Chapter 32

Synthesis and Reactivity of Cyclic Hydroxamic Acids

Resistance Factors in the Gramineae

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General synthetic methods have been developed which make available a variety of analogues of the maize derived cyclic hydroxamic acid 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA). Analogues include the following structural changes: aryl ring substitution, removal of the phenolic oxygen (to form a tetrahydroquinoline analogue), and removal of the lactol and the hydroxamic hydroxyl groups. Analogues with substituents *para-* to the hydroxamic acid nitrogen have been used to produce linear free energy relationships (LFERs) for the hydroxamic and phenolic pK_as, and for the unimolecular decomposition of these compounds to benzoxazolinones. Reactivity with mercaptoethanol as a model biological nucleophile was also examined. Feeding trials with larvae of *Ostrinia nubilalis* (Hübner) show that biological activity is not adequately described by the *in vitro* reduction by thiols. The demonstrated inhibition of larval gut proteases by DIMBOA is also discussed.

Over thirty years ago the first reports were published on suspected insect resistance factors in maize (*1-5*)and rye (*6*). This early work focused attention on the benzoxazolinones such as 6-MBOA (Figure 1). When it was shown that these were the degradation products of cyclic hydroxamic acid glucosides, (*6,7*) the latter were then suspected to be the true resistance factors. The levels of hydroxamic acids in some cereal grains have been correlated with resistance to some important pests such as the European corn borer, *Ostrinia nubilalis* (Hübner); the western corn rootworm *Diabrotica virgifera virgifera* (LeConte) (*8*); the corn leaf aphid *Rhopalosiphum maidis*; the bird cherry oat aphid *Rhopalosiphum padi* (L.) (*9,10*); the fungi *Helminthosporium turcicum* and *Erwinia* species of bacteria. A thorough discussion of these correlations is included in a recent review on the biology and chemistry of these cyclic hydroxamic acids(*11*). The precise mechanism of action of these allelochemicals, however, has not been completely elucidated. Knowledge of

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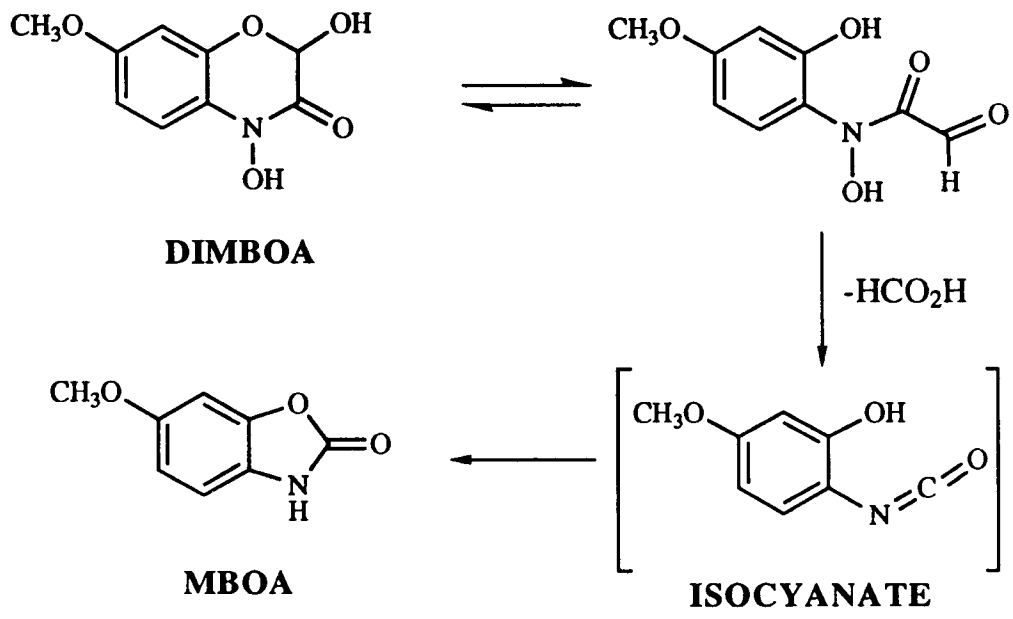


Figure 1. Decomposition of DIMBOA to 7-methoxy-benzoxazolinone, MBOA. From J. Org. Chem. **1991,** *56(5),* 1788. Copyright 1991 American Chemical Society.

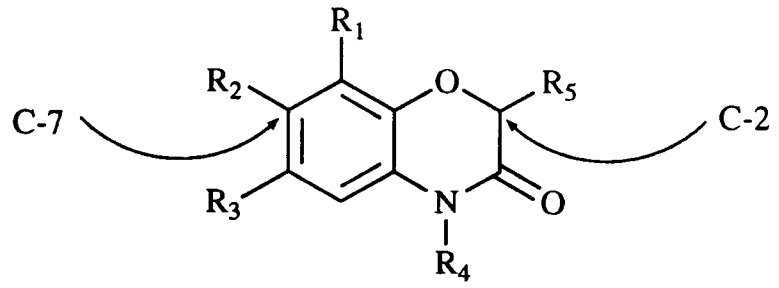
their chemistry is required to interpret accurately feeding trials with insect larvae, and ultimately to support a case for maintaining high levels in cultivated crop varieties. Recent work at the University of Ottawa, (Canada) and the Universidad de Chile, (Santiago, Chile) has delineated several key aspects of the chemistry and biology of this class of compounds. The synthesis of adequate quantities of the naturally occurring materials, as well as various analogues, has enabled detailed investigations of solution stability, reactions with nucleophiles, reduction by thiols, feeding and behavioural trials. This chapter will summarize these results.

Syntheses

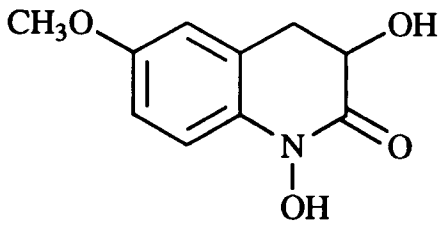
Reductive Cyclizations Honkanen and Virtanen (*12*) reported the first synthesis in this class (DIBOA, Table I) starting from protected nitrophenols, but the yield for a simple four step synthesis was only 3.5 %. The synthesis is problematic since it demanded the use of the carcinogenic chloromethyl methyl ether (MOM-Cl) to protect the phenol and it required that an intermediate arylhydroxylamine be isolated from the zinc/ammonium chloride reduction of the nitro-precursor. This sort of reduction procedes poorly with electron donating substituents such as methoxy on the aryl ring and thus does not allow an efficient synthesis of DIMBOA. The hydroxamates are also recovered as their zinc chelates which must be hydrolysed in strongly acidic media which may limit the choice of substituents.

In 1975 a patent was issued to Hoffman-La Roche (*13*) for the synthesis of DIMBOA and a small number of analogues. The construction of the benzoxazinone ring was accomplished by reductive cyclization of suitably functionalized nitroesters either with zinc/ammonium chloride or a method first developed by Coutts (*14*) using palladium on charcoal and sodium borohydride in aqueous dioxane (Figure 2). The latter method is quite general. (The catalyst should be handled as a suspension in water since any loose particles that adhere to the reaction flask can ignite the hydrogen that is

Table I. Cyclic hydroxamic acids and lactams available by synthesis



Compound	R_1	R_2	R_3	R_4	R_5	Acronym
1	H	MeO	H	OH	OH	DIMBOA
2	H	MeO	H	H	OH	HMBOA
3	H	-OCH_2O-		OH	OH	
4	H	MeO	MeO	OH	OH	
5	MeO	MeO	H	OH	OH	DIM_2BOA
6	H	*t*-Bu	H	OH	OH	
7	H	Me	H	OH	OH	
8	H	Me	H	OH	H	
9	H	H	H	OH	OH	DIBOA
10	H	Cl	H	OH	OH	
11	H	Cl	H	OH	H	
12	H	F	H	OH	OH	
13	H	F	H	OH	H	
14	H	CO_2Me	H	OH	OH	
15	H	CO_2Me	H	OH	H	
16	H	NO_2	H	OH	OH	
17	H	CF_3	H	OH	MeO	
18	H	CN	H	OH	OH	
19	H	CN	H	OH	H	
20	H	MeO	H	OH	MeO	
21	H	MeO	H	OH	H	
22	H	H	H	OH	H	
23	H	H	H	H	OH	



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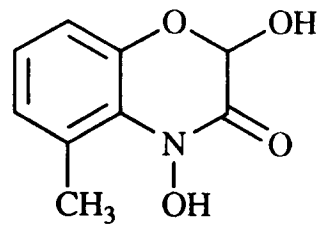

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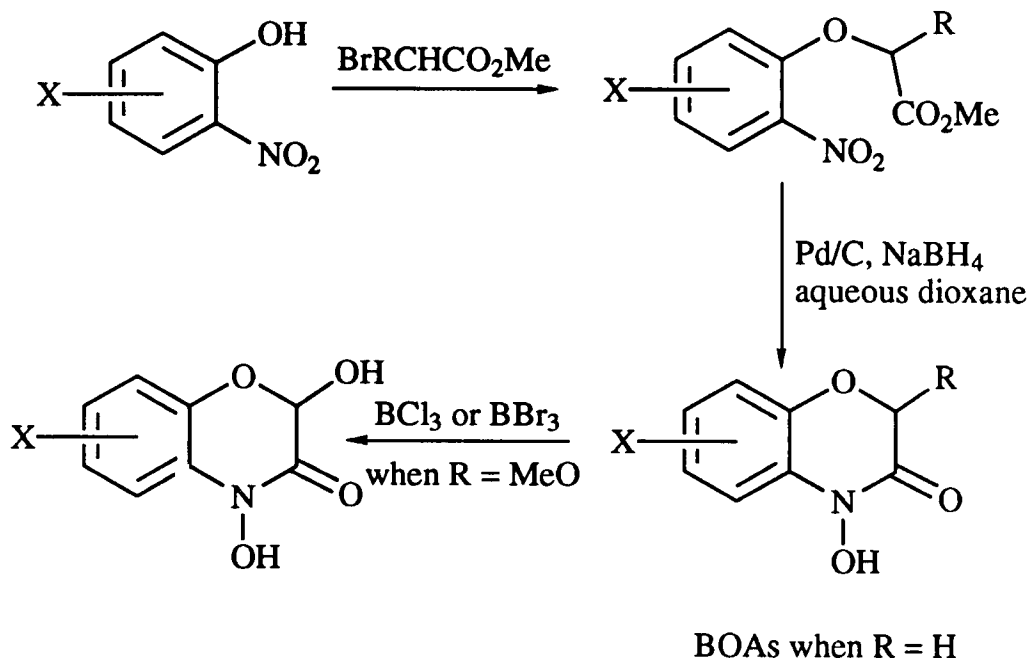


Figure 2. Synthesis of cyclic hydroxamic acids by reductive cyclization. From J. Org. Chem. **1991,** *56(5),* 1788. Copyright 1991 American Chemical Society.

evolved from the mixture.) Once cyclized the lactol at C-2 is unmasked by demethylation with boron trichloride or tribromide in methylene chloride. We have synthesized several new analogues (*15*) (Table I) most of which had varied substituents para to the hydroxamic acid nitrogen (R_2) since this would most directly affect the electronic nature of the nitrogen. In Table I, compounds **1**, **9** and **20** have been described in the literature (*13*) as have **2**, **21-23** (*16,17*). A series of 7-substituted 4-hydroxy-1,4-benzoxazin-3-ones (R_5=H in Table I) have also been synthesized by this method (*15,18*). The only substrates that could not be successfully cyclized to the hydroxamic acid had strong electron donating groups on the aryl ring. When R_2 was dimethylamino or acetamido (and R_5=MeO) the reactions were highly coloured and only amides, products of over reduction, were isolated. This is a common problem with attempts at partial reduction of electron rich nitroaromatics. Even the rather selective technique of transfer hydrogenation (*19*) is most efficiently performed on unsubstituted or halogenated nitroaromatics when hydroxylamine products are desired.

Compound **24** has had the phenolic oxygen replaced by a methylene group which removed the lactol moiety in a different manner to those analogues where R_5=H. It was readily synthesized (*15*) by acylation of the benzyl anion (potassium tert-butoxide) of 5-methoxy-2-nitrotoluene with diethyloxalate, followed by an identical reductive cyclization as mentioned above, which concomitantly reduces the carbonyl of the initial α-ketoester.

Demethylation With Boron Trihalides The demethylation of the acetals was generally very efficient with yields usually in the 60 -90% range. The boron trihalides can be purchased commercially as solutions in methylene chloride and can be safely handled by common syringe techniques for moisture sensitive materials. In the case of compounds **3**, **4** and **5** control of temperature and reaction time were essential since the aryl methoxy and methylenedioxy groups are also easily cleaved by these reagents. Yields in these cases were 20-40% after purification on Fe^{3+}-Sephadex (20). As R_2

becomes increasingly electron withdrawing the ease of demethylation decreases to the point where with the cyano and trifluoromethyl analogues no reaction occurred, even with the more reactive boron tribromide. For compound **14** it is best to work with the ethyl ester since this functionality reacts very slowly with these reagents, unlike the methyl esters. It should be noted that these acetals are not amenable to preparative hydrolysis with aqueous acids. Prolonged exposure to a variety of acids (hydrochloric, hydrobromic, sulfuric, perchloric, acetic, trifluoroacetic) produced a number of coloured products in minor amounts, but the bulk of the acetal remained unchanged.

Lactol Formation Via Bromination The C-2 lactol has also been elaborated by bromination and hydrolysis after reductive ring closure to form the benzoxazinone hydroxamic acid (*21*). DIBOA could be prepared in this manner starting from 4-hydroxy-1,4-benzoxazin-3-one (**22**) by bromination (bromine in carbontetrachloride) and silver(I) carbonate assisted hydrolysis. Unfortunately, this method is not effective for the synthesis of DIMBOA. Bromination of 4-hydroxy-7-methoxy-1,4-benzoxazin-3-one (**21**) occurred exclusively on the aromatic ring at C-6 rather than at C-2, due to the activating nature of the 7-methoxy group. Similar results occurred when the bromination was performed with N-bromosuccinimide in acidic media.

N-Oxidation of Lactams Hydroxamic acids can also be produced by the oxidation of amides and lactams. Sammes (*22*) has reported the synthesis of DIBOA by the oxidation of the silylated lactam with a peroxo-molybdenum complex with dimethylformamide. The yield for the oxidation was 33%. This method can be used to overcome the limitations of the demethylation with boron trihalides; in particular it allows the synthesis of a 7-nitro analogue which would not be possible using the reductive cyclization/demethylation methods (*15,23*). In the event, the benzoxazinone ring is formed by condensation of the appropriate o-hydroxyaniline with dichloroacetylchloride followed by hydrolysis of the isolable dichloroacetanilide derivative to generate the 2-hydroxy-1,4-benzoxazin-3-one (Figure 3). Oxidation of the silylated derivative (formed by heating the lactam in neat bis(trimethylsilyl)acetamide) then forms the hydroxamic acid. Unlike the demethylations mentioned above, the yields for the N-oxidation of lactams decreased as the electron donating ability of ring substituents increased (Table II). This would seem to be a result of the substituent's effect on the silylation of the lactam. Sammes has shown that silylation of p-methoxyacetanilide gave predominantly N-silylated products and that p-chloroacetanilide was mostly O-silylated (*24*). It is only the O-silylated amides (O-silyl imino ethers) that are oxidized by the electrophilic peroxo-molybdenum complexes.

Chemical Reactivity

pka's and Unimolecular Decomposition The 2,4-dihydroxy-1,4-benzoxazin-3-ones decompose in organic and aqueous solvents to give benzoxazolinones with concomitant liberation of formic acid (*25,26*). For DIMBOA the major product of decomposition is MBOA. Figure 1 shows a 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, (*25*) the approximate pH of the larval lepidopteran gut (*27*). With the aid of the C-7 substituted analogues (**1**, **6**, **7**, **9**,

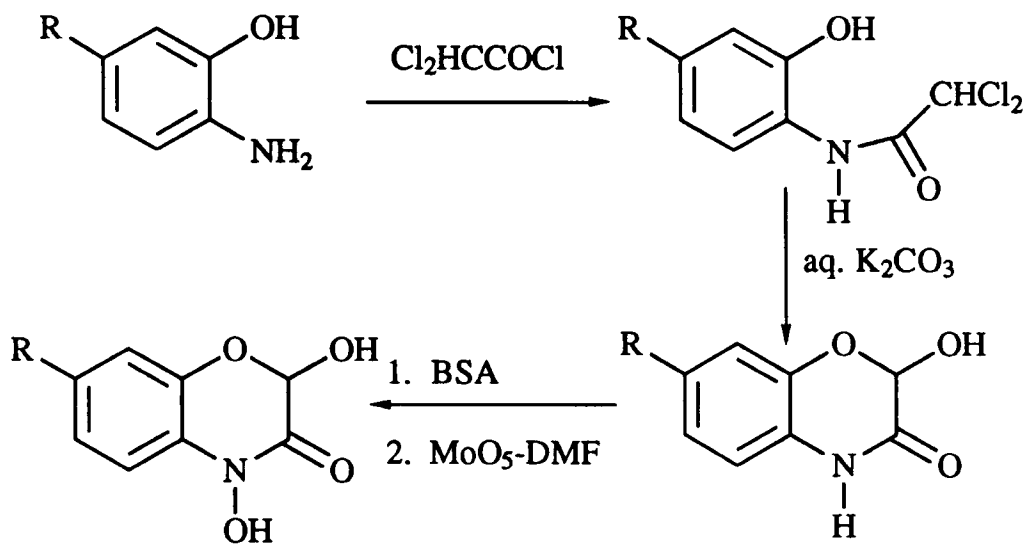
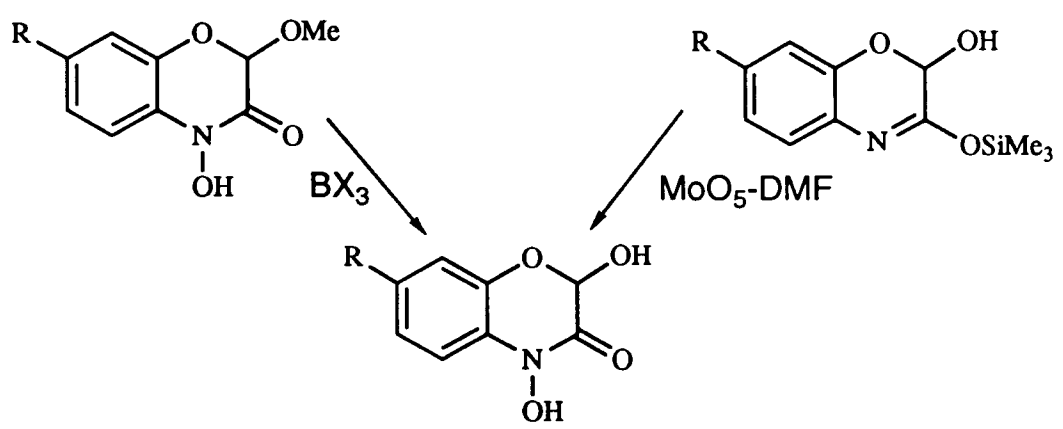


Figure 3. Synthesis of substituted 2-hydroxy-1,4-benzoxazin-3-ones and their oxidation to hydroxamic acids.

Table II. Comparison of isolated yields for the syntheses of lactol containing hydroxamic acids by demethylation and oxidation methodologies



	Percent Yields	
R	Demethylation	Oxidation
NO_2	no rxn	60
CN	no rxn	40
F	25	35
CH_3	96	10
CH_3O	70	< 5

10, 14, and **16**) it has been possible to construct a linear-free-energy-relationship (LFER) for both pK_as (the hydroxamic acid and the lactol/phenol), as well as the observed pseudo-first-order rate constants for the decomposition (*15*).

When the pK_a of the hydroxamic acid (pK_a^1) is plotted versus the substituent constant σ_p a LFER is obtained with $\rho = 0.71$ (r^2=0.86, n=8). This is considerably higher than rho values reported for acylic N-phenyl substituted hydroxamic acids (ρ = 0.1)(*28*) and may be a result of the hydroxamic acid moiety being constrained in the benzoxazine ring structure. The second ionization, which actually represents the product of the dissociation constant of the phenol and the equilibrium constant of the lactol/phenol-aldehyde, also generates a LFER when plotted against σ_m. The ρ-value of 1.61 (r^2=0.85, n=8) resembles what one might expect for a phenol where the ρ–values are generally around two.

A less strict LFER exists for the rates of decomposition. The rates of decomposition were determined at specific pHs such that all compounds were ionized to the same degree. The 7-F and the 7-NO_2 compound could not be included in the relationship because of uncertain products in the 7-F case and because of poorly defined isosbestic points in the 7-NO_2 case. Recent work with the 7-NO_2 compound has shown that the decomposition is biphasic (*23*). 5-nitro-2-aminophenol was shown to be a product of the reaction suggesting that an intermediate carbamic acid is formed by attack of solvent water on an isocyanate. The nitro-group has changed the rate determining step of the decomposition and slowed the rate of intramolecular attack of the phenol on the isocyanate (Figure 1). For the remaining compounds a plot of log pseudo first-order rate constants versus σ^+, which takes into account resonance effects of the substituent, yields a LFER with $\rho = -1.1$ ($r^2 = 0.72$, n=6). The negative ρ-value means that, during the transition state for formation of the isocyanate, electron density at nitrogen decreases with respect to reactants.

Reaction Mechanism for Decomposition 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,(*25*) or (ii) that it is a leaving group (*26,29*). Unfortunately, the present work does not distinguish between the two mechanisms since, in the LFER for the psuedo-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.

Reduction by Thiols The hydroxamic acid moiety of DIMBOA reacts with excess thiols in aqueous media to give the corresponding lactams as the main isolable product (*30*). 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 maximum at pH approximately 8.3 and is kinetically described by the reaction of the unionized hydroxamic acid with the thiolate anion (*31*). This reduction reaction can be monitored spectrophotometrically since the hydroxamate anion and the product amide have different absorbance spectra. These studies with DIMBOA have been extended to include some of the analogues shown in Table I (*15*). It was found that only those compounds with electron rich aromatic rings were reduced at appreciable rates at 23° C. The methylenedioxy analogue **3** had the highest rate constant (6720 L mol-1 min-1 followed by **4** (1480), DIMBOA (**1**) (227) and **5** (88.5). It is not clear why the methylenedioxy ring of **3** affects the rate of reduction to such a degree since it is

doubtful that the electronic nature of the aromatic ring and the nitrogen atom are greatly different than in **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.

Aside from an electron rich aromatic ring, the lactol at C-2 is also necessary for facile reduction by thiol. Those analogues in which this functionality was blocked (**20**) or removed (**21**, **22**, and **24**) were not easily reduced. Small amounts of the lactams (<2-3%) could be detected by GC/MS if the conditions were forcing (excess thiol, 45°C, 16 hr), but most of the starting material remained. These experiments, combined with the observation by 1H-NMR that the aldehyde tautomer of those componds having a lactol were quickly titrated by the thiol to form hemithioacetals, suggested that only those analogues that could exist in a ring opened form (and that had electron rich aromatic rings) could be reduced by thiols in aqueous solution. A ring-opened structure would allow extended resonance between a methoxy substituent at C-7 and the partially positively charged nitrogen of a putative ion pair.

Mode of Action of Hydroxamic Acids in European Corn Borer

Feeding trials with laboratory cultures of the European corn borer have extended the work of Klun (32-35) and shown that DIMBOA increased larval mortality, decreased the pupal and adult weights, lengthened the time to pupation, and decreased the number of eggs and egg masses deposited by surviving adult females (*36*). Similar effects were found with MBOA (without the lowering of pupal and adult weights) but at much higher concentrations (*37*).

The nutritional indices of Waldbauer (*38*) have been utilized (*39*) to distinguish whether DIMBOA and MBOA manifest toxicity in a predigestive manner or postingestive (physiological effects taking place after crossing the gut wall). Studies with a range of concentrations have established that DIMBOA reduced the approximate digestibility and the efficiency of conversion of ingested food while MBOA reduced the efficiency of conversion of digested food . That is, DIMBOA manifested its effect as a larval toxin by interfering with digestive processes within the gut and MBOA, in some manner, affected the ability of the insect to utilize nutrients that have already been absorbed.

The log P values [log (octan-1-ol/water) partition coefficients] have been estimated for MBOA and un-ionized DIMBOA (*40*). Even when un-ionized, DIMBOA is more hydrophilic (log P=0.30) than MBOA (log P=0.91) and this difference would certainly be exaggerated at higher pH where DIMBOA is largely ionized. Consequently, assuming that DIMBOA is not actively transported across the gut wall at an appreciable rate, MBOA would more easily cross membrane barriers to other potential sites of interaction. Tracer studies with dietary 3H-DIMBOA or 3H-MBOA indicate in both cases that 3H-MBOA and other metabolites are detected in the insect internal tissues, while 3H-DIMBOA was not detectable in internal tissues after dietary administration (*40*).

Structure-Activity Relationships To date, feeding trials with *O. nubilalis* larvae have been conducted with most of the analogues in Table I except for **8**, **11**, **13**, **15**, **19** (R_5 = H in Table I) and **16** and **18** which became available later. Table III compares the rates of reduction, rates of unimolecular decomposition, pK_as and growth inhibitory activity of the various analogues. Of the four compounds (**1**,**3-5**) that yielded kinetic results for reaction with mercaptoethanol, the two with the largest rate constants (**3** and **4**) did not inhibit the growth of larvae to an extent that was significantly different from control ($P<0.05$) Compounds **1** and **5** (DIMBOA and DIM_2BOA respectively, the aglucones of the naturally occurring 2-O-ß-D-glucosides) were less reactive towards reduction by mercaptoethanol but did inhibit growth,

DIM_2BOA to a lesser extent than DIMBOA. All of the 7-substituted series except for 7-CO_2Me, **14**, inhibited the growth of larvae during the feeding trials and all had activites equal to or surpassing that of DIMBOA. The remaining compounds tested (**2**, **20-24** and MBOA) did not significantly reduce growth.

Table III. Comparison of rates of reduction by mercaptoethanol, k_2, rate constants for unimolecular decomposition, k_{obs}, hydroxamic acid pK_a and growth inhibition in diets of *Ostrinia nubilalis*, for a series of cyclic hydroxamic acids

Compound		Reduction	Decomposition		
Number	Substituent	k_2 (M^{-1} min^{-1})[a]	k_{obs} (min^{-1})[b]	pK_a[1]	% Growth[c]
25	5-Me	---	$> 10^{-1}$		NS
3	6,7-MDO	6720	4.0×10^{-2}	6.91	NS
4	6,7-diMeO	1480	2.9×10^{-2}	6.91	NS
1	DIMBOA	227	8.6×10^{-3}	6.92	49
5	DIM_2BOA	88.5	7.2×10^{-3}	7.03	79
12	7-F	no rxn	9.0×10^{-3}	6.63	57
10	7-Cl	no rxn	3.0×10^{-3}	6.78	38
9	DIBOA	no rxn	$< 10^{-3}$	6.91	56
7	7-Me	no rxn	$< 10^{-3}$	6.83	27
6	7-*t*--Bu	no rxn	$< 10^{-3}$	6.94	53
14	7-CO_2Me	no rxn	$< 10^{-4}$	6.52	NS

a) True second order rate constants were obtained at 23 ± 0.3 ° C, pH 9.00 (Tris) and ionic strength 0.15. b) Pseudo first-order rate constants for unimolecular decomposition were determined at 37 ± 0.3 ° C, pH 9.00 (Tris-HCl) and ionic strength 0.15. c) Growth is expressed as percent of control. NS: not statistically different from control (χ^2 test, $\alpha = 0.10$). All compounds were tested at 0.5 mM in fresh meridic diets.

It is unlikely that the benzoxazolinones are responsible for a large measure of the biological activity of consumed hydroxamic acid since concentrations of MBOA ten to twenty times that of DIMBOA are necessary in feeding trials to show comparable toxicity. The rates at which the hydroxamic acids convert to benzoxazolinones, however, is likely very important. Hydroxamic acids that decompose in solution to produce less toxic benzoxazolinones more quickly than they react with nucleophiles on vital enzymes or proteins would not be expected to be active in an experiment such as a feeding trial. The diets for the feeding trials were made to approximately pH 4, a pH at which hydroxamic acids react or decompose very slowly at ambient temperatures. After three days in the diet nearly 70% of the added DIMBOA remained(*36*).

Once the hydroxamic acids are consumed and enter the high pH of the larval gut, the rates of reaction with nucleophiles and the rate of unimolecular decomposition

to benzoxazolinones will compete. Compounds that are not hydroxamic acids (the lactams HMBOA and HBOA, **2** and **23**), or that have had the lactol blocked (**20**) or removed (**21**, **22** and **24**) did not inhibit the growth of the larvae in a manner significantly different from control. One might suspect then that those analogues which unimolecularly decay the slowest should have a greater opportunity to manifest toxicity. Table III compares the rate constants for reduction and decomposition reactions with growth inhibition as recorded during feeding trials. The pseudo-first order rate constants for decomposition given here do not represent the rate maxima for each compound since all measurements were made at pH 9.00 and the compounds have different pKa's. For the sake of comparison, the assumption has been made that pH 9 is near the maximum rate for all. Regardless of this assumption, all of the compounds would experience the same pH once ingested and present in the gut. It would seem that any compound which would unimolecularly decompose to benzoxazolinones with a rate constant greater than ≈2x10-2 min-1 at pH 9 does not persist long enough in the larval gut to manifest toxicity. Remarkably, DIMBOA and DIM_2BOA strike a balance between the different reactivities. They both decompose relatively fast in basic solution yet both are active in the growth study. They also represent the lower limit for reactivity towards reduction by mercaptoethanol. Those compounds that react faster with mercaptoethanol (**3** and **4**) decompose too quickly in solution, apparently, to show toxicity in the growth study. Compound **25** decomposes so fast at pH 9 that it was impossible to determine accurate absorbance data for it. The 7-CO_2Me analogue **14** is an anomaly since it undergoes unimolecular decomposition very slowly and yet showed no activity in the growth study. Perhaps the ester is hydrolysed in the insect gut and the revealed charged group (CO_2^-) interferes with the 'normal' toxicokinetics. It appears that the hydroxamic acid moiety and the lactol are necessary for activity, but there is no correlation between solution reactivity with mercaptoethanol and inhibition of the growth of *O. nubilalis* larvae. It is not possible with the information at hand to understand the variation within the growth inhibitory data.

Protease Inhibition Incubation of 3H-DIMBOA with partially purified trypsin from European corn borer, followed by gel filtration, showed that label co-eluted with protein. These protein containing fractions also had decreased proteolytic activity (*40*). Recent work (*39*) has shown that DIMBOA is an noncompetitive inhibitor of partially purified tryptic and chymotrypic activities from gut homogenates of the European corn borer. It is argued that an noncompetitive mode of inhibition would overcome the conditions of substrate saturation that exist in the gut of a feeding larva. DIMBOA has also been observed to inhibit in vitro the cysteine protease papain (*41*) and the serine protese α-chymotrypsin (*42*) by covalent interaction with the active site cysteine and serine respectively. Lower concentrations of DIMBOA were needed for inhibition of papain suggesting that the observed reactivity of DIMBOA and analogues with low molecular weight thiols may extend to proteins and enzymes as well. Further work is in progress to define the nature of enzyme inhibition and the events that affect larval fitness and mortality.

Role of Hydroxamic Acids in Crop Protection

Since the discovery of hydroxamic acids in corn and other cereal grains an extensive literature has developed correlating the occurrence and levels of these chemicals to plant resistance to insects herbivory and other pathogens (see ref. 11 and references therein). Varieties of maize with high contents of hydroxamic acids are resistant to the European corn borer and, as has been recently demonstrated, the western corn

rootworm (*Diabrotica virgifera virgifera* [LeConte]) (*8*). Thus, maintenance of sufficient hydroxamic acid levels in new maize cultivars should be an important concern in breeding programs. Interestingly, DIMBOA has also been identified as the agent responsible for poor growth of *Agrobacterium tumefaciens*, a bacterial vector being used to study the viability of transfecting genes responsible for herbicide resistance into corn (*43*). It has been suggested that it may be necessary to select plants with *low* DIMBOA content to allow this technology to procede. Hopefully other ways can be found to overcome these obstacles rather than to select against one of corn's demonstrably effective chemical defenses.

Conclusions

Synthetic methodologies now exist for the production of a variety of analogues of the general class of cyclic hydroxamic acids typified by DIMBOA. Generation of the hydroxamic moiety by reductive cyclization is limited to those aryl substituents that do not affect the ability of the boron trihalides to reveal the lactol. Oxidative generation of the hydroxamic acids from amides works best for strongly electron withdrawing aryl substituents and thus compliments the above method. The detailed mechanism of the unimolecular decomposition of DIMBOA and analogues is still not known, but clearly electron donating substituents such as methoxy greatly accelerate the reaction. Only four of the analogues were reduced by mercaptoethanol at appreciable rates (**1**, **3**-**5**) and feeding trials show that this reactivity does not correlate with the growth inhibitory activity in *O. nubilalis*. although several analogues had equal or better activity than DIMBOA. Work describing DIMBOA as a protease inhibitor, in conjuction with the known reactivity of the aldehyde tautomer of the lactol, suggests that both the hydroxamic acid and the aldehyde may play a role in association with macromolecules. This remains a goal of future work.

Acknowledgments

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Literature Cited

(1) Beck, S. D.; Stauffer, J. F. *Ann. Entomol. Soc. Am.* **1957**, *50*, 166-70.
(2) Smissman, E. E.; LaPidus, J. B.; Beck, S. D. *J. Org. Chem.* **1957**, *22*, 220.
(3) Loomis, R. S.; Beck, S. D.; Stauffer, J. F. *Plant Physiol.* **1957**, *32*, 379.
(4) Beck, S. D. *J. Insect Physiol.* **1957**, *1*, 158.
(5) Beck, S. D.; Smissman, E. E. *Ann. Entomol. Soc. Am.* **1961**, *54*, 53.
(6) Virtanen, A. L.; Hietala, P. K. *Acta Chem. Scand.* **1960**, *14*(2), 499-502.
(7) Brendenberg, J.-B.; Honkanen, E.; Virtanen, A. *Acta Chem. Scand.* **1962**, *16*, 35.
(8) Xie, Y. S.; Arnason, J. T.; Philogéne, B. J. R.; Lambert, J. D. H.; Atkinson, J. K.; Morand, P. *Can. Entomol.* **1990**, *122*, 1177-86.
(9) Thackray, D. J.; Wratten, S. D.; Edwards, P. J.; Niemeyer, H. M. *Ann. Appl. Biol.* **1990**, *116*, 573-82.

(10) Givovich, A.; Niemeyer, H. M. *Ent. Exp. Appl.* **1991**, *59*, 79-85.
(11) Niemeyer, H. M. *Phytochemistry* **1988**, *27*(11), 3349-58.
(12) Honkanen, E.; Virtanen, A. I. *Acta Chem. Scand.* **1960**, *14*(2), 504-7.
(13) Jernow, J. L.; Rosen, P. United States Patent 3,862,180, 1975.
(14) Coutts, R. T.; Hindmarsh, K. W. *Can. J. Pharm. Sci.* **1966**, *1*, 11-7.
(15) Atkinson, J.; Morand, P.; Arnason, J. T.; Niemeyer, H., M; Bravo, H. *J. Org. Chem.* **1990**, *56*(5), 1788-800.
(16) Hashimoto, Y.; Shudo, K.; Okamoto, T.; Nagao, M.; Takahashi, Y.; Sugimura, T. *Mutation Res.* **1979**, *66*, 191-194.
(17) Coutts, R. T.; Pound, N. J. *J. Chem. Soc. (C)* **1971**, 2696-2771
(18) Quiroz, A.; Niemeyer, H. M. *Heterocycles* **1991**, *32*, 1681-5.
(19) Johnstone, R. A. W.; Wilby, A. W.; Entwistle, I. D. *Chem. Rev.* **1985**, *85*(2), 129-70.
(20) Corbett, M. D.; Chipko, B. R. *J. Chromatogr.* **1978**, *151*, 379-83.
(21) Sicker, D.; Prätorius, B.; Mann, G.; Meyer, L. *Synthesis* **1989**, 211-2.
(22) Matlin, S. A.; Sammes, P. G.; Upton, R. M. *J. Chem. Soc. Perkin I* **1979**, 2481-7.
(23) Bravo, H. R.; Niemeyer, H. M. *Heterocycles* **1991**, *32*, 1687-91.
(24) Matlin, S. A.; Sammes, P. G.; Upton, R. M. *J. Chem. Soc. Perkin I* **1979**, 2478-80.
(25) Bravo, H. R.; Niemeyer, H. M. *Tetrahedron* **1985**, *21*, 4983-6.
(26) Smissman, E. E.; Corbett, M. D.; Jenny, N. A.; Kristiansen, O. *J. Org. Chem.* **1972**, *37*(11), 1700-4.
(27) Dow, J. A. T. In "Advances in Insect Physiology"; Academic Press, Inc.: London, 1986; Vol. 19.
(28) Brink, C. P.; Crumbliss, A. L. *J. Org. Chem.* **1982**, *47*(7), 1171-6.
(29) Grambow, H. J.; Lückge, J.; Klausener, A.; Müller, E. Z. *Naturforsch.* **1986**, *41c*, 684-90.
(30) Niemeyer, H. M.; Corcuera, L. J.; Pèrez, F. J. *Phytochemistry* **1982**, *21*(9), 2287-9.
(31) Pèrez, F. J.; Niemeyer, H. M. *Phytochemistry* **1985**, *24*(12), 2963-6.
(32) Robinson, J. F.; Klun, J. A.; Guthrie, W. D.; Brindley, T. A. *J. Kansas Entomol. Soc.* **1982**, *55*(2), 357-64.
(33) Klun, J. A.; Robinson, T. A. *J. Econ. Entomol.* **1969**, *62*, 214-20.
(34) Klun, J. A.; Tipton, C. L.; Brindley, T. A. *J. Econ. Entomol.* **1967**, *60*, 1529-33.
(35) Klun, J. A.; Brindley, T. A. *J. Econ. Entomol.* **1966**, *59*, 711-8.
(36) Campos, F.; Atkinson, J.; Arnason, J. T.; Philogene, B. J. R.; Morand, P.; Werstiuk, N. H.; Timmins, G. *J. Chem. Ecol.* **1989**, *15*(7), 1989-2001.
(37) Campos, F.; Atkinson, J.; Arnason, J. T.; Philogène, B. J. R.; Morand, P.; Werstiuk, N. H.; Timmins, G. *J. Chem. Ecol.* **1988**, *14*(3), 989-1002.
(38) Waldbauer, G. P. *J. Insect Physiol.* **1968**, *5*, 229-88.
(39) Houseman, J. G.; Campos, F.; Thie, N. M. R.; Philogène, B. J. R.; Atkinson, J.; Morand, P.; Arnason, J. T. *J. Econ. Entomol.* **1991**. (in press)
(40) Campos, F. Ph.D. Dissertation, University of Ottawa, Ottawa, 1990.
(41) Pérez, F. J.; Niemeyer, H. M. *Phytochemistry* **1989**, *28*(6), 1597-600.
(42) Cuevas, L.; Niemeyer, H. M.; Pérez, F. J. *Phytochemistry* **1990**, *29*(5), 1429-32.
(43) Sahi, S. V.; Chilton, Mary-D.; Chilton, W. S. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 3879-83.

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