

Registry No. L-1, 35597-44-5; L-1a, 73777-49-8; D-1a, 104361-75-3; 4, 140-88-5; 5, 15090-27-4; 6, 79778-02-2; Z-7a, 131131-78-7; Z-7b, 131131-79-8; E-7b, 131131-83-4; Z-7c, 131131-80-1; 8, 63135-95-5; 9, 131131-76-5; 10, 37002-48-5; 11,

67884-32-6; 12, 71042-54-1; 13, 64896-28-2; 14, 12257-42-0; 15, 112066-74-7; L-16a, 125280-42-4; D-16a, 131232-92-3; L-16b, 131131-81-2; L-16c, 131131-82-3; 17, 131131-77-6; MePH(O)OEt, 16391-07-4; CNCH₂COOEt, 2999-46-4; AcNH₂, 60-35-5.

Analogues of the Cyclic Hydroxamic Acid 2,4-Dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one: Decomposition to Benzoxazinones and Reaction with β -Mercaptoethanol

Jeffrey Atkinson, Peter Morand,* and John T. Arnason†

Departments of Chemistry and Biology, University of Ottawa, Ottawa, Canada, K1N 6N5

Hermann M. Niemeyer and Héctor R. Bravo

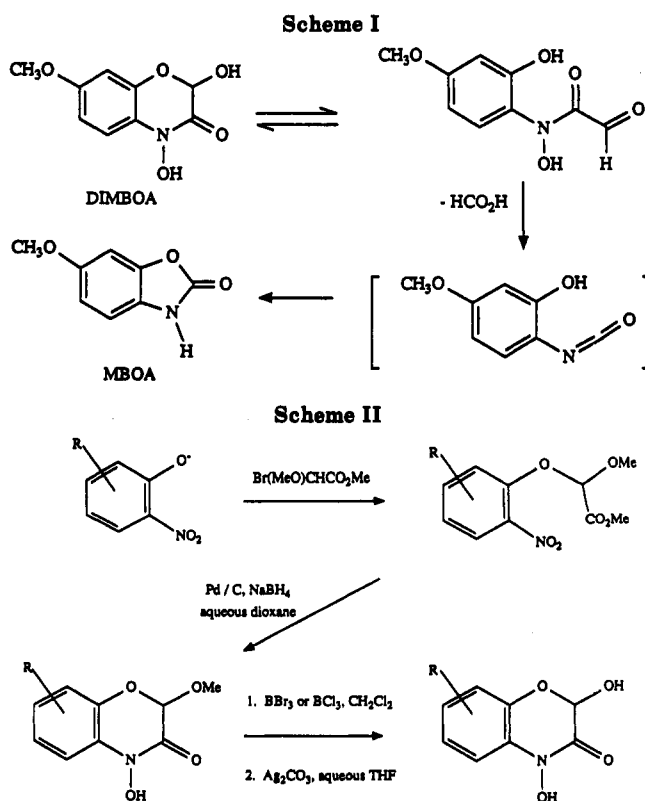
Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

Received February 27, 1990

Analogues of the aglucones of naturally occurring cyclic hydroxamic acids (2,4-dihydroxy-1,4-benzoxazin-3-ones) from Gramineae (Poaceae) have been synthesized by the reductive cyclization of the ring-substituted methyl α -(*o*-nitrophenoxy)- α -methoxyacetates, followed by demethylation of the C-2 methoxy group with BBr₃ or BCl₃ to reveal the 2-hydroxy group. A structure-activity series was produced by varying the substituent at C-7 on the aromatic ring [R = MeO (1), *t*-Bu (6), Me (7), H (8), Cl (9), F (10), CO₂Me (11a)]. The pK_a values for the hydroxamic acid and the phenol moieties were determined for each member of the C-7 series. They correlated well with σ in a linear free energy relationship (LFER) yielding values of $\rho = 0.71$ (with σ_p) for pK_{a1} (the hydroxamic acid) and $\rho = 1.6$ (with σ_m) for pK_{a2} (the phenol). A LFER also existed between the rate constants for the unimolecular decomposition of these hydroxamic acids to benzoxazinones and σ^+ ($\rho = -1.1$). The rates of hydroxamic acid reduction to lactams by β -mercaptoethanol were also investigated. It was found that only compounds with electron-rich aromatic rings and specifically an oxa functionality para to the hydroxamic acid nitrogen atom (compounds 1 and 3-5) had measurable rates of reduction. ¹H NMR spectra recorded during this reaction in D₂O buffers (pD 9), however, showed that compounds 1, 2, 6-9 (the only ones investigated) formed a hemithioacetal at C-2 even though only 1 has a measurable rate of reduction by the same thiol. The remarkable rate enhancement provided by an oxa functionality suggests that reduction occurs by direct attack of thiolate on the hydroxamic nitrogen of a resonance-stabilized ion pair.

Over 400 species of insects are now known to be resistant to insecticides.^{1,2} Because of the problems of resistance and environmental contamination, most researchers in the area of crop protection agree on the urgency of developing new pest management strategies that reduce our dependence on pesticides. One such important area of research is the development of plant varieties resistant to pest attack.^{2,3} A plant may be resistant to attack for a number of reasons, including morphological characteristics such as shape, toughness of tissues, presence of trichomes (leaf hairs), or silica.⁴ Much recent research⁵⁻¹³ demonstrates that the presence of secondary chemicals has an important role in protecting the plant against pest attack.

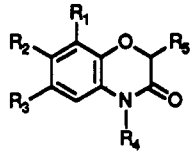
Cyclic hydroxamic acids with the 1,4-benzoxazin-3-one skeleton are secondary metabolites found in several grasses (Gramineae) of which maize (corn), wheat, and rye are important crop plants. These hydroxamic acids exhibit a wide variety of biological activities and have recently been reviewed.¹⁴ The most abundant hydroxamic acid in maize is 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one, DIMBOA.¹⁵ The presence of this allelochemical in plant tissues has been correlated with resistance toward herbivory by the European corn borer (*Ostrinia nubilalis*, Lepidoptera: Pyralidae).¹⁶⁻²² Our laboratories have investigated the toxicity and toxicokinetics of hydroxamic acids in corn borer larvae^{23,24} and an endoparasitoid²⁵ of the larvae. In parallel with this work the chemistry of DIMBOA itself has also been investigated, including its reaction with thiols²⁶ and with amines²⁷ and its decom-



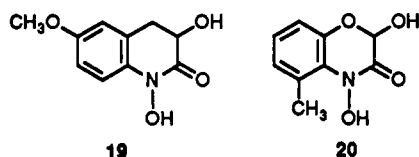
position-rearrangement to MBOA in organic and aqueous solvents²⁸ (Scheme I). We report here the synthesis of

† Department of Biology, University of Ottawa.

Table I. Compounds Synthesized or Utilized in This Work



compd	R ₁	R ₂	R ₃	R ₄	R ₅
1 ^a	H	MeO	H	OH	OH
2 ^a	H	MeO	H	H	OH
3	H	-OCH ₂ O-	H	OH	OH
4	H	MeO	MeO	OH	OH
5	MeO	MeO	H	OH	OH
6	H	<i>t</i> -Bu	H	OH	OH
7	H	Me	H	OH	OH
8	H	H	H	OH	OH
9	H	Cl	H	OH	OH
10	H	F	H	OH	OH
11a	H	CO ₂ Me	H	OH	OH
11b	H	CO ₂ Et	H	OH	OH
12	H	NO ₂	H	OH	OH
13	H	CF ₃	H	OH	MeO
14	H	CN	H	OH	MeO
15 ^a	H	MeO	H	OH	MeO
16 ^a	H	MeO	H	OH	H
17 ^a	H	H	H	OH	H
18 ^a	H	H	H	H	OH

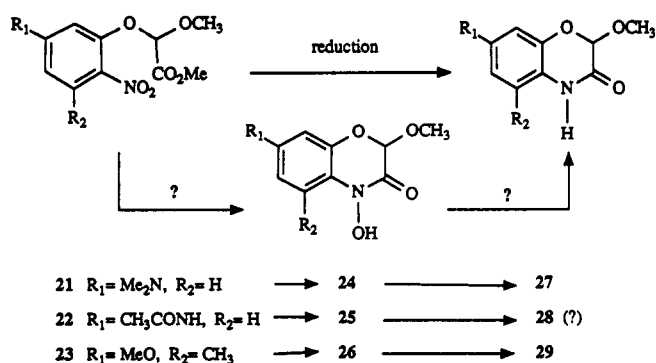


^a The synthesis of compounds 1 and 15 has been reported previously.³⁰ Compounds 2, 16–18 have been mentioned in the literature.^{51,56}

a number of analogues of DIMBOA, a linear free energy relationship for their decomposition, and their reaction

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Scheme III



with thiols.

Results and Discussion

Synthesis of Hydroxamic Acids. Most of the hydroxamic acids in this study (Table I) were synthesized by a reductive cyclization methodology first reported by Coutts²⁹ and later utilized in a patent³⁰ for the synthesis of DIMBOA and a few analogues. (For details on the synthesis of the 7-NO₂ analogue 12 see the Experimental Section.) The reaction (Scheme II) involves the reductive cyclization of an appropriately substituted methyl (*o*-nitrophenoxy)acetate, available from the reaction of a potassium *o*-nitrophenoxide with an α -haloacetate. The nitrophenols in the present work were synthesized by standard methods.

For the synthesis of DIMBOA and analogues the bromoacetate must incorporate the functionality necessary to form the hemiacetal moiety. Thus, for this series of compounds, methyl α -bromo- α -methoxyacetate was used (R = MeO in Scheme II). It is easily prepared by brominating methyl methoxyacetate with Br₂ in boiling CCl₄.

The reductive cyclization³¹ proceeded well for most compounds, the isolated yields depending strongly on the substituent para to the nitro group. Electron-withdrawing alkyl, and methoxy groups allowed facile reduction to the hydroxamic acid, but stronger electron donors such as

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dimethylamino and acetamido did not. The reduction products of **21**, **22**, **23** (Scheme III) were highly colored, usually orange or red. On attempted extraction of the acidified reaction mixture with EtOAc a deep blue-purple color developed at the interface which intensified on shaking and aeration, suggesting the presence of an air-oxidizable species. The color could be made to disappear by reduction with dithionite ($\text{Na}_2\text{S}_2\text{O}_4$) but was extremely difficult to remove from the other organic products. The cyclic amide **29**, a product of the reduction of **23**, was readily isolated as well as a small amount of the dimethylamino compound **27**, but products from the acetamido-substituted material were very water soluble and purification and characterization were not achieved.

The reagents and conditions of this method do not easily reduce the product hydroxamic acids to their respective amides. After addition of the nitro precursor the reaction mixture could be stirred for 15 min or 1 h, and the isolated yield of hydroxamic acid did not change. It is unclear whether the hydroxamic acids **24**, **25**, and **26** are intermediates or not. It may be that these particular substituents enhance the ease of reduction of the hydroxamic acid already produced, or there may not be a stable aryl-hydroxylamine produced which can cyclize (Scheme III).

The methyl group could be removed to reveal the hemiacetal by treatment with a boron trihalide, either BCl_3 or BBr_3 ^{32,33} (Scheme II). The boron reagents were generally adequate for this transformation; however, they did have some limitations. As the electron-withdrawing nature of R increased the reaction proceeded more slowly. By comparison, the methyl acetal of DIMBOA (**15**) reacts completely with BCl_3 within 2.5 h from 0 °C to room temperature; the 7-Cl analogue **9**, even with the more reactive BBr_3 , requires 3 h at room temperature for complete reaction; for strongly electron withdrawing substituents such as CN and CF_3 no reaction occurred after exposure to a 10 M excess of BBr_3 at 20 °C for 16 h. For compounds **10** and **11** it was found that the use of Ag_2CO_3 was not absolutely necessary. An aqueous workup was all that was needed to hydrolyze the unisolated bromohydroxamic acid when BBr_3 was used. All of these acetals were resistant to preparative acid hydrolysis. After treatment with a variety of aqueous acids (HCl , HBr , HClO_4 , H_2SO_4 , AcOH , $\text{CF}_3\text{CO}_2\text{H}$), TLC showed that the starting acetal largely remained. The only change observed was the formation of colored products both more and less polar on TLC than the hydroxamic acid acetal.

Decomposition of Hydroxamic Acids. The 2,4-dihydroxy-1,4-benzoxazin-3-ones (DIBOAs) decompose in organic and aqueous solvents to give benzoxazolinones (BOAs) with concomitant liberation of formic acid (Scheme I).^{28,34-38} For DIMBOA the major product of decomposition is MBOA. Scheme I shows the proposed mode of decomposition for the un-ionized hydroxamic acid—the species expected in most organic solvents. The pH dependence of the rate of decomposition in aqueous solution describes a bell-shaped curve with a maximum at pH 9. At pH ~ 9 (the approximate pH of the larval lepidopteran gut³⁹) one would expect all ionized forms of the hydroxamic

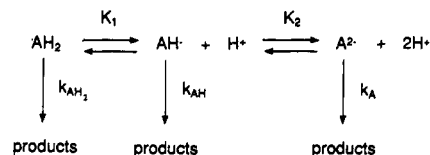


Figure 1. Decomposition of 2,4-dihydroxy-1,4-benzoxazin-3-ones and their two conjugate bases in aqueous solution. AH_2 represents these cyclic hydroxamic acids as diprotic acids.

acid to be present to some degree. They would then have their own pathways for decomposition to products. This is shown in Figure 1. Taking into account the possible reactive species shown in Figure 1, and their respective rates of decomposition, k_{AH_2} , k_{AH^-} , and $k_{\text{A}^{2-}}$, where AH_2 represents a cyclic hydroxamic acid as a diprotic acid, one can write an expression for the observed pseudo-first-order rate constant k_{obs} ^{28a} such that

$$k_{\text{obs}} = \frac{k_{\text{AH}_2}(\text{H}^+)^2 + k_{\text{AH}^-}K_1(\text{H}^+) + k_{\text{A}^{2-}}K_1K_2}{(\text{H}^+)^2 + K_1(\text{H}^+) + K_1K_2} \quad (1)$$

where the constants K_1 and K_2 are the first and second dissociation constants for the hydroxamic acids. In practice, at ca. pH 8.5 at which decomposition experiments were performed, the observed rate constants can be directly equated to the rate constants for the monodissociated hydroxamic acid (or the kinetically equivalent undissociated acid and hydroxide ion at this pH), i.e. $k_{\text{obs}} \approx k_{\text{AH}^-}$, since it is clear that this species fully dominates the rate profile. These measurements were very "clean"; the UV spectra of the completed reaction matching those of standard benzoxazolinones.⁴⁰

The dissociation constants K_1 and K_2 ($\text{p}K_{\text{a}}$ values) were determined spectrophotometrically by recording the differences in absorbance between the monoionized and un-ionized forms at various pH values spanning the $\text{p}K_{\text{a}}$. The absorbance differences between the dianion and the monoanion were about one-fourth of those between the un-ionized and the monoanion. At pH 9 (the maximum of the pH range used to determine $\text{p}K_{\text{a}1}$) the dianion accounts for <10% of the ions in solution if a $\text{p}K_{\text{a}2}$ of 10 is assumed. Thus, the total absorbance by the dianion did not contribute significant error in the determination of $\text{p}K_{\text{a}1}$. These values are listed in Table II.

The $\text{p}K_{\text{a}2}$ values (actually the product of the $\text{p}K_{\text{a}}$ of the phenol and the log of the equilibrium constant for the lactol/phenol-aldehyde equilibrium) showed greater variation than those for $\text{p}K_{\text{a}1}$ (1.6 $\text{p}K_{\text{a}}$ units versus 0.8). A measured value for the $\text{p}K_{\text{a}2}$ of the 7-F compound **10** was not obtained, but could be estimated at ~ 10.1 . This compound was not sufficiently stable at high pH to allow reliable absorbance differences to be recorded. It appeared that at high pH a reaction was occurring at such a rate that even during the short time (~ 45 s) that it took to prepare a sample cuvette the absorbance maxima had diminished and reproducible spectra could not be obtained. A similar, although much less severe, phenomenon occurred with the 7- CO_2Me compound **11a**. This problem could be minimized by recording the spectrum of the doubly ionized species at pH 11.5 rather than at 12.5. Consequently, a $\text{p}K_{\text{a}2}$ value could be obtained for **11a**.

Linear Free Energy Relationships (LFERs). The C-7 substituted compounds in Table I were chosen (within

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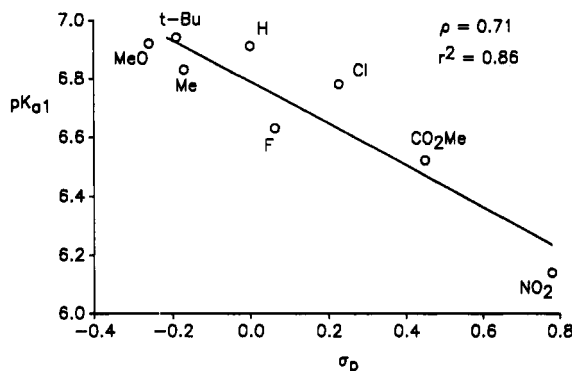
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Table II. σ Constants, pK_a 's, Ultraviolet Absorbance Maxima, and Pseudo-First-Order Rate Constants for the Decomposition of C-7 Substituted 2,4-Dihydroxy-1,4-benzoxazin-3-ones to Benzoxazolinones

compd	7-X	σ_m^a	σ_p	σ^+	pK_{a1}^b	pK_{a2}	λ , nm		10^3k , min ⁻¹ (pH) ^d
							λ_1^c	λ_2	
1	MeO	0.10	-0.26	-0.78	6.92	10.1	263	288	75.9 (8.5)
6	<i>t</i> -Bu	-0.10	-0.19	-0.26	6.94	11.00	258	283	6.61 (9.0)
7	Me	-0.06	-0.17		6.83	10.56	260	283	8.94 (8.7)
8	H	0	0		6.91	10.55	254	282	6.01 (8.7)
9	Cl	0.37	0.23	0.11	6.78	10.22	255	284	17.4 (8.5)
10	F	0.34	0.06		6.63	~10.1	249	280	-
11a	CO ₂ Me	0.37	0.45		6.52	9.90	273	298	2.25 (8.2)
12	NO ₂	0.71	0.78		6.14	9.40	300	335	3.03 (7.8)

^a σ constants were taken from ref 42-44. ^b pK_a 's were determined spectrophotometrically. The value of pK_{a2} for the 7-F compound 10, was estimated to be approximately 10.1, though attempts to measure this were unsuccessful due to 10's instability at high pH. ^cUV spectra were recorded in MeOH at concentrations around 7×10^{-5} M. ^dRate constants were determined at 48 °C in 100 mM phosphate or 200 mM carbonate. The pH was chosen so as to be halfway between the two pK_a 's, thus keeping the concentration of monoionized hydroxamic acid constant for all compounds tested. The analytical wavelengths were chosen such that the absorbance differences between starting material and products were the largest possible.

**Figure 2.** Plot of pK_{a1} (the hydroxamic acid group) versus the substituent constants σ para (σ_p) for the C-7 substituted series of 2,4-dihydroxy-1,4-benzoxazin-3-ones.

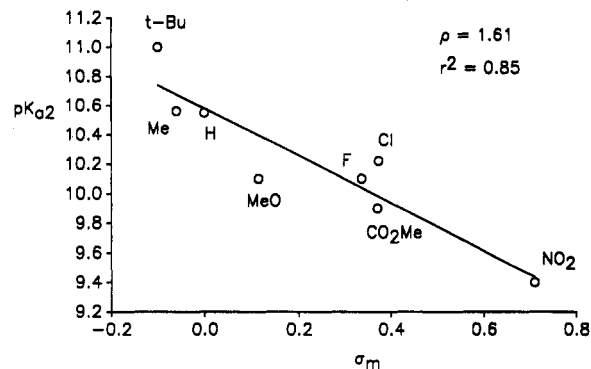
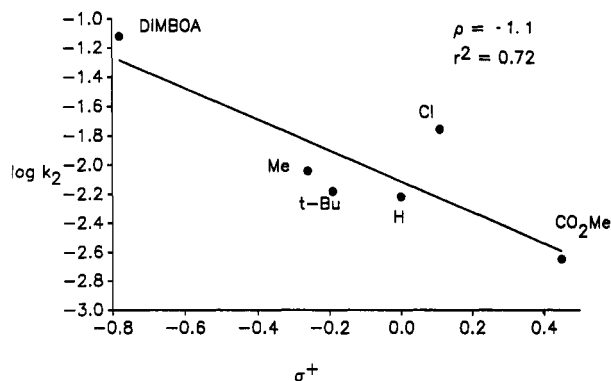
the bounds of synthetic availability) to offer the widest possible variation in physical parameters, such as pK_a , within a structurally defined set. The aim was to similarly affect the rates of their decomposition.

In MeOH the UV spectra of the compounds in this series tend to show two maxima, one as a shoulder. Table II lists the σ constants and the pseudo-first-order rate constants for the decomposition of the members of this series.

Figure 2 shows that the pK_{a1} values correlate reasonably well with σ_p ($r^2 = 0.86$) though the ρ value ($\rho = 0.71$) is much higher than that reported for acyclic *N*-phenylhydroxamic acids ($\rho = 0.1$).⁴¹ Brink and Crumbliss⁴¹ observed that the hydroxamic acid pK_a values are more sensitive to variations in the substituted phenyl group when attached to the carbonyl carbon than when attached to the nitrogen of the hydroxamic acid moiety. The larger ρ values for the compounds reported here are likely due to the hydroxamic moiety being constrained in a ring.

Figure 3 shows that the pK_{a2} values also correlate well with σ_m ($r^2 = 0.85$), and the magnitude of the ρ value ($\rho = 1.6$) resembles that for ionization of a phenol where $\rho = 2$.⁴²

The pseudo-first-order rate constants for decomposition (see Table II) are also amenable to this sort of correlation analysis. The correlation was poor if σ_p was used, but if the modified constant σ^+ was used,⁴³⁻⁴⁵ those substituents

**Figure 3.** Plot of pK_{a2} (phenol) versus the substituent constants σ meta (σ_m) for the C-7 substituted series of 2,4-dihydroxy-1,4-benzoxazin-3-ones.**Figure 4.** Plot of the observed pseudo-first-order rate constants for the decomposition of the C-7 substituted series of 2,4-dihydroxy-1,4-benzoxazin-3-ones versus the substituent constants σ plus (σ^+). The rates were measured at the pH values listed in Table II at 48 °C.

that could exhibit resonance interactions with a partial positive charge at the reactive center, such as MeO, Cl, and *t*-Bu, became part of a LFER. The relation, shown in Figure 4, is not strict, but the trend is clear. The presence of the strong electron-donating MeO group greatly enhances the rate of the reaction. A strong electron-withdrawing substituent such as CO₂Me retards the rate considerably. This correlation has $\rho = -1.1$ meaning that, during the transition state for formation of the isocyanate (see Scheme I), electron density at nitrogen decreases with respect to reactants. The 7-NO₂ compound did not show obvious isobestic points in its UV spectra taken during its decomposition in buffer. It seems that there is a buildup of an intermediate, likely the isocyanate (Scheme I). Since the rate-limiting step has changed, this compound

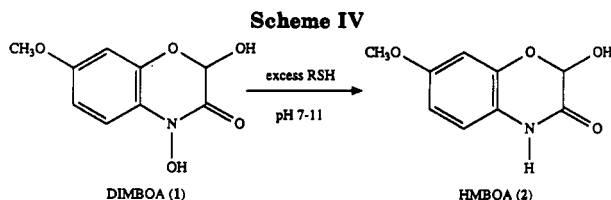
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was omitted from this correlation.

Reaction Mechanisms. A number of mechanisms have been postulated to describe the decomposition of 2,4-dihydroxy-1,4-benzoxazin-3-ones to benzoxazolinones. They are of essentially two forms: (i) that the hydroxamic acid hydroxyl group is acting as an internal nucleophile,^{28,34} or (ii) that it is a leaving group.^{38,46} Unfortunately, the present work does not distinguish between the two mechanisms since, in the LFER for the pseudo-first-order rate constants of decomposition, the ρ value of -1.1 describes a developing positive charge on nitrogen, and this would be expected for both mechanisms as the N-O bond cleaves. Our data show that analogues of DIMBOA with electron-withdrawing aryl substituents significantly slow the rate of decomposition. At 25 °C the compound with two oxygen substituents in the 7,8-positions (DIM₂BOA, 5) decomposed only slightly slower than DIMBOA, and the 6,7-dioxy-substituted compounds 3 and 4 nearly 4 times faster than DIMBOA (data not shown). This supports the conclusion that an electron-rich phenyl ring stabilizes the developing positive charge on nitrogen in the transition state and thus accelerates the rate of decomposition.

One of the analogues prepared in this work (20, 5-Me-DIBOA) has a Me group on the aryl ring at C-5. It was impossible to measure a pK_a for this compound because of its instability in the buffers. Inspection of the reaction products by GC/MS showed that it had completely converted to 4-Me-BOA. Presumably, steric overlap between the methyl group and the hydroxamic acid moiety accelerated the rate of decay. Decomposition to the 4-Me-BOA with scission of the N-OH bond would remove this strain.

It is worth noting that the methyl acetal of DIMBOA, 15, is unreactive to the conditions that normally decompose those cyclic hydroxamic acids having a free hydroxyl group at C-2. It is not unreasonable to suggest then that the 2-O- β -D-glucoside moiety of the naturally occurring hydroxamic acids serves to protect against decomposition within the plant. Damage to plant tissues (for example, by feeding insects) releases hydrolytic enzymes⁴⁷ that cleave their sugar moiety from the toxic hydroxamic acid.

The relative rates of decomposition of the analogues is very valuable information. It is crucial for interpreting the results of biological tests (feeding trials) and assessing which molecules are present during the course of the trial. Results from feeding trials and from studies of insect protease inhibition by cyclic hydroxamic acids will be presented elsewhere.

Reaction with Thiols. The hydroxamic acid moiety of DIMBOA reacts with excess thiols in aqueous media to give the corresponding lactams as the main isolable product^{26a} (Scheme IV). For the reaction of DIMBOA with mercaptoethanol the pH dependence of the apparent second-order rate constant, k^*_2 , describes a bell-shaped curve with a maxima at pH approximately 8.3.^{26b} This reduction reaction can be monitored spectrophotometrically since the hydroxamate anion and the product amide

Table III. Calculated Second-Order Rate Constants for the Reduction of Cyclic Hydroxamic Acids with Mercaptoethanol^a

compound	k^*_2 (L mol ⁻¹ min ⁻¹)	k_2 (L mol ⁻¹ min ⁻¹)
6,7-MDO (3)	9.00 \pm 0.72	6720 \pm 800
6,7-di-MeO (4)	1.98 \pm 0.10	1480 \pm 135
DIMBOA (1) (7-MeO)	0.312 \pm 0.016	227 \pm 20
7,8-di-MeO (5)	0.156 \pm 0.009	88.5 \pm 9

^a Pseudo-first-order rate constants were determined at pH 9.0, ionic strength $I = 0.15$, at 23 \pm 0.4 °C, spanning a concentration of thiol from 80- to 400-fold molar excess. These values of k_{obs} were then plotted against concentration of thiol yielding lines whose slopes were k^*_2 . True second-order rate constants were then calculated using eq 2.

have different absorbance spectra. The true second-order rate constant k_2 is expressed in eq 2 where K_1 and K_2 are

$$k_2 = k^*_2 \left(\frac{[H^+]^2 + K_1[H^+] + K_1K_2}{[H^+]^2} \right) \left(\frac{[H^+] + K_3}{K_3} \right) \quad (2)$$

the dissociation constants of DIMBOA (1.20×10^{-7} and 7.94×10^{-11} , respectively) and K_3 the dissociation constant of the thiol. It was shown^{26b} that the reactive species that predominantly determine the reaction rate are the undissociated hydroxamic acid and the thiolate anion. Thus, a graph of the log k_2 values for the reaction of DIMBOA with several thiols versus the pK_a of the thiols was linear.^{26b}

N-Acetoxy derivatives of 1,4-benzoxazin-3-ones have also been shown to react with thiols.^{48,49} In this instance the thiol was found to attack both the aromatic ring and the nitrogen atom, and these thiol adducts could be isolated. For DIMBOA the net reaction is reduction of the hydroxamic acid to the lactam. An ESR experiment performed when DIMBOA was reduced with ethanethiol^{26b} resulted in no detectable single-electron species, although this admittedly only rules out long-lived radicals and not a fast single electron transfer process. In theory, an ionic mechanistic pathway exists for this reaction by direct attack of thiolate on nitrogen. The rate of the reaction should be affected by changing the electronic nature of the nitrogen atom. This was the guiding rationale for synthesizing compounds with a range of substituents on C-7 para to the hydroxamic acid nitrogen (1, 6-11, see Table I). A further goal of this structure-activity study was to explore those structural features necessary for the reaction to occur. The two amides 2 and 18 (which also occur naturally¹⁴) were included as controls for the hydroxamic moiety. The known 2-deoxy compounds⁵⁰ 16 and 17, as well as the tetrahydroquinolin-2-one, 19, and the methyl acetal of DIMBOA, 15, were included as tests for the necessity of the lactol group for chemical or biological activity. Examining the lactol moiety was important since it had been shown that this group was necessary for toxicity in carcinogenicity studies⁵¹ and for anti-inflammatory activity.⁵²

Measurement of Rate Constants. For the kinetic experiments with DIMBOA and analogues the chosen pH was 9.0. At this pH the measured rates are near their maxima (which varied little from compound to compound)

(48) Hashimoto, Y.; Ohta, T.; Shudo, K.; Okamoto, T. *Heterocycles* 1978, 9(1), 109.

(49) Hashimoto, Y.; Ohta, T.; Shudo, K.; Okamoto, T. *Tetrahedron Lett.* 1979, 1611.

(50) Kugai, N.; Hashimoto, Y.; Shudo, K. *Heterocycles* 1984, 22, 277.

(51) Hashimoto, Y.; Shudo, K.; Okamoto, T. *Mutation Res.* 1979, 66, 191.

(52) Otsuka, H.; Hirai, Y.; Nagao, T.; Yamasaki, K. *J. Nat. Prod.* 1988, 51(1), 74.

(46) Grambow, H. J.; Lückge, J.; Klausener, A.; Müller, E. *Z. Naturforsch.* 1986, 4c, 684.

(47) Virtanen, A. J.; Hietala, P. K. *Acta Chem. Scand.* 1960, 14(2), 499.

and the absorbance differences between the hydroxamate anion and the product lactams were sufficient for reproducible results. Unfortunately, of all the analogues only three compounds other than DIMBOA showed measurable rates of reduction under the conditions of the experiments. The results are listed in Table III.

Similar experiments with other synthetic analogues were unsuccessful. When the substituent on C-7 was *t*-Bu, Me, H, Cl, F, or CO₂Me (6–11a) the observed pseudo-first-order rate constants for the decay of the UV absorbance were constant despite different concentrations of thiol and temperatures as high as 55 °C. Evidently, a reaction (or reactions) other than reduction by thiol are responsible for the decreasing absorbance over time.

The reactivities of compounds 13, 14, and 17 were also investigated. At the same conditions as the other kinetic trials their UV spectra did not change and thus rate constants were not calculable. When small amounts (3 mg) of the analogues were allowed to react with a 40-fold molar excess of mercaptoethanol at 45 °C, pH 9.0, for 16 h, GC/MS analysis of the product mixtures showed the presence of small amounts of lactam in the case of compounds 6–9 (and also, of course, the oxy-substituted analogues 3, 4, and 5 which had measurable rate constants for reaction with mercaptoethanol). It was impossible to accurately quantify these analyses because of broad, poorly integrated peaks at long retention times and problems of analytical reproducibility. When compounds 15, 16, and 19 were subjected to the same conditions and analyzed by GC/MS, only 16 showed a small amount of the lactam, approximately 3% relative to the starting hydroxamic acid peak. Also detected in the case of 15 and 16 were peaks whose *m/z* suggest the addition of a molecule of mercaptoethanol with concomitant loss of water. The products of the reaction of 16 and mercaptoethanol on a preparative scale (50 mg of 16) could not be obtained. Only the starting hydroxamic acid was recovered. The conclusion must be that the analogues of DIMBOA do react with mercaptoethanol in pH 9.0 buffer, but at a much reduced rate. For the 7-substituted series attempts to force the reaction with more extreme conditions, such as higher temperatures, serve only to accelerate the decomposition of the hydroxamic acids by other means.

¹H NMR in D₂O Buffers. The masked aldehyde of the lactol is another electrophilic center in these molecules. We thought it likely that thiols in the reduction experiments were also reacting at this center, but the UV absorption spectra could not detect this.^{26a} Consequently, the reaction of a few of the analogues with mercaptoethanol were inspected by ¹H NMR.

¹H NMR spectra were recorded in 2 M carbonate buffer (D₂O) at "pD" ~9.⁵³ The spectrum of the amide 2 (HMBOA) alone in buffer was not complicated (see Figure 5a). The sample had been dissolved in the buffer for about 15 min before the first spectrum was run. The spectrum showed two sets of aromatic protons, one set being about 10 times more intense than the other. The characteristic doublet for the C-5 proton (*J*_{5,6} = 7.1 Hz) occurred at δ 6.9 for the more abundant species and at δ 7.3 for the less intense signals. On addition of a 7.5-fold excess of mercaptoethanol and inspection of the spectrum after 5 min (Figure 5b), the original higher intensity aromatic signals had almost completely disappeared and the lower intensity ones had increased. In addition, the 1 H singlet at δ 5.62 was absent and a new 1 H singlet at 5.45

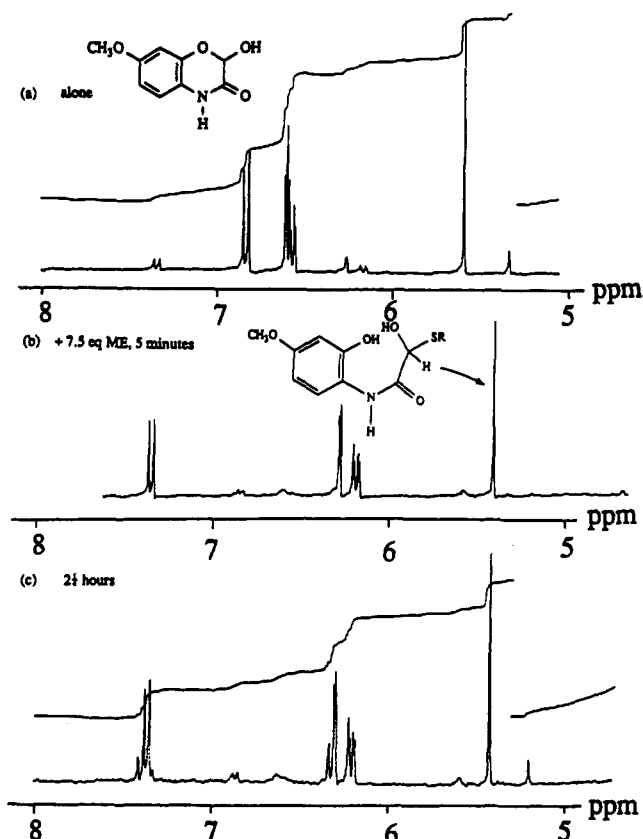


Figure 5. NMR spectra of HMBOA 2 in D₂O buffer (2 M carbonate, pD 9.0) alone (a) and during the reaction with 7.5 molar equiv of mercaptoethanol (ME), (b) and (c). *T* = 20 ± 1 °C.

had appeared. The 3 H singlet was now slightly changed at δ 3.69. After 2.25 h at 30 °C the only change in the spectrum was the presence of peaks (~15%) at δ 7.46, 6.36, 5.23.

A similar experiment was also performed with DIMBOA, but the amount of mercaptoethanol added was restricted to a 2.4 molar excess so that spectral changes could be followed over the time necessary to take several spectra. Nonetheless, the changes were relatively fast.

The spectrum of DIMBOA alone in buffer was clean and uncomplicated (Figure 6a) and exhibited the expected splitting pattern for the aromatic protons and a 1 H singlet at δ 5.60. A spectrum taken 5 min after addition of the thiol was much more complex (Figure 6b). At least 20 new peaks had formed and were observed to increase in intensity in successive spectra while the original peaks decreased. The original solitary resonance at δ 5.60 was now accompanied by four other major signals; one whose appearance was rapid and seemingly complete after 5 min (δ 5.34), and three others (δ 5.55, 5.50 and 5.48) that increased more slowly over the 10 min when the next spectrum was taken (Figure 6c).

In contrast, a similar experiment with the methyl acetal of DIMBOA, 15, and a 2.2 molar excess of mercaptoethanol showed no changes from the original spectrum after 2 h.

This same sort of experiment was performed with the 7-Cl (9) and 7-Me (7) analogues, with parallel results. Peaks not visible in the original spectrum ca. δ 5.4 were observed to increase in intensity slowly. The changes occurred much more slowly than in the case of HMBOA or DIMBOA however. The original resonances did not completely disappear until after 20 h at room temperature.

Equilibrium of Species in Solution. Most cyclic hemiacetals exist in solution predominantly in the closed form,⁵⁴ but are usually in equilibrium with the open form.

(53) (a) Covington, A. K.; Paabo, M.; Robinson, R. A.; Bates, R. G. *Ann. Chem.* 1968, 40, 700. (b) Forcés, R. K.; Carr, J. D. *Ann. Chem.* 1974, 46, 2049.

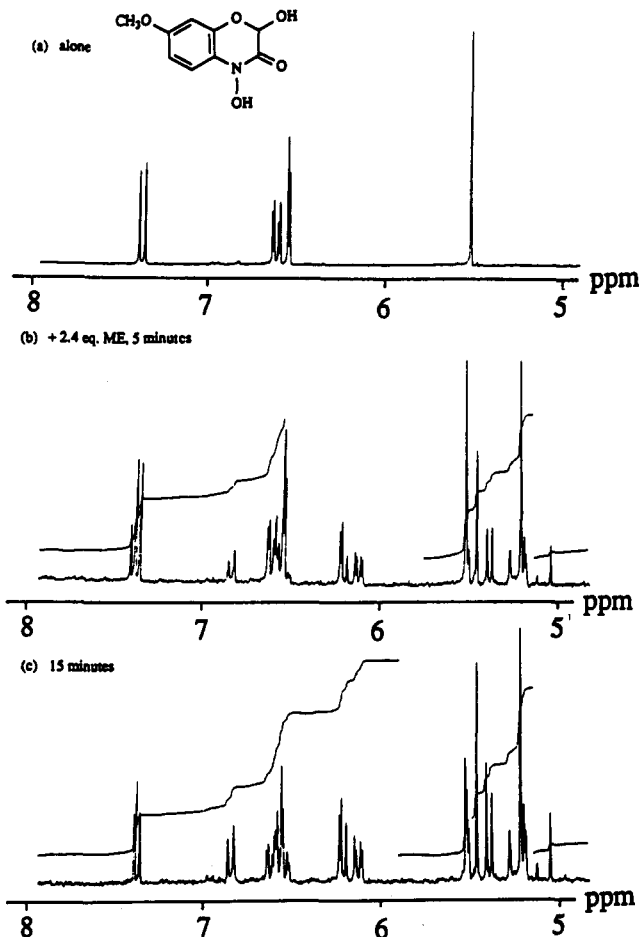


Figure 6. NMR spectra of DIMBOA 1 in D₂O buffer (2 M carbonate, pD 9.0) alone (a) and during the reaction with 2.4 molar equiv of mercaptoethanol (ME) (b and c). $T = 20 \pm 1^\circ\text{C}$.

The NMR experiments show that the cyclic form of these hydroxamic acids predominates. This is clear from the 1 H singlet at ca. δ 5.6, corresponding to the hemiacetal proton. For HMBOA in buffer small peaks were visible in the aromatic region (Figure 5a) whose chemical shifts support the ring-opened structure.⁵⁵ No aldehydic proton

(54) Eliel, E. L.; Allinger, N. L.; Angyal, S. J.; Morrison, G. A. *Conformational Analysis*; John Wiley and Sons, Inc.: New York, 1977; pp 380–81.

(55) The chemical shift changes observed in the aromatic region of the spectrum can be explained empirically by the degree of delocalization of the nitrogen lone pair in the lactam and the (acyclic) amide isomers. Inspection of models, and a reported X-ray crystal structure of the 2-O- β -D-glucoside of HMBOA,⁵⁶ suggest that in the cyclic form (2), the nitrogen lone pair has diminished overlap with the aromatic π -system. Only in the open form is this possible. A comparison of the assigned H-5 chemical shifts for both isomers of HMBOA with the simple derivative (1) is shown below.

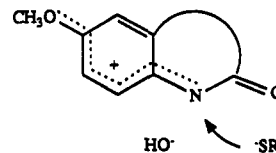
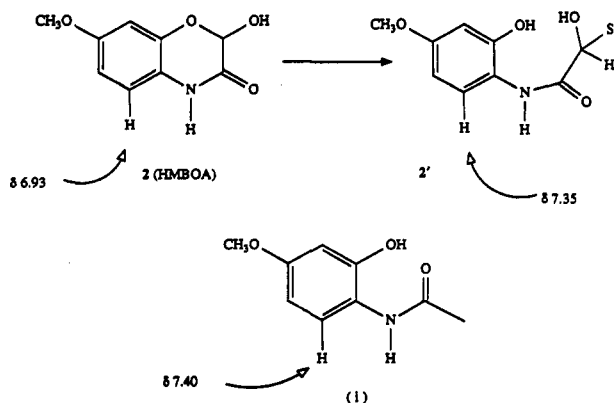


Figure 7. Hypothetical ion pair after scission of the N–O bond of a 7-methoxy-substituted cyclic hydroxamic acid. The *p*-methoxy group stabilizes the species bearing the positive charge. It is this species that would then be attacked by thiolate.

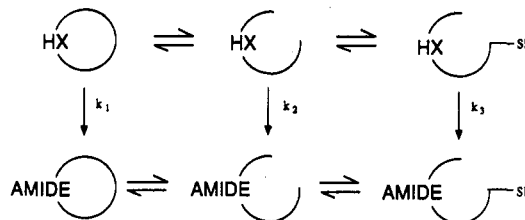


Figure 8. Schematic diagram of the equilibrating species and reaction products in solution during the reaction of a cyclic hydroxamic acid with a thiol. The rate constants k_1 , k_2 , and k_3 represent the overall reduction of each of the hydroxamic acid species that conceivably exist in solution. Closed rings are the lactols, open rings the phenol–aldehyde, and species containing SR are hemithioacetals at C-2.

is visible in this spectrum, presumably because of the low intensity of the spectrum and the possibility that the signal is blurred by a hydration–dehydration equilibrium.

On production of a hemithioacetal one would expect the chemical shift of the proton attached to C-2 to move upfield since one of the strongly electronegative oxygens of the lactol/aldehyde has been replaced by the less electronegative sulfur. Indeed, the NMR spectrum of HMBOA, 2, after addition of thiol showed the rapid appearance of a signal upfield from the original hemiacetal proton, δ 5.45 vs 5.60. This is likely the hemithioacetal produced by attack of thiolate on the acetal/aldehyde carbon. Also, the pattern of the chemical shifts of the aromatic protons is consistent with the open form of the ring.⁵⁵ The lactol/phenol–aldehyde equilibrium has been effectively trapped in the open form. These sort of spectral changes also occurred with the hydroxamic acids investigated.

It has been suggested that the reduction of these hydroxamic acids by thiols proceeds via attack of thiolate directly at the nitrogen atom.^{26b} This is supported by the remarkable increase in rate when a 7-MeO group is present, since it would stabilize a positive charge on nitrogen in a hypothesized ion pair which would then be attacked by thiolate (Figure 7). The production of the ion pair is facilitated by this resonance stabilization and by the strong tendency of oxygen to retain the electron pair that constitutes the N–O bond.⁵⁷ The reaction can also be discussed utilizing the hard soft acid base (HSAB) principle.⁵⁸ As described by Saville⁵⁹ one expects a substitution reaction to proceed more easily if the nucleophilic and the electrophilic species are of the same (hard or soft) category. For attack of thiolate on the hydroxamic acid nitrogen, the thiolate, a soft nucleophile, attacks the soft electrophilic center at nitrogen. In a reciprocal fashion, the leaving group HO[−], a hard base, attacks the hard acid H⁺ in the buffered solvent. Attack of sulfur on nitrogen is not

(56) Nagao, T.; Otsuka, H.; Kohda, H.; Sato, T.; Yamasaki, K. *Photochemistry* 1985, 24(12), 2959.

(57) Stirling, C. J. *Acc. Chem. Res.* 1979, 12, 198.

(58) Ho, T.-L. *Hard and Soft Acids and Bases Principle in Organic Chemistry*; Academic Press: New York, 1977; esp. pp 26–54.

(59) Saville, B. *Angew. Chem., Int. Ed. Engl.* 1967, 6(11), 928.

without precedent. Thiols are known to attack the nitrogen atom of nitrate esters during reactions catalyzed by glutathione transferase.⁶⁰

With the ring-opening equilibria and the thiol addition products in mind, one can envision the six species in Figure 8 occurring in solution over the course of the reaction. Attack by thiolate at nitrogen can theoretically occur on either the closed (k_1) or open (k_2) hydroxamic acid, or on the hydroxamic hemithioacetal (k_3). Judging from the rate at which the hemithioacetal signal appeared in the case of HMBOA, 2 (Figure 5), the eventual product in solution in the presence of excess thiol must be the amide hemithioacetal. In effect, all equilibria drain toward this product. That the free amides (without the hemithioacetal moiety) are isolated from reactions is simply a matter of the hemithioacetal's instability on removal from a basic medium. Extraction of neutralized reaction solutions, and chromatography on silica would cleave this group. The hemithioacetals of both the lactam and the hydroxamic acid have been isolated in small amounts ($\approx 3\%$ unoptimized yields^{26a}) from the reaction of DIMBOA with excess ethanethiol. The UV spectral properties of the isolated amide hemithioacetal differ from the free lactam by only a couple of nanometers at the maxima and so legitimize the kinetic analysis by UV absorbance changes.

When the reaction of DIMBOA and 2.4 molar equiv of mercaptoethanol was followed by ¹H NMR no resonances were visible that corresponded to the HMBOA hemithioacetal. This is expected, since three molecules of mercaptoethanol are needed to fully reduce the hydroxamic acid moiety and make the hemithioacetal. However, when the reaction was repeated with 8 molar equiv of mercaptoethanol the spectrum after 2.5 h (data not shown) clearly showed the presence of HMBOA hemithioacetal.

The rate enhancement provided by the 7-MeO group implies that the nitrogen atom is in conjugation with this ring substituent, and this is most likely to occur from the open form where the nitrogen lone pair can more easily overlap with the aromatic π -system. Support for this conclusion is found in the observation that 15 and 16 show no perceivable rates of reaction with mercaptoethanol at those conditions (20–37 °C, pH 9.0, 1–2 h) where DIMBOA showed measurable kinetics. With more extreme conditions (45 °C, 16 h) 16 is forced to react in the closed form and after extraction from the reaction buffer some of the lactam was detected. The carbocyclic compound 19 also yields some lactam at these more extreme conditions, but considerable ($\sim 93\%$ by GC) starting hydroxamic acid remained.

Given the rapidity in which the signals in the NMR spectra ca. δ 5.6 change upon addition of mercaptoethanol to the buffer solutions of DIMBOA, it is likely that the attack at nitrogen occurs on the already formed hydroxamic acid hemithioacetal. It has recently been shown²⁷ that DIMBOA is an inhibitor of the thiol protease papain (EC 3.4.2.2) and that the inactivation is reversible by added dithiothreitol. The non-hydroxamic acid HMBOA, completely analogous to DIMBOA in structure except that it lacks a N-OH group, is not an inhibitor. This suggests that it is the nitrogen atom of the hydroxamic acid moiety that is susceptible to attack by thiol (Cys-25 in the case of papain) which leads to inactivation of the enzyme.

It is not clear why the methylenedioxy ring of 3 affects the rate of reduction to such a degree. It is doubtful that the electronic nature of the aromatic ring and the nitrogen

atom are greatly different than in 4, yet 3 is reduced almost 5 times faster than 4. Clearly the C-6 oxy substituent is greatly enhancing the rate since the 7,8-dimethoxy-substituted 5 is reduced more slowly than DIMBOA. It is worth noting that both DIMBOA and 5 are naturally occurring (as their 2-O- β -D-glucosides) and that they are at the boundary between the two forms of reactivity available to these compounds. Their unimolecular decomposition is slower than 3 and 4 (unpublished results), yet faster than other analogues when *t*-Bu, Me, H, or Cl are substituted for the methoxy at C-7. A more detailed discussion of the biological structure-activity results for these compounds will be published elsewhere.

Experimental Section

All reagent chemicals were purchased from the Aldrich Chemical Co. except where indicated. Solvents were glass distilled, initial and final fractions being discarded. Mercaptoethanol was distilled and stored under nitrogen. The bottle was periodically checked by GC for the presence of impurities. Melting points are uncorrected.

Thin-layer chromatography (TLC) was performed on aluminum backed silica plates (Whatman, AL-SIL-G/UV, 250 μ m). Visualization of hydroxamic acids and their respective amides was achieved by dipping the plate in a 5% H₂SO₄ solution in MeOH and heating on a hot plate. Most of the hydroxamic acids turned a purplish-brown color. The amides gave less intense colors and were beige to brown.

Column chromatography (gravity flow) for purification of the nitrophenols was performed on Terochem silica (normal type) equivalent to Merck 7734, 60–200 mesh, in a 6 \times 20 cm column.

Chromatographic purification of hydroxamic acids and of lactams was best performed using radial chromatography with either 1- or 2-mm silica covered rotors. The plates were made using Merck Kieselgel 60 PF₂₅₄ silica for preparative TLC.

Gas chromatographic analyses were performed on a 10-m length of a vitreous silica capillary column (Varian, BP5-0-25, i.d. 0.22 mm). Samples were derivatized with one drop of bis(trimethylsilyl)acetamide (BSA) at room temperature. Most injections were run with the same temperature program; 150 °C for 2 min, then warmed at 10 °C/min up to 250 °C, and maintained there for 10–25 min. The carrier gas was helium and the detector a FID.

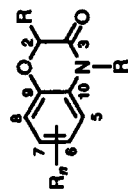
¹H NMR (300 MHz) and ¹³C NMR (80 MHz) spectra were recorded in acetone-*d*₆ or DMSO-*d*₆. All spectra were referenced on tetramethylsilane (TMS). Infrared spectra were recorded in KBr pellets or in chloroform solution. Ultraviolet (UV) spectra were recorded in MeOH using quartz cuvettes.

pK_a Measurements. Acid dissociation constants (pK_a's) were determined spectrophotometrically⁶¹ at 37 \pm 0.3 °C, $I = 0.15$. The temperature within the spectrophotometer was maintained by a circulating thermostated water bath (Haake, D1 L). Isoestic points were clearly present in the determination of pK_{a1} (hydroxamic acid), confirming the presence of two species in equilibrium. They were less obvious in the determination of pK_{a2} (phenol), because the absorbance differences in this case were much smaller.

Stock Solutions. Stock solutions of the hydroxamic acids were made at concentrations such that 100 μ L of stock, diluted in 5.00 mL of buffer, gave an absorbance of approximately 0.8–0.9. This necessitated dissolving 8–10 mg of hydroxamic acid in 10.00 mL of MeOH, giving final concentrations ca. 8 \times 10⁻⁵ M. Beer's law was shown to hold for DIMBOA and the 7-Cl compound, 9, through this concentration range. The stock solutions were monitored by TLC (CH₂Cl₂/MeOH, 10:1) over the course of the experiments. Even after several months in solution (the well-sealed solutions were stored at -10 °C when not in use) no re-acetalization of the lactol was evident. On the contrary, the amides HMBOA (2) and HBOA (16) slowly re-acetalized on standing in MeOH. Fresh stock solutions of these compounds were made as needed.

(60) (a) Pabst, M. J.; Habig, W. H.; Jakoby, W. B. *J. Biol. Chem.* 1974, 249, 7140. (b) Keen, J. H.; Habig, W. H.; Jakoby, W. B. *J. Biol. Chem.* 1976, 251, 6183.

(61) Albert, A.; Serjeant, E. P. *The Determination of Ionization Constants*, 3rd ed.; Chapman and Hall: London, 1984; Chapter 4.

Table IV. ^{13}C NMR Chemical Shifts (δ) of Selected Compounds in $\text{DMSO}-d_6$ 

carbon	1	2	3	4	5	6	7	8	9	10	11a	12	15	16	17	18 ^c	19	20
1	92.4	90.5	92.1	92.2	92.2	92.1	92.0	92.0	92.2	92.3	91.8	92.1	98.2	68.1	67.7	90.3	33.6	92.5
2	156.7	161.9	157.0	156.8	157.0	157.2	157.2	157.4	157.2	159.7	157.8	158.2	156.1	159.1	159.8	162.5	55.2	159.0
3	113.6	120.0	99.7	98.4	106.0	113.9*	112.7	112.9	114.1	113.7	112.9	113.2	113.8	123.1	115.7	115.6	113.4*	126.4*
5	107.6	108.1	143.1	144.8	107.2	119.0	122.7	123.6	122.4	108.8	124.1*	119.2	108.1	107.5	122.3	123.1	111.9	126.1*
6	155.9	155.5	142.4	143.9	149.3	146.7	133.2	122.4	127.1*	156.7	124.7*	143.1	155.0	155.9	123.4	122.4	154.8	124.1
7	103.4	103.6	94.9	102.7	138.3	112.5*	117.4	117.0	117.1	105.0	117.5	112.7	103.5	102.5	112.8	117.4	113.8*	115.2
8	141.5	141.0	134.8	133.8	134.9	140.2	140.4	140.5	141.4	141.5	140.2	140.7	140.9	144.5	143.3	140.6	142.1	142.1
9	122.4	116.0	122.6	121.1	123.7	126.2	126.3	128.6	127.9*	125.5	132.7	134.7	122.3	113.8	129.3	126.6	132.7	127.4
7-MeO	55.5	55.6	101.4	56.1*	56.2	34.1 ^b	20.4				165.2 ^c		55.9*	55.6			66.4	
			(MDO) ^d	56.0*	60.7	31.1	(7-Me)				52.1		55.4*					20.6
				(6-MeO)	8-MeO								(2-MeO)					(5-Me)

^aThe numbering of the hydroxamic acid parent structure has been used for the tetrahydroquinolin-2-one analogue 19 (carbocyclic). Assignment of the aromatic carbons was based on additivity rules for benzene substituents and are thus tentative. Values followed by an asterisk (*) may be interchanged within a vertical column.

^bResonance of the quaternary carbon in the *tert*-butyl group was 34.1 ppm and of the methyl groups 31.1 ppm. ^cResonance of the carbonyl in the 7-CO₂Me group was 165.2 ppm and of the associated methoxy 52.1 ppm. ^dMDO refers to the methylenedioxy group in compound 3.

Reaction of Hydroxamic Acids with Mercaptoethanol. (i) Product Analysis by GC/MS. Each analogue (3 mg) was dissolved in 8 mL of buffer (0.5 M Tris, pH 9.0) to which was added 50 μL of mercaptoethanol (~ 40 -fold molar excess). The solutions in 10-mL vials were well sealed and left in a thermostated oven at 45 $^{\circ}\text{C}$ for 16 h. After this time some of the solutions had developed faint colors, from orange and pink (3 and 4) to a greenish hue (9). Each solution was then titrated with ~ 1.5 mL of 1 N HCl (whereupon colors, if present, lightened) and immediately extracted with EtOAc. The organics were dried over anhydrous MgSO_4 , and the solvent was removed in vacuo. The oils that remained were derivatized with bis(trimethylsilyl)-acetamide and analyzed by GC/MS immediately. Waiting before injection for any more than an hour gave poor chromatograms with few recognizable peaks. Samples were worked up individually to minimize the time spent in the acidified solution. Not surprisingly, much of the starting mercaptoethanol remained. It occasionally interfered with the observation of benzoxazinone products (BOA's $t_R = 4$ –9 min) versus 4.8 min for bis-silylated mercaptoethanol, but not with observation of the lactams or starting hydroxamic acids ($t_R = 6$ –11 min). The temperature program was always 150 $^{\circ}\text{C}$ for 2 min and then increased to 250 $^{\circ}\text{C}$ at the rate of 10 $^{\circ}\text{C}/\text{min}$.

(ii) Measurement of the Kinetics of Reaction with Mercaptoethanol. Five test tubes each containing 10.00 mL of buffer (0.1 M TRIS, pH = 9.00, $I = 0.15$) were equilibrated in a water bath that was generally 8–10 $^{\circ}\text{C}$ higher than the thermostated temperature in the turrets of the spectrophotometer. This was done to account for cooling of the buffer while measuring, mixing with hydroxamic acid, and filling a cuvette, prior to placement in the spectrophotometer. To the test tubes were added 5, 10, 15, 20, and 25 μL of mercaptoethanol (80–400-fold excess) such that a series of buffer solutions of increasing concentration of mercaptoethanol was at hand. To each of five 10-mL vials was added 100 μL of stock hydroxamic acid solution. To begin the experiment, 5.00 mL of the most dilute ME buffer solution (5 μL of mercaptoethanol in 10.00 mL of buffer) was added to one of the vials. It was capped and shaken briefly to ensure mixing. Approximately 1 mL of this solution was transferred via a Pasteur pipette to a quartz cuvette and this was placed in the spectrophotometer. Each reaction cuvette (with hydroxamic acid and mercaptoethanol) was blanked against the mercaptoethanol containing buffer of the appropriate concentration. When all cuvettes had been loaded into the machine (this took ~ 5 min) acquisition of absorbance data was not begun for another 1.5 min to allow for equilibration. The analytical wavelength was chosen such that the absorbance difference between starting material and final product was as large as possible. Generally, these were in the range 290–310 nm. Data was acquired and manipulated by a Varian DS-15 data station and the 2200/2300 Series/DS-15 Kinetics storage and calculation programs (enhanced). Values for A_{inf} and A_{init} as well as an estimation of the rate constant are supplied by the experimenter. Each run was repeated at least twice the second as confirmation of the first. The calculated standard deviations for each determination of a rate constant was less than $\pm 2.5\%$.

Decomposition Reaction. The pH of solutions was adjusted to equalize the concentrations of the monodissociated hydroxamic acid for each member of the series. Buffers were either phosphate or carbonate and were warmed to ~ 55 $^{\circ}\text{C}$ (7 $^{\circ}\text{C}$ higher than the trial temperature of 48 ± 0.3 $^{\circ}\text{C}$) and 5.00 mL of it was added to a vial containing 100 μL of the stock solution of the hydroxamic acid. It was thoroughly mixed by briefly shaking. Approximately 1 mL of this was transferred to a cuvette and then placed in the spectrophotometer, and the sample was blanked with buffer. The analytical wavelength was chosen at the maximal absorbance difference between the initial spectrum and one judged to be the "infinity" spectrum. At this temperature the product spectrum for the 7-F compound 10 did not resemble a benzoxazinone so a rate constant has not been reported. Data were acquired and manipulated as described above. Each run was repeated at least twice—the second as confirmation of the first. The calculated standard deviations for each determination of a rate constant were less than $\pm 2.5\%$.

Nitrophenols. 3-Hydroxy-4-nitrobenzoic acid, also purchased, was readily esterified to either the ethyl or methyl ester by re-

fluxing the acid in a 1:1 solution of CH_2Cl_2 -MeOH or EtOH (absolute) with H_2SO_4 as catalyst. The yields for both esters were 93 and 97%, respectively. 5-Methoxy-, 5-*tert*-butyl-, 5-(trifluoromethyl)-, and 5-cyano-2-nitrophenol were synthesized by nitration of the appropriate phenol in acetic acid. The phenol, as a solution in acetic acid, was added dropwise to a cooled solution ($\sim 10^\circ\text{C}$, ice bath) of glacial acetic acid and a 1.1 molar excess of 70% nitric acid with rapid stirring. In the case of the CF_3 and CN compounds, the resulting mixture was then warmed to 40°C for several hours. The highly colored solutions were then poured into ice water and the resulting crystals filtered, or the oil extracted with CHCl_3 . Yields were between 25 and 40% after recrystallization or column chromatography (SiO_2 , hexane/ CH_2Cl_2) to remove contaminating isomers. In all cases the desired isomer had the higher R_f . 2,3-Dimethoxy-6-nitrophenol, 4,5-dimethoxy-2-nitrophenol, and 4,5-(methylenedioxy)-2-nitrophenol were synthesized using the method of Orphanos and Taurins.⁶² 5-(Dimethylamino)-2-nitrophenol was prepared from the acetate ester of the corresponding phenol following the procedure outlined in ref 62. 5-Acetamido-2-nitrophenol was synthesized by a similar literature method.⁶³ 5-Chloro-2-nitrophenol was prepared by a phase transfer catalyzed aromatic nucleophilic substitution of 2,4-dichloronitrobenzene by base.⁶⁴ 5-Methoxy-3-methyl-2-nitrophenol was prepared from nitrocinol⁶⁵ which was first methylated at both phenol groups (K_2CO_3 , 2 mol of MeI, reflux in acetone, 87% yield), and then the *o*-methoxy group was selectively demethylated with 1 equivalent of BBr_3 in CH_2Cl_2 at 0°C for 1 h to give the *o*-nitrophenol in 97% yield after recrystallization and silica chromatography of the mother liquors.

Synthesis and Reductive Cyclization of Methyl α -(*o*-Nitrophenoxy)- α -methoxyacetates. All compounds (except for the 7- NO_2 analogue 12) were synthesized by the reaction of suspensions of a substituted potassium nitrophenoxide in ether or THF with a CCl_4 solution of freshly prepared methyl α -bromo- α -methoxyacetate (MBMA). Most of the reactions were complete within 1–3 h as observed by the disappearance of the brightly colored nitrophenoxides. Yields were dependent on the completeness of removal of HBr from the solutions of MBMA. A representative procedure for the bromination of methyl methoxyacetate and subsequent coupling with potassium 5-methoxy-2-nitrophenolate is given below.

Bromination of Methyl Methoxyacetate. A modification of the method of Bendich et al.⁶⁵ was used. Fresh MBMA was made for each reaction since isolation by distillation was time consuming, resulted in partial degradation and the product did not store well. A 1.1 molar excess of methyl methoxyacetate (with respect to the potassium nitrophenoxide) was brominated to ensure as complete coupling as possible with the more valuable nitrophenoxide.

In a 500-mL three-necked round-bottomed flask equipped with an efficient condenser, stirring bar, and a dropping funnel, methyl methoxyacetate (11.3 g, 10.8 mmol) was set to reflux in 200 mL of dry CCl_4 . Over 4 h, Br_2 (17.3 g, 10.8 mmol) in 25 mL of CCl_4 was added dropwise to the solution. After the addition of Br_2 was complete the solution was allowed to cool slightly and was bubbled with a vigorous stream of N_2 (sintered glass bubbler) with rapid stirring to remove HBr. After ~ 20 min this could be completed by briefly evaporating a small portion of the solution at reduced pressure.

Preparation of Potassium Nitrophenoxides. All of the potassium nitrophenoxides used in these syntheses were made by titration of ethereal solutions of the nitrophenol with ethanolic KOH.

Typical Coupling Procedure: α -Bromo- α -methoxyacetate + Potassium 5-Methoxy-2-nitrophenoxide. Potassium 5-methoxy-2-nitrophenoxide (20.7 g, 0.1 mol) was rapidly stirred in 100 mL of THF until the crystals formed a fine suspension. The CCl_4 solution from the bromination of methyl methoxyacetate (11.3 g, 0.11 mol), after removal of HBr, was added all at once, and the mixture stirred for 2 h.

The dull orange slurry was poured into a separatory funnel, and a half volume of CH_2Cl_2 was added to keep the CCl_4 and THF from forming two layers. The organics were then washed $3 \times 10\%$ Na_2CO_3 to remove any starting nitrophenol, once with water, and once with brine, and finally dried over anhydrous Na_2SO_4 or MgSO_4 . Evaporation of the solvent gave an orangish oil (24.7 g, 92%) that slowly crystallized on standing. GC analysis of this and all other (*o*-nitrophenoxy)acetates produced by this method showed purities of $>98\%$.

Yields reported for this coupling reaction are corrected for recovered starting material.

General Procedure for Reductive Cyclization. The Pd/C- NaBH_4 reductive cyclization method described by Coutts^{29,66} for similar α -(*o*-nitrophenoxy) esters was used with slight modifications. For all reactions the vessel was immersed in a water bath and kept at 15 – 20°C , since the reaction was mildly exothermic and this also contributed to color formation. This was especially important for reactions on a scale larger than a few grams.

An α -methoxy- α -(*o*-nitrophenoxy)acetate (2 g, 6–9 mmol) was dissolved in ~ 10 mL of dioxane and added dropwise to a rapidly stirring suspension of NaBH_4 (1 g) and 10% Pd on charcoal (100 mg) in 1:1 water/dioxane (80 mL). Addition of a drop of the α -methoxy- α -(*o*-nitrophenoxy)acetate tinted the reaction mixture with a yellow to red color. More was not added until this color disappeared, which usually took about 15 s, longer as addition of the nitro ester proceeded. After complete addition of the nitro ester the mixture was stirred for another 15–30 min. It was then filtered and the filtrate acidified with 1 N HCl until no more foaming was evident and the pH, as judged by pH paper, was ~ 4 . The solution was immediately extracted $3 \times$ EtOAc, the organics washed once with brine, dried with Na_2SO_4 (treated with activated charcoal to decolorize if necessary), and evaporated. Purification of the products so obtained usually involved trituration with hexane/ethyl acetate or hexane/acetone mixtures, though occasionally chromatography on silica or Fe^{3+} -Sephadex⁶⁷ was necessary.

2,7-Dimethoxy-4-hydroxy-2H-1,4-benzoxazin-3-one (DIM-BOA Methyl Acetal, 15). Methyl α -(5-methoxy-2-nitrophenoxy)- α -methoxyacetate (9.48 g, 34.9 mmol) was cyclized following the general method. Dilution of the dried EtOAc extracts with hexane provided, after cooling in a refrigerator overnight, slightly pink crystals (3.27 g). Trituration of the residual oil after evaporation of the mother liquor with hexane-chloroform (4:1) gave two more lots of crystals (0.820 g and 0.670 g): total yield 4.76 g (60%); mp 150 – 152°C dec (lit.³⁰ mp 148 – 150°C); ^1H NMR (300 MHz, acetone- d_6) δ 7.26 (d, 1 H, $J_{5,6} = 9.3$ Hz), 6.72 (m, 2 H), 5.39 (s, 1 H), 3.78 (s, 3 H, Ar-OCH₃), 3.50 (s, 3 H, 2-OCH₃); MS (EI) m/z 225 (12) M^+ , 209 (100), 178 (12), 166 (11), 165 (18), 150 (54), 149 (49), 134 (24), 106 (16); IR (KBr) ν_{max} 1675 (br, s), 1600 (m), 1503 (s) cm^{-1} .

Demethylation of Hydroxamic Acid Methyl Acetals. Demethylation of the acetals followed the method reported by Jernow and Rosen.³⁰ Temperatures, reaction times, and reagents used were particular to each analogue synthesized and are detailed below under the individual compound names.

General Procedure for Demethylation: 2,4-Dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one (1, DIMBOA). 15 (5.31 g, 23.6 mmol) was suspended in CH_2Cl_2 (150 mL) and cooled to -50°C under N_2 in a dry ice/acetone bath; 1 M BCl_3 in CH_2Cl_2 (70.9 mL, 3 molar equiv) was added precooled. The flask was removed from the bath and stirred at room temperature for 2.5 h. THF (25 mL) was added via pipette, and then 40 mL of water was added all at once in a separatory funnel. The CH_2Cl_2 layer was removed, and the aqueous layer was extracted once with EtOAc. The combined organics were washed once with a small volume of water and evaporated *without drying* to a dark oil. This was resuspended in THF (40 mL), and this solution added over 10 min to a rapidly stirring suspension of Ag_2CO_3 (13 g, ~ 2 -fold molar excess) in 2:1 H_2O -THF and stirred for 20 min. The

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mixture was then filtered and extracted with EtOAc until the aqueous showed no color with FeCl_3 . The organics were washed with brine, dried with anhydrous MgSO_4 , and evaporated to ~50 mL. Addition of hexane and cooling in a fridge for 1 day gave 1.9 g of a tan solid. Concentration of the mother liquor gave another 0.43 g of a beige solid. The residual oil on evaporation could be triturated with hexane-acetone to give 1.15 g of beige solid: total yield 3.48 g (70%); mp 163–164.5 dec (lit.³⁰ mp 162–163 °C); $^1\text{H NMR}$ (300 MHz, acetone- d_6) δ 7.25 (d, 1 H, $J_{5,6} = 8.8$ Hz), 6.68 (dd, 1 H, $J_{6,5} = 8.8$ Hz, $J_{6,8} = 2.6$ Hz), 6.61 (s, 1 H, $J_{6,6} = 2.6$ Hz), 5.72 (s, 1 H), 3.57 (s, 3 H), 2.85 (br s, OH); GC/MS (TMS deriv) m/z 355 (75) M^+ , 340 (45), 238 (100), 210 (9), 194 (50), 191 (33), 165 (7); UV λ_{max} (MeOH) 288 nm (sh), 262 nm ($\epsilon = 10300$); IR (KBr) ν_{max} 1662 (vs), 1605 (m), 1519 (s) cm^{-1} . Anal. Calcd for $\text{C}_{10}\text{H}_9\text{NO}_5$: C, 51.19; H, 4.30; N, 6.63. Found: C, 51.34; H, 4.63; N, 6.60.

2,7-Dimethoxy-2H-1,4-benzoxazin-3-one (HMBOA) (2), methyl acetal: 59%; mp 149–150 °C; $^1\text{H NMR}$ (300 MHz, acetone- d_6) δ 9.62 (weak and broad, NH), 6.93 (d, 1 H, $J_{5,6} = 8.7$ Hz), 6.69 (d, 1 H, $J_{6,5} = 2.6$ Hz), 6.62 (dd, 1 H, $J_{6,5} = 2.6$ Hz, $J_{6,8} = 8.7$ Hz), 5.21 (s, 1 H), 3.76 (s, 3 H), 3.49 (s, 3 H); MS (EI) m/z 209 (100) M^+ , 181 (10), 178 (14), 166 (13), 150 (68), 149 (56), 134 (30), 110 (11), 107 (10), 106 (18); IR (KBr) ν_{max} 1692 (vs), 1611 (m), 1513 (s) cm^{-1} .

2-Hydroxy-7-methoxy-2H-1,4-benzoxazin-3-one (2, HMBOA): 90%; BCl_3 , 0 °C, 3 h; mp 198–199.5 °C (lit.⁶⁶ mp 196–198 °C, MeOH); $^1\text{H NMR}$ (300 MHz, acetone- d_6) 9.5 (br s, NH), 6.92 (dd, 1 H, $J_{6,5} = 7.1$ Hz, $J_{6,8} = 1.8$ Hz), 6.58 (m, 2 H), 5.55 (s, 1 H), 3.74 (s, 3 H). GC/MS (TMS deriv) m/z 339 (100) M^+ , 324 (24), 310 (8), 296 (9), 250 (13), 222 (39), 206 (10), 194 (11), 191 (26); UV λ_{max} (MeOH) 287 nm (sh), 256 nm; IR (KBr) ν_{max} 1693 (s), 1610 (w), 1514 (s) cm^{-1} .

4-Hydroxy-2-methoxy-6,7-(methylenedioxy)-2H-1,4-benzoxazin-3-one (3 methyl acetal): 33% after purification on Fe^{3+} -Sephadex; mp ~150 °C slow dec; $^1\text{H NMR}$ (300 MHz, acetone- d_6) δ 6.91 (s, 1 H), 6.72 (s, 1 H), 6.01 (d, 1 H, $J_{\text{gem}} = 1$ Hz), 5.97 (d, 1 H, $J_{\text{gem}} = 1$ Hz), 5.34 (s, 1 H), 3.48 (s, 3 H), 2.81 (s, 1 H, OH); MS (EI) m/z 239 (18) M^+ , 223 (100), 194 (15), 192 (17), 180 (12), 179 (43), 164 (29), 163 (84), 162 (37), 152 (45), 123 (14); IR (KBr) ν_{max} 1668 (s), 1510 (m), 1491 (s) cm^{-1} . Anal. Calcd for $\text{C}_{10}\text{H}_9\text{NO}_6$: C, 50.22; H, 3.79; N, 5.86. Found: C, 50.17; H, 4.00; N, 5.91.

2,4-Dihydroxy-6,7-(methylenedioxy)-2H-1,4-benzoxazin-3-one (3): 38%; BCl_3 , -50 °C to -20 °C, 1 h; mp 152–153 °C (decomposition to red-black tar, bubbling); $^1\text{H NMR}$ (300 MHz, acetone- d_6) δ 6.90 (s, 1 H), 6.62 (s, 1 H), 5.99 (d, 1 H, $J_{\text{gem}} = 1.0$ Hz), 5.95 (d, 1 H, $J_{\text{gem}} = 1.0$ Hz), 5.66 (s, 1 H); GC/MS (TMS deriv) m/z 369 (33) M^+ , 354 (13), 341 (100), 325 (28), 310 (71), 297 (41), 266 (9), 251 (84), 236 (29), 208 (83), 179 (25), 163 (27); UV λ_{max} (MeOH) 308 nm ($\epsilon = 8600$), 264 nm ($\epsilon = 4000$); IR (KBr) ν_{max} 1680 (s), 1500 (s) cm^{-1} .

4-Hydroxy-2,6,7-trimethoxy-2H-1,4-benzoxazin-3-one (4 methyl acetal): 30% after purification on Fe^{3+} -Sephadex; mp 96–97 °C; $^1\text{H NMR}$ (300 MHz, acetone- d_6) δ 6.98 (s, 1 H), 6.79 (s, 1 H), 5.34 (s, 1 H), 3.81 (s, 3 H), 3.79 (s, 3 H), 3.48 (s, 3 H), 2.83 (br s, OH); MS (EI) m/z 255 (2) M^+ , 239 (100), 208 (14), 196 (13), 180 (14), 179 (26), 168 (12), 164 (30), 136 (17), 109 (14); IR (KBr) ν_{max} 1698 (s), 1507 (s) cm^{-1} . Anal. Calcd for $\text{C}_{11}\text{H}_{13}\text{NO}_6$: C, 51.77; H, 5.13; N, 5.49. Found: C, 51.96; H, 5.34; N, 5.48.

2,4-Dihydroxy-6,7-dimethoxy-2H-1,4-benzoxazin-3-one (4): 22% after purification on Fe^{3+} -Sephadex; BCl_3 , -45 °C to 0 °C, 2 h; mp 154–155 °C (decomposition to red-black tar, bubbling); $^1\text{H NMR}$ (300 MHz, acetone- d_6) δ 6.96 (s, 1 H), 6.68 (s, 1 H), 5.67 (s, 1 H), 3.80 (s, 3 H), 3.77 (s, 3 H); GC/MS (TMS deriv) m/z 385 (52) M^+ , 357 (79), 341 (43), 326 (100), 283 (19), 267 (58), 252 (51), 224 (31), 179 (27), 164 (24); UV λ_{max} (MeOH) 302 nm ($\epsilon = 8100$), 264 nm ($\epsilon = 5100$); IR (KBr) ν_{max} 1664 (s), 1515 (s) cm^{-1} .

4-Hydroxy-2,7,8-trimethoxy-2H-1,4-benzoxazin-3-one (5 methyl acetal): 90%; mp 143–146 °C dec; $^1\text{H NMR}$ (300 MHz, acetone- d_6) δ 7.02 (d, 1 H, $J_{5,6} = 10.1$ Hz), 6.79 (d, 1 H), 5.44 (s, 1 H), 3.84 (s, 3 H), 3.83 (s, 3 H), 3.53 (s, 3 H), 2.85 (s, 1 H, OH) MS (EI) m/z 255 (33) M^+ , 239 (100), 210 (23), 208 (11), 195 (30), 181 (16), 180 (26), 179 (38), 164 (39), 140 (13), 136 (13), 109 (16); IR (KBr) ν_{max} 1664 (s), 1605 (w), 1500 (s) cm^{-1} . HRMS (using m/z 239 as reference mass) calcd for $\text{C}_{11}\text{H}_{13}\text{NO}_5$ 239.0792, found 239.0780.

2,4-Dihydroxy-7,8-dimethoxy-2H-1,4-benzoxazin-3-one (5 methyl acetal): 31% after purification on Fe^{3+} -Sephadex; BCl_3 , -50 °C to room temperature, 2 h; mp 145–146 °C (decomposition to red tar with bubbling); $^1\text{H NMR}$ (300 MHz, acetone- d_6) δ 7.01 (d, 1 H, $J_{5,6} = 9.0$ Hz), 6.76 (d, 1 H, $J_{6,5} = 9.0$ Hz), 5.77 (s, 1 H), 3.81 (s, 3 H), 3.78 (s, 3 H); GC/MS (TMS deriv) m/z 385 (85) M^+ , 370 (42), 357 (7), 326 (7), 297 (13), 268 (100), 237 (83), 224 (13), 191 (46); UV λ_{max} (MeOH) 259 nm ($\epsilon = 11300$); IR (KBr) ν_{max} 1660 (s), 1604 (w), 1500 (s) cm^{-1} .

7-tert-Butyl-4-hydroxy-2-methoxy-2H-1,4-benzoxazin-3-one (6 methyl acetal): 56%; mp 141–143 °C dec; $^1\text{H NMR}$ (300 MHz, acetone- d_6) δ 7.28 (d, 1 H, $J_{5,6} = 8.4$ Hz), 7.16 (m, 2 H), 5.40 (s, 1 H), 3.49 (s, 3 H), 2.86 (s, 1 H, OH), 1.30 (s, 9 H); MS (EI) m/z 251 (11) M^+ , 235 (45), 220 (100), 192 (45), 176 (24), 160 (29), 132 (15), 120 (16); IR (KBr) ν_{max} 1685 (s), 1665 (s), 1517 (m) cm^{-1} . Anal. Calcd for $\text{C}_{13}\text{H}_{16}\text{NO}_4$: C, 62.14; H, 6.42; N, 5.57. Found: C, 62.24; H, 6.50; N, 5.57.

7-tert-Butyl-2,4-dihydroxy-2H-1,4-benzoxazin-3-one (6): 86%; BCl_3 , -60 °C to room temperature; mp 135–138 °C (decomposition to red-black tar, bubbling); $^1\text{H NMR}$ (300 MHz, acetone- d_6) δ 7.26 (d, 1 H, $J_{5,6} = 8.4$ Hz), 7.14 (dd, 1 H, $J_{6,5} = 8.4$ Hz, $J_{6,8} = 2.1$ Hz), 7.04 (d, 1 H, $J_{6,8} = 2.1$ Hz), 5.73 (s, 1 H), 1.29 (s, 9 H); GC/MS (TMS deriv) m/z 381 (58) M^+ , 368 (10), 367 (34), 366 (81), 292 (9), 264 (100), 220 (43), 191 (55); UV λ_{max} (MeOH) 290 nm (sh), 283 nm ($\epsilon = 5500$), 258 nm ($\epsilon = 7100$); IR (KBr) ν_{max} 1694 (s), 1670 (s), 1650 (s), cm^{-1} .

4-Hydroxy-2-methoxy-7-methyl-2H-1,4-benzoxazin-3-one (7 methyl acetal): 76%; mp 168–171 °C dec; $^1\text{H NMR}$ (300 MHz, acetone- d_6) δ 7.23 (d, 1 H, $J_{5,6} = 8.7$ Hz), 6.94 (m, 2 H), 5.38 (s, 1 H), 3.48 (s, 3 H), 2.83 (br s, OH), 2.29 (s, 3 H). MS (EI) m/z 209 (5), 193 (18), 164 (3), 149 (9), 134 (15), 133 (12), 104 (4); IR (KBr) ν_{max} 1672 (vs), 1510 (s) cm^{-1} ; HRMS calcd for $\text{C}_{10}\text{H}_{11}\text{NO}_4$ 209.0687, found 209.0687.

2,4-Dihydroxy-7-methyl-2H-1,4-benzoxazin-3-one (7): 96%; BCl_3 , -60 °C to room temperature, 3.5 h; mp 163–164 °C (decomposition to a red-black tar, bubbling); $^1\text{H NMR}$ (300 MHz, acetone- d_6) δ 7.22 (d, 1 H, $J_{5,6} = 8.2$ Hz), 6.92 (m, 1 H), 6.84 (d, 1 H, $J_{6,6} = 1$ Hz), 5.71 (s, 1 H), 2.28 (s, 3 H); GC/MS (TMS deriv) m/z 339 (68) M^+ , 324 (100), 311 (15), 250 (7), 222 (74), 206 (21), 193 (23), 192 (15), 191 (49), 179 (18), 178 (47), 150 (19); UV λ_{max} (MeOH) 283 nm (sh), 260 nm ($\epsilon = 11900$); IR (KBr) ν_{max} 1648 (s), 1620 (m), 1604 (m) cm^{-1} .

4-Hydroxy-2-methoxy-2H-1,4-benzoxazin-3-one [DIBOA (8) methyl acetal]: 83%; mp 136–138 °C (lit.³⁰ mp 121–123 °C, impure); $^1\text{H NMR}$ (300 MHz, acetone- d_6) δ 7.37 (dd, 1 H, $J_{5,6} = 7.9$ Hz, $J_{5,7} = 1.7$ Hz), 7.13 (m, 3 H), 5.42 (s, 1 H), 3.49 (s, 3 H), 2.88 (br s, OH); MS (EI) m/z 195 (17), 179 (86), 148 (13), 136 (17), 135 (38), 120 (91), 119 (100), 92 (13), 91 (34), 90 (14); IR (KBr) ν_{max} 1678 (s), 1610 (m), 1500 (s) cm^{-1} . Anal. Calcd for $\text{C}_9\text{H}_9\text{NO}_4$: C, 55.39; H, 4.65; N, 7.18. Found: C, 55.18; H, 4.84; N, 7.05.

2,4-Dihydroxy-2H-1,4-benzoxazin-3-one (8, DIBOA): 62%; BCl_3 , -30 °C to room temperature, 2 h; mp 155–157 °C (lit.⁵¹ mp 155–56 °C) (decomposition to red-black tar, bubbling); $^1\text{H NMR}$ (300 MHz, acetone- d_6) δ 7.36 (m, 1 H), 7.06 (m, 3 H), 5.74 (s, 1 H); GC/MS (TMS deriv) m/z 325 (44) M^+ , 310 (100), 297 (29), 282 (6), 236 (8), 208 (42), 192 (35), 179 (25), 164 (32); UV λ_{max} (MeOH) 254 nm ($\epsilon = 7800$), 282 nm ($\epsilon = 5100$); IR (KBr) ν_{max} 1666 (s), 1655 (s), 1601 (m) cm^{-1} .

7-Chloro-4-hydroxy-2-methoxy-2H-1,4-benzoxazin-3-one (9 methyl acetal): 66%; mp 163–165 °C dec; $^1\text{H NMR}$ (300 MHz, acetone- d_6) δ 7.37 (d, 1 H, $J_{5,6} = 9.3$ Hz), 7.17 (m, 2 H), 5.46 (s, 1 H), 3.52 (s, 3 H), 2.85 (br s, OH); MS (EI) m/z 229 (40) M^+ , 215 (17), 213 (50), 198 (18), 184 (17), 171 (25), 170 (16), 169 (70), 156 (15), 155 (22), 154 (39), 153 (47), 141 (17); IR (KBr) ν_{max} 1675 (s) cm^{-1} ; HRMS calcd for $\text{C}_9\text{H}_8\text{ClNO}_4$ 229.0140, found 229.0126.

7-Chloro-2,4-dihydroxy-2H-1,4-benzoxazin-3-one (9): 49% after purification on Fe^{3+} -Sephadex; mp 173–174 °C (decomposition to red-black tar, bubbling); $^1\text{H NMR}$ (300 MHz, acetone- d_6) δ 7.35 (d, 1 H, $J_{5,6} = 8.6$ Hz), 7.14 (dd, 1 H, $J_{6,5} = 8.6$ Hz, $J_{6,8} = 2.2$ Hz), 7.07 (d, 1 H, $J_{6,8} = 2.3$ Hz), 5.78 (s, 1 H); GC/MS (TMS deriv) m/z 359 (55) M^+ , 344 (100), 242 (42), 226 (19), 213 (30), 198 (40), 191 (54), 150 (47); UV λ_{max} (MeOH) 291 nm (sh), 284 nm ($\epsilon = 5900$), 255 nm ($\epsilon = 9600$); IR (KBr) ν_{max} 1675 (s), 1600 (m), 1520 (s) cm^{-1} .

7-Fluoro-4-hydroxy-2-methoxy-2H-1,4-benzoxazin-3-one (10 methyl acetal): 93%; mp 162–165 °C dec; $^1\text{H NMR}$ (300

MHz, acetone- d_6) δ 7.37 (dd, 1 H, $J_{5,6} = 8.8$ Hz, $J_{mF} = 5.4$ Hz), 6.95 (m, 2 H), 5.46 (s, 1 H), 3.52 (s, 3 H), 2.87 (br s, OH). MS (EI) m/z 213 (80) M^+ , 197 (65), 182 (21), 168 (25), 153 (100), 138 (49), 137 (61), 125 (38), 109 (22), 108 (26); IR (KBr) ν_{max} 1672 (s), 1604 (m), 1509 (s) cm^{-1} . Anal. Calcd for $C_9H_9FNO_4$: C, 50.72; H, 3.78; N, 6.57; F, 8.91. Found: C, 50.42; H, 4.02; N, 6.33; F, 9.06.

2,4-Dihydroxy-7-fluoro-2H-1,4-benzoxazin-3-one (10): 25%; BBR_3 , -45 °C to 10 °C, 2 h; mp 145–146 °C (decomposition to black tar with bubbling); 1H NMR (300 MHz, acetone- d_6) δ 7.35 (m, 1 H), 6.88 (m, 2 H), 5.77 (s, 1 H); GC/MS (TMS deriv) m/z 343 (41) M^+ , 328 (100), 315 (16), 226 (32), 210 (17), 197 (18), 191 (33), 182 (37), 154 (20); UV λ_{max} (MeOH) 280 nm ($\epsilon = 4700$), 249 nm ($\epsilon = 6500$); IR (KBr) ν_{max} 1659 (m), 1615 (m), 1504 (s) cm^{-1} .

7-Carbomethoxy-4-hydroxy-2-methoxy-2H-1,4-benzoxazin-3-one: 54%; mp 125–131 °C (slow decomposition over this range); 1H NMR (300 MHz, acetone- d_6) δ 7.81 (dd, 1 H, $J_{6,5} = 8.4$ Hz, $J_{6,8} = 1.8$ Hz), 7.67 (d, 1 H, $J_{6,6} = 1.8$ Hz), 7.48 (d, 1 H, $J_{5,6} = 8.4$ Hz), 5.51 (s, 1 H), 3.87 (s, 3 H), 3.54 (s, 3 H), 2.87 (br s, OH); MS (EI) m/z 253 (14), 237 (72), 209 (11), 206 (21), 194 (12), 193 (19), 178 (81), 177 (14), 167 (12), 146 (100), 136 (21); IR (KBr) ν_{max} 1725 (s), 1672 (s), 1612 (m), 1599 (m), 1510 (m), cm^{-1} . Anal. Calcd for $C_{11}H_{11}NO_6$: C, 52.18; H, 4.38; N, 5.53. Found: C, 51.99; H, 4.60; N, 5.39.

7-Carbomethoxy-2,4-dihydroxy-2H-1,4-benzoxazin-3-one (11a): 7% after purification on Fe^{3+} -Sephadex; BBR_3 , -60 °C to room temperature, 1 h; mp 186–187 °C (decomposition to red-black tar with bubbling); 1H NMR (300 MHz, acetone- d_6) δ 7.78 (dd, 1 H, $J_{6,5} = 8.4$ Hz, $J_{6,8} = 1.7$ Hz), 7.59 (d, 1 H, $J_{6,6} = 1.7$ Hz), 7.46 (d, 1 H, $J_{5,6} = 8.3$ Hz); GC/MS (TMS deriv) m/z 383 (43) M^+ , 368 (100), 355 (38), 352 (11), 294 (13), 266 (30), 250 (27), 222 (37), 209 (40), 191 (34), 190 (34), 162 (45); UV λ_{max} (MeOH) 298 nm ($\epsilon = 8000$), 273 nm ($\epsilon = 9600$); IR (KBr) ν_{max} 1685 (shoulder), 1673 (s), 1607 (m), 1593 (m) cm^{-1} .

7-Carbomethoxy-4-hydroxy-2-methoxy-2H-1,4-benzoxazin-3-one (93%): This amorphous solid, one spot by TLC (10:1 $CHCl_3$ -MeOH), >97% by GC, "melted" over 80–90 °C; 1H NMR (300 MHz, acetone- d_6) δ 7.81 (dd, 1 H, $J_{6,5} = 8.4$ Hz, $J_{6,8} = 1.8$ Hz), 7.68 (d, 1 H, $J_{6,6} = 1.8$ Hz), 7.47 (d, 1 H), 5.51 (s, 1 H), 4.31 (q, 2 H), 3.54 (s, 3 H), 2.88 (br s, OH), 1.35 (t, 3 H); MS (EI) m/z 267 (3) M^+ , 262 (16), 251 (58), 223 (6), 220 (7), 206 (16), 192 (39), 176 (7), 164 (16), 163 (26), 146 (49), 129 (10); IR (KBr) ν_{max} 1720 (s), 1678 (s), 1618 (m), 1600 (m) cm^{-1} .

7-Carbomethoxy-2,4-dihydroxy-2H-1,4-benzoxazin-3-one (11b): 46%; BCl_3 , room temperature, 48 h; mp 163–165 °C (decomposition to red-black tar with bubbling); 1H NMR (300 MHz, acetone- d_6) δ 7.79 (dd, 1 H, $J_{6,5} = 8.4$ Hz, $J_{6,8} = 1.8$ Hz), 7.60 (d, 1 H, $J_{6,6} = 1.8$ Hz), 7.46 (d, 1 H, $J_{5,6} = 8.4$ Hz), 5.82 (s, 1 H), 4.32 (q, 2 H), 1.35 (t, 3 H); GC/MS (TMS deriv) m/z 397 (57) M^+ , 382 (100), 369 (42), 354 (13), 352 (18), 309 (11), 308 (16), 280 (38), 264 (33), 236 (30), 223 (21), 222 (75), 191 (52), 162 (70); IR (KBr) ν_{max} 1690 (s), 1615 (m), 1600 (m) cm^{-1} .

2,4-Dihydroxy-7-nitro-2H-1,4-benzoxazin-3-one (12): This synthesis was achieved by oxidation of the corresponding lactam as described for the unsubstituted compound.⁶⁹ To 5-nitro-2-aminophenol (2.0 g, 13 mmol) in 250 mL of anhydrous CH_2Cl_2 was added Et_3N (2 mL) under N_2 . Freshly distilled dichloroacetyl chloride (2.0 g, 13 mmol) in CH_2Cl_2 (20 mL) was added dropwise. After 2 h the mixture was filtered and evaporated to dryness. The residue was dissolved in water and extracted three times with diethyl ether (Et_2O) and then treated with petroleum ether. A yellow solid was obtained (1.86 g), mp 134–135 °C.

The solid (1.6 g) was refluxed with 60 mL of 0.015 M $NaHCO_3$ for 1 h and extracted with Et_2O . The aqueous phase was neutralized with concentrated HCl and extracted with Et_2O . The ethereals were concentrated to dryness and crystallized from ether-petroleum ether. A yellow solid (0.85 g) was obtained, mp 200–205 °C.

The solid (0.85 g) was heated for 1 h with 3.6 g of *N,O*-bis(trimethylsilyl)acetamide. The excess of silylating agent was evaporated, the residue was dissolved in CH_2Cl_2 (20 mL), and 2.3 g of the complex (*N,N*-dimethylformamido)oxidiperoxo-

molybdenum(VI) was added. After 4 h at room temperature the solvent was evaporated and the residue dissolved in 25 mL of 1 M EDTA- Na_2 . After filtering and washing with Et_2O the aqueous phase was acidified to pH 6.5 and was continuously extracted with Et_2O for 24 h. Evaporation gave a yellow solid (0.18 g, 6% from the 5-nitro-2-aminophenol), which was a single spot on TLC (10:1 $CHCl_3$ -MeOH), R_f 0.75, and gave a positive ferric chloride test: mp 158 °C; 1H NMR (300 MHz, acetone- d_6) δ 8.09 (dd, 1 H, $J_{6,5} = 8.9$ Hz, $J_{6,8} = 2.4$ Hz), 7.87 (d, 1 H, $J_{6,6} = 2.3$ Hz), 7.60 (d, 1 H, $J_{5,6} = 9.0$ Hz), 5.93 (s, 1 H), 2.85 (br s, OH); MS (EI, direct probe) m/z 226 (5) M^+ , 208 (6), 192 (5), 181 (47), 180 (86), 164 (10), 153 (13), 150 (45), 134 (35), 122 (14), 106 (77); UV λ_{max} (MeOH) nm 335, 235; IR (KBr) ν_{max} 1690 (s), 1600 (s), 1450, 1370, 1290 cm^{-1} .

4-Hydroxy-2-methoxy-7-(trifluoromethyl)-2H-1,4-benzoxazin-3-one (13) (99%). On first melt this material liquifies over the range 80–90 °C. When the same sample was cooled it solidified, and a second heating yielded a melting point of 94–96 °C; 1H NMR (300 MHz, acetone- d_6) δ 7.51 (m, 3 H), 5.54 (s, 1 H), 3.55 (s, 3 H), 2.86 (br s, OH). MS (EI) m/z 263 (11) M^+ , 247 (78), 217 (10), 216 (12), 204 (14), 203 (28), 188 (100), 187 (98), 177 (26), 159 (19), 158 (11), 148 (18), 147 (24); IR (KBr) ν_{max} 1705 and 1690 (s), 1630 (m), 1520 (m) cm^{-1} ; HRMS calcd for $C_{10}H_8F_3NO_4$ 263.0408, found 263.0429.

7-Cyano-4-hydroxy-2-methoxy-2H-1,4-benzoxazin-3-one (14) (93%): mp 155–158 °C dec; 1H NMR (300 MHz, acetone- d_6) δ 7.56 (m, 3 H), 5.55 (s, 1 H), 3.55 (s, 3 H); MS (EI) m/z 220 (15) M^+ , 204 (70), 176 (10), 161 (13), 160 (31), 145 (100), 144 (88), 116 (23), 105 (19), 104 (36); IR (KBr) ν_{max} 2240 (m), 1700 (s), 1672 (m), 1607 (m), 1507 (m), 1502 (m) cm^{-1} . Anal. Calcd for $C_{10}H_8N_2O_4$: C, 54.55; H, 3.66; N, 12.72. Found: C, 54.31; H, 3.75; N, 12.46.

Synthesis of 2-Deoxy Compounds 16 and 17. 4-Hydroxy-7-methoxy-2H-1,4-benzoxazin-3-one (16). 5-Methoxy-2-nitrophenol (2.53 g, 15.0 mmol) was dissolved in acetone (80 mL) to which had been added 6 g of anhydrous K_2CO_3 . Methyl bromoacetate (1.43 mL, 15.0 mmol) was added, and the mixture was refluxed overnight. After cooling, the solids were filtered, and the acetone solution was poured into 200 mL of water. After extraction with chloroform the combined organics were washed with 10% Na_2CO_3 , dried, and evaporated to an oil which slowly crystallized on standing. This could be recrystallized from $EtOH$ and gave 2.31 g (64%) of a yellowish solid.

Reductive cyclization of this nitro ester (1.15 g, 4.51 mmol) followed the usual procedure. The acidified reduction medium was extracted with ether and concentrated by evaporation. Addition of hexane to this solution and storing in the freezer for a day gave 16 (0.589 g, 64%) as a pinkish solid: mp 121–123 °C (decomposition to dark tar) (lit.⁵¹ mp 125–127 °C); 1H NMR (300 MHz, acetone- d_6) δ 7.21 (d, 1 H, $J_{6,5} = 8.8$ Hz), 6.63 (dd, 1 H, $J_{6,5} = 8.8$ Hz, $J_{6,8} = 2.6$ Hz), 6.55 (d, 1 H, $J_{6,6} = 2.6$ Hz), 4.67 (s, 2 H), 3.76 (s, 3 H); GC/MS (TMS deriv) m/z 267 (67) M^+ , 252 (100), 194 (26), 150 (71), 136 (14), 120 (6); UV λ_{max} (MeOH) 292 nm (sh) ($\epsilon = 6200$), 268 nm ($\epsilon = 8100$); IR (KBr) ν_{max} 1665 (s), 1620 (m), 1601 (m), 1513 (s), 1502 (s) cm^{-1} .

4-Hydroxy-2H-1,4-benzoxazin-3-one (17). *o*-Nitrophenol (10.0 g, 72 mmol) was coupled with ethyl bromoacetate (8.0 mL, 12.00 g, 1 equiv) in 150 mL of warm ethanol and 1 equiv of KOH for 20 h. The mixture was poured into 300 mL of water and extracted 3 times with chloroform, washed with 10% Na_2CO_3 and with brine, dried, and evaporated to an orange oil. This material recrystallized rather poorly from MeOH, giving crude ethyl α -(2-nitrophenoxy)acetate (10.55 g, 65%) as an oily orange solid.

A portion of this nitro ester (5.38 g, 24.0 mmol) was reductively cyclized following the general procedure. Acidification of the filtrate, after removal of the catalyst, spontaneously gave a mass of fluffy, fibrous crystals. Filtering and cooling of the mother liquor allowed the collection of three crops: 1.13, 0.433, and 0.320 g. Extraction of the filtrate with $EtOAc$ and purification on a silica column (CH_2Cl_2 with 1% MeOH) gave another 0.246 g of the title compound: total yield of 17 2.13 g (54%); mp 162–165 °C (slow decomposition) (lit.⁵¹ mp 167–169 °C); 1H NMR (300 MHz, acetone- d_6) δ 7.32 (dd, 1 H, $J_{6,5} = 7.8$ Hz, $J_{5,7} = 1.6$ Hz), 7.05 (m, 3 H), 4.69 (s, 2 H); GC/MS (TMS deriv) m/z 237 (28) M^+ , 222 (100), 180 (7), 178 (7), 164 (13), 149 (7), 146 (7), 120 (27); UV λ_{max} (MeOH) 282 nm ($\epsilon = 4400$), 255 nm ($\epsilon = 5600$); IR (KBr,

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cm⁻¹) 1680 (s), 1646 (s), 1608 (m), 1499 (s) cm⁻¹.

2-Methoxy-2H-1,4-benzoxazin-3-one [HBOA (18) Methyl Acetal]. Methyl α -(2-nitrophenoxy)- α -methoxyacetate (3.00 g, 12.4 mmol) in EtOAc (40 mL) was hydrogenated (1 atm) with 350 mg of 10% Pd/C. After 6 h (900 mL of H₂) a FeCl₃ test gave a strong positive (blue-purple color) for the presence of a hydroxamic acid. The reaction mixture was filtered of catalyst, the solvent was evaporated, and the residual semisolid was purified on Fe³⁺-Sephadex; 0.215 g of the hydroxamic acid **8** was recovered as well as 1.15 g (52%) of the title lactam, which crystallized from the 25% aqueous MeOH used to rinse the gel of non-hydroxamic acids: mp 168–171 °C; ¹H NMR (300 MHz, acetone-*d*₆) δ 7.05 (m, 4 H), 5.24 (s, 1 H), 3.49 (s, 3 H); MS (EI) *m/z* 179 (100) M⁺, 151 (7), 148 (15), 136 (12), 120 (85), 119 (97), 91 (24), 80 (18); IR (KBr) ν_{\max} 1704 (s), 1613 (m), 1501 (s) cm⁻¹.

2-Hydroxy-2H-1,4-benzoxazin-3-one (18, HBOA) (71%): BCl₃, 0 °C to room temperature, 2 h; mp 208–208.5 °C (lit.⁵⁶ mp 201–203 °C); ¹H NMR (300 MHz, acetone-*d*₆) δ 9.65 (br s, <1 H, NH), 6.94 (m, 4 H), 5.58 (s, 1 H); GC/MS (TMS deriv) *m/z* 309 (100) M⁺, 294 (27), 280 (13), 266 (20), 237 (7), 220 (10), 208 (13), 192 (22), 179 (11), 165 (14); UV λ_{\max} (MeOH) 282 nm (ϵ = 4300), 250 nm (ϵ = 8900); IR (KBr) ν_{\max} 1698 (s), 1686 (s), 1610 (m), 1502 (s) cm⁻¹.

Synthesis of Carbocyclic Analogue 19 (1,3-Dihydroxy-6-methoxy-1,2,3,4-tetrahydroquinolin-2-one). Following the procedure of Allen et al.,⁷⁰ diethyl oxalate was coupled to the benzyl anion of 5-methoxy-2-nitrotoluene giving ethyl 3-(5-methoxy-2-nitrophenyl)pyruvate as a stiff oil which was nearly pure (>99% by GC) in 43% yield.

The nitro ester (5.6 g, 21.0 mmol) was then reductively cyclized by the standard procedure. The so produced red-brown oil was dissolved in CHCl₃/EtOAc and diluted with hexane to give several crops of brown crystals of total mass 2.26 g. This was then purified on Fe³⁺-Sephadex (prepared from 30 g of Sephadex-SP) and yielded 1.10 g (25%) of pure **19**: mp 159–160 °C; ¹H NMR (300 MHz, acetone-*d*₆) δ 7.22 (d, 1 H, *J*_{5,6} = 9.7 Hz), 6.85 (m, 2 H), 4.28 (ABX quartet, 1 H, *J*_{2,1} = 15.2 and 6.2 Hz), 3.77 (s, 3 H), 3.11 (q, 1 H), 2.85 (q, 1 H, revealed by D₂O addition); MS (EI) *m/z* 209 (67) M⁺, 193 (100), 191 (49), 176 (15), 164 (69), 150 (23), 148 (25), 136 (66), 122 (23); UV λ_{\max} (MeOH) 259 nm (ϵ = 12600); IR (KBr) ν_{\max} 1670 (s), 1647 (s), 1590 (m), 1494 (s) cm⁻¹.

4-Hydroxy-2-methoxy-5-methyl-2H-1,4-benzoxazin-3-one (20 methyl acetal) (94%): mp 92–95 °C dec; ¹H NMR (300 MHz, acetone-*d*₆) δ 6.96 (m, 3 H), 5.40 (s, 1 H), 3.46 (s, 3 H), 2.87 (br s, OH), 2.55 (s, 3 H); MS (EI) *m/z* 209 (5) M⁺, 193 (100), 162 (18), 150 (17), 149 (14), 134 (92), 133 (75), 132 (26), 106 (13), 104 (18); IR (KBr) ν_{\max} 1670 (s), 1470 (br, m) cm⁻¹. Anal. Calcd for C₁₀H₁₁NO₄: C, 57.41; H, 5.30; N, 6.70. Found: C, 57.26; H, 5.57; N, 6.59.

2,4-Dihydroxy-5-methyl-2H-1,4-benzoxazin-3-one (20) (36%): BCl₃, -55 °C to 15 °C, 2.5 h; mp 138–141 °C (decomposition to red-black tar with bubbling); ¹H NMR (300 MHz, acetone-*d*₆) δ 9.55 (br s, <1 H, aldehyde?), 6.92 (m, 3 H), 5.70 (s, 1 H), 2.55 (s, 3 H); GC/MS (TMS deriv) *m/z* 339 (27) M⁺, 324 (100), 250 (12), 222 (46), 206 (45), 193 (11), 191 (37), 178 (40), 149 (18); UV λ_{\max} (MeOH) 287 (sh) (ϵ = 2600), 257 nm (ϵ = 6000);

IR (KBr) ν_{\max} 1690 (vs), 1670 (s), 1609 (w) cm⁻¹.

7-(Dimethylamino)-2-methoxy-2H-1,4-benzoxazin-3-one (27). Potassium 5-(dimethylamino)-2-nitrophenoxide (2.22 g, 10.1 mmol) gave, after reaction with MBMA, methyl α -(5-(dimethylamino)-2-nitrophenoxy)- α -methoxyacetate (2.69 g, 94%) as a yellowish-green oil which slowly crystallized at room temperature. Numerous attempts at reductive cyclization were hampered by the production of colors and tars. An attempt at partial reduction using H₂/Pt poisoned with DMSO⁷¹ did not produce a hydroxamic acid but gave the best isolated yield of the title lactam.

Hydrogenation (1 atm, 10 mg of 10% Pt/C) of 100 mg (0.35 mmol) of the nitro ester with 1 μ L of DMSO in 12 mL of EtOAc was very slow (2 mL H₂/45 min). After uptake of 2 equiv of H₂ (17 mL) the solution was filtered. On aeration the filtrate turned green. Radical chromatography (1 mm plate, silica) eluting with CH₂Cl₂-MeOH gave 56.5 mg (72%) of the title compound as a pinkish solid: mp 174–175 °C; ¹H NMR (300 MHz, CDCl₃) δ 8.28 (br s, NH), 6.72 (d, 1 H, *J*_{5,6} = 8.6 Hz), 6.46 (d, 1 H, *J*_{8,6} = 1.5 Hz), 6.37 (dd, 1 H, *J*_{6,5} = 8.6 Hz, *J*_{6,8} = 1.5 Hz), 5.25 (s, 1 H), 3.54 (s, 3 H), 2.90 (s, 6 H); MS (EI) *m/z* 222 (100) M⁺, 194 (11), 192 (17), 191 (18), 190 (14), 163 (30), 162 (28), 161 (37), 151 (12), 135 (12), 123 (14); IR (KBr) ν_{\max} 1690 (s), 1637 (m), 1600 (m), 1528 (s) cm⁻¹. Anal. Calcd for C₁₁H₁₄N₂O₃: C, 59.45; H, 6.35; N, 12.60. Found: C, 59.26; H, 6.37; N, 12.51.

2,7-Dimethoxy-5-methyl-2H-1,4-benzoxazin-3-one (29). Potassium 5-methoxy-3-methyl-2-nitrophenoxide (2.15 g, 9.73 mmol) gave, after reaction with MBMA following the usual procedure, methyl α -(5-methoxy-3-methyl-2-nitrophenoxy)- α -methoxyacetate (2.72 g, 98% based on 68 mg starting nitrophenol recovered) as a yellow-orange oil. Workup was hampered by oxidative color formation. Evaporation of the dried EtOAc extracts gave a dark oil (1.66 g), which was purified on 4 g of Fe³⁺-Sephadex, giving, after EtOAc extraction of the column rinses and trituration (hexane-CHCl₃) of the oil remaining on evaporation, 90 mg of beige crystals in a first crop and 143 mg of slightly impure darker beige crystals in a second crop: Total 233 mg (31% from nitro ester); mp 215–217 °C; ¹H NMR (300 MHz, acetone-*d*₆) δ 6.54 (m, 2 H), 5.36 (s, 1 H), 3.76 (s, 3 H), 3.45 (s, 3 H), 2.51 (s, 3 H); MS (EI) *m/z* 223 (100) M⁺, 192 (14), 180 (13), 164 (69), 163 (62), 149 (12), 148 (24), 129 (9), 120 (18), 109 (7); IR (KBr) ν_{\max} 1703 (s), 1632 (w), 1511 (s) cm⁻¹. Anal. Calcd for C₁₁H₁₃NO₄: C, 59.19; H, 5.87; N, 6.27. Found: C, 59.04; H, 5.84; N, 6.49.

Acknowledgment. We wish to acknowledge to assistance of Dr. Clem Kazakoff for mass spectral analyses and Mr. Raj Capoor for recording the NMR spectra. J.A. kindly acknowledges Dr. B. J. R. Philogène, Dean of Science, University of Ottawa, for providing funds for a visit to Dr. Niemeyer's laboratory in Santiago, Chile. Funding was provided by the Natural Sciences and Engineering Research Council of Canada, Ontario Ministry of Agriculture and Food (P.M. and J.T.A.) and the Canadian International Development Agency and FONDECYT (H.M.N.) and is gratefully acknowledged.

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