Inhibitors of Glycolic Acid Oxidase. 4-Substituted 3-Hydroxy-1*H*-pyrrole-2,5-dione Derivatives

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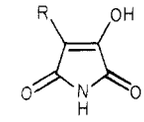
An extensive series of novel 4-substituted 3-hydroxy-1*H*-pyrrole-2,5-dione derivatives has been prepared and studied as inhibitors of glycolic acid oxidase (GAO). Compounds possessing large lipophilic 4-substituents are, in general, potent, competitive inhibitors of porcine liver GAO in vitro. Methylation of the nitrogen or the 3-hydroxy substituent reduced potency dramatically, indicating the requirement for the two acidic functions on the 1*H*-pyrrole-2,5-dione nucleus. In rat liver perfusion studies, with three representative compounds, concentration-dependent inhibition of the conversion of [1-^{14}C]glycolate to [^{14}C]oxalate was observed. Chronic oral administration to ethylene glycol fed rats of the 4-(4′-bromo[1,1′-biphenyl]-4-yl) derivative (**83**) was shown to effect a significant reduction in urinary oxalate levels over a 58-day period.

The majority of kidney stones in humans contain calcium oxalate and, with many stones, it is the predominate component.[1] Furthermore, calcium oxalate may serve as the nidus for the crystallization of stones of mixed composition. The anion component of this highly insoluble crystalloid may be derived from the diet as well as from endogenous metabolism of precursors such as glycolic acid, glyoxylic acid, glycine, ascorbic acid, hydroxyproline, and tryptophan.[2] In the genetic primary hyperoxalurias types I and II, enzyme defects preventing the efficient utilization of glyoxylate in normal pathways result in the metabolic production of excessive amounts of oxalate, leading to serious and frequently lethal consequences.[3] Glyoxylate is the most important immediate biosynthetic precursor of oxalate[3] (see Scheme I). There are two enzymes considered to be functionally important for carrying out the conversion of glyoxylate to oxalate.[2,3] One of these, glycolic acid oxidase (glycolate:O_2 oxidoreductase, EC 1.1.3.1) (GAO) also catalyzes the production of glyoxylate from glycolate. The second enzyme, lactic dehydrogenase, is able to catalyze the reversible conversion of glyoxylate to glycolate, with the equilibrium for this reaction being far in the direction of the reduced substrate.[3] Inhibitors of GAO are of interest as potentially useful drugs for the treatment of those disease states (calcium oxalate renal lithiasis and the primary hyperoxalurias) in which the pathological consequences are due to the crystallization of calcium oxalate. Inhibitors of GAO could act to reduce both the production of glyoxylate from glycolate as well as the subsequent oxidation of glyoxylate to oxalate (Scheme I). The purpose of this investigation was to develop potent, orally absorbable inhibitors of GAO and to examine their effects on oxalate production in animals.

Inhibitors of GAO reported in the literature include phenyllactic acid,[4] alkanoic and alkanedioic acids,[4,5] substituted glycolic and glyoxylic acids,[6] oxamic acids,[7] 2-hydroxy-3-butynoic acid,[7,8] and hydroxymethanesulfonic acid derivatives.[9] The last two are not specific for GAO, while many of the others are relatively weakly active. The product, oxalic acid, is a competitive inhibitor of GAO.[5] Schumann and Massey[5,10] have reported evidence that the active site of porcine GAO may contain two positively charged groups in close proximity, plus a hydrophobic bonding region. Based on this information, we have investigated a variety of diacidic molecules containing lipophilic substituents as potential inhibitors of GAO. In this report we describe a series of 4-substituted 3-hydroxy-1*H*-pyrrole-2,5-dione derivatives (structure A)



A

that are potent, orally active competitive inhibitors of GAO. Rat liver perfusion studies with three of the most potent representatives have demonstrated effective inhibition of the conversion of glycolate to oxalate in this organ. One of these compounds, when administered orally to ethylene glycol treated rats, also resulted in a significant reduction in oxalate excretion in the urine.

Chemistry. The most direct route to 4-substituted 3-hydroxy-1*H*-pyrrole-2,5-dione derivatives involves the reaction of 2-monosubstituted acetamide derivatives with dialkyl oxalates and 2 mol of strong base in solvents such as dimethylformamide, ethanol, or benzene[11] (method A, Scheme II). This method is applicable only if the substituent attached to the methylene of the acetamide is activating for carbanion formation. (It is reasonable to

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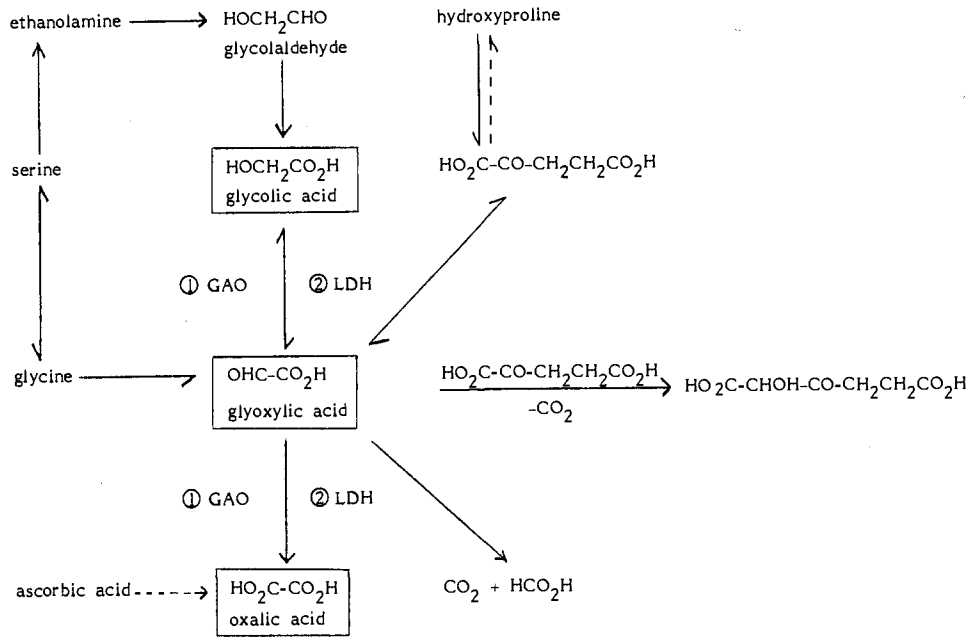
Scheme I. Major Pathways of Oxalate Biosynthesis



Scheme II. Synthetic Routes to 4-Substituted 3-Hydroxy-1*H*-pyrrole-2,5-diones

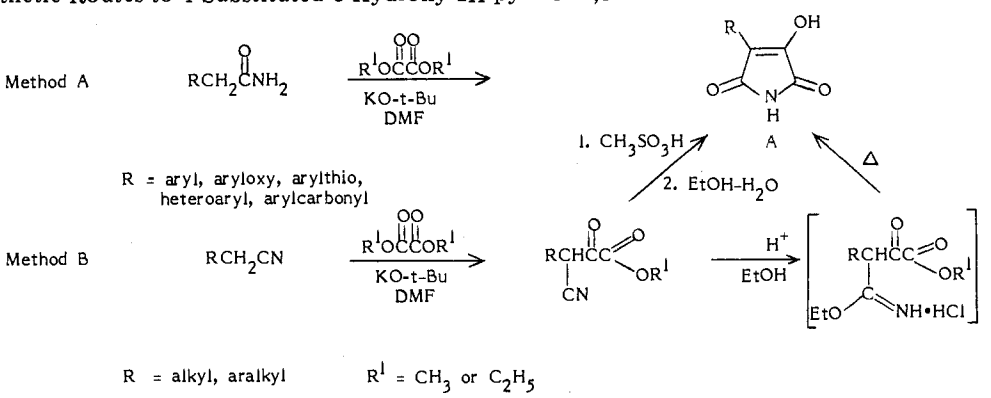


Table I. Aryl Methyl Ketone Intermediates[a]

compd	R	catalyst/solvent	mp, °C	solvent	yield, %	formula	anal.
1	4-(c-C_6H_{11})C_6H_4	$AlCl_3/CH_2Cl_2$	65–67	pet. ether	61	$C_{14}H_{18}O$	C, H
2	4-(3,4-$Cl_2C_6H_3CH_2$)C_6H_4	$AlCl_3/CH_2Cl_2$	37.5–39		75	$C_{15}H_{12}Cl_2O$	C, H, Cl

[a] New methyl ketone derivatives prepared by Friedel–Crafts procedure.

assume that the dianion is the reactive species for acylation.) Thus, the reaction proceeds well with aryl, heteroaryl, aryloxy, and arylthio substituents but not with alkyl or aralkyl substituents. An alternative route with greater generality begins with a monosubstituted acetonitrile (method B, Scheme II). Reaction of the nitrile with dialkyl oxalate and 1 mol of strong base, in solvents such as dimethylformamide, ethanol, ether, benzene, etc., results in acylation at the carbon adjacent to the nitrile. The 3-cyano-2-keto ester intermediate thus formed can be converted to the desired pyrrole ring system by reaction with ethanolic hydrogen chloride.[12] This reaction presumably proceeds through an imino ether intermediate. Reacting the 3-cyano-2-keto ester with concentrated sulfuric or methanesulfonic acids, followed by quenching in ethanol, also results in conversion to the 3-hydroxy-1*H*-pyrrole-2,5-dione derivative.[13] The 4-substituted 3-hydroxy-1*H*-pyrrole-2,5-dione derivatives, lacking substituents on either the ring nitrogen or the 3-hydroxy group, are acidic and require quite strongly acidic conditions for their isolation in the free acid form. The first acidic pK_a of 2.8–5.3 is due to the hydroxy group, the imide function being much less acidic ($pK_a \approx 10$). These assignments are supported by the pK_a value of 10.7 for the 3-*O*-methyl derivative **82** and the pK_a of 3.95 for the N-methylated derivative 81. pK_a values for three related derivatives, **96–98**, are also in agreement with these conclusions.

The 4-alkyl derivatives **92** and **93** and the aralkyl derivative **77** in Table VII were prepared from the corresponding nitriles by method B. The 4-aryl-substituted derivatives of Table VII were prepared from the corre-

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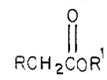
Table II. Methyl (or Ethyl) Arylacetate Intermediates



compd	R	R^1	mp, °C	solvent	yield,[a] %	formula	anal.
3	4-(4-BrC_6H_4)C_6H_4	CH_3[b]	58–60	diisopropyl ether	99	$C_{15}H_{13}BrO_2$	C, H, Br
4	4-(c-C_6H_{11})C_6H_4	CH_3[b]	oil		98	$C_{15}H_{20}O_2$[c]	
5	4-(1-Ad)C_6H_4[d]	CH_3[b]	62–65	diisopropyl ether	88	$C_{19}H_{24}O_2$	C, H
6	4-(3,4-$Cl_2C_6H_3CH_2$)C_6H_4	CH_3[b]	41–43[e]		100	$C_{16}H_{14}Cl_2O_2$	C, H; Cl[f]
7	4-(1,2,3,4-H_4-1-$C_{10}H_7$)C_6H_4	CH_3[b]	oil		89	$C_{19}H_{20}O_2$[c]	
8	4-(2-C_6H_5-1-C_5H_6N)C_6H_4	CH_3[b]	77–79	pet. ether	56	$C_{23}H_{19}NO_2$	H, N; C[g]
9	6-OCH_3-2-$C_{10}H_7$	CH_3[b]	75–77	pet. ether	88	$C_{14}H_{14}O_3$	C, H
10	6-(3-HDBOM)-2-$C_{10}H_7$	CH_3[b]	106–108	diisopropyl ether	61	$C_{23}H_{22}O_6$	C, H
11	5-(4-ClC_6H_4)-2-C_4H_3S	CH_3[b]	84–85	MeOH	82	$C_{13}H_{11}ClO_2S$	C, H, S
12	4-(C_6H_5)C_6H_4S	C_2H_5[i]	52–55	EtOH	78	$C_{16}H_{16}O_2S$	C, H, S

[a] In most cases these are crude yields, as the products were utilized in the next step without purification. [b] Methyl esters were prepared by the Taylor–McKillop $Tl(NO_3)_3$–MeOH procedure.[14] [c] Crude compound was not analyzed. ^1H NMR and IR indicated structure was as assigned. [d] Ad = 1-adamantyl. [e] The product was purified by short-path distillation at high vacuum. [f] Cl: calcd, 22.93; found, 23.77. [g] C: calcd, 80.91; found, 80.46. [h] 3-HDBOM = (3-hydroxy-3,4-dihydro-2*H*-1,5-benzodioxepin-3-yl)methyloxy. [i] The ethyl ester was prepared by alkylation of the sodium salt of the corresponding thiophenol with ethyl bromoacetate in ethanol at room temperature.

sponding acetamide intermediates by method A. The generally employed route to the arylacetamide intermediates that were not commercially available or were unknown in the literature was as follows: (1) Friedel–Craft acetylation of the arene (or thiophene) with acetyl chloride and Lewis acid catalyst, (2) oxidative rearrangement to the methyl arylacetate by the thallium trinitrate–methanol procedure of McKillop, Swann, and Taylor[14] and (3) conversion of the methyl ester to the amide with saturated methanolic ammonia. Data for new ketone, ester, and amide intermediates are given in Tables I–III. For the 4-(2-phenyl-1-indolyl)phenyl-substituted derivative **86**, a different synthesis of the required acetyl intermediate was employed as outlined under Experimental Section. In the synthesis of **91**, 2-acetyl-6-hydroxynaphthalene was allowed to react with 3,4-dihydro-2*H*-1,5-benzodioxepin-3-spirooxirane in the presence of base to obtain the starting acetyl compound. The spirooxirane reagent was available to us from an earlier, unrelated project.[15]

For the 4-aryloxy and 4-arylthio derivatives **88**, **101**, **102**, and **89**, the required oxy- and thioacetamide intermediates were prepared from ethyl esters obtained from the reactions of the corresponding phenols and thiophenols with ethyl or methyl bromoacetate and base. The 2-(4-phenyl-1-piperidyl)acetamide intermediate for **125** was prepared in a similar manner. In the synthesis of the hydroxyindan-containing derivative **100** (Table VIII), the requisite 2-[(1-hydroxy-2,2-disubsituted-6,7-dichloroindan-5-yl)oxy]acetamide intermediate was obtained as a mixture of racemates by sodium borohydride reduction of the corresponding indanone[16] in 2-propanol. Attempted conversion of the latter compound to the 4-substituted 3-hydroxy-1*H*-pyrrole-2,5-dione system under the conditions of method A resulted only in an intramolecular rearrangement.[16] The multistep preparation of the 3-oxo-3-([1,1′-biphenyl]-4-yl)propionamide intermediate required for the synthesis of the 4-biphenylylcarbonyl derivative **87** is outlined under Experimental Section. The 3-methoxy derivative **82** was prepared by diazomethane methylation of the parent **80**. The isomeric 1-methyl derivative **81** was prepared by method A employing the *N*-methylacetamide intermediate.

For the synthesis (Scheme I) of the 4-(4-thiazolyl)-3-hydroxy-1*H*-pyrrole-2,5-dione derivatives **105** to **123** (Table X), the Hantzsch procedure was utilized for construction of the thiazole ring. Thus, reaction of the appropriate thio amide wth ethyl 4-chloroacetoacetate produced the thiazolylacetic ester intermediate. The ester, on reaction with concentrated ammonium hydroxide, gave the thiazolylacetamide (see method C), which was then treated according to method A. The melting points, yields, and recrystallization solvents for new intermediates in the thiazole series are presented in Tables IV–VI. In the synthesis of **123**, the initial step involved reaction of 1-(4-bromophenyl)-2-bromoethanone with ethyl 3-amino-3-thioxopropanoate[17] in ethanol. Reaction of the resulting ethyl [4-(4-bromophenyl)thiazol-2-yl]acetate with ammonia provided the amide intermediate for processing by method A.

^{13}C NMR spectra for compounds **80**, **82**, **83**, **92**, 114, and 118 support the assignment of 4-substituted-3-hydroxy-1*H*-pyrrole-2,5-dione structures rather than the tautomeric 2,3,5-triketopyrrolidine alternative.[18] The ^{13}C NMR data for **123** are anomalous, suggesting that this compound exists as the triketopyrrolidine tautomer but with the acidic hydrogen attached to the thiazole ring nitrogen and with a double bond connecting the thiazole and pyrrolidine nuclei.[18]

Results and Discussion

In Vitro Enzyme Inhibition. The 4-substituted 3-hydroxy-1*H*-pyrrole-2,5-dione derivatives reported herein were all tested for their ability in vitro to inhibit purified pig liver GAO, employing the dye reduction procedure described previously.[6] The enzyme inhibitory activities of the compounds are reported as molar concentrations that produced an inhibition of 50% (I_{50} values). As can be seen from examination of the data in Table VII, the 4-phenyl derivative **72** (possessing free NH and OH groups) exhibited an I_{50} value of 1.35×10^{-5} M. Since **72** was the first compound in the series to be tested, it was of immediate interest to examine the effects on potency of the introduction of a variety of substituents, as well as

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(18) ^{13}C NMR data for these compounds determined by D. W. Cochran will be published elsewhere.

Table III. Arylacetamide Intermediates[a]

compd	R	mp, °C	solvent	yield, %	formula	anal.
13	4-(4-BrC$_6$H$_4$)C$_6$H$_4$	265–267	DMF/MeCN	50	$C_{14}H_{12}BrNO$	C, H, N
14	4-(c-C$_6$H$_{11}$)C$_6$H$_4$	164–166	MeOH	50	$C_{14}H_{19}NO$	C, H, N
15	4-(1-Ad)C$_6$H$_4$[b]	154–156	MeCN	46	$C_{18}H_{23}NO$	C, H, N
16	4-(3,4-Cl$_2$C$_6$H$_3$CH$_2$)C$_6$H$_4$	151–153	C$_6$H$_6$	42	$C_{15}H_{13}Cl_2NO$	H, Cl, N; C[c]
17	4-(1,2,3,4-H$_4$-1-C$_{10}$H$_7$)C$_6$H$_4$	124–126	diisopropyl ether	27	$C_{18}H_{19}NO$	C, H, N
18	4-(2-C$_6$H$_5$-1-C$_8$H$_6$N)C$_6$H$_4$	195–196	MeCN	95	$C_{22}H_{18}N_2O$	C, H, N
19	6-OCH$_3$-2-C$_{10}$H$_7$	242–245	DMF	55	$C_{13}H_{13}NO_2$	C, H, N
20	6-(3-HDBOM)-2-C$_{10}$H$_7$[d]	201–202	MeOH	62	$C_{22}H_{21}NO_5$	C, H, N
21	5-(4-ClC$_6$H$_4$)-2-C$_4$H$_3$S	208.5–209	MeCN	95	$C_{12}H_{10}ClNOS$	C, H, Cl, N, S
22	4-IC$_6$H$_4$	207.5–208.5	EtOH	86	C_8H_8INO	C, H, N
23	(6,7-dichloro-2-methyl-2-cyclopentyl-1-oxoindan-5-yl)	foam		96	$C_{17}H_{19}Cl_2NO_2$	C, H, N
24	(6,7-dichloro-2-methyl-2-cyclopentyl-1-oxoindan-5-yl)oxy	103–106	C$_6$H$_5$CH$_3$	92	$C_{17}H_{19}Cl_2NO_3 \cdot 0.5C_7H_8$	C, H, N
25	4-(C$_6$H$_5$)C$_6$H$_4$O	192–193	EtOH	67[e]	$C_{14}H_{13}NO_2$	C, H, N
26	4-(C$_6$H$_5$)C$_6$H$_4$S	188.5–189.5	EtOAc	86	$C_{14}H_{13}NOS$	C, N, S; H[f]
27	4-(C$_6$H$_5$)-1-C$_5$H$_{10}$N	112–114	pet. ether	76	$C_{13}H_{18}N_2O$	C, H, N
28	(10,11-dihydro-5*H*-5-oxodibenzo[*a*,*d*]cycloheptan-3-yl)oxy	133–135	EtOAc	77[e]	$C_{17}H_{15}NO_3$	C, H, N
29	(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptan-3-yl)oxy	147–150	MeOH	59[e]	$C_{17}H_{17}NO_2$	C, H, N

[a] Amides were prepared by reacting the ester intermediates with methanol saturated with ammonia at room temperature. [b] 1-Ad = 1-adamantyl. [c] C: calcd, 61.24; found, 60.80. [d] 3-HDBOM = (3-hydroxy-3,4-dihydro-2*H*-1,5-benzodioxepin-3-yl)methyloxy. [e] The amide was prepared from the corresponding phenol intermediate by reaction of its sodium salt with ethyl bromoacetate in ethanol at room temperature, followed by treatment of the crude ester with methanolic ammonia. [f] H: calcd, 5.39; found, 5.84.

to establish if conjugation between the aromatic ring and the acidic heterocyclic nucleus was important. The I_{50} value of 9.3×10^{-6} M determined for the benzyl analogue **77** indicated that conjugation was not a requirement. Introduction into the para position of **72** of halogen (**73** and **74**), isopropyl (**95**), and nitro (**75**) substituents served to increase potency somewhat, whereas a *p*-amino group (**76**) resulted in reduced activity. The larger, lipophilic substituents adamantyl (**79**), cyclohexyl (**78**), and 1,2,3,4-tetrahydro-1-naphthyl (**85**) afforded compounds with potencies close to that of the iodo derivative **74** (i.e., I_{50} values in the $1–2 \times 10^{-6}$ M range). However, introduction of a *p*-phenyl substituent to give the biphenyl derivative **80** resulted in a further elevation of potency to the 10^{-7} M range. Since this result represented an increase in activity of nearly two orders of magnitude over the simple phenyl derivative **72**, it was decided to carry out a more extensive structure–activity study with this lead. Insertion of an oxygen atom between the biphenyl system and the pyrroledione ring (**88**) increased potency further, while a sulfur atom in that position reduced activity (**89**). Placement of a carbonyl group between the biphenyl and pyrrole-2,5-dione nuclei (**87**) reduced activity considerably. Methylation on either the nitrogen or 3-hydroxy of the pyrroledione system (**81** and **82**) rendered the compounds essentially inactive, indicating the requirement for two acidic functions. The inactivities of the three compounds **96–98**, in which the heterocyclic ring is also modified, lend further support to this conclusion. Introduction of a bromine substituent at the 4′-position of the biphenyl group (**83**) gave the most potent compound in the biphenyl series, with an I_{50} value of 8.7×10^{-8} M against pig liver enzyme. (This compound, when tested against purified human liver GAO,[7] gave an I_{50} of 1.1×10^{-7} M). In contrast, addition of two chlorine atoms combined with insertion of a methylene group between the two rings of the biphenyl system gave a compound (**84**) with only slightly greater potency than the unsubstituted biphenyl **80**. In an effort to explore the question of whether activity could be modified significantly in derivatives containing two aromatic rings in fixed geometry, the tricyclic compounds **101–103** (Table IX) were prepared. Their potencies are comparable to those of the corresponding unsubstituted biphenyls, suggesting that lipophilicity is more important than the shape of the substituent group. Two other types of fused ring analogues were also examined. The hydroxyindan derivative **100** was quite potent, whereas the closely related indan **99** and the two naphthalene derivatives **90** and **91** were about an order of magnitude less active. The rather large 3-hydroxy-3,4-dihydro-2*H*-1,5-benzodioxepin-3-yl substituent on the naphthalene ring of **91** was introduced in an effort to determine whether there might be a physical limit to the size of the hydrophobic binding pocket at the active site of GAO.

In an extension of the study of substituents that would impart high GAO inhibitory potencies, a number of heterocyclic modifications of the biphenyl system were investigated. Compound **86**, with a 4-(2-phenylindol-1-yl)-phenyl substituent, exhibited inhibitory activity in the 10^{-7} M range, as did **124** and **106**, compounds with thiophene and thiazole rings adjacent to the pyrroledione nucleus. Because of the ease of synthesis, as well as the close analogy with the biphenyl lead, the thiazole system was selected for a systematic SAR investigation. The conclusions that may be drawn from the data on thiazole compounds **105–123** in Table X are as follows: (1) Inhibitory potencies in the 10^{-7} M range against porcine liver GAO are realized with 2-(halophenyl)thiazol-4-yl and 2-(halo-

Table IV. Thioamide Intermediates[a]

compd	R	mp, °C	solvent	yield, %	formula	anal.
30	2,3-$Cl_2C_6H_3$	123–125	Et_2O–pet. ether	100[b]	$C_7H_5Cl_2NS$	C, H, N
31	4-CH_3-3-ClC_6H_3	158–160	Et_2O	95	C_8H_8ClNS	C, H, N
32	4-thiazolyl	195–197	EtOH	92	$C_4H_4N_2S_2$	C, H, N
33	10,11-dihydro-5*H*-5-oxodibenzo[*a*,*d*]cyclohepten-3-yl	141–143	EtOH	74	$C_{16}H_{13}NOS$	C, H, N
34	4-CH_3O-2,6-$Cl_2C_6H_2$	196–198	Et_2O	60[b]	$C_8H_7Cl_2NOS$	C, H, N

[a] Prepared from the corresponding nitrile by the procedure of Fairfull et al.[30] [b] Crude yield.

benzyl)thiazol-4-yl substituents at the 4-position of the 3-hydroxy-1*H*-pyrrole-2,5-dione system. (2) Introduction of two chloro substituents into the 3,4- or 2,6-positions of the phenyl ring attached to thiazole increases I_{50} values to the 10^{-8} M range (**114** and **112**). Chloro substituents are more effective than methyl substituents (**115** and **116**). (3) Reversal of the positions on the thiazole of the phenyl and pyrrole nuclei can be done with little change in potency (**123** vs. **106**). (4) Introduction of a methyl substituent at the 5-position of the thiazole greatly reduces potency (**107**). This is thought to be due to the steric effect of the methyl group. (5) A tricyclic substituent at the 2-position of the thiazole imparts good activity (**104**). (6) Pyridine and substituted pyridine substituents at the 2-position of the thiazole also result in good inhibitory potencies (**118–120**). Other heterocyclic nuclei, such as pyrazine (**121**) and thiazole (**122**), gave rise to less potent compounds.

The results from the phenyl-, biphenyl-, and thiazole-substituted 3-hydroxypyrrole-2,5-diones and related analogues support the conclusions advanced by Schumann and Massey[5,10] regarding the active site of porcine GAO. Our work demonstrates that the interaction of a variety of quite large lipophilic substituents with the hydrophobic binding region of the enzyme active site can impart very high inhibitory potencies to derivatives of the 3-hydroxy-1*H*-pyrrole-2,5-dione ring system. After completion of the extensive structure–activity study with aromatic and heteroaromatic nuclei, it occurred to us that the effect of simple long-chain alkyl substituents on this diacidic ring system should also be explored. (In the work of Schumann and Massey, investigation of the inhibitory activities of alkanoic acids had indicated the existence of the hydrophobic bonding region at the active site of GAO.[5,10]) Testing of the *n*-decyl and *n*-dodecyl derivatives **92** and **93** (Table VII) showed them to have I_{50} values in the 10^{-8} M range. These representatives were selected so as to have a total number of carbon atoms roughly comparable to that in the biphenyl series. The I_{50} values for **92** and **93** should be compared to those of the corresponding straight-chain aliphatic acids, *n*-dodecanoic acid and *n*-tetradecanoic acid, which were determined to be 1×10^{-5} M and 3.4×10^{-6} M, respectively. This comparison constitutes additional evidence for the need for two acidic centers in close proximity for high in vitro inhibitory potencies against GAO, as seen with the 3-hydroxy-1*H*-pyrrole-2,5-dione derivatives.

As an independent check on the screening assay procedure, in which the reduction of the dye 2,4-dichlorophenolindophenol (DCIP) provides the read out, results are presented for six compounds that were also assayed against GAO by using spinach glyoxylate reductase enzyme coupled to NADH as the electron acceptor. This modification afforded the advantages that substrate concentrations remained constant, no product accumulated, and subsequent oxidation of glyoxylate to oxalate did not occur.

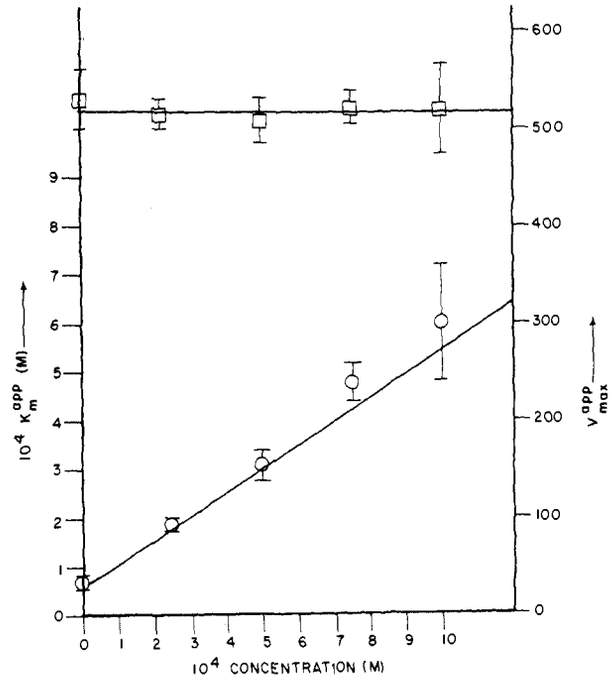


Figure 1. V_{max}^{app} (□) and K_m^{app} (○) parameter values were calculated by nonlinear least-squares fits of enzyme rate data to the Michaelis–Menten equation at the indicated concentrations of **80**. The vertical lines represent estimates of the standard deviations of these parameters. The solid horizontal line through the V_{max}^{app} parameters indicates their weighted average. The solid line through the K_m^{app} parameters represents the best fit to a model of competitive inhibition by **80**.

The data are presented in Tables VII, IX, and X.

Quantitative Structure–Activity Correlations. The pattern of inhibition of glycolate oxidase produced by a selected group of compounds from Tables VII and X was determined for fixed DCIP and variable sodium glycolate concentrations. For all compounds investigated, the apparent maximum velocity V_{max}^{app}, was found to be independent of glycolate concentration, and the apparent Michaelis constant, K_m^{app}, was found to increase linearly with glycolate concentration. A representative plot of these parameters for the 4-biphenyl derivative **80** is shown in Figure 1. Such an inhibition pattern is indicative of compounds that are competitive inhibitors with respect to glycolate.[19] From plots of K_m^{app} vs. [glycolate], estimates of the inhibition constant, K_I, may be obtained. Values of K_I are listed in Table XI.

The K_I value determined for **80** was checked by a direct study of its binding using circular dichroism spectropolarimetry. The observed change in ellipticity at 380 nm vs. inhibitor concentration is shown in Figure 2. A Lowry

(19) Segel, I. H. "Enzyme Kinetics"; Wiley-Interscience: New York, 1975.

Table V. Substituted Ethyl Thiazol-4-ylacetate Intermediates

compd	R_2	R_5	mp or bp (mm), °C	solvent	yield, %	formula	anal.
35	4-FC_6H_4	H	44–47[b]		100	$C_{13}H_{12}FNO_2S$	C, H, N
36	3-BrC_6H_4	H	68–70	pet. ether	65	$C_{13}H_{12}BrNO_2S$	C, H, N
37	4-BrC_6H_4	CH_3	89–91	Et_2O–pet. ether	66	$C_{14}H_{14}BrNO_2S$	C, H, N
38	4-ClC_6H_4[a]	H	114–116	$CHCl_3$–Et_2O	11	$C_{13}H_{12}ClNO_2S$	C, H, N
39	3-$CF_3C_6H_4$	H	68–69	pet. ether	57	$C_{14}H_{12}F_3NO_2S$	C, H, N
40	4-$BrC_6H_4CH_2$	H	160–163	EtOH	61	$C_{14}H_{14}BrNO_2S$·HCl	C, H, N
41	2,6-$Cl_2C_6H_3$	H	183–185 (0.2)		29	$C_{13}H_{11}Cl_2NO_2S$	C, H, N
42	2,3-$Cl_2C_6H_3$	H	68–70	Et_2O–pet. ether	75	$C_{13}H_{11}Cl_2NO_2S$	C, H, N
43	3,4-$Cl_2C_6H_3$	H	74–77	Et_2O–pet. ether	72	$C_{13}H_{11}Cl_2NO_2S$	C, H, N
44	4-CH_3-3-ClC_6H_3	H	53–55	pet. ether	89[c]	$C_{14}H_{14}ClNO_2S$	C, H, N
45	2,6-$(CH_3)_2C_6H_3$	H	152–154	EtOH–Et_2O	80	$C_{15}H_{17}NO_2S$·HCl	C, H, N
46	4-CH_3O-2,6-$Cl_2C_6H_2$	H	76–78	pet. ether	100[c]	$C_{14}H_{13}Cl_2NO_3S$	C, H, N
47	4-C_5H_4N	H	165 (0.1 mm)		47	$C_{12}H_{12}N_2O_2S$	C, H, N
48	3-C_5H_4N	H	123–125[d]	acetone–pet. ether	87	$C_{12}H_{12}N_2O_2S$·HCl	C, H, N
49	2,6-$(CH_3)_2$-4-C_5H_2N	H	94–96	dioxane	20	$C_{14}H_{16}N_2O_2S$·HCl	C, H, N, Cl
50	2-pyrazinyl	H	oil		100[c]	$C_{11}H_{11}N_3O_2S$	
51	4-thiazolyl	H	88–90	Et_2O–pet. ether	86	$C_{10}H_{10}N_2O_2S_2$	C, H, N
52	10,11-dihydro-5*H*-5-oxodibenzo[*a*,*d*]cyclohepten-3-yl	H	108–110	EtOH	82	$C_{22}H_{19}NO_3S$	C, H, N

[a] Reference 31. [b] Bp 135–138 °C (0.2 mm). [c] Crude yield. Intermediate was utilized without purification. [d] Characterized as the hydrochloride salt. The free base was an oil.

protein measurement[20] yields an estimated maximum glycolate oxidase concentration of $7.0 \pm 0.5 \times 10^{-7}$ M. When this fixed enzyme concentration is used, the parameters that best fit the binding data are:

$$K_I = 1.1 \pm 0.8 \times 10^{-7} \text{ M}$$

$$[\theta]^{380} = 1.07 \pm 0.1 \times 10^4 \text{ deg cm}^2/\text{dmol}$$

If the enzyme concentration is allowed to vary (to allow for the contribution of protein impurities to the Lowry results), a slightly better fit of the data is obtained:

$$K_I = 3.1 \pm 1.9 \times 10^{-7} \text{ M}$$

$$[\theta]^{380} = 2.4 \pm 1.8 \times 10^4 \text{ deg cm}^2/\text{dmol}$$

$$[\text{enzyme}] = 3.4 \pm 2.3 \times 10^{-7} \text{ M}$$

In either case, the value of K_I obtained from the above titration agrees well with that obtained from the steady-state kinetics analysis.

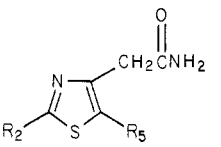
In order to facilitate a search for quantitative structure–activity relationships within the series of compounds reported in Tables VII and X, the I_{50} values were converted to pI_{50} ($= -\log_{10} I_{50}$, molar concentrations) (see Table XII). Estimates of the hydrophobic parameter, π, and the "steric" parameter, MR (group molar refractivity), for the substituents were obtained from compilations of these parameters[21,22] or calculated from their additivity characteristics.[23] MR is only an approximate measure of steric effects and is known to be also related to electronic polarizability.[24] However, the heterogeneous nature of the substituents employed in this work ruled out the use of more theoretically justified parameters, such as Es,[25] which have been tabulated for only a limited number of substituents.[26] Of the 53 final products, **72** through **124**, 12 were excluded from the QSAR analyses. Five were not included because they represented modifications of the parent pyrrole-2,5-dione ring system through substitution at the N or O positions (**81**, **82**, and **96**) or through the removal of the 3-hydroxy group (**97** and **98**). All such modifications caused a fundamental reduction in glycolate oxidase inhibitory activity independent of the nature of the 4-substituent. The remaining seven compounds, **91** and **99–104**, were not considered, since it was felt that the extremely complicated nature of these substituents prevented an accurate estimation of their π or MR substituent values.

Regression equations were then calculated in order to determine if any relationship exists between the biological response, pI_{50}, and these physical parameters. In the following equations the error term for each coefficient is the estimate of its standard deviation. The figure in parentheses below each parameter is its associated Student's t test. We have also listed the number of compounds employed, N, the regression coefficient, R, the standard deviation of the regression, S, and the value of the F test, F.

For the compounds of Table XII, the steric and hydrophobic parameters fit the data nearly equally well (eq

(20) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.
(21) Hansch, C.; Leo, A.; Unger, S. H.; Kim, K. H.; Nikaitani, D.; Lien, E. J. *J. Med. Chem.* **1973**, *16*, 1207.
(22) Hansch, C.; Rockwell, S. D.; Jow, P. Y. C.; Leo, A.; Steller, E. E. *J. Med. Chem.* **1977**, *20*, 304.
(23) Leo, A.; Hansch, C.; Elkins, D. E. *Chem. Rev.* **1971**, *71*, 525.
(24) LeFevre, R. J. W. *Adv. Phys. Org. Chem.* **1965**, *3*, 1–90.
(25) Hancock, C. K.; Meyers, E. A.; Yager, B. J. *J. Am. Chem. Soc.* **1961**, *83*, 4211.
(26) Unger, S. H.; Hansch, C. *Prog. Phys. Org. Chem.* **1976**, *12*, 91–118.

Table VI. Substituted Thiazol-4-ylacetamide Intermediates



compd	R_2	R_5	mp, °C	solvent	yield, %	formula	anal.
53	4-FC_6H_4	H	164-166	EtOH-H_2O	58	$C_{11}H_9FN_2OS$	C, H, N
54	4-BrC_6H_4 [c]	H	181.5-182.5	EtOH	65 [a]	$C_{11}H_9BrN_2OS$	C, H, S
55	4-BrC_6H_4	CH_3	182-184	toluene	40	$C_{12}H_{11}BrN_2OS$	C, H, N
56	3-BrC_6H_4	H	150-152	EtOH	78	$C_{11}H_9BrN_2OS$	C, H, N
57	4-ClC_6H_4 [c]	H	168-170	EtOH	54	$C_{11}H_9ClN_2OS$	C, H, N
58	3-$CF_3C_6H_4$	H	160-162	EtOH-Et_2O-pet. ether	70	$C_{12}H_9F_3N_2OS$	C, H, N
59	4-$BrC_6H_4CH_2$	H	123-126	EtOH	44	$C_{12}H_{11}BrN_2OS$	H, N; C [a]
60	2,6-$Cl_2C_6H_3$	H	128-130	Et_2O	85	$C_{11}H_8Cl_2N_2OS$	C, H, N
61	2,3-$Cl_2C_6H_3$	H	153-154	EtOH-Et_2O	85	$C_{11}H_8Cl_2N_2OS$	C, H, N
62	3,4-$Cl_2C_6H_3$	H	175-177	THF	85	$C_{11}H_8Cl_2N_2OS$	C, H, N
63	4-CH_3-3-ClC_6H_3	H	175-177	EtOH	61	$C_{12}H_{11}ClN_2OS$	C, H, N
64	2,6-$(CH_3)_2C_6H_3$	H	114-116	toluene	85 [b]	$C_{13}H_{14}N_2OS$	C, H, N
65	4-CH_3O-2,6-$Cl_2C_6H_2$	H	152-154	THF-Et_2O-pet. ether	35	$C_{12}H_{10}Cl_2N_2O_2S$	C, H, N
66	4-C_5H_4N	H	197-199	EtOH	49	$C_{10}H_9N_3OS$	C, H, N
67	3-C_5H_4N	H	175-177	acetone	29	$C_{10}H_9N_3OS$	C, H, N
68	2,6-$(CH_3)_2$-4-C_5H_2N	H	208-212	toluene	85 [b]	$C_{12}H_{13}N_3OS$	C, H, N
69	2-pyrazinyl	H	169-171	EtOH-Et_2O-pet. ether	36	$C_9H_8N_4OS$	C, H, N
70	4-thiazolyl	H	175-177	H_2O	79	$C_8H_7N_3OS_2$	C, H, N
71	10,11-dihydro-5*H*-5-oxodibenzo[*a*,*d*]cyclohepten-3-yl	H	171-173	EtOH	71	$C_{20}H_{16}N_2O_2S$	C, H, N

[a] C: calcd, 46.31; found, 46.97. [b] Crude yield. [c] Reference 31.

1 and 2). The equivalence between eq 1 and 2 is reflected in the large correlation between π and MR of 0.824.

$$pI_{50} = \underset{(5.84)}{(0.45 \pm 0.08)\pi} + 4.54 \quad (1)$$

$$N = 41;\ R = 0.68;\ S = 0.736;\ F = 34.13$$

$$pI_{50} = \underset{(5.62)}{(0.056 \pm 0.010)MR} + 3.32 \quad (2)$$

$$N = 41;\ R = 0.67;\ S = 0.749;\ F = 31.64$$

An examination of the residuals from eq 1 and 2 reveals a significant fact: 16 of 18 (eq 1) of the substituents containing a thiazolyl group connected to the pyrrole-2,5-dione ring system (**105–123**, excluding **107**, of Table XII) had observed pI_{50} values larger than predicted. Consequently, a dummy parameter (D) was created and given a value of 1.0 to signal the presence of a thiazole group and 0.0 to signal its absence. A multiple regression fit revealed that the data were best fit by a relationship involving π and D (eq 3).

$$pI_{50} = \underset{(9.26)}{(0.50 \pm 0.05)\pi} + \underset{(6.62)}{(1.1 \pm 0.2)D} + 3.91 \quad (3)$$

$$N = 41;\ R = 0.87;\ S = 0.508;\ F = 57.76$$

The equation relating MR and D to pI_{50} (eq 4) does not fit the data as well, and an equation employing π, MR, and D is not significantly better than eq 3.

$$pI_{50} = \underset{(5.52)}{(0.051 \pm 0.009)MR} + \underset{(2.98)}{(0.65 \pm 0.22)D} + 3.28 \quad (4)$$

$$N = 41;\ R = 0.74;\ S = 0.683;\ F = 23.48$$

The substituents in the compounds of Table XII constitute an extremely heterogeneous collection whose diversity might mask the significance of the contribution of any single parameter. Accordingly, a subset of substituents consisting of hydrocarbon or substituted hydrocarbon radicals in which no heteroatom is near the pyrrole-2,5-dione moiety (**72–80**, **83–85**, **90**, **92**, **93**, and **95** of Table XII) was selected for further consideration.

An equation relating pI_{50} to π (eq 5) is seen to give a reasonably good fit. Over 72% of the variance for this restricted subset of data is now explained by the hydrophobic parameter alone.

$$pI_{50} = \underset{(6.02)}{(0.44 \pm 0.07)\pi} + 4.24 \quad (5)$$

$$N = 16;\ R = 0.85;\ S = 0.446;\ F = 36.27$$

The fit employing the molar refractivity parameter is not as good (eq 6).

$$pI_{50} = \underset{(3.71)}{(0.04 \pm 0.01)MR} + 3.94 \quad (6)$$

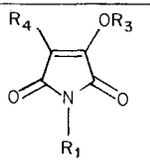
$$N = 16;\ R = 0.70;\ S = 0.601;\ F = 13.74$$

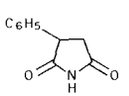
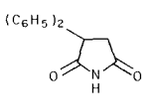
The standard deviations of the regressions for eq 1–6 are in all cases considerably greater than the error in the pI_{50} values (conservatively estimated to be less than ±0.1 from the I_{50} errors listed in Tables VII and X). This implies that π, MR, and D are not sufficient to completely explain the biological results. A similar finding has been reported for other series of glycolic acid oxidase inhibitors.[6] While additional parameters would undoubtedly improve the fit of the data, substituent values are not available for the heterogeneous collection of compounds considered in this investigation.

Rat Liver Perfusion Studies. In a typical single pass experiment with **114**, inhibition of oxalate formation from glycolate in the perfused rat liver was shown to be dependent on the concentration of **114** in the perfusate. Inhibitions of 43, 67, and 79% were measured at 0.035, 0.43, and 0.85 μM, respectively. A similar result was obtained with **92**.

The inhibition of oxalate from glycolate by **83** was also demonstrated to be concentration dependent. In one experiment, 22% inhibition was obtained at 0.6 μM and 92% at 3 μM. However, the absolute value of the observed response was variable since, in other experiments, inhibitions of 69–81% were achieved with a 0.1 μM concen-

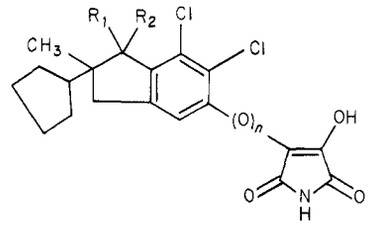
Table VII. 4-Aryl- and 4-Alkyl-3-hydroxy-1*H*-pyrrole-2,5-dione Derivatives



compd	R_1	R_3	R_4	method	mp, °C	solvent	yield, %	formula	anal.	pK_a[a]	I_{50}, M × 10^{-7}, for inhibn of GAO in vitro: DCIP proced	I_{50}, M × 10^{-7}, for inhibn of GAO in vitro: spinach glyoxylate reductase procedure
72	H	H	C_6H_5	12 (a)							135 ± 4	
73	H	H	4-BrC_6H_4	11 (b)							25 ± 3	
74	H	H	4-IC_6H_4	A	269–271	MeCN	32	$C_{10}H_6INO_3$	C, H, N		20 ± 3	
75	H	H	4-$NO_2C_6H_4$	A	277–278[j]						80 ± 6	
76	H	H	4-$NH_2C_6H_4$	*b*	>310	DMF–MeCN	66	$C_{10}H_8N_2O_3$	C, H, N		250 ± 20	
77	H	H	$C_6H_5CH_2$	B	140–142	$CHCl_3$	4	$C_{11}H_9NO_3$	C, H, N		93 ± 6	
78	H	H	4-(c-C_6H_{11})C_6H_4	A	275–278	MeCN	63	$C_{16}H_{17}NO_3$	C, H, N		10.6 ± 1.3	
79	H	H	4-(1-Ad)C_6H_4[c]	A	313–316	MeCN	63	$C_{20}H_{21}NO_3$	C, H, N		18 ± 2	
80	H	H	4-(C_6H_5)C_6H_4	A	305–307 dec	DMF–MeCN	62	$C_{16}H_{11}NO_3$	C, H, N	4.13	6.5 ± 0.5	6.4
81	CH_3	H	4-(C_6H_5)C_6H_4	A	275–277	MeCN	42	$C_{17}H_{13}NO_3$	C, N; H[d]	3.95	<10% @ 1 × 10^{-3} M	
82	H	CH_3	4-(C_6H_5)C_6H_4	*e*	182–183	toluene		$C_{17}H_{13}NO_3$	C, H, N	10.7	14% @ 1 × 10^{-3} M	
83	H	H	4-(4-BrC_6H_4)C_6H_4	A	326–328 dec	DMF–MeCN	50	$C_{16}H_{10}BrNO_3$	C, H, N, Br	4.00	0.87 ± 0.08	1.03
84	H	H	4-(3,4-$Cl_2C_6H_3CH_2$)C_6H_4	A	333–334	MeCN	54	$C_{17}H_{11}Cl_2NO_3$	C, H, N		2.2 ± 0.2	
85	H	H	4-(1,2,3,4-H_4-1-$C_{10}H_7$)C_6H_4	A	177–179	MeOH	36	$C_{20}H_{17}NO_3$	C, H, N		13 ± 1	
86	H	H	4-(2-C_6H_5-1-C_8H_6N)(C_6H_4)	A	233–235	EtOH	37	$C_{24}H_{16}N_2O_3 \cdot C_2H_5OH$	C, H, N		5 ± 1	
87	H	H	4-(C_6H_5)C_6H_4CO	A	265–266 dec	MeCN	36	$C_{17}H_{11}NO_4$	C, H, N		172 ± 13	
88	H	H	4-(C_6H_5)C_6H_4O	A	247–248	2-PrOH	48	$C_{16}H_{11}NO_4$	C, H, N	3.80[f]	2.7 ± 0.2	
89	H	H	4-(C_6H_5)C_6H_4S	A	220 dec	2-PrOH	63	$C_{16}H_{11}NO_3S$	C, H, S	2.85[f]	30.2 ± 1.7	
90	H	H	6-OCH_3-2-$C_{10}H_7$	A	265–267	2-PrOH	58	$C_{15}H_{11}NO_4$	C, H, N		22 ± 2	
91	H	H	6-(3-HDBOM)-2-$C_{10}H_7$[g]	A	285–287	THF–MeCN	42	$C_{24}H_{19}NO_7$	C, H, N		20 ± 2	
92	H	H	*n*-$C_{10}H_{21}$	B	109–111	c-C_6H_{12}	33[h]	$C_{14}H_{23}NO_3$	C, H, N	5.30	0.59 ± 0.09	
93	H	H	*n*-$C_{12}H_{25}$	B	108–109	c-C_6H_{12}	17[h]	$C_{16}H_{27}NO_3$	C, H, N	5.30	0.81 ± 0.09	
94	H	H	$NH_2C(=O)$	11 (a)							65000 ± 7000	
95	H	H	4-$(CH_3)_2CHC_6H_4$	*i*							46 ± 2	
96	C_6H_5	H	C_6H_5	11 (c)						3.80	7% @ 1 × 10^{-3} M	
						Related Compounds						
97				29						9.44	10% @ 1 × 10^{-3} M	
98				*i*						9.25	8% @ 1 × 10^{-3} M	

[a] pK_a determinations were done in 50% EtOH unless otherwise indicated. [b] Prepared by low-pressure catalytic reduction of the corresponding nitro derivative in EtOH with 10% Pd/C catalyst. [c] 1-Ad = 1-adamantyl. [d] H: calcd 4.69; found, 4.25. [e] See Experimental Section. [f] Determined in H_2O. [g] 3-HDBOM = (3-hydroxy-3,4-dihydro-2*H*-1,5-benzodioxepin-3-yl)methyloxy. [h] Overall yield for two steps starting from the alkyl nitrile. [i] Supplied by W. B. Gall, Chemical Data Department, Merck Sharp & Dohme Research Laboratories, Rahway, NJ. [j] Reference 13.

Table VIII. 4-Indanyl-3-hydroxy-1*H*-pyrrole-2,5-dione Derivatives



compd	R^1	R^2	n	method	mp, °C	solvent	yield, %	formula	anal.	I_{50}, M × 10^{-7}, for inhibn of GAO in vitro (DCIP proced)
99	=O		0	A	213.5–214	DMF–MeCN	58	$C_{19}H_{17}Cl_2NO_4 \cdot C_5H_{12}N_2$	C, H, N	67 ± 4
100	H	OH	1	A	259–261	DMF–MeCN	67	$C_{19}H_{19}Cl_2NO_5 \cdot C_{12}H_{23}N \cdot 0.5H_2O$[a]	C, H, N	4.0 ± 0.2

[a] Isolated as the dicyclohexylammonium salt.

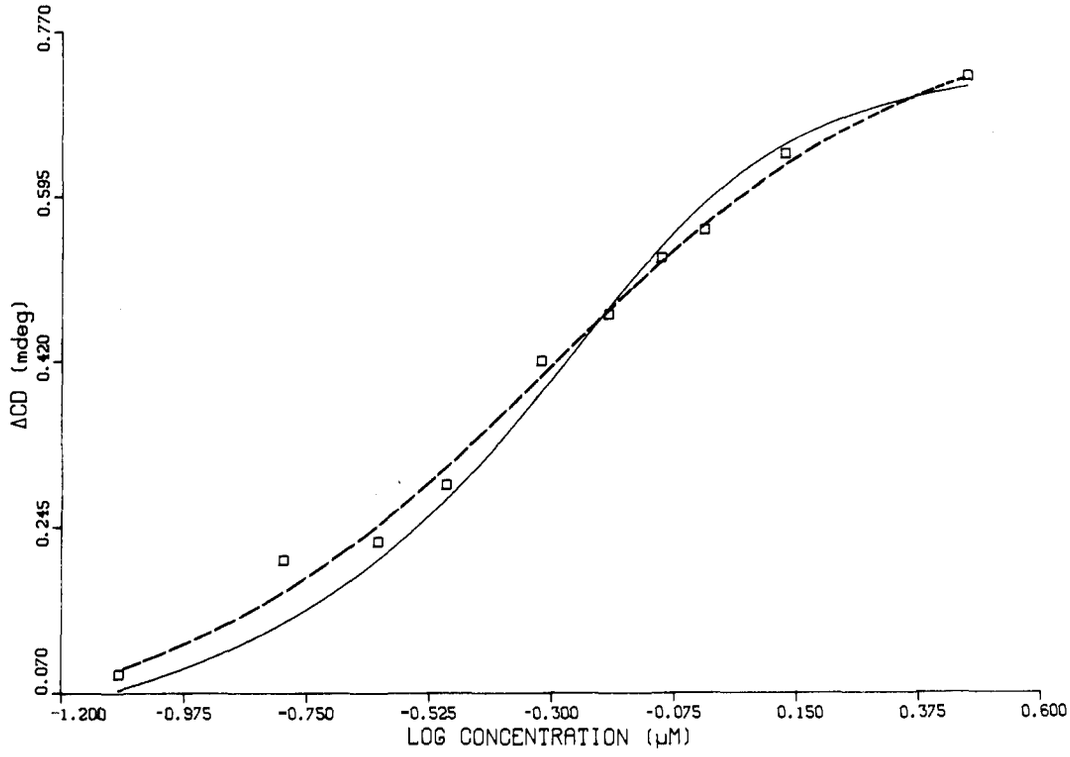


Figure 2. Plot of the change in observed CD with the log of compound (**80**) concentration: observed data (□); two-parameter fit (—); three-parameter fit (- - -).

tration of **83**. When the inhibitor was washed from the system and a fresh glycolate solution perfused, the rate of formation of oxalate approached preinhibitor values. For comparison, in the same protocol, the irreversible inhibitor, racemic ammonium 2-hydroxy-3-butynoate,[7,8] at 1×10^{-3} M resulted in 95% inhibition; however, after the washout, the rate was only 22% of the preinhibitor value.

In Vivo Studies in Rats. The ability of **83** to reduce oxalate biosynthesis in vivo was examined by chronic treatment in normal rats and in rats rendered hyperoxaluric by chronic ingestion of ethylene glycol (Tables XIII and XIV). In normal animals, there was no significant effect through 58 days on treatment with inhibitor at 10 (mg/kg)/day. In ethylene glycol fed animals, however, the inhibitor effected a time-dependent reduction, so that by 58 days, oxalic acid output was reduced to near normal levels. When oxalate excretion was normalized on the basis of endogenous urinary creatinine (Table XIV), significant reduction was achieved by the first sampling interval and by day 43 reached the level of $p < 0.001$.

Toxicity Data. Compounds **80**, **83**, **88**, **92**, and **114** when examined in the Ames test showed no evidence for an increase in the number of revertants over that seen with controls. Approximate LD_{50} values in mice by ip and po administration are presented for five compounds in Table XV. This determination is done in our laboratories on selected compounds in order to establish that a series is not inherently highly toxic and also to have an early indication of oral activity. Thus, the results for **118** (po/ip ratio of ~10) suggest that this compound is not well absorbed orally in this species.

Conclusion

The 4-substituted 3-hydroxy-1*H*-pyrrole-2,5-dione derivatives reported herein represent the most potent reversible competitive inhibitors of mammalian GAO yet discovered. Structure–activity correlations are consistent with the conclusions of Schumann and Massey[5,10] that the

Table IX. 4-Tricyclic-Substituted 3-Hydroxy-1*H*-pyrrole-2,5-dione Derivatives

compd	X	Y	Z	method	mp, °C	solvent	yield, %	formula	anal.	I_{50}, M × 10^{-7}, for inhibn of GAO in vitro: DCIP proced	I_{50}, M × 10^{-7}, for inhibn of GAO in vitro: spinach glyoxylate reductase proced
101	CH_2	O	O	A	115–117	EtOAc	58	$C_{19}H_{13}NO_5 \cdot C_3H_7NO$	C, H, N	2.6 ± 0.2	8.0
102	CH_2	H_2	O	A	90–105	toluene	20	$C_{19}H_{15}NO_4 \cdot 1.25H_2O$	C, H, N	5 ± 1	
103	O	O		A	278–280	EtOAc	35	$C_{18}H_{11}NO_5$	C, H; N[a]	7 ± 1	
104	CH_2	O		A	284–285 dec	DMF–MeCN	52	$C_{22}H_{14}N_2O_4S$	C, H, N	0.9 ± 0.2	

[a] N: calcd, 4.36; found, 3.90.

active site of porcine GAO contains two positively charged groups in close proximity, plus a hydrophobic bonding region. In rat liver perfusion studies with **83**, **92**, and **114**, three of the most potent compounds in vitro, it was demonstrated that the conversion of [^{14}C]glycolate to [^{14}C]-oxalate was inhibited in a concentration-dependent manner. Administering **83** orally to rats, consuming ethylene glycol in the drinking water, for a 58-day period resulted in a significant reduction in urinary oxalate output. On the other hand, normal rats treated with **83** over the same period of time without ethylene glycol showed no reduction in urinary oxalate levels. Compounds from this investigation should prove useful in determining the extent to which GAO contributes to oxalate production in humans. If this enzyme should be proven to be a major catalyst for oxalate formation in humans, compounds of this type may be of therapeutic value in the treatment of oxalate disorders.

Experimental Section

Chemistry. Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were determined in $CDCl_3$ with Varian Associates T-60, EM-360, and EM-390 instruments. Chemical shifts were recorded in parts per million (δ) relative to Me_4Si as internal standard. Infrared spectra were determined with Perkin-Elmer 257 and 297 spectrophotometers. Elementary analyses were determined in our West Point, PA, laboratories with a Perkin-Elmer Model 240 elemental analyzer or by Dr. C. Daessle, Organic Microanalysis, Montreal, Quebec, and are within ±0.4% when indicated by the symbols for the elements. Low-resolution mass spectra were determined by the Morgan-Schaffer Corp., Montreal, Quebec, or by Mr. R. E. Rhodes and Dr. H. Ramjit in our West Point, PA, laboratories. Thin-layer chromatography was carried out with Uniplates from Analtech coated with 250 μm of silica gel GF or with Eastman chromogram sheets, No. 13181, precoated with silica gel.

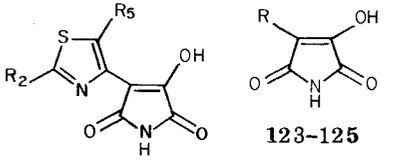
4-(4′-Bromo[1,1′-biphenyl]-4-yl)-3-hydroxy-1*H*-pyrrole-2,5-dione (83). Method A. To a mixture of 2-(4′-bromo[1,1′-biphenyl]-4-yl)acetamide (3.81 g, 0.013 mol) and diethyl oxalate (2.01 g, 0.014 mol) in dry DMF (40 mL), under a nitrogen atmosphere and cooled to ice temperature, was added potassium *tert*-butoxide (3.24 g, 0.029 mol) in two equal portions, 15 min apart. The reaction mixture, after stirring at room temperature overnight, was poured into water (200 mL). Acidification with 6 N HCl gave the product as a yellow solid, which was recrystallized from hot DMF (30 mL).

4-*n*-Decyl-3-hydroxy-1*H*-pyrrole-2,5-dione (92). Method B. To a solution of *n*-dodecanenitrile (36.28 g, 0.20 mol) and diethyl oxalate (30.8 g, 0.21 mol) in DMF (350 mL) was added in portions potassium *tert*-butoxide (24.4 g, 0.20 mol), and the mixture was stirred at room temperature overnight. Following evaporation of most of the solvent and addition of Et_2O and H_2O, the mixture was made acidic with concentrated HCl. The Et_2O extract was washed with H_2O, dried ($MgSO_4$), and evaporated. The residue was crystallized from petroleum ether to give 41.6 g (73.8%), mp 53–56 °C, of ethyl 3-cyano-2-hydroxytridec-2-enoate: IR (Nujol) 3320, 2210, 1728 cm^{-1}. Anal. ($C_{16}H_{27}NO_3$) C, H, N.

A mixture of the above intermediate (41.6 g, 0.15 mol) and methanesulfonic acid (200 mL) was allowed to stir overnight at room temperature. The reaction mixture was poured into 80% EtOH and allowed to stand for 4 h. Evaporation of the EtOH, followed by filtration, gave a crude yellow solid (28.0 g). Recrystallization from hot cyclohexane gave pure **92** (17.2 g, 45.3%).

4-(Phenylmethyl)-3-hydroxy-1*H*-pyrrole-2,5-dione (77). Alternative Method B. Sodium (0.92 g, 0.04 mol) was allowed to react completely with EtOH (45 mL). After cooling in an ice bath, there were then added diethyl oxalate (4.66 g, 0.032 mol) and 3-phenylpropionitrile (4.17 g, 0.032 mol). The mixture was stirred at room temperature for 1 h, refluxed for 3 h, and then allowed to stand at room temperature overnight. The solvent was removed, and the residue was partitioned between $MeCl_2$ and dilute HCl. After the solution was dried, the $MeCl_2$ was evapo-

Table X. 4-Heterocyclic-Substituted 3-Hydroxy-1*H*-pyrrole-2,5-dione Derivatives



105-122

123-125

compd	R_2	R_5	method	mp, °C	solvent	yield, %	formula	anal.	pK_a[a]	I_{50}, M × 10^{-7}, for inhibn of GAO in vitro: GAO in vitro DCIP proced	I_{50}, M × 10^{-7}, for inhibn of GAO in vitro: spinach glyoxylate reductase proced
105	4-FC_6H_4	H	A	243-246	aq NH_4OH-dil HCl[b]	44	$C_{13}H_7FN_2O_3S$	C, H, N		1.76 ± 0.05	
106	4-BrC_6H_4	H	A	273-274	dioxane	62	$C_{13}H_7BrN_2O_3S \cdot 0.5H_2O$	C, H, N		1.0 ± 0.1	7.5
107	4-BrC_6H_4	CH_3	A	250-252	acetone	13	$C_{14}H_9BrN_2O_3S$	C, H, N	4.13	260 ± 20	
108	3-BrC_6H_4	H	A	258-261	THF-acetone	42	$C_{13}H_7BrN_2O_3S$		3.90	1.5 ± 0.1	
109	4-ClC_6H_4	H	A	248-250	DMF-MeCN	49	$C_{13}H_7ClN_2O_3S$	C, H, N	4.20	1.37 ± 0.06	
110	3-$CF_3C_6H_4$	H	A	262-264	aq $HOCH_2CH_2NH_2$-HOAc[b]	69	$C_{14}H_7F_3N_2O_3S$	C, H, N	4.14	2.7 ± 0.4	
111	4-$BrC_6H_4CH_2$	H	A	199-202	acetone	21	$C_{14}H_9BrN_2O_3S$	C, H, N		6.6 ± 0.3	8.5
112	2,6-$Cl_2C_6H_3$	H	A	244-246	95% EtOH	31	$C_{13}H_6Cl_2N_2O_3S$	C, H, N		0.8 ± 0.1	
113	2,3-$Cl_2C_6H_3$	H	A	291-293	DMF-MeCN	49	$C_{13}H_6Cl_2N_2O_3S$	C, H, N		1.6 ± 0.3	
114	3,4-$Cl_2C_6H_3$	H	A	287-288 dec	DMF-MeCN	56	$C_{13}H_6Cl_2N_2O_3S$	C, H, N	3.8[c]	0.78 ± 0.08	
115	3-Cl-4-$CH_3C_6H_3$	H	A	273-276 dec	DMF-MeCN	51	$C_{14}H_9ClN_2O_3S$	C, H, N		1.2 ± 0.1	
116	2,6-$(CH_3)_2C_6H_3$	H	A	185-188	EtOH-H_2O	51	$C_{15}H_{12}N_2O_3S$	C, H, N		1.2 ± 0.1	
117	4-CH_3O-2,6-$Cl_2C_6H_2$	H	A	275-276	DMF	73	$C_{14}H_8Cl_2N_2O_4S$	C, H, N		1.7 ± 0.2	
118	4-C_5H_4N	H	A	290 dec	aq $HOCH_2CH_2NH_2$-HOAc[b]	44	$C_{12}H_7N_3O_3S$	C, H, N	3.2, 5.0[d]	3.4 ± 0.3	
119	3-C_5H_4N	H	A	269-270	DMF-MeCN	55	$C_{12}H_7N_3O_3S$	C, H, N		6.9 ± 0.3	
120	2,6-$(CH_3)_2$-4-C_5H_2N	H	A	288 dec	EtOH-H_2O	51	$C_{14}H_{11}N_3O_3S$	C, H, N		5.5 ± 0.3	
121	2-pyrazinyl	H	A	288-291 dec	DMF-MeCN	48	$C_{11}H_6N_4O_3S$	C, H, N		47 ± 4	100
122	4-thiazolyl	H	A	279-280 dec	DMF-MeCN	77	$C_{10}H_5N_3O_3S_2$	C, H, N		64 ± 4	
123	4-(4-BrC_6H_4)-2-C_3H_2NS		A	>310	aq $HOCH_2CH_2NH_2$-HOAc[b]	19	$C_{13}H_7BrN_2O_3S$	C, H, N	5.73[d]	2.4 ± 0.2	
124	5-(4-ClC_6H_4)-2-C_4H_3S		A	279-281 dec	dioxane	51	$C_{14}H_8ClNO_3S$	C, H, N		2.6 ± 0.2	
125	4-C_6H_5-1-$C_5H_{10}N$		A	227-229	MeCN	35	$C_{15}H_{16}N_2O_3$[e]	C, N; H[e]		*f*	

[a] pK_a's were determined in 50% EtOH, except where otherwise noted. [b] The compound was precipitated from dilute aqueous base solution by addition of acid. [c] Determined in 30% EtOH. [d] Determined in H_2O. [e] H: calcd, 5.92; found, 6.37. [f] Decolorized the indicator and could not be assayed by the standard procedure.

Table XI. Mechanism of Inhibition of Glycolate Oxidase by Selected 4-Substituted 3-Hydroxy-1*H*-pyrrole-2,5-dione Derivatives

compd	K_I, M × 10^{-7}	I_{50}, M × 10^{-7}
78	3.6 ± 1.4	10.6 ± 1.3
80	1.0 ± 0.5	6.5 ± 0.5
83	0.3 ± 0.2	0.87 ± 0.08
92	0.45 ± 0.12	0.59 ± 0.09
107	110 ± 20	260 ± 20
117	0.4 ± 0.3	1.7 ± 0.2

rated to give ethyl 3-cyano-2-hydroxy-4-phenylbut-2-enoate (2.81 g, 38%). Recrystallization from petroleum ether gave mp 80–83 °C. Anal. ($C_{13}H_{13}NO_3$) C, H, N.

The above intermediate (0.46 g, 0.002 mol) was added to EtOH saturated with hydrogen chloride (10 mL). After the solution was left standing at room temperature overnight, the solvent was evaporated, and the residue was dried at high vacuum. Dry $CHCl_3$ (20 mL) was added, and the mixture was heated under reflux for 3 h. Extraction of the $CHCl_3$ solution with excess 2 N NaOH, acidification of the basic extract with dilute HCl, and reextraction with $CHCl_3$ afforded, after evaporation of the $CHCl_3$, **77**.

2-[2-(3,4-Dichlorophenyl)thiazol-4-yl]acetamide (62). Method C. A mixture of 3,4-dichlorothiobenzamide (12.4 g, 0.06 mol) and ethyl 4-chloroacetoacetate in EtOH (75 mL) was heated at reflux for 4 h. While the solution was standing overnight at room temperature, the product crystallized. The mixture was diluted with water and filtered to give crude ethyl 2-[(3,4-dichlorophenyl)thiazol-4-yl]acetate (**43**). A mixture of this intermediate (13.5 g, 0.043 mol), concentrated NH_4OH (75 mL), and dioxane (25 mL) was stirred at room temperature for 5 days, when TLC showed a single component. The mixture was poured into ice–water, and the solid **62** was removed by filtration.

Methyl (4′-Bromo[1,1′-biphenyl]-4-yl)acetate (3). To a mixture of MeOH (600 mL), 70% perchloric acid (25 mL), and thallium trinitrate (20.3 g, 0.046 mol), cooled in an ice bath, was added 4-acetyl-4′-bromo[1,1′-biphenyl] (12.55 g, 0.046 mol). After the mixture was stirred overnight at room temperature, the solids were removed by filtration and washed well with $MeCl_2$. The filtrate plus washes was diluted with H_2O (500 mL), and the product was extracted into $MeCl_2$. After the extract was dried ($MgSO_4$) and evaporated, **3** was obtained.

2-(4′-Bromo[1,1′-biphenyl]-4-yl)acetamide (13). A mixture of **3** (12.28 g, 0.04 mol) in MeOH saturated with ammonia (75 mL) was stirred at room temperature for 4 days. Filtration, followed by recrystallization of the solid product, gave pure **13**.

***N*-Methyl-2-([1,1′-biphenyl]-4-yl)acetamide.** Methyl ([1,1′-biphenyl]-4-yl)acetate (0.75 g, 0.0033 mol) was added to a ~5 N solution of methylamine in MeOH (5 mL). After the solution was left standing at room temperature for 24 h, the desired *N*-methyl amide, mp 178–179.5 °C, was obtained by filtration (0.7 g, 93%). Anal. ($C_{15}H_{15}NO$) C, H, N.

4-([1,1′-Biphenyl]-4-yl)-3-methoxy-1*H*-pyrrole-2,5-dione (82). To a solution of **80** (0.265 g, 0.001 mol) in dry THF (5 mL) was added a slight excess of ethereal diazomethane. After 30 min, the solvent was evaporated, and the residue was recrystallized to afford **82** (0.22 g, 79%).

1-(4-Acetylphenyl)-2-phenylindole. A mixture of *p*-bromoacetophenone (19.9 g, 0.1 mol), 2-phenylindole (20.26 g,

Table XII. Physical Parameters for a Series of 4-Substituted 3-Hydroxy-1*H*-pyrrole-2,5-dione Derivatives

				pI_{50}				
compd	π	MR	D	obsd	calcd[a]	Δ	calcd[b]	Δ
72	1.96	25.36	0	4.87	5.42	−0.55	4.89	−0.02
73	2.82	33.21	0	5.60	5.81	−0.21	5.31	0.29
74	3.08	38.27	0	5.70	5.92	−0.22	5.44	0.26
75	1.68	31.69	0	5.10	5.29	−0.19	4.75	0.35
76	0.73	29.75	0	4.60	4.87	−0.27	4.28	0.32
77	2.01	30.01	0	5.03	5.44	−0.41	4.91	0.12
78	4.47	51.02	0	5.98	6.55	−0.57	6.13	−0.15
79	5.26	64.96	0	5.74	6.90	−1.16	6.52	−0.78
80	3.92	49.69	0	6.38	6.30	0.08	5.86	0.52
83	4.78	57.54	0	7.06	6.69	0.37	6.28	0.78
84	5.39	64.34	0	6.66	6.96	−0.30	6.58	0.07
85	5.31	65.19	0	5.89	6.93	−1.04	6.55	−0.66
86	4.23	65.40	0	6.30	6.44	−0.14	6.01	0.29
87	3.01	54.66	0	4.76	5.89	−1.13	5.41	−0.65
88	4.04	52.01	0	6.57	6.35	0.22	5.92	0.65
89	4.28	58.62	0	5.52	6.46	−0.94	6.04	−0.52
90	3.32	47.61	0	5.66	6.03	−0.37	5.56	0.10
92	5.06	47.41	0	7.23	6.81	0.42	6.42	0.81
93	6.06	56.68	0	7.09	7.26	−0.17	6.92	0.17
94	−1.49	9.81	0	2.19	3.87	−1.68	3.18	−0.99
95	3.49	39.31	0	5.34	6.11	−0.77	5.64	−0.30
105	2.91	45.56	1	6.75	5.85	0.90	6.42	0.33
106	3.63	53.52	1	7.00	6.17	0.83	6.78	0.22
107	3.81	58.14	0	4.58	6.25	−1.67	5.80	−1.22
108	3.63	53.52	1	6.82	6.17	0.65	6.78	0.04
109	3.48	50.67	1	6.86	6.10	0.76	6.71	0.15
110	3.65	49.66	1	6.57	6.18	0.39	6.79	−0.22
111	4.09	58.17	1	6.18	6.38	−0.20	7.01	−0.83
112	4.19	55.67	1	7.07	6.42	0.65	7.06	0.01
113	4.19	55.67	1	6.77	6.42	0.35	7.06	−0.29
114	4.19	55.67	1	7.11	6.42	0.69	7.06	0.05
115	4.04	55.24	1	6.92	6.35	0.57	6.98	−0.06
116	3.83	54.19	1	6.92	6.26	0.66	6.88	0.04
117	4.17	62.51	1	6.77	6.41	0.36	7.05	−0.28
118	1.13	43.34	1	6.48	5.05	1.43	5.54	0.94
119	1.13	43.34	1	6.16	5.05	1.11	5.54	0.62
120	2.60	52.58	1	6.26	5.71	0.55	6.27	−0.01
121	0.59	42.97	1	5.33	4.80	0.53	5.28	0.05
122	1.62	41.65	1	5.19	5.27	−0.08	5.79	−0.60
123	3.63	53.52	1	6.63	6.17	0.46	6.78	−0.15
124	4.28	54.40	0	6.58	6.46	0.12	6.03	0.54

[a] Calculated from eq 1. [b] Calculated from eq 3.

Table XIII. Effect of 83 Administered Orally on Urinary Oxalate in Normal and Ethylene Glycol Loaded Rats

expt no.	day	no. of animals	control	83	p
			Oxalic Acid (μg per 6 h) in Normal Rats		
1	8	15	70 ± 25	93 ± 43	NS
	16	15	99 ± 34	79 ± 48	NS
	24	15	114 ± 36	119 ± 31	NS
	30	15	109 ± 35	126 ± 25	NS
	37	15	100 ± 27	154 ± 43	NS
	44	15	95 ± 29	102 ± 24	NS
	51	15	159 ± 45	175 ± 46	NS
	58	15	144 ± 36	127 ± 30	NS
			Oxalic Acid (μg per 6 h) in Ethylene Glycol Treated Rats		
2	13	15	619 ± 159	546 ± 307	NS
	16	15	676 ± 116	583 ± 270	NS
	23	15	489 ± 234	373 ± 253	NS
	43	15	878 ± 296	587 ± 260	<0.01
	50	14	442 ± 262	283 ± 163	<0.10
	58	11	557 ± 213	171 ± 161	<0.001

Table XIV. Urinary Oxalate/Creatinine Ratios of Ethylene Glycol Treated Rats Administered 83 Orally

day	no. of animals	oxalate/creatinine control	83	p
13	15	0.43 ± 0.12	0.32 ± 0.19	<0.1
16	15	0.42 ± 0.09	0.31 ± 0.15	<0.025
23	15	0.34 ± 0.19	0.25 ± 0.15	<0.2
43	15	0.49 ± 0.11	0.12 ± 0.04	<0.001
50	14	0.18 ± 0.10	0.05 ± 0.03	<0.001
58	11	0.28 ± 0.11	0.03 ± 0.02	<0.001

Table XV. Approximate LD_{50} in Mice

compd	LD_{50}, mg/kg ip	po
80	439	555
83	374	479
88	216	845
114	521	1044
118	531	5000

0.105 mol), cuprous oxide (0.5 g), potassium carbonate (13.82 g), and pyridine (20 mL) was heated in a sealed Carius tube at 175 °C for 24 h in a rocking oven. The cooled reaction mixture was poured into water (1 L), and the product was extracted with $CHCl_3$ (800 mL). The extract was washed with 1 N HCl (80 mL), H_2O (250 mL), 1 N NaOH (80 mL), and finally H_2O (250 mL). The $CHCl_3$ solution was dried ($MgSO_4$) and evaporated. Recrystallization from MeCN of the residues from two batches gave 40.3 g (64.8%) of 1-(4-acetylphenyl)-2-phenylindole, mp 147–150 °C. The pure solid crystallized in large prisms from acetonitrile, mp 153–155 °C. Anal. ($C_{22}H_{17}NO$) C, H, N.

2-Acetyl-6-[(3-hydroxy-3,4-dihydro-2*H*-1,5-benzodioxepin-3-yl)methoxy]naphthalene. A mixture of 6-hydroxyacetonaphthone (186 mg, 0.001 mol), 3,4-dihydro-2*H*-1,5-benzodioxepin-3-spirooxirane[15] (0.178 g, 0.001 mol), and *n*-BuOH (2 mL) containing 1 drop of 4% Triton B in MeOH was heated at 120 °C for 72 h, during which time 2 more drops of the base were added at 24-h intervals. The mixture was evaporated to dryness, and the residue was purified by passing a solution in $CHCl_3$ down a short column of silica gel (6 g). The eluate was evaporated to dryness, and the residue crystallized from MeCN (1 mL) to give 0.19 g (53%) of product, mp 131–132.5 °C. Anal. ($C_{22}H_{20}O_5$) C, H.

Methyl (6,7-Dichloro-2-cyclopentyl-2-methyl-1-oxoindan-5-yl)acetate. Acetyl chloride (20 mL) was added slowly to ice-cold MeOH (50 mL), and the resulting mixture was added to (6,7-dichloro-2-cyclopentyl-2-methyl-1-oxoindan-5-yl)acetic acid[16] (3.9 g, 0.011 mol). The mixture was allowed to stand for 24 h and then evaporated under reduced pressure. The residue was dissolved in $MeCl_2$ (50 mL), and the solution was washed with H_2O (2 × 10 mL), dried ($MgSO_4$), and evaporated to a pale amber oil (4.1 g, 100%).

2-[(6,7-Dichloro-2-cyclopentyl-1-hydroxy-2-methylindan-5-yl)oxy]acetamide. 2-[(6,7-Dichloro-2-cyclopentyl-2-methyl-1-oxoindan-5-yl)oxy]acetamide[16] hemitoluene solvate (5.85 g, 0.015 mol) was added to a mixture of 2-PrOH (75 mL) and MeOH (15 mL). To the stirred mixture at room temperature was added sodium borohydride (0.55 g, 0.015 mol). After 1 h, a second portion of sodium borohydride (0.55 g, 0.015 mol) was added. After a further 3 h, the reaction mixture was diluted with ice–water (250 mL) and extracted with EtOAc (2 × 100 mL). The extract was washed with H_2O (250 mL) and then with saturated NaCl solution (50 mL) before drying ($MgSO_4$). Evaporation of the solvent and recrystallization of the residue from MeCN gave the product as a mixture of the two racemates, mp 168–179 °C. Anal. ($C_{17}H_{21}Cl_2NO_3$) C, H, N.

5-([1,1′-Biphenyl]-4-yl)isoxazole (127). To 50% sodium hydride in mineral oil (2.40 g, 0.05 mol) under nitrogen was added dry Et_2O (180 mL) and then absolute EtOH (2.14 g, 0.05 mol). The reaction mixture was cooled (ice bath), and a solution of 4-acetylbiphenyl (9.81 g, 0.05 mol) in a mixture of ethyl formate (4.24 g) and dry THF (75 mL) was added with stirring. Another 25 mL of dry Et_2O was added, and the stirring was continued at ice bath temperature for 2 h. The mixture was then allowed to stand at room temperature overnight. The solid was collected, washed with Et_2O, and dried to give 11.45 g (93%) of 3-([1,1′-biphenyl]-4-yl)-3-oxopropionaldehyde sodium salt.

The crude sodium salt was placed in a 500-mL flask, and ice–water (60 mL) was added. The mixture was stirred mechanically in an ice bath, and to the suspension were added sodium acetate trihydrate (6.53 g) in ice–water (50 mL) and hydroxylamine hydrochloride (6.3 g) in ice–water (30 mL). The mixture was stirred in the ice bath for 45 min and then filtered. The solid was collected, washed with cold H_2O, and dried in vacuo to give 9.80 g (91%) of the oxime of 3-([1,1′-biphenyl]-4-yl)-3-oxopropionaldehyde, mp 144–146 °C.

The oxime (77.2 g, 0.32 mol) was suspended in THF (400 mL), and the suspension was cooled in an ice bath while a mixture of acetyl chloride (50.9 g, 0.64 mol) and THF (100 mL) was added slowly with stirring. The solid dissolved and a new precipitate came out. The mixture was stirred for 1 h at room temperature, and the solid was filtered off and washed with a little THF. Evaporation of the filtrate and crystallization of the residue from 1:4 benzene/petroleum ether gave 41.65 g (59%) of 5-([1,1′-biphenyl]-4-yl)isoxazole. An analytical specimen had mp 122–124 °C (from benzene). Anal. ($C_{15}H_{11}NO$) H, N; C: calcd, 81.42; found, 80.75.

3-([1,1′-Biphenyl]-4-yl)-3-oxopropionitrile (128). A solution of **127** (28.76 g, 0.13 mol) in benzene was added slowly to a cooled solution of sodium (4.48 g, 0.195 mol) in MeOH (60 mL) with stirring under nitrogen. A yellow solid precipitated during the addition. The suspension was stirred at room temperature for 45 min, then H_2O was added, and the mixture was acidified with 6 N HCl. The product was extracted with EtOAc, and the extract was washed with water, dried ($MgSO_4$), and evaporated. The yellow solid was recrystallized from a mixture of CCl_4 (400 mL) and benzene (100 mL) to give 17.53 g (61%) of product, mp 110–112 °C. An analytical sample recrystallized from benzene had mp 112–113 °C. Anal. ($C_{15}H_{11}NO$) C, H, N.

3-([1,1′-Biphenyl]-4-yl)-3-oxopropionamide. To **128** (6.00 g, 0.027 mol) suspended in a mixture of HOAc (60 mL) and H_2O (9 mL) was passed a stream of BF_3 gas. When saturated, the mixture was cooled, and water (50 mL) was added, followed by 10 N NaOH (150 mL) until the pH rose to ~9. The mixture was heated on a steam bath for 1 h, and the solid was collected, washed with H_2O, and dried. The solid (containing a large amount of inorganic salts) was extracted with hot nitromethane (150 mL), and the filtered solution was concentrated under vacuum to afford a BF_3 derivative (5.96 g, 76%), mp 238–241 °C. An analytical specimen recrystallized from EtOAc had mp 240–242 °C. Anal. ($C_{15}H_{12}BF_2NO_2$) C, H, N.

A mixture of the BF_3 derivative (5.70 g, 0.02 mol), MeOH (350 mL), and H_2O (17.5 mL) was heated under reflux for ~14 h and then evaporated to ~100 mL, and the solid (3.71 g, 78%) was

collected. An analytical sample of the title compound was obtained by recrystallization from EtOAc, mp 176.5–177.5 °C. Anal. ($C_{15}H_{13}NO_2$) C, H, N.

Ethyl (4-Phenyl-1-piperidyl)acetate. A mixture of 4-phenylpiperidine (0.81 g, 0.005 mol), diisopropylamine (0.68 g, 0.005 mol), and dry THF (5 mL) was treated with ethyl bromoacetate (0.88 g, 0.005 mol) in dry THF (5 mL), and the mixture was allowed to stand at room temperature for 3 days. H_2O (20 mL) and then 10% sodium carbonate were added to a strongly alkaline reaction. The crude product (0.20 g) was separated and used as such to prepare the amide.

Ethyl 2-[4-(4-Bromophenyl)thiazol-2-yl]acetate Hydrobromide (126). A mixture of ethyl 3-amino-2-thioxopropanoate[17] (13.9 g, 0.05 mol) and 1-(4-bromophenyl)ethanone in EtOH (65 mL) was heated under reflux for 2.5 h. After the mixture was cooled, the solid product was filtered. Recrystallization from EtOH gave 7.2 g (35.4%), mp 191–193 °C.[31] Anal. ($C_{13}H_{12}BrNO_2S$·HBr) C, H, Br, N.

2-[4-(4-Bromophenyl)thiazol-2-yl]acetamide. A mixture of **126** (10 g, 0.025 mol), concentrated NH_4OH (50 mL), and dioxane (10 mL) was stirred at room temperature for 2 days. The mixture was poured into ice–water, the product was filtered, and the filtrate was washed with water. Recrystallization from EtOH gave 4.63 g (63.4%), mp 166–168 °C.[31] Anal. ($C_{11}H_9BrN_2OS$) C, H, N.

3-Hydroxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene. To a solution of 3-hydroxy-10,11-dihydro-5*H*-5-oxodibenzo[*a*,*d*]cycloheptene in dry THF (500 mL), stirred under nitrogen and cooled in an ice bath, was added BF_3·Et_2O (31.9 g, 0.225 mol), and then 0.9 M diborane in THF (333 mL) was added slowly. The mixture was stirred in the cooling bath for 20 min and then at room temperature overnight. MeOH (50 mL) was added slowly with cooling, after which the solvent was distilled off. To the residue was added MeOH (1 L), and the mixture was boiled briefly and then evaporated. The residue was treated with aqueous sodium bicarbonate and extracted with EtOAc. The organic layer was washed with H_2O, dried ($MgSO_4$), and evaporated. Recrystallization of the crude product from benzene/petroleum ether gave 20.4 g (92%), mp 105–109 °C. Further recrystallization gave product of mp 111–112 °C. Anal. Calcd for $C_{15}H_{14}O$: C, 85.68; H, 6.71. Found: C, 85.20; H, 6.79.

In Vitro Assay Procedure for Inhibition of GAO. The standard assay of enzyme inhibition has been described previously.[6] Briefly, this employs the spectrophotometric determination (600 nm) of the enzyme-mediated reduction of 2,6-dichlorophenolindophenol (DCIP) by glycolate at 25 °C. Initial substrate concentrations of DCIP and glycolate were 5×10^{-5} and 2×10^{-4} M, respectively. Three inhibitor concentrations were tested concurrently with a control containing no inhibitor, and the resulting rates were expressed as a fraction of the control.

The determination of enzyme inhibition patterns for a selected set of compounds was done at the same DCIP concentrations as above. For these determinations, the initial velocity of the reaction was determined as a function of the initial glycolate concentration at a series of fixed inhibitor concentrations.

Spectropolarimetric Determination of Inhibitor Binding. A Jasco J-41A circular dichroism spectropolarimeter was employed to determine directly the binding of compound **80** to GAO. Small, measured volumes of a 4×10^{-4} M solution of **80** in a pH 7.4, 0.1 ionic strength phosphate buffer were added to a 10-cm path-length cuvette containing 30 mL of a 33 ± 3 μg/mL GAO solution in the same buffer. The change in observed ellipticity was followed at 380 nm. Protein concentrations were determined by the method of Lowry.[20]

Methods for Analysis of in Vitro Data. Data were fitted to equations by the method of nonlinear least squares.[27] This method provides the values of the adjustable parameters that would best fit an equation to experimental data, as well as estimates of the standard deviations of these parameters.

Values of I_{50}, the inhibitor molar concentration required to reduce the enzyme activity to one-half the control value, were determined from the equation

$$f_I = \frac{1}{1 + I_{50}/[I]_t}$$

In this equation, f_I is the fractional inhibition (= R_I/R_c, where R_I and R_c are the initial rates in the presence and absence of inhibitor, respectively) and $[I]_t$ is the total inhibitor concentration.

Apparent maximum velocities and Michaelis constants were determined from initial rates by the standard Michaelis–Menten equation

$$V = \frac{V_{max}^{app}}{1 + K_m^{app}/[\text{glycolate}]}$$

V_{max}^{app} and K_m^{app} were then related to the inhibitor concentration by the appropriate equation for competitive, uncompetitive, or noncompetitive inhibition.[19]

The CD spectropolarimetric titration data were fitted to the equation:

$$\Delta\theta^{380} = (\Delta[\theta^{380}]/10)([GAO]_t + [I]_t + K_I - [([GAO]_t + [I]_t + K_I)^2 - 4[GAO]_t[I]_t]^{1/2})/2$$

where $\Delta\theta^{380}$ is the observed change of ellipticity produced by the addition of a total concentration ($[I]_t$) of inhibitor, $\Delta[\theta^{380}]$ is the difference in molar ellipticity of the enzyme–inhibitor complex and the enzyme, $[GAO]_t$ is the total enzyme concentration, and K_I is the enzyme–inhibitor dissociation constant.

QSAR Calculations. Substituent π and MR values were taken from tabulated values[21,22] or calculated by means of the additivity principle.[23] As an example, **86** contains the 4-(2-phenylindol-1-yl) substituent, which was represented as

1-pyrrole	+ 3,4-(CH=CHCH=CH)	+ C_6H_5	
π = 0.95	+ 1.32	+ 1.96 =	4.23
MR = 22.57	+ 17.47	+ 25.36 =	65.40

In Vitro Assay Procedure for Inhibition of GAO Employing Spinach Glyoxylate Reductase. The oxidation of glycolic acid to glyoxylic acid by GAO, and inhibition thereof, was measured spectrophotometrically by coupling to excess spinach glyoxylate reductase (Calbiochem) and NADH. The reaction mixture contained glycolic acid (2×10^{-4} M), NADH (2×10^{-4} M), spinach glyoxylate reductase (0.1 mL), and varying concentrations of test inhibitor in 0.9 mL of 0.1 M potassium phosphate buffer, pH 7.0. The reaction was started by the addition of 0.1 mL of porcine glycolate oxidase stock solution, and the rate of decrease in absorbance at 340 nm was measured.

Rat Liver Perfusion Studies. Livers from Charles River rats (370–450 g) were removed and perfused via the hepatic portal vein and the inferior vena cava with balanced salt solution (BSS). Viability of the preparation was assessed on the basis of perfusion pressure (measured with a Statham P-23 Dc transducer) and oxygen consumption (measured with a Yellow Springs Instruments oxygen monitor). Oxygenation, temperature control, and circulation of perfusate (1 mL min^{-1} g^{-1}) were maintained with an Ambec Extracorporeal Perfusion unit.

Two types of perfusion experiments were performed: recirculating and single pass. In recirculating experiments, [1-^{14}C]-glycolate at 1 mM, 0.05 μCi/μmol (Amersham Searle), was added to the BSS for control and experimental periods. Each period was 30 min. The concentration of [^{14}C]oxalate formed was measured in samples of perfusion fluid by the method described under "In Vivo Studies in Rats". The increase in [^{14}C]oxalate in the recirculating perfusate in control experiments was linear with time up to 3 h (<10% conversion of glycolate).

In the single-pass experiments, the perfusate contained the same components, except for the ^{14}C tracer. This was added to the perfusate via a syringe infusion pump only during the time a measurement of oxalate formation was being made. A 10-min pulse of label was found to be optimal.

Both methods permitted measurement of the effects of GAO inhibitors on oxalate production in the intact liver. The single-pass method reduced the [1-^{14}C]glycolate required and eliminated the possible buildup of metabolic products in the perfusate.

In Vivo Studies in Rats. Female Sprague–Dawley rats of starting body weight 140–150 g were allowed free access to pulverized laboratory chow with or without **83** at a concentration calculated to deliver 10 (mg/kg)/day and drinking water without

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(experiment 1) or with (experiment 2) ethylene glycol at a 1% concentration (v/v). On days indicated, all animals were placed in individual metabolism cages for collection of urine between 0800 and 1400 h. Urinary oxalic acid was determined as its dichloroethyl ester by electron-capture gas chromatography according to the procedure of Tocco and co-workers.[28]

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Studies on *v*-Triazoles. 9.[1] Antiallergic 4,9-Dihydro-4,9-dioxo-1*H*-naphtho[2,3-*d*]-*v*-triazoles

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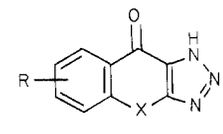
A short series of the title compounds was prepared and evaluated for antiallergic activity in the rat passive cutaneous anaphylaxis screen. All but the two N-methylated derivatives were active in this screen by the intravenous route, the most potent being the symmetrical dimethyl compound, 4,9-dihydro-6,7-dimethyl-4,9-dioxo-1*H*-naphtho[2,3-*d*]-*v*-triazole, and its 5-nitro derivative. The latter two compounds were noticeably more potent than disodium cromoglycate, and one of these, the unnitrated material, was selected for further evaluation as a potential antiasthmatic drug.

In a previous paper in this series,[2] we reported the antiallergic activity of a novel series of benzopyranotriazoles of type 1 that resulted from our studies on the triazoloquinolines **2**.[3] As part of this program, we also investigated the analogous 4,9-dihydro-4,9-dioxo-1*H*-naphtho[2,3-*d*]-*v*-triazoles **3** which constitute a potent class of antiallergic compounds as assessed by their ability to inhibit the IgE-mediated passive cutaneous anaphylaxis (PCA) reaction in the rat. This paper describes the synthesis and activity of these compounds.



1, X = O
2, X = NH
3, X = CO

Chemistry. Several methods are available for the synthesis of simple naphthotriazoles **3**, the method of

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