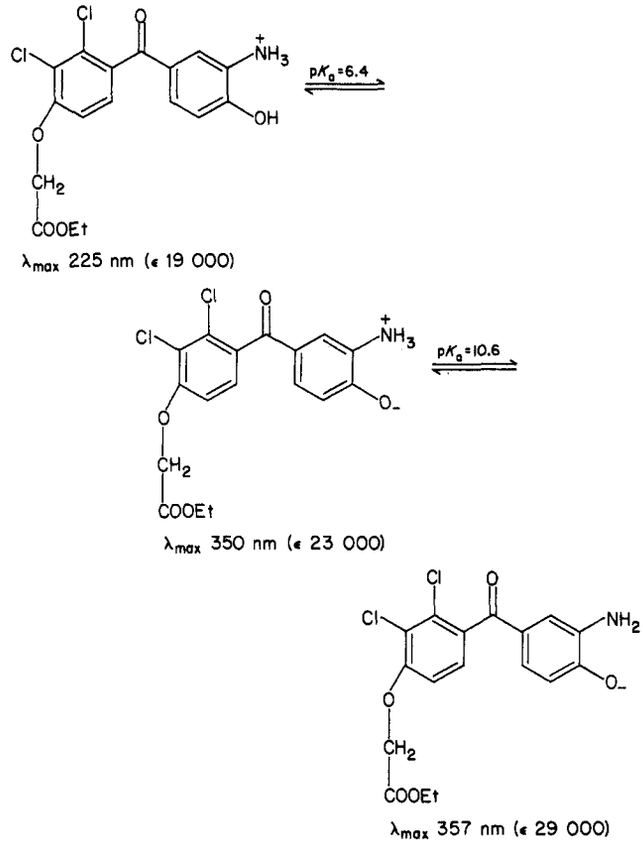
values for the substituent constants for the compounds. The equation that relates pK_a to Hammet σ constants is the following:

$$pK_a = 8.47\ (\pm 0.05) - 2.48\ (\pm 0.09)\ \sigma \qquad (1)$$

$$R^2 = 0.996,\ s = 0.077,\ n = 5$$

For this equation the usual Hammett constants were used for compounds containing the $-CH_2-$, $-O-$, and $-SO_2-$ bridges as well as for compound **53**[2b] that has a >C=O bridge but lacks the two chloro substituents. However, compound **2**, which contains both chloro substituents, has a pKa of 6.38. In order for it to fit the relationship in Equation 1, it was necessary to use its σ^- value--a sustituent constant that includes the effect of direct resonance between the >C=O and the phenolate anion. Greater direct resonance interaction of the ketone in this compound with the phenolate anion is substantiated by the observation that the extinction coefficient of the phenolate form of compound **2** is 1.5 times that of the phenol, whereas the corresponding change in extinction coefficient of compound **53** upon ionization is only 1.1 times. We conclude that the chlorine atom in **2** prevents the >C=O from being coplanar with the oxyacetate aromatic ring. As a consequence it has relatively less resonance interaction with this ring and more resonance interaction with the aminophenol ring.

Scheme X



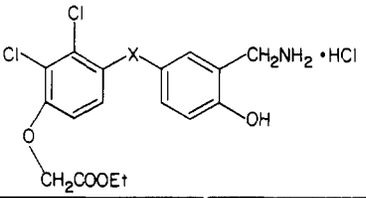
Hammett σ values and/or the Hansch–Fujita π values are not related to either the ED_2 values of the active compounds or to the classification of a compound as active or inactive. For example, the most potent bridge analogue, compound **11a**, has a σ value of –0.03 and a π value of 2.08, whereas compound **52** is inactive in spite of a σ value of 0.0 and a π value of 1.96. Thus, we examined other physical properties to explain the structure–activity relationships.

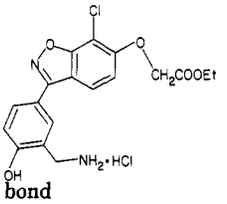
C. Relationship between Biological Activity and Conformational Properties. Because the traditional linear free-energy properties do not correlate with potency, we suspected that the conformational properties are important. The validity of the parameters used for the conformational energy calculations were verified by comparison with the crystal structure of tienilic acid.[10] The conformation of **2** that corresponds to the crystal conformation of tienilic acid in rotation about the C(Cl)–C–C(O)–C is less than 0.5 kcal/mol above its calculated global minimum. The other ketone torsional angle is 15° larger for compound **2**. This difference is probably due to the lack of a hydrogen atom on the sulfur and to the larger ring angles in the thiophene. The former removes a steric interaction with the oxygen of the ketone and the latter decreases the ortho–ortho hydrogen interactions between the two rings. Figure 1 shows a stereopair of the comparison of tienilic acid in the crystal conformation and **2** in the conformation in which the C(Cl)–C–C(O)–C bond has been rotated to the local minimum that matches tienilic acid.

Most of the active compounds in Table II have eight rotatable bonds. If each were rotated in 10° increments

(10) Carpy, A.; Goursolle, A.; Leger, J-M. *Acta Crystallogr., Sect. B* **1980**, *36*, 1706.

Table I. 4-Substituted (2,3-Dichlorophenoxy)acetates



no.	reaction scheme	X	mp, °C	yield,[a] %	formula[b]	rat ED_2,[c] mg/kg
2		CO[d]	218–221	35[d]	$C_{18}H_{18}Cl_3NO_5$	2.5
5	I	CH_2	220–222	25	$C_{18}H_{20}Cl_3NO_4$	7.5
11a	II	O	198–201	22	$C_{17}H_{18}Cl_3NO_5$	0.8
11b	II	S	203–205	26	$C_{17}H_{18}Cl_3NO_4S \cdot {}^1/_2H_2O$	1.0
14	III	SO[e]	223–225 dec	30	$C_{16}H_{16}Cl_3NO_5S$	16.3
20	IV	SO_2	229–230	30	$C_{17}H_{18}Cl_3NO_6S$	inactive
27	V	CH_2O	227–229	80[f]	$C_{18}H_{20}Cl_3NO_5 \cdot H_2O$	inactive
33	VI	COCO	238–240 dec	28	$C_{19}H_{18}Cl_3NO_6$	inactive
38	VII	CONH	228–230	90[g]	$C_{18}H_{19}Cl_3N_2O_5 \cdot H_2O$	inactive
41	VIII		257–260	24	$C_{18}H_{18}Cl_2N_2O_5$	35.0
52	IX	bond	244–246	71[f]	$C_{17}H_{18}Cl_3NO_4$	inactive

[a] These yields are for the amidomethylation/hydrolysis except where otherwise noted. [b] All compounds gave satisfactory C, H, and N analyses. [c] The natriuretic potency of the compounds listed in the above table is reported as an ED_2. This is the oral dose in mg/kg necessary to produce an excretion of 2 mequiv of Na^+/kg in the rat urine during the 4-h period after dosing. Compounds reported as inactive showed a Na^+ excretion no different from the control value at the high dose of 100 mg/kg. Details of the test protocol are described in ref 2. [d] Reference 2a. [e] Compound **14** is a methyl ester. [f] Yield for oxime formation and hydrogenation. [g] Yield for hydrolysis of trifluoroacetyl group.

Table II. Physical Properties of Compounds

no.	X	pK_a	σ^a	log D^b	π^c	size,[d] Å
2	CO	6.38 ± 0.07	0.88[e]	1.41 ± 0.01	1.05	2.10
5	CH_2	8.73 ± 0.05	−0.09	1.66 ± 0.03	2.01	2.50
11a	O	8.55 ± 0.05[f]	−0.03	1.82[f]	2.08	1.70
11b	S		0.00[g]		2.32	2.15
14	SO		0.49[g]		0.27[h]	
20	SO_2	6.67 ± 0.07	0.70	1.25 ± 0.01	0.27	3.60
27	CH_2O		−0.15[i]		2.50	
33	COCO					
38	CONH		−0.19		0.49	
41			0.33[j]		1.32[k]	3.20
52	bond		−0.01		1.96	
53	C=O[l]	7.34 ± 0.06	0.43	0.30 ± 0.01	(1.05)	

[a] Hammett σ value for XC_6H_5: Martin, Y. C., "Quantitative Drug Design"; Marcel Dekker: New York, 1978. [b] Octanol–pH 7.0 phosphate buffer distribution coefficient measured at room temperature. [c] Calculated π value from Martin, Y. C., "Quantitative Drug Design"; Marcel Dekker: New York, 1978. [d] The width calculated as described in the text. [e] σ^- rather than σ (0.43). [f] Measured on the analogue for which the oxyacetate was replaced on OCH_2CH_2OH. The log P is corrected for the observed difference between the OCH_2COOEt and OCH_2CH_2OH (log D = 0.03) analogue of compound **2**. [g] Estimated from the methyl rather than the phenyl analogue. The σ value for $SO_2C_6H_5$ is 0.70 whereas that for SO_2CH_3 is 0.72. [h] Estimated from π $SOCH_3$ (−1.58), SO_2CH_3 (−1.63), and $SO_2C_6H_5$ (0.27). [i] Estimated from OMe (σ = −0.27) and OC_6H_5 (σ = −0.03). [j] Estimated from benzoxazoyl-2-yl (0.33), CH=NC_6H_5 (0.42), and 3-furyl (0.25). [k] Estimated from compound **2** (1.05) plus the difference between the aromatic π value of CHO (−0.65) and CH=NOH (−0.38). [l] The analogue in which the Cl's are replaced by H.

for energy calculations we would investigate 8^{36} energies. Fortunately, all of these do not need to be evaluated. The reason for this is that there are three rather distant regions of the molecule: the aminomethyl side chain, the bridge, and the oxyacetate side chain. The contribution to the energy of each region is essentially independent of the conformation (and energy contribution) of the other two regions. Note, however, that the *distance* between the essential carboxylate and the ammonium groups does depend on seven of these eight torsion angles.

Rotation about the Aminomethyl Group and about the Phenol. Structure–activity studies reported earlier showed that the (dimethylamino)methyl analogue **54** and the α-aminoethyl analogue **55** are both active compounds.[2a] We propose that the receptor-bound conformation must be one common to these molecules and **2**. For **2** there are no forbidden rotations about the aminomethyl side chain. However, both **54** and **55** show a definite restriction of rotation. Figure 2 shows the calculated low-energy conformations of the aminomethyl groups of **2**, **54**, and **55**. The conformations are similar; all three compounds show a definite preference for the nitrogen to be out of the plane of the aromatic ring. The energy to be gained by forming a tighter hydrogen bond between the amine and phenolic hydroxyl is not enough to overcome the unfavorable steric contacts. Because neither **54** nor **55** can easily rotate such that the nitrogens exactly overlap, and because any proposed electrostatic interactions between these molecules and the receptor would not be strongly distance dependent, we decided to do calculations and comparisons on the other

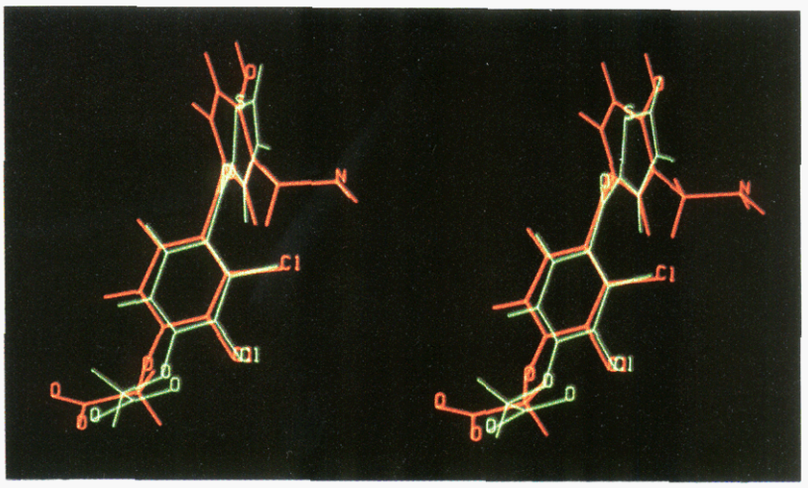


Figure 1. Stereoview of the superposition of the crystal structure of tienilic acid (green) and the corresponding low-energy conformation of **2** (red).

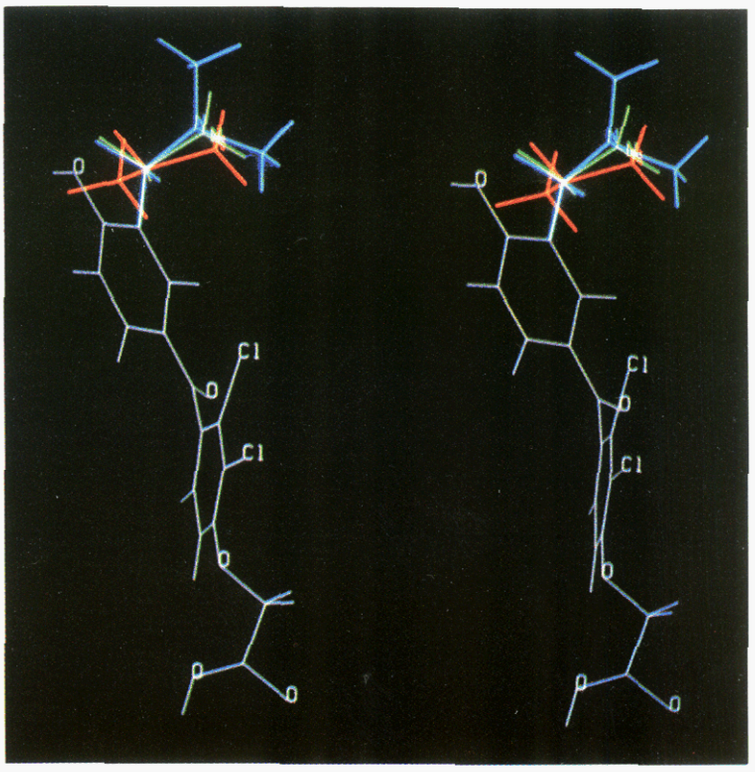
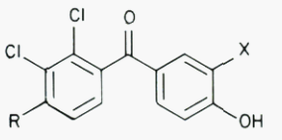
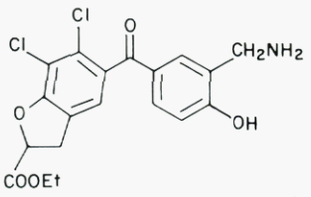


Figure 2. Stereoview of the superposition of the low-energy conformation of the aminomethyl side chain of **55** (blue), **2** (green), and **54** (red).

two regions of the molecules with the aminomethyl group in its low-energy conformation.





56

54, R = OCH_2COOEt; X = CH_2NMe_2
55, R = OCH_2COOEt; X = $CH(Me)NH_2$
57, R = $OCH(Me)COOEt$; X = CH_2NH_2
58, R = $OC(Me)_2COOEt$; X = CH_2NH_2
59, R = CH_2CH_2COOEt; X = CH_2NH_2

Rotation about the Oxyacetate Side Chain. The dihydrobenzofuran analogue **56** is 30 times more potent than the noncyclic analogue **2**.[2b] This increase in potency also applies to the carboxylates when administered intravenously. From this observation we propose that **56** successfully mimics the receptor-bound conformation of the oxyacetate side chain of **2** and the other analogues. Indeed, because it is active at all means that it can assume the "active" conformation. Accordingly, we carefully modeled the conformation of **56** with the molecular mechanics program MM2.[11] Although this compound has two low-energy conformations, they differ only very slightly in the steric relationship between the carboxylate and the dichlorophenyl ring. Therefore, we choose the conformation of slightly lower energy for comparison. We calculated the conformational energy maps for **2**, the various analogues in which the ring chlorine atoms had been replaced,[2a] and for compounds **57**,[2b] **58**,[2b] and **59**.[2b] Although none of the compounds superimposes exactly with the rigid analogue, in every case investigated it takes less than 2.0

(11) Allinger, N.; Yuh, Y. H. *QCPE* **1980**, 395.

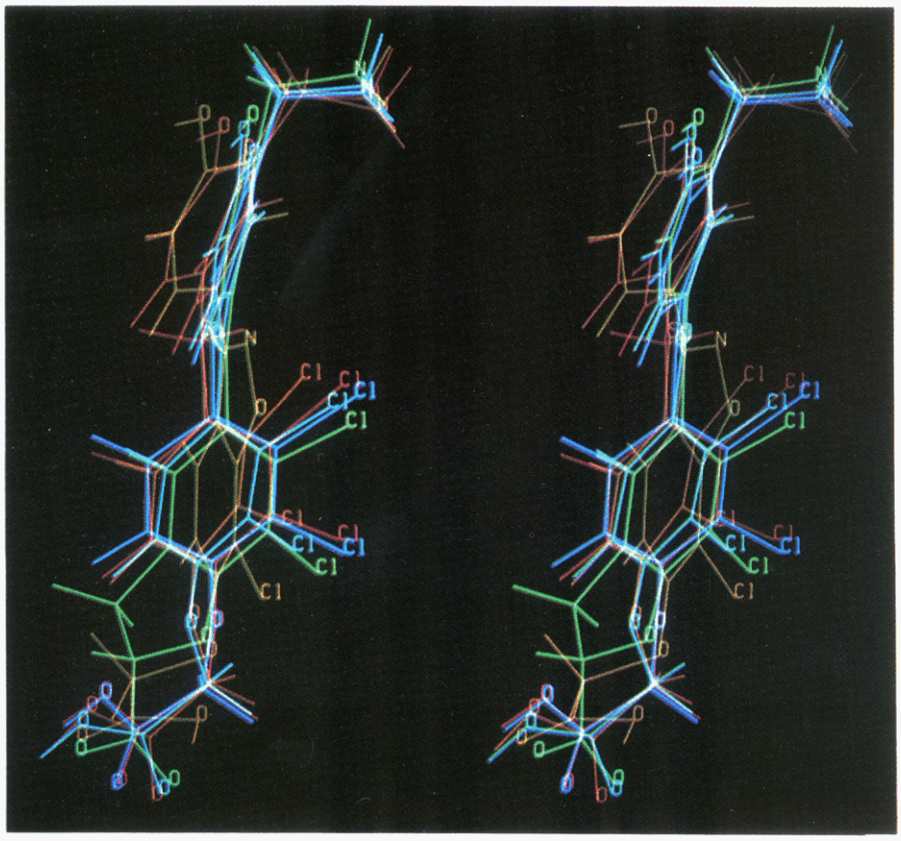


Figure 3. Stereoview of the superposition of all active bridge-substituted analogues in a low-energy conformation. Note that the oxyacetate side chains of the dihydrofuran (green) and benzisoxazole (purple) analogues do not perfectly align with the other analogues.

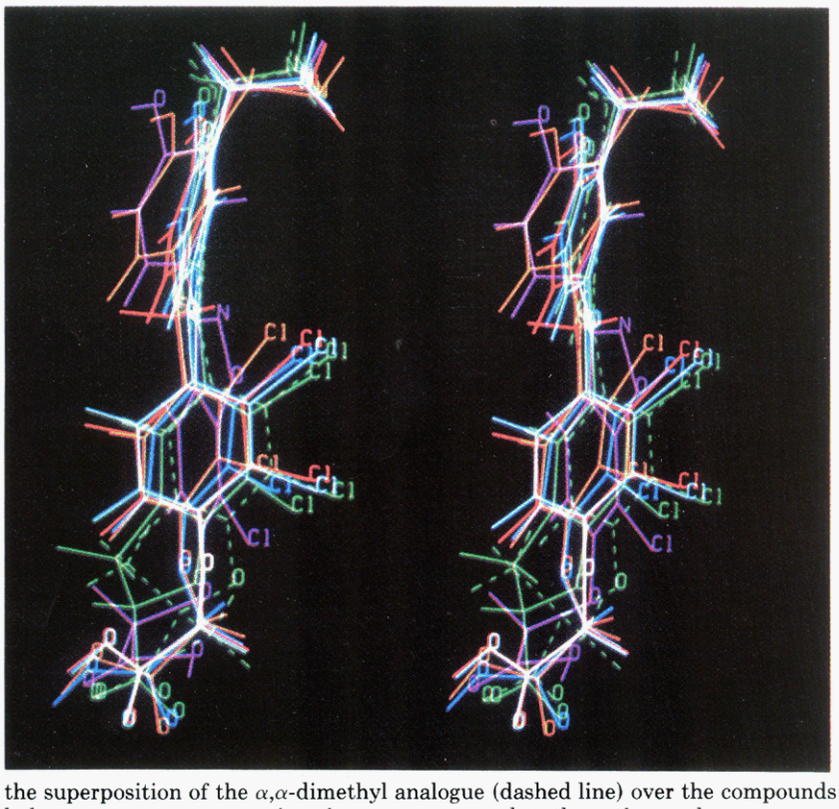


Figure 4. Stereoview of the superposition of the α,α-dimethyl analogue (dashed line) over the compounds in Figure 3 (solid lines). Note that the added methyl groups occupy new regions in space compared to the active analogues.

kcal/mol to rotate from the minimum-energy conformation to one in which the carbon atom of the carboxylate and that of the ketone bridge overlap that of the rigid analogue. The superposition of the active flexible analogues with the rigid analogue is shown in Figure 3 (the conformation about the bridge is discussed below). In Figure 4 is shown the superposition of these compounds plus analogue **58**. It can be seen that the inactive analogue **58** occupies a different region in space than any of the active analogues and so it is not unexpected that it is inactive.

Rotation about the Bridging Atom. Finally, the conformation of the analogues about the bridging atoms was explored. We postulated that variations in the bridge atoms serve to position the two rings in such a way as to

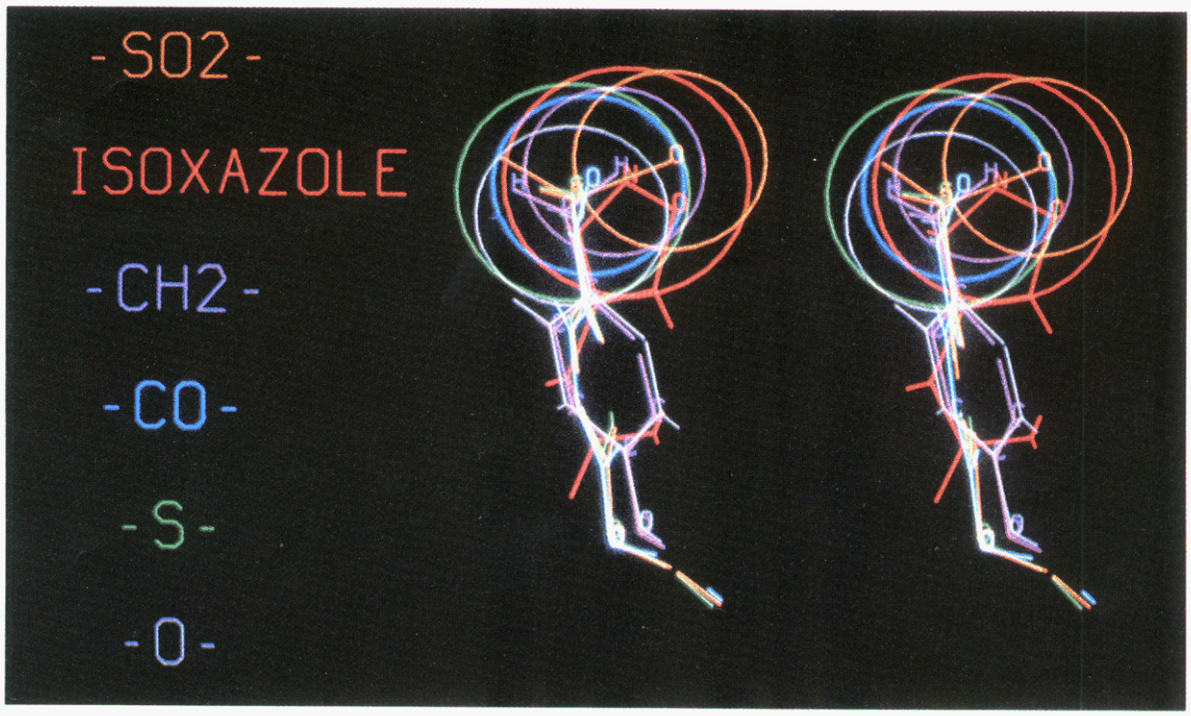


Figure 5. Stereoview of the superposition of the bridge analogues clipped to show the bridge only. The circles represent the van der Waals radius of that atom of the bridge that penetrates farthest to the top of the figure.

regulate the distance between the key atoms and hoped to demonstrate the required distance with our calculations. In order to investigate this hypothesis, we fixed the aminomethyl group in its low-energy conformation and the oxyacetate side chain of the compounds in the conformation that best mimics that of **56** and then calculated the energy of rotation about the bridge. From these energy values we calculated a Boltzmann probability. At the same time we recorded the distance between the carboxylate carbon and the amino nitrogen atoms. These calculations showed that, for every active analogue, the distance between the nitrogen and carbon has a finite probability of being at most distances between 9.5 and 13.0 Å. The analogues prepared do not allow us to decide what is the required distance.

It was at this point that we prepared the biphenyl analogue **52**. For this compound the possible distance is restricted to be less than 11.2 Å. If it had been active we would have a better idea of the active conformation of the compounds. However, the compound is essentially inactive. Since it occupies a different region in space near the bridge between the two aromatic rings than does any of the active analogues, we cannot be sure if it is inactive because it has the wrong distances between the carboxylate and the nitrogen or if it is inactive because of unfavorable steric interactions with the receptor.

Because one of the angles about the bridge is fixed by cyclization, analogue **41** is quite different conformationally from the other bridge analogues. Compound **41** has two low-energy conformations that are at the bottom of rather steep energy wells. The first has the aminomethyl pointed toward the carboxylate (a C–N distance of 10.3 Å) and the other has it pointed away from the carboxylate (a C–N distance of 13.0 Å). Although we have no direct information as to which of these is closest to the receptor-bound conformation, we have arbitrarily chosen the latter to show in Figure 3. The flexible analogues have three energy minima that contain a conformation with ca. 12.8 Å C–N distance. One of these is absent in two analogues. From the remaining two we chose the one that has the best match of total surfaces with **40** to show in Figure 3. It is our tentative proposal of one possible conformation of these molecules when bound to the target biomolecule. The compounds were superimposed by minimizing the sum of squared differences between each analogue and **41**. The carboxylate carbon atom, the bridge atom, and the carbon atom of the aminomethyl side chain were used for the fit. The latter atom was chosen in preference to the nitrogen atom because of the flexibility around the aminomethyl chain discussed above. Analogue **41** clearly is different in shape from the other compounds, so the fact that it is quite low in potency is not inconsistent with this figure. Analogues **33** and **38** also have conformations with ca. 10.3 and 13.0 Å N–O distance. However, in these conformations they occupy new volume not occupied by active compounds.

The information presented above can be used to propose why the sulfone is inactive. First notice that the order of activity of these ring analogues is **11a** > **11b** > **2** > **5** > **41** > **20**. There appears to be a general trend for more bulky bridging groups to result in lowered diuretic potency. For example, compare the –O– and $-CH_2-$ compounds (**11a** and **5**), which differ by 10 times in potency. Since in section B we ruled out electronic or hydrophobic effects on potency, the difference between these two compounds may be due to the steric repulsion between the receptor and hydrogen atoms of the CH_2 group.

Figure 5 shows the superposition of the sulfone analogue over the active bridge analogues. Notice that its oxygen atoms penetrate even more into the space that the hydrogen atoms of the CH_2 analogue just start to touch. In order to quantify this observation, we compared the location in space of the van der Waals surface of the molecule along the S–O bond axis. The circles in Figure 5 illustrate this. The numbers are listed in Table II. Regression analysis of this data resulted in the following equation:

$$\log 1/c = 2.07\ (\pm 0.49) - 1.13\ (\pm 0.21)\ \text{size}$$

$$R^2 = 0.908,\ s = 0.233,\ n = 5$$

In this equation, c is the ED_2, R^2 is the squared correlation

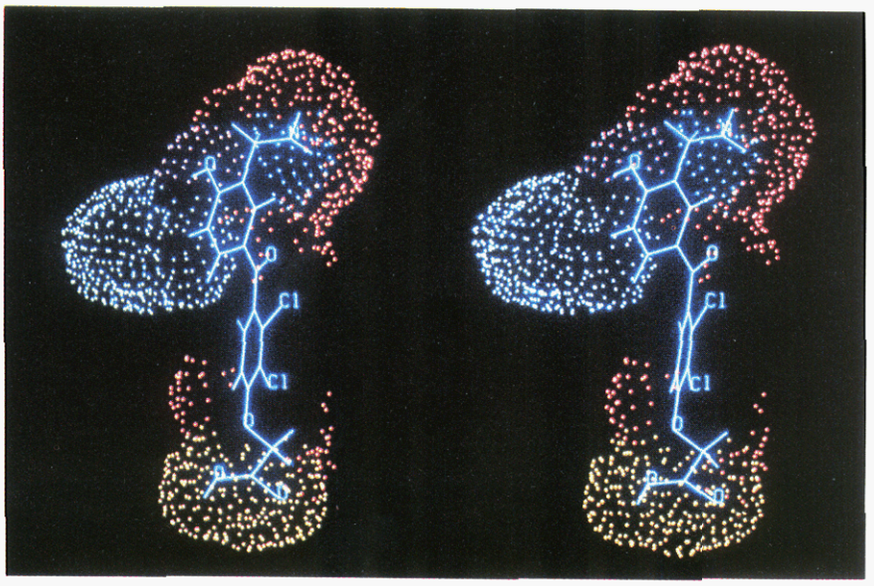


Figure 6. A summary of the regions explored in the receptor cavity and their chemical properties. The yellow region represents the proposed anionic site; the dark blue, the cationic site; the red, areas that are limited in size (i.e., for which larger substituents result in inactivity); the purple, a hydrophilic site; and the light blue, a hydrophobic site. For those regions in space not enclosed by a surface, we have no information on size or chemical properties. The color and shape of the aminophenol binding site was based on eq i. I_3 is an indicator variable equal to 1.0 if position 3 is CH_2NH_2 and 0.0 if it is $CH_2N(CH_3)_2$.

$$\log 1/C = -1.64\ (\pm 0.12) + 0.59\ (\pm 0.11)\ I_3 - 0.94\ (\pm 0.12)\ \pi_4 + 0.48\ (\pm 0.08)\ \pi_5$$

$$R^2 = 0.95,\ s = 0.15,\ n = 10 \qquad \text{(i)}$$

coefficient, and s is the standard deviation.

This equation correctly predicts the inactivity of the sulfone: its pED_2 value is predicted to be −1.98 (ED_2 = 100 mg/kg) with a 95% confidence interval −3.15 to −0.82. We conclude that the sulfone is inactive because its oxygen atoms penetrate into space required by the receptor. The exact three-dimensional location of this space cannot be exactly defined because (a) we do not know which enantiomer of the sulfoxide is active and (b) we do not know whether the "long" or "short" carbon–nitrogen conformation is the active one. If we knew both of these, we could verify our hypothesis. If we knew one or the other, we could propose more exact location of the forbidden region in space.

Discussion

Compound **2** has eight major conformational degrees of freedom. We were able to propose an active conformation about three of these bonds because we had an active cyclic analogue. The "active" conformation about the remaining rotatable bonds has been more elusive. Inactive cyclic or sterically constrained analogues, such as the biphenyl compound **52**, provide such ambiguous answers. If one wishes to answer conformational or distance questions with a set of analogues, the set must be designed for this purpose.

This data set is instructional to ponder vis-a-vis QSAR series design criteria.[5] There are four clusters of points. The first is the –CONH– analogue with low π and σ values, the second contains the –SO– and $-SO_2-$ analogues with low π and high σ values, the third is the –CO– analogue with intermediate π and high σ values, and the fourth cluster contains the biphenyl, $-CH_2-$, –O–, –S–, and $-CH_2O-$ analogues. Clusters 1, 2, and 4 contain inactive compounds; clusters 2, 3, and 4 contain active analogues. Thus, if only one member from each cluster had been synthesized, one could come to the erroneous conclusion that either σ constants determine activity (**2** and **14** active but **38** and **52** inactive) or that π values determine potency (**11a** and **2** active but **20** and **38** inactive). Only when such preliminary relationships were explored with more compounds would the lack of correlation be apparent. Alternatively if the original set had been (**2**, **20**, **38**, and **52**) one might have concluded that there is one special requirement for the carbonyl bridge. Although series design strategies are helpful, one must still guard against jumping to conclusions.

The combination of QSAR, molecular modeling, and regression analysis has allowed us to propose the receptor cavity shown in Figure 6. For reference, the lead compound **2** is shown in the cavity. It is provisional in the sense that various regions of the receptor were explored while others were kept constant. The full matrix of all possible combinations has not been explored.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. The NMR spectra were recorded on a Varian T-60 spectrometer using tetramethylsilane as an internal standard. Mass spectra were recorded on Kratos MS-50 mass spectrometer. Microanalyses were performed by the Abbott Analytical Department.

Ethyl [2,3-Dichloro-4-(4-hydroxybenzyl)phenoxy]acetate (4). To a suspension of 85.4 g (0.25 mol) of [2,3-dichloro-4-(4-hydroxybenzoyl)phenoxy]acetic acid[2a] in 34.5 g (0.75 mol) of EtOH and 100 mL of ethylene dichloride was added 3.5 mL of H_2SO_4 and the mixture was refluxed with stirring overnight while the acid gradually went into solution. The cooled reaction mixture was washed successively with water, twice with $KHCO_3$ solution, and finally with water. The dried ethylene dichloride was evaporated to dryness to give an oil which solidified to give 86 g of ethyl ester on trituration with pentane, mp 127–129 °C (93%). This material was used without further purification for the reduction. Anal. ($C_{17}H_{14}Cl_2O_5$) C, H. To trifluoroacetic acid (50 mL) under a nitrogen atmosphere was added 2.27 g (0.06 mol) of $NaBH_4$ pellets over a period of 30 min at 5 °C. A solution of the above ester (3 g, 0.0081 mol) in 30 mL of CH_2Cl_2 was added dropwise at 15–20 °C over a period of 20 min. The reaction

mixture was stirred overnight at room temperature while the $NaBH_4$ pellets slowly dissolved. At this time the reaction mixture was poured into water and the resulting solution extracted with CH_2Cl_2. The organic extract was washed with aqueous NaCl and dried over $MgSO_4$. Evaporation of the CH_2Cl_2 furnished a residue which was chromatographed on a silica gel column eluting with increasing amounts of MeOH in CH_2Cl_2. There was obtained 2 g (69%) of **4**, mp 102–103 °C. Anal. ($C_{17}H_{16}Cl_2O_4$) C, H.

Ethyl [2,3-Dichloro-4-[3-(aminomethyl)-4-hydroxybenzyl]phenoxy]acetate Hydrochloride (5). 2-Chloro-*N*-(hydroxymethyl)acetamide[12] (0.7 g, 0.0056 mol) was added, in small portions, to a stirred solution of **4** (2 g, 0.0056 mol) in 20 mL of acetic acid and 2 mL of concentrated H_2SO_4 at 10–15 °C. The mixture was stirred at room temperature overnight and poured in 200 mL of ice water. The solid that formed was extracted into EtOAc and the resulting solution washed with aqueous NaCl and dried over $MgSO_4$. The residue obtained by evaporating the EtOAc was dissolved in 150 mL of absolute EtOH containing 0.75 mL of concentrated H_2SO_4. After the solution stood overnight at room temperature, the EtOH was partially evaporated under reduced pressure and the residue distributed between CH_2Cl_2 and aqueous NaCl. The organic layer was washed several times with aqueous NaCl, dried over $MgSO_4$, and evaporated. The crude ethyl ester was chromatographed on a silica gel column eluting with benzene/EtOAc (3:1) to give 1.35 g (52%) of pure compound as a glass. A 1-g sample of the chloroacetyl derivative was heated at reflux in 10 mL of EtOH and 10 mL of concentrated HCl. After 4 h, the mixture was cooled to room temperature and the reaction mixture was filtered. The resulting white solid was washed with 50% EtOH/Et_2O and dried. There was obtained 0.8 g (88%) of **5**, mp 220–222 °C. Anal. ($C_{18}H_{20}Cl_3NO_4$) C, H, N.

2,3-Dichloro-4-(4-nitrophenoxy)anisole (8a). To a suspension of NaH (2.5 g, 0.052 mol of a 50% mineral oil suspension) in 60 mL of DMF was added portionwise 2,3-dichloro-4-methoxyphenol[13] (9.0 g, 0.047 mol). The mixture was stirred at room temperature under nitrogen for 15 min and then 4-fluoronitrobenzene (7.3 g, 0.052 mol) was rapidly added. The resulting mixture was heated at 100 °C for 2.5 h, cooled to room temperature, and poured into ice water. The precipitate was filtered, washed with MeOH, and dried to give 13 g (88%) of **8a**, mp 165–166 °C. Anal. ($C_{13}H_9Cl_2NO_4$) C, H, N.

Ethyl [2,3-Dichloro-4-(4-nitrophenoxy)phenoxy]acetate (9a). A solution of **8a** (13.5 g, 0.043 mol) in 135 mL of acetic acid and 80 mL of 48% HBr was heated at reflux for 30 h. After cooling, the product that had crystallized was filtered, washed with water, and dried. There was obtained 11.6 g (90%) of 2,3-dichloro-4-(4-nitrophenoxy)phenol, mp 147–150 °C. A mixture of this phenol (11.5 g, 0.038 mol), ethyl bromoacetate (9.51 g, 0.057 mol), and pulverized K_2CO_3 (7.9 g, 0.057 mol) in 100 mL of 2-butanone was heated at reflux for 2 h. The reaction mixture was then filtered and the filtrate concentrated under reduced pressure. The residue was taken into CH_2Cl_2 and the resulting solution was washed with aqueous NaCl and dried over $MgSO_4$. Evaporation of the solvent was followed by trituration with hexane to furnish the solid product. Recrystallization from cyclohexane gave 12.5 g (84%) of **9a**, mp 90–91 °C. Anal. ($C_{16}H_{13}Cl_2NO_6$) C, H, N.

Ethyl [2,3-Dichloro-4-(4-hydroxyphenoxy)phenoxy]acetate (10a). A solution of **9a** (30 g, 0.078 mol) in 1000 mL of EtOH was hydrogenated on a Parr apparatus over prewashed Raney nickel catalyst (12 g). After the hydrogenation was complete, the catalyst was removed by filtration through Celite and the filtrate mixed with ethanolic hydrogen chloride. Evaporation of the EtOH gave the hydrochloride salt (27.5 g). To a stirred suspension of this salt in 425 mL of aqueous H_2SO_4 (4 parts H_2SO_4 to 1 part H_2O) was added 5.1 g (0.074 mol) of $NaNO_2$ in 16 mL of H_2O, while the internal temperature was kept at 5 °C or below. The resulting solution was stirred for 30 min at 0–5 °C and then an additional 0.75 g of $NaNO_2$ in 2 mL of H_2O was added. Stirring at 0–5 °C was continued for 15 min and then 0.5 g of $NaNO_2$ in 1 mL of H_2O was added. After stirring an additional 1.25 h at 0–5 °C, the slurry of the diazoniam salt was slowly poured into a boiling mixture of H_2O (990 mL) and H_2SO_4 (595 mL). The resulting clear solution was heated at reflux for 1.25 h, then cooled, and extracted with EtOAc. The organic extract was dried over $MgSO_4$ and evaporated to an oil which crystallized upon standing to give 21.5 g of acid, mp 157–161 °C. The crude [2,3-dichloro-4-(4-hydroxyphenoxy)phenoxy]acetic acid was dissolved in 300 mL of EtOH containing 1.0 mL of concentrated H_2SO_4 and heated at reflux for 2.5 h. After cooling, the EtOH was partially evaporated and the residue distributed between aqueous $NaHCO_3$ and CH_2Cl_2. Drying and evaporation of the CH_2Cl_2 gave the solid ester. Recrystallization from cyclohexane/CH_2Cl_2 gave 17 g (61%) of **10a**, mp 105–106 °C. Anal. ($C_{16}H_{14}Cl_2O_5$) C, H.

Ethyl [2,3-dichloro-4-[3-(aminomethyl)-4-hydroxyphenoxy]phenoxy]acetate hydrochloride (11a) was obtained from **10a** in the same manner as **5** in 38% yield, mp 198–201 °C. Anal. ($C_{17}H_{18}Cl_3NO_5$) H, N; C: calcd, 48.31; found, 43.79.

2,3-Dichloro-4-ethoxybenzenethiol (6b). 2,3-Dichloro-4-ethoxybenzenesulfonyl chloride[14] (20.0 g, 0.069 mol) was dissolved in 200 mL of anhydrous Et_2O and added by dropwise addition to 6.29 g (0.166 mol) of $LiAlH_4$ suspended in 150 mL of Et_2O. After stirring overnight at room temperature, the mixture was refluxed for 1 h, cooled in an ice bath, and excess $LiAlH_4$ destroyed with water. Acidification with concentrated HCl was followed by extraction of the mixture with Et_2O. The ethereal extract was dried and evaporated to dryness to give 13.5 g (87%) of **6b** as an oil.

2,3-Dichloro-4-[(4-nitrophenyl)sulfenyl]phenetole (8b). A mixture of **6b** (13.5 g, 0.06 mol), *p*-fluoronitrobenzene (6.4 mL, 0.06 mol), and anhydrous K_2CO_3 (10.87 g, 0.079 mol) in 140 mL of DMF was stirred at room temperature overnight and then poured into water. The resulting solid product was filtered and washed well with EtOH to give 14.8 g (71%) of **8b**, mp 164–166 °C.

Ethyl [2,3-Dichloro-4-[(4-nitrophenyl)sulfenyl]phenoxy]acetate (9b). A solution of **8b** (10 g, 0.029 mol) in 400 mL of CH_2Cl_2 was treated with 8.52 g of $AlCl_3$ all at once at 0 °C. The reaction mixture was stirred overnight at room temperature and then an additional 3.87 g of $AlCl_3$ was added. Stirring was continued for an additional 3 h at which time 3.87 g of $AlCl_3$ was again added. After stirring overnight, the mixture was poured into 800 mL of crushed ice and the precipitate filtered. The solid was washed with EtOH and dried to give 8.67 (95%) of demethylated product, mp 202–204 °C. Anal. ($C_{12}H_7Cl_2NO_3S$) C, H, N. Alkylation of 2,3-dichloro-4-[(4-nitrophenyl)sulfenyl]phenol with ethyl bromoacetate was carried out in 88% yield as described for **9a** to give **9b**, mp 126–127 °C. Anal. ($C_{16}H_{13}Cl_2NO_5S$) C, H, N.

Ethyl [2,3-Dichloro-4-[(4-hydroxyphenyl)sulfenyl]phenoxy]acetate (10b). The procedure described for **10a** was used for **9b** with several modifications. The hydrogenation catalyst employed was 5% sulfided platinum on carbon rather than Raney nickel, and the product was isolated as the free base. The diazotization reaction was also modified as follows. The aniline derivative obtained from the hydrogenation (7.52 g, 0.02 mol) was dissolved in 50 mL of concentrated H_2SO_4. To this solution was added over a period of 20 min 72 mL of nitrosylsulfuric acid (4.9 g of $NaNO_2$ dissolved in 72 mL of concentrated H_2SO_4) and the reaction mixture stirred overnight at room temperature. The mixture was then poured onto 850 mL of ice and treated with sufficient urea to destroy excess nitrosylsulfuric acid. The aqueous mixture was then added to a refluxing mixture of 1.7 g of Na_2SO_4 dissolved in 25 mL of concentrated H_2SO_4 and 25 mL of water. After refluxing for 2 h, the mixture was cooled and the solid product filtered and dried to give the desired phenol. The corresponding ethyl ester **10b** was prepared in the same manner as described for **10a** in 85% yield and had mp 154.5–155 °C. Anal. ($C_{16}H_{14}Cl_2O_4S$) C, H.

Ethyl [2,3-dichloro-4-[[3-(aminomethyl)-4-hydroxyphenyl]sulfenyl]phenoxy]acetate hydrochloride (11b) was obtained from **10b** in the same manner as **5** in 24% yield, mp 205–207 °C dec. Anal. ($C_{17}H_{18}Cl_3NO_4S \cdot {}^1/_2H_2O$) C, H, N.

Methyl [2,3-Dichloro-4-[[3-(aminomethyl)-4-hydroxyphenyl]sulfinyl]phenoxy]acetate Hydrochloride (14). A

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solution of **11b** (1.19 g, 0.0027 mol) in 7 mL of DMF was treated successively with 0.55 g (0.0054 mol) of triethylamine and 0.65 g (0.003 mol) of di-*tert*-butyl dicarbonate. The reaction was stirred at room temperature for $2^1/_2$ h and then poured into brine solution. Extraction with CH_2Cl_2 and evaporation gave 1.36 g of N-*t*-Boc derivative. This material was dissolved in 5 mL of CH_2Cl_2 and treated successively with 0.51 g (0.004 mol) of MEM-Cl and 0.53 g (0.004 mol) of diisopropylethylamine. The mixture was stirred for 1 h, the CH_2Cl_2 solution was diluted with an additional 50 mL of CH_2Cl_2, and the organic layer was washed with brine solution. Evaporation of the CH_2Cl_2 furnished 1.55 g (97%) of **12** as a viscous oil. Anal. ($C_{26}H_{33}Cl_2NO_8S$) C, H, N. This material was oxidized to the sulfoxide by dissolving 1.5 g of (0.0025 mol) in 3.6 mL of pyridine and 0.3 mL of H_2O and adding 0.7 g (0.0025 mol) of iodobenzene dichloride. After the solution was stirred for 2 h, 50 mL of H_2O was added and the reaction mixture then stirred overnight at room temperature. The supernatant liquid was decanted away from the gummy product (**13**), which weighed 1.46 g. The gummy sulfoxide was allowed to stand overnight in 25 mL of saturated methanolic HCl. Evaporation to dryness furnished methyl ester **14**. Trituration with ether gave 0.96 g (90%) of pure product, mp 223–225 °C dec. Anal. ($C_{16}H_{16}Cl_3NO_5S$) C, H, N.

2,3-Dichloro-4-[(4-methoxyphenyl)sulfonyl]anisole (17). 4-Methoxybenzenesulfonyl chloride (20.6 g, 0.1 mol) and 2,3-dichloroanisole (17.7 g, 0.1 mol) were dissolved in 100 mL of CH_2Cl_2 at 0–5 °C, and $AlCl_3$ (13.3 g, 0.1 mol) was added portionwise. The reaction was stirred at 23 °C for 20 h and then poured into ice and 6 N HCl. Evaporation of the CH_2Cl_2 gave sulfone **17** which was triturated with MeOH to give 28.5 g (82%) of pure product, mp 148–149 °C. Anal. ($C_{14}H_{12}Cl_2O_4S$) C, H.

Ethyl [2,3-Dichloro-4-[(4-methoxyphenyl)sulfonyl]phenoxy]acetate (18). A mixture of **17** (18 g, 0.052 mol) and $AlCl_3$ (13.8 g, 0.104 mol) was refluxed for 3 h in 250 mL of CH_2Cl_2. After cooling, the reaction was poured onto ice and the resulting mixture extracted with CH_2Cl_2. The organic layer was extracted with 2 N NaOH and discarded. The aqueous portion was acidified with concentrated HCl and extracted with CH_2Cl_2. Evaporation of the CH_2Cl_2 gave 7.3 g of 2,3-dichloro-4-[(4-methoxyphenyl)-sulfonyl]phenol, mp 207–208 °C. Alkylation of this material with ethyl bromoacetate was carried out in the same manner as described for **9a** to give **18** in 80% yield after recrystallization from methyl ethyl ketone, mp 159–161 °C.

Ethyl [2,3-Dichloro-4-[(4-hydroxyphenyl)sulfonyl]phenoxy]acetate (19). A mixture of **18** (10 g, 0.024 mol) in 250 mL of 48% HBr and 100 mL of acetic acid was heated at reflux for 22 h and then evaporated to one-half the original volume on the rotary evaporator. The residue was diluted with water and extracted with EtOAc. The organic extract was washed with aqueous $NaHCO_3$ and evaporated to give a 70% yield of [2,3-dichloro-4-[(4-hydroxyphenyl)sulfonyl]phenoxy]acetic acid. Esterification of this material as described for **4** gave **19** in 90% yield after trituration with 50% CH_2Cl_2 in hexane, mp 154–156 °C. Anal. ($C_{16}H_{14}Cl_2O_6S$) C, H.

Ethyl [2,3-Dichloro-4-[[3-(aminomethyl)-4-hydroxyphenyl]sulfonyl]phenoxy]acetate Hydrochloride (20). Amidomethylation of **19** was carried out in concentrated H_2SO_4 for 4 h at 15 °C in an analogous manner to the procedure described for **5** to give **20** in 29% yield, mp 229–230 °C. Anal. ($C_{17}H_{18}Cl_3NO_6S$) C, H, N.

Ethyl [2,3-Dichloro-4-(hydroxymethyl)phenoxy]acetate (22). A suspension of ethyl [2,3-dichloro-4-formylphenoxy]-acetate[15] (20 g, 0.072 mol) in 200 mL of EtOH was cooled in an ice bath and treated with $NaBH_4$ (1 g, 0.026 mol) portionwise over a period of 5 min. The reaction mixture was stirred for an additional 15 min at 5 °C and then poured into ice water. After careful addition of acetic acid (1 mL), the aqueous solution was extracted with CH_2Cl_2. Evaporation of the CH_2Cl_2 gave 19 g (94%) of **22** after trituration with hexane, mp 95–96 °C. Anal. ($C_{11}H_{12}Cl_2O_4$) C, H.

2,2-Dimethyl-6-hydroxy-1,3-benzodioxane (23). To a solution of 2.5 g (0.018 mol) of gentisyl alcohol in 10 mL of DMF and 5 mL of 2,2-dimethoxypropane was added 10 mg of *p*-TsOH. After stirring overnight at room temperature, the reaction mixture was poured into aqueous NaCl solution and extracted with Et_2O. The organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$, and evaporated. Chromatography of the residue on silica gel eluting with 5/1 hexane–EtOAc gave 2.3 g (71%) of **23**, mp 78–79 °C. Anal. ($C_{10}H_{12}O_3$) C, H.

Ethyl [2,3-Dichloro-4-[[(2,2-dimethyl-1,3-benzodioxan-6-yl)oxy]methyl]phenoxy]acetate (24). A 17-g (0.061 mol) sample of **22** was dissolved in 85 mL of $CHCl_3$ and treated all at once with 8.5 mL of $SOCl_2$. After 1 h the $CHCl_3$ and excess $SOCl_2$ were evaporated, and the residual solid was triturated with hexane to give a quantitative yield of the corresponding chloromethyl compound, mp 68–69 °C. A mixture of this chloride (14.2 g, 0.05 mol), phenol **23** (8.7 g, 0.048 mol), and pulverized K_2CO_3 (13.8 g, 0.1 mol) in 70 mL of DMF was heated at 65 °C for 6 h. The reaction was poured into aqueous NaCl and extracted with CH_2Cl_2. The organic layer was washed with aqueous NaCl, dried over $MgSO_4$, and evaporated. Chromatography of the residue on silica gel eluting with CH_2Cl_2 gave 12.6 g (60%) of pure **24**, mp 89–90 °C. Anal. ($C_{21}H_{22}Cl_2O_6$) C, H.

Ethyl [2,3-Dichloro-4-[[3-(hydroxymethyl)-4-(benzyloxy)phenoxy]methyl]phenoxy]acetate (25). A solution of 12.5 g (0.028 mol) of **24** in 50 mL of THF and 300 mL of 65% aqueous HOAc was heated at 50 °C for 3 h. The mixture was evaporated to dryness under reduced pressure and the residue triturated with hexane to give 11.1 g of the deprotected derivative, mp 138–139 °C. A 12.5-g (0.031 mol) sample of this phenol was heated at reflux in 100 mL of acetone in the presence of benzyl bromide (10.6 g, 0.062 mol) and pulverized K_2CO_3 (9.45 g, 0.07 mol). After 4 h, the reaction mixture was filtered through Celite and the filtrate concentrated under reduced pressure. The residue was taken into CH_2Cl_2 and the resulting solution was washed with aqueous NaCl and dried over $MgSO_4$. Evaporation of the solvent was followed by chromatography of the residue on silica gel eluting with 4/1 benzene–EtOAc to give 9.0 g (59%) of **25**. A sample recrystallized from CCl_4 had mp 101–103 °C. Anal. ($C_{25}H_{24}Cl_2O_6$) C, H.

Ethyl [2,3-Dichloro-4-[[3-formyl-4-(benzyloxy)phenoxy]-methyl]phenoxy]acetate (26). Pyridinium chlorochromate (1.52 g, 7 mmol) was suspended in 10 mL of anhydrous CH_2Cl_2, and **25** (2.31 g, 4.7 mmol) in 7.5 mL of CH_2Cl_2 was added in one portion to the magnetically stirred solution. After $1^1/_2$ h, the supernatant was decanted from the gummy chromium salts and applied directly to a silica gel column. Elution with CH_2Cl_2 gave 1.6 g (69%) of aldehyde **26**, mp 125–126 °C. Anal. ($C_{25}H_{22}Cl_2O_6$) C, H.

Ethyl [2,3-Dichloro-4-[[3-(aminomethyl)-4-hydroxyphenoxy]methyl]phenoxy]acetate Hydrochloride (27). A solution of **26** (1.4 g, 2.1 mmol) and $NH_2OH{\cdot}HCl$ (1.5 g, 21 mmol) in a mixture of pyridine (5.5 mL), EtOH (15 mL), and CH_2Cl_2 (4 mL) was stirred for 20 h at room temperature. The reaction mixture was partially concentrated under reduced pressure and the residue distributed between EtOAc and H_2O. The organic extract was washed with 1 N HCl and aqueous NaCl and then dried over $MgSO_4$. Evaporation of the EtOAc furnished the oxime, which crystallized upon standing, mp 135–137 °C. A solution of this oxime dissolved in MeOH (99 mL) containing 1.55 mL of 6 N HCl was hydrogenated in a Parr apparatus over 5% Pd–C (0.3 g). After the uptake of hydrogen was complete, the catalyst was filtered and the MeOH evaporated. The residue was triturated with Et_2O to give the hydrochloride salt **27**, mp 227–229 °C. Anal. ($C_{18}H_{20}Cl_3NO_5{\cdot}H_2O$) C, H, N.

2,3-Dichloro-4-[(4-nitrophenyl)acetyl]anisole (29). (4-Nitrophenyl)acetyl chloride[16] (20 g, 0.1 mol) and 2,3-dichloroanisole (17.7 g, 0.1 mol) were dissolved in 150 mL of CH_2Cl_2, and $AlCl_3$ (13.4 g, 0.1 mol) was added portionwise. The reaction mixture was stirred overnight at room temperature, 2 h at reflux, and then poured into ice and 6 N HCl. Evaporation of the CH_2Cl_2 gave a solid which was recrystallized from EtOAc to give 22 g (65%) of pure **29**, mp 132–133 °C. Anal. ($C_{15}H_{11}Cl_2NO_4$) C, H, N.

2,3-Dichloro-4-[(4-nitrophenyl)acetyl]phenol (30). A solution of **29** (112.25 g, 0.33 mol) in 1100 mL of 48% HBr and 1100 mL of acetic acid was heated at reflux for 22 h. After partial

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concentration and then cooling, the product that had crystallized was filtered, washed with water, and dried. Recrystallization from CH_3CN gave 71 g (66%) of **30**, mp 191–193 °C. Anal. ($C_{14}H_9Cl_2NO_4$) C, H, N.

Ethyl [2,3-Dichloro-4-[(4-nitrophenyl)acetyl]phenoxy]acetate 1,2-Propylene Ketal (31). A mixture of **30** (32.6 g, 0.1 mol), propylene glycol (20 mL), and *p*-TsOH (0.5 g) was heated at reflux in 150 mL of toluene with a Dean–Stark trap. After 19 h the reaction mixture was evaporated to ca. one-half the original volume and the residue cooled in an ice bath. The resulting solid was filtered to give 34 g (89%) of the ketal, mp 171–173 °C. An analytical sample was obtained by recrystallization from benzene and had mp 175–177 °C. Anal. ($C_{17}H_{15}Cl_2NO_5$) C, H, N. Alkylation of this material with ethyl bromoacetate was carried out in 86% yield as described for **9a** to give **31**, mp 117–118 °C. Anal. ($C_{21}H_{21}Cl_2NO_7$) C, H, N.

4-(Carboxymethoxy)-2,3-dichloro-4′-hydroxybenzil (32). A solution of **31** (83.6 g, 0.18 mol) in methyl Cellosolve (1000 mL) was hydrogenated in a Parr apparatus with Raney nickel catalyst. The reaction mixture was filtered to remove the catalyst, and the filtrate was evaporated under reduced pressure. The residue (70 g) was dissolved in 1000 mL of 6 N HCl and heated at reflux for 20 h. After cooling, a solid crystallized and was filtered and dried. There was obtained 59.7 g (96%) of [2,3-dichloro-4-[(4-aminophenyl)acetyl]phenoxy]acetic acid hydrochloride, mp 240 °C dec. Anal. ($C_{16}H_{14}Cl_3NO_4$) C, H, N. To a solution of this amine (40 g, 0.102 mol) in 800 mL of H_2O was added concentrated H_2SO_4 (100 mL) with cooling. The resulting solution was cooled in an ice bath and treated by dropwise addition with $NaNO_2$ (8.01 g, 0.116 mol) dissolved in 35 mL of H_2O. After stirring for 3 h at room temperature, the entire reaction mixture was added by dropwise addition to a boiling mixture of H_2O (1000 mL) and H_2SO_4 (500 mL). The reaction was heated at reflux for 1 h and then cooled to room temperature. The solid that had precipitated was filtered and dissolved in EtOAc and the resulting solution was washed with 2 N HCl. Evaporation of the EtOAc gave a solid which was triturated with hexane to give 33 g (90%) of **32**, mp 191–194 °C. Anal. ($C_{16}H_{10}Cl_2O_6$) C, H.

2,3-Dichloro-4-[(ethoxycarbonyl)methoxy]-3′-(aminomethyl)-4′-hydroxybenzil Hydrochloride (33). Amidomethylation of **32** was carried out in 3/1 HOAc–H_2SO_4 in an analogous manner to the procedure described for **5** to give **33** in 21% yield, mp 238 °C dec. Anal. ($C_{19}H_{18}Cl_3NO_6$) C, H, N.

4-Hydroxy-3-[(trifluoroacetamido)methyl]aniline Hydrochloride (35). To a solution of *p*-nitrophenol (13.9 g, 0.1 mol) in concentrated H_2SO_4 (50 mL) cooled to 5 °C was added 2-chloro-*N*-(hydroxymethyl)trifluoroacetamide (15 g, 0.105 mol) portionwise over a period of 20 min. After the mixture was stirred at 22 °C for 16 h, the resulting solution was poured onto ice. The crude amide separated as a viscous gum, which was removed from the water and then dissolved in EtOAc. The EtOAc solution was washed with H_2O, dried, and evaporated to give a solid. Recrystallization from aqueous MeOH gave 19 g (72%) of 2-[(trifluoroacetamido)methyl]-4-nitrophenol, mp 165–167 °C. A solution of this nitro compound (5.62 g, 0.021 mol) in MeOH (100 mL) containing 1.9 mL of 6 N HCl was hydrogenated in a Parr apparatus with 5% Pt–C as catalyst. The reaction mixture was filter to remove the catalyst, and the filtrate was evaporated under reduced pressure. The residue was triturated with CH_2Cl_2 to give 5.05 g (8.7%) of **35**, mp 188–190 °C. Anal. ($C_9H_{10}ClF_3N_2O_2$) C, H, N.

***N*-[4-Hydroxy-3-[(trifluoroacetamido)methyl]phenyl]-2,3-dichloro-4-[(ethoxycarbonyl)methoxy]benzamide (37).** To a mixture of **36**[17] (3.8 g, 0.0122 mol) and **35** (3.3 g, 0.0122 mol) in CH_3CN (100 mL) was added a solution of $KHCO_3$ (2.56 g, 0.0256 mol) in H_2O (25 mL) at 0–5 °C. The reaction mixture was allowed to warm to room temperature and stirred an additional 1.5 h. The resulting two-phase mixture was placed in a separatory funnel and the aqueous layer removed. The organic layer was evaporated and the residue dissolved in CH_2Cl_2. The CH_2Cl_2 solution was washed with aqueous $NaHCO_3$ and dried over $MgSO_4$. Evaporation of the CH_2Cl_2 furnished the crude product as a dark solid. Recrystallization from CH_3CN–H_2O gave 4.0 g of pure **37**, mp 209–211 °C. Anal. ($C_{20}H_{17}Cl_2F_3N_2O_6$) C, H, N.

***N*-[4-Hydroxy-3-(aminomethyl)phenyl]-2,3-dichloro-4-[(ethoxycarbonyl)methoxy]benzamide Hydrochloride (38).** A mixture of **37** (3 g, 5.9 mmol) and saturated ethanolic HCl (75 mL) was heated at reflux for 7 h. After cooling, the product was filtered and triturated with ether to give 2.47 g (93%) of **38**, mp 228–230 °C. Anal. ($C_{18}H_{19}Cl_3N_2O_5 \cdot H_2O$) H, N; C: calcd, 46.23; found, 47.00.

2,3-Dichloro-4-(carboxymethoxy)-4′-hydroxybenzophenone Oxime (39). A mixture of **3** (34.15 g, 0.1 mol) and $NH_2OH \cdot HCl$ (68.3 g, 0.982 mol) was refluxed for 24 h in 350 mL of pyridine and 350 mL of absolute EtOH. The solvents were evaporated, and the residue was partitioned between EtOAc and 5% HCl. From the organic phase was obtained 35 g of **39** as a mixture of isomers. Recrystallization from aqueous MeOH gave 28 g, mp 190–192 °C. Anal. ($C_{15}H_{11}Cl_2NO_5$) C, H, N.

3-(4-Hydroxyphenyl)-6-(carboxymethoxy)-7-chloro-1,2-benzisoxazole (40). A mixture of **39** (7.48 g, 0.02 mol) and KOH (5.9 g) was refluxed for $2^1/_2$ h in 100 mL of absolute EtOH. After cooling, the precipitated bis-potassium salt of **40** was filtered and dried. Anal. ($C_{15}H_8ClNO_5K_2$) C, H, N. Treatment of an aqueous solution of the salt with concentrated HCl precipitated **40** (3.0 g 45%), which was used without additional purification.

3-[3-(Aminomethyl)-4-hydroxyphenyl]-6-[(ethoxycarbonyl)methoxy]-7-chloro-1,2-benzisoxazole Hydrochloride (41). Amidomethylation of **40** was carried out in concentrated H_2SO_4 as described for **20** to give **41** in 62% yield, mp 257–260 °C. Anal. ($C_{18}H_{18}Cl_2N_2O_5$) C, H, N.

(2,3-Dichloro-4-methoxyphenyl)acetaldehyde (43). To a stirred solution of $NaOCH_3$ (24.8 g, 0.46 mol) in 100 mL of MeOH cooled to 0 °C was added by dropwise addition a solution of **42**[18] (61.7 g, 0.3 mol) and methyl chloroacetate (50.5 g, 0.46 mol) in 350 mL of THF over a period of $2^1/_2$ h. The reaction mixture was then stirred at 0–5 °C for 2 h and poured into 2000 mL of H_2O containing 20 mL of HOAc. The solid product was filtered and dried to give 66 g of epoxy ester. To a solution of this ester in 650 mL of THF was added at 5 °C a solution of 25 g (0.625 mol) of NaOH in 30 mL of H_2O. After stirring for 1 h at room temperature, the solid sodium carboxylate salt was filtered and then suspended in a mixture of 275 mL of toluene, 25 mL of HOAc, and 165 mL of H_2O. After heating at 80 °C for 2 h, the cooled mixture was extracted with aqueous $NaHCO_3$. The organic layer was dried and evaporated to give 35 g (53%) of **43**, mp 85–87 °C. Anal. ($C_9H_8Cl_2O_2$) C, H.

Ethyl 2-Hydroxy-5-(2,3-dichloro-4-methoxyphenyl)-1,5-cyclohexadienecarboxylate (44). To 15.3 g (0.096 mol) of *N*-(trimethylsilyl)morpholine[19] were added 7.0 g (0.032 mol) of **43** and a few crystals of *p*-TsOH. The mixture was stirred overnight at room temperature and then diluted with CH_2Cl_2. The solution was washed successively with H_2O and brine and then dried over Na_2SO_4. Evaporation gave a quantitative yield of the crude enamine as a yellow solid. NMR ($CDCl_3$) δ 2.85 (m, 4 H), 3.55 (m, 4 H), 3.70 (s, 3 H), 5.45 (d, 1 H, J = 14 Hz), 6.34 (d, 1 H, J = 14 Hz), 6.55 (d, 1 H, J = 8 Hz), 7.05 (d, 1 H, J = 8 Hz). To a solution of 3.5 g (0.016 mol) of the enamine in 20 mL of benzene was added 5.0 g (0.032 mol) of ethyl 3-oxo-4-pentenoate (Nazorov's reagent)[7] and the solution was refluxed for 2 h. The benzene was evaporated and the residue was dissolved in a 1:1 mixture of THF and 1 N HCl and the mixture was refluxed for 45 min. The cooled reaction mixture was extracted with EtOAc and the organic extract was washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave a crude product, which was applied to a silica gel column and allowed to stand overnight. Elution with hexane–CH_2Cl_2 (2/1) gave 2.4 g of **44**, mp 93–95 °C. Anal. ($C_{16}H_{16}Cl_2O_4$) C, H.

Ethyl 2-Hydroxy-5-(2,3-dichloro-4-methoxyphenyl)-benzoate (45). To a solution of 0.75 g (0.002 mol) of **44** in 6 mL of CCl_4 were added 0.40 g (0.002 mol) of NBS and a catalytic amount of benzoyl peroxide. The mixture was refluxed for 5 min. The cooled reaction mixture was filtered and the filtrate was evaporated to provide 0.71 g of **45**, mp 119–121 °C. Anal.

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($C_{16}H_{14}Cl_2O_4$) C, H.

Ethyl 2-Methoxy-5-(2,3-dichloro-4-methoxyphenyl)-benzoate (46). A mixture of **45** (4.5 g, 0.013 mol), methyl iodide (10 mL), and pulverized K_2CO_3 (2 g) in 40 mL of 2-butanone was heated at reflux for 20 h. The reaction mixture was then filtered and the filtrate concentrated under reduced pressure. The residue was taken into CH_2Cl_2 and the resulting solution was washed with aqueous NaCl and dried over $MgSO_4$. Evaporation of the solvent was followed by trituration with hexane to furnish 4.5 g (96%) of **46**. Recrystallization from EtOH gave the analytical sample, mp 141–142 °C. Anal. ($C_{17}H_{16}Cl_2O_4$) C, H.

2-Methoxy-5-(2,3-dichloro-4-methoxyphenyl)benzyl Alcohol (47). A solution of **46** (5.7 g, 0.016 mol) in 100 mL of THF was treated by dropwise addition with 33 mL of a 1 M solution of diborane in THF. After heating at reflux for 6 h, the cooled reaction mixture was quenched with MeOH and then evaporated to dryness. The residue was triturated with MeOH to give 3.71 g (74%) of **47**, mp 151–152 °C. Anal. ($C_{15}H_{14}Cl_2O_3$) C, H.

2-Methoxy-5-(2,3-dichloro-4-methoxyphenyl)benzaldehyde (48). Oxidation of **47** was carried out in an analogous manner to the procedure described for **26** to give **48** in 90% yield, mp 192–193 °C. Anal. ($C_{15}H_{12}Cl_2O_3$) C, H.

2-Hydroxy-5-(2,3-dichloro-4-hydroxyphenyl)benzaldehyde (49). To a solution of **48** (3.75 g, 0.012 mol) in 57 mL of CH_2Cl_2 cooled to 0 °C was added BBr_3 (2.31 mL, 0.0244 mol) by dropwise addition. The mixture was stirred for 3.5 h at 0–5 °C and then poured onto ice–H_2O. The aqueous mixture was extracted with EtOAc and the organic layer was washed with aqueous NaCl and dried over $MgSO_4$. Evaporation gave a solid product which was triturated with hexane to give 2.8 g of **49**, mp 187–188 °C. Anal. ($C_{13}H_8Cl_2O_3$) C, H.

Ethyl [2,3-dichloro-4-(3-formyl-4-hydroxyphenyl)phenoxy]acetate (50) was obtained from **49** in the same manner as **9a** in 66% yield after chromatography on silica gel followed by recrystallization from EtOH, mp 121–122 °C. Anal. ($C_{17}H_{14}Cl_2O_5$) C, H.

Ethyl [2,3-Dichloro-4-[3-(aminomethyl)-4-hydroxyphenyl]phenoxy]acetate Hydrochloride (52). Aldehyde **50** was converted in 95% yield to oxime **51** by the procedure described for **27**, mp 164–165 °C. Catalytic hydrogenation of the oxime as described for **27** gave **52** in 74% yield, mp 244–246 °C. Anal. ($C_{17}H_{18}Cl_3NO_4$) C, H, N.

Measurements of Octanol–Water Distribution Coefficients. These were done by standard methods.[20] Because of the zwitterionic nature of the compounds it was necessary to measure the partition coefficient at a pH at which the molecule is ionized. pH 7.0 phosphate buffer was used as the aqueous phase. Quantitation of the compounds was accomplished by measurement of the absorption at the wavelength of maximum absorbance in the ultraviolet.

Measurement of the pK_a Values. This was accomplished by recording the ultraviolet absorption spectra in a series of buffers that spanned the pH range over which the phenolic hydroxyl is protonated to that at which it is fully deprotonated.[21] The absorbance vs. pH curves were fit by nonlinear regression analysis to an equation that fit both the extinction coefficients for the protonated and nonprotonated forms as well as the pK_a value. By similar methodology except that the pH range of the buffers was extended to pH 12.0, it was possible to estimate that the pK_a of the amino group of compound **2** is 10.6.

Conformational Calculations. The structures were built with a Dreiding Model Builder,[22] rotated to a sterically allowed conformation,[23] minimized with a molecular mechanics program,[11] and then systematically rotated in 30° increments (oxyacetate side chain) or 10° increments (bridge between rings and aminomethyl side chain) with a potential energy program.[23] For the latter rotations the default steric parameters and electrostatic function were used with CNDO/2 charges. A tortional potential was used for rotation about the >C=O and isoxazolyl bridge; the barrier of 2.8 kcal was estimated from the CNDO/2 energy for rotation of benzaldehyde corrected for the CAMSEQ steric energy. Hydrogen bonding was included by setting to 0.0 the steric repulsion of a potential H-bonding hydrogen atom with oxygen or nitrogen atom.[24]

In the final rigid rotation both energy and distance between atoms of interest (usually the carbon atom of the carboxylate and the amino nitrogen atom) were recorded as a function of the rotation angle. For the studies on the conformation of the oxyacetate side chain, we prepared contour diagrams of energy as a function of rotation angle and also of distance between the two atoms of interest as a function of the same rotation angle. This allowed a visualization of the allowed distances between the atoms. Because this type of comparison was of value, a computer program was written to fit the energy-distance data.[25] This program was used to analyze the data on the relative energies of conformations that differ in rotation about the bridge atom and also in rotation about the amino methyl groups.

The conformations were examined on an Evans and Sutherland Multipicture System computer terminal using the program CMD. CMD was written at Abbott. It is based on the graphics program GRAMPS,[26] which the user calls as a subprocess. The figures are photographed directly from the screen using EKTACHROME 400 film.

Registry No. **3**, 62967-00-4; **4**, 87181-08-6; **5**, 87181-10-0; **5** (free base), 93134-86-2; **6a**, 39542-65-9; **6b**, 87181-26-8; **7**, 350-46-9; **8a**, 87181-12-2; **8b**, 87181-25-7; **9a**, 87181-14-4; **9b**, 87181-28-0; **10a**, 87181-15-5; **10b**, 87181-29-1; **11a**, 87181-19-9; **11a** (free base), 87181-56-4; **11b**, 87181-33-7; **11b** (free base), 87181-58-6; **12**, 93134-87-3; **13**, 93134-88-4; **14**, 87181-37-1; **14** (free base), 93184-24-8; **15**, 1984-59-4; **16**, 98-68-0; **17**, 93134-89-5; **18**, 93134-90-8; **19**, 93134-91-9; **20**, 93134-92-0; **20** (free base), 93134-93-1; **21**, 16861-23-7; **22**, 93134-94-2; **23**, 93134-95-3; **24**, 93134-96-4; **25**, 93134-97-5; **26**, 93134-98-6; **27**, 93134-99-7; **27** (free base), 93135-00-3; **28**, 50434-36-1; **29**, 93135-01-4; **30**, 93184-25-9; **31**, 93184-26-0; **32**, 93135-02-5; **33**, 93135-03-6; **33** (free base), 93135-04-7; **34**, 100-02-7; **35**, 93135-05-8; **36**, 66883-43-0; **37**, 93135-06-9; **38**, 93135-07-0; **38** (free base), 93135-08-1; **39**, 93135-09-2; **40**, 93135-10-5; **41**, 93135-11-6; **41** (free base), 93135-12-7; **42**, 41827-86-5; **43**, 93135-13-8; **44**, 93135-14-9; **45**, 93135-15-0; **46**, 93135-16-1; **47**, 93135-17-2; **48**, 93135-18-3; **49**, 93135-19-4; **50**, 93135-20-7; **51**, 93135-21-8; **52**, 93135-22-9; **52** (free base), 93135-23-0; ethyl [2,3-dichloro-4-(4-hydroxybenzoyl)-phenoxy]acetate, 78235-15-1; [2,3-dichloro-4-[4-hydroxy-3-[(2-chloroacetyl)aminomethyl]benzyl]phenoxy]acetic acid, 93135-24-1; ethyl [2,3-dichloro-4-[4-hydroxy-3-[(2-chloroacetyl)aminomethyl]benzyl]phenoxy]acetate, 87181-09-7; 2,3-dichloro-4-(4-nitrophenoxy)phenol, 87181-13-3; 2,3-dichloro-4-(4-nitrophenythio)phenol, 87181-27-9; ethyl [2,3-dichloro-4-(4-aminophenoxy)phenoxy]acetate hydrochloride, 93184-27-1; ethyl [2,3-dichloro-4-(4-aminophenylthio)phenoxy]acetate, 93135-25-2; [2,3-dichloro-4-(4-hydroxyphenoxy)phenoxy]acetic acid, 87181-16-6; [2,3-dichloro-4-(4-hydroxyphenylthio)phenoxy]acetic acid, 87181-30-4; [2,3-dichloro-4-[4-hydroxy-3-[(2-chloroacetyl)aminomethyl]phenoxy]phenoxy]acetic acid, 93135-26-3; [2,3-dichloro-4-[4-hydroxy-3-[(2-chloroacetyl)aminomethyl]phenylthio]phenoxy]acetic acid, 93135-27-4; ethyl [2,3-dichloro-4-[4-hydroxy-3-[(2-chloroacetyl)aminomethyl]phenoxy]phenoxy]acetate, 87181-17-7; ethyl [2,3-dichloro-4-[4-hydroxy-3-[(2-chloroacetyl)aminomethyl]phenylthio]phenoxy]acetate, 87181-31-5; ethyl [2,3-dichloro-[4-hydroxy-3-[*tert*-butoxycarbonyl)aminomethyl]phenylthio]phenoxy]acetate, 93135-28-5; 2,3-dichloro-4-[(4-methoxyphenyl)sulfonyl]phenol, 93135-29-6; [2,3-dichloro-4-[(4-hydroxyphenyl)sulfonyl]phenoxy]acetic acid, 93135-30-9; [2,3-dichloro-4-[[4-hydroxy-3-[(2-chloroacetyl)aminomethyl]phenyl]sulfonyl]-phenoxy]acetic acid, 93135-31-0; ethyl [2,3-dichloro-4-[[4-

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hydroxy-3-[(2-chloroacetyl)aminomethyl]phenyl]sulfonyl]phenoxy]acetate, 93135-32-1; ethyl[2,3-dichloro-4-[[4-hydroxy-3-(hydroxymethyl)phenoxy]methyl]phenoxy]acetate, 93135-33-2; ethyl [2,3-dichloro-4-[[3-(methyliminohydroxy)-4-(benzyloxy)phenoxy]methyl]phenoxy]acetate, 93135-34-3; 2,3-dichloro-4-[(4-nitrophenylacetyl]phenol 1,2-propylene ketal, 93135-35-4; [2,3-dichloro-4-[(4-aminophenylacetyl]phenoxy]acetic acid hydrochloride, 93135-36-5; 4-(carboxymethoxy)-2,3-dichloro-4′-hydroxy-3-[(chloroacetyl)aminomethyl]benzil, 93135-37-6; ethyl 4-(carboxymethoxy)-2,3-dichloro-4′-hydroxy-3-[(chloroacetyl)-aminomethyl]benzil, 93135-38-7; 2-[(trifluoroacetamido)-methyl]-4-nitrophenol, 93135-39-8; 3-[4-hydroxy-3-[(2-chloroacetyl)aminomethyl]phenyl]-6-(carboxymethoxy)-7-chloro-1,2-benzisoxazole, 93135-40-1; ethyl 3-[4-hydroxy-3-[(2-chloroacetyl)aminomethyl]phenyl]-6-(carboxymethoxy)-7-chloro-1,2-benzisoxazole, 93135-41-2; methyl 3-(2,3-dichloro-4-methoxyphenyl)oxirane-2-carboxylate, 93135-42-3; sodium 3-(2,3-dichloro-4-methoxyphenyl)oxirane-2-carboxylate, 93135-43-4; 1-morpholino-2-(2,3-dichloro-4-methoxyphenyl)ethene, 93135-44-5; 2,3-dichloro-4-ethoxybenzenesulfonyl chloride, 93135-45-6; gentisyl alcohol, 495-08-9; *N*-(hydroxymethyl)trifluoroacetamide, 50667-69-1; ethyl 3-oxo-4-pentenoate, 22418-80-0.

Diterpenoid Sweeteners. Synthesis and Sensory Evaluation of Stevioside Analogues with Improved Organoleptic Properties

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Congeneric series of stevioside (1) and rebaudioside A (3) analogues have been prepared. It was found that the bitter-taste component endogenous in the natural compounds 1 and 3 may be eliminated by increase in molecular hydrophilic character. Through the series of compounds prepared, bitter-taste character was correlated with k', a chromatographic indicator of gross hydrophilicity. An analogue (11) of stevioside, shown chromatographically to be of increased hydrophilicity, was prepared and found to exhibit no bitter-taste character. Similarly an analogue (13) of rebaudioside A, having increased polarity, was prepared and found not to exhibit any bitter taste. The rebaudioside A analogue 13 was determined to have higher potency than 11 and is suggested as a potential nonnutritive sweetener for food applications.

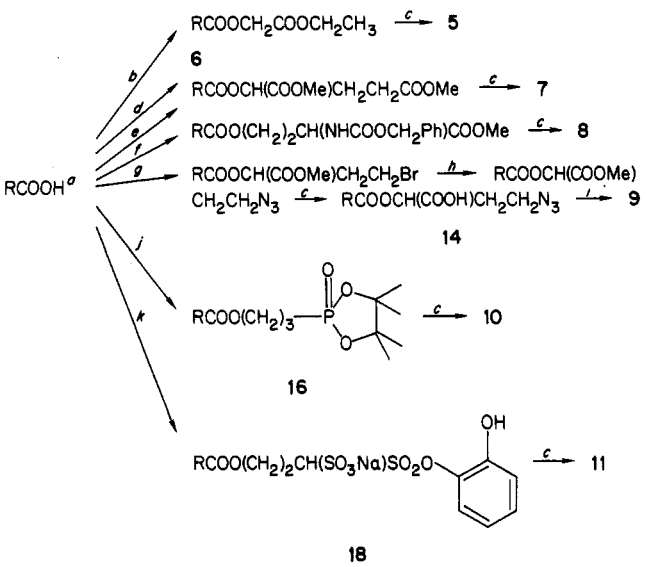
Interest in safe, high-sweetness quality, nonnutritive sweeteners is very high. The record 1983 sales of the dipeptide sweetener, aspartame, document the public's willingness to pay a premium price for a very good sucrose mimic.[1] In our sensory investigations on the well-known nonnutritive sweeteners,[2] only sodium cyclamate and aspartame were found to consistently exhibit the high sweet-taste quality mandated by the consumer. Recently, we reported that the marginal taste quality of the sweet diterpenoid triglucoside, stevioside (1), could be improved dramatically by replacement of the 19-*O*-glucosyl substituent by a (sodiosulfo)propyl moiety to give **2**.[3] Although this stevioside analogue **2** exhibits taste quality similar to that of sodium cyclamate, it reproducibly exhibits a weak, bitter-taste component. In the interest of obtaining a nonnutritive sweetener devoid of bitter taste, we have prepared a congeneric series of 19-*O*-substituted analogues of 1 and also of the related diterpenoid tetraglucoside, rebaudioside A (3). This work provides the subject for the following report.

Sensory Evaluation. The experimental compounds described below were evaluated by a human sensory panel. The same criteria, regarding purity and absence of toxicity which were applied in our earlier work,[4] were applied to these materials. Since none of them showed any toxicity, they were subjected to sensory analysis by a trained panel of judges. Panelists were required to carry out magnitude estimation (vs. 10% sucrose) and taste quality determination (percent sweet, sour, salty, bitter, and other) in one sensory session. From this analysis, comparative taste potency data, calculated on both weight (P_w) and molar (P_m) bases, and taste quality data were obtained.

Results

Analogue Design, Synthesis, and Sensory Evaluation. The mechanism responsible for the substantially improved taste quality of **2** over stevioside (1) is not known.

Scheme I



[a] RCOOH = steviolbioside (4). [b] K_2CO_3-DMF-$ClCH_2COOCH_2CH_3$. [c] NaOH. [d] Potassium *tert*-amyl oxide/toluene-DMF-1,4-butanesultone. [e] K_2CO_3-DMF-$COOMeCHBrCH_2CH_2COOMe$. [f] K_2CO_3-DMF-$Br(CH_2)_2CH(NHCOOCH_2Ph)COOMe$. [g] Potassium *tert*-amyl oxide/toluene-DMF-$Br(CH_2)_2CHBrCOOMe$. [h] NaN_3-DMF. [i] $NaBH_4$-$NiCl_2 \cdot 6H_2O$-MeOH. [j] K_2CO_3-DMF-15. [k] Potassium *tert*-amyl oxide/toluene-DMF-17.

Clearly, however, the 19-*O*-glucosyl substituent in 1 is not involved in any essential receptor binding interaction. Evidence has been put forth by Koyama and Kurihara[5]

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