A Structure-Activity Study of Naturally Occurring and Synthetic Cyclic Hydroxamic Acids

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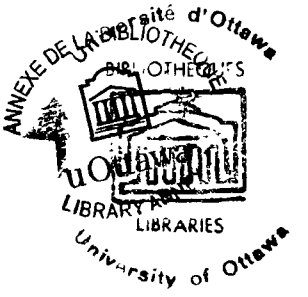
A thesis submitted in partial fulfillment
of the requirements for the degree of

Doctor of Philosophy

in the
Department of Chemistry
University of Ottawa
Ottawa, Canada.



Candidate

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Peter Morand, Chemistry

John T. Arnason, Biology

To Mom and Dad

To be is to do.

Jean-Paul Sartre

"*Scoobee doobee doo.*"

Frank Sinatra

ABSTRACT

Analogues of the aglucones of naturally occurring cyclic hydroxamic acids (2,4-dihydroxy-1,4-benzoxazin-3-ones) from Graminae have been synthesized by the reductive cyclization of ring-substituted methyl α-(o-nitrophenoxy)-α-methoxyacetates, followed by demethylation of the C-2 methoxy group with BBr_3 or BCl_3 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**), CO_2Me (**11a**).] The C-2 methoxy group could not be demethylated when the C-7 substituent was CF_3 or CN. Hydroxamic acids with nitrogen substituents at C-7 (Me_2N and CH_3CONH) could not be obtained; only the lactam **25** could be isolated from the highly coloured reductive cyclization reaction mixtures of methyl α-(5-dimethylamino-2-nitrophenoxy)-α-methoxyacetate. Similarly, only the lactam **27** was recovered during the attempted synthesis of a 7-MeO-5-Me analogue, although a 5-Me compound (**17**) was successfully synthesized. Three compounds bearing two oxygen substituents on the aromatic ring [7,8-dimethoxy (**2**), 6,7-dimethoxy (**3**), and 6,7-methylenedioxy (**4**)] and a compound lacking the phenolic oxygen (1,3-dihydroxy-6-methoxy1,2,3,4-tetrahydroquinolin-2-one, **18**) were also synthesized.

The pK_a values for the hydroxamic acid and phenol moieties were determined for each member of the C-7 series. They correlated well with σ_p in a linear free energy relationship (LFER) yielding values of ρ=0.706 for pK_{a1} (the hydroxamic acid) and ρ=1.62 for pK_{a2} (the phenol). A LFER also existed between the rate constants for the unimolecular decomposition of these hydroxamic acids to benzoxazolinones and σ^+ (ρ= -0.81).

The rates of hydroxamic acid reduction to lactams by mercaptoethanol (ME) were investigated. Only compounds **1-4** had measurable rates of reaction. NMR spectra of this reaction in D_2O buffers (pD 9), however, showed that compounds **1**, **6**, **7**, **9**, and **13** (the only ones investigated) formed a hemithioacetal at C-2 although only **1** has a measurable rate of reduction by the same thiol. The biological activity of all analogues, as determined by feeding trials with the larvae of the European corn borer (ECB, *Ostrinia nubilalis*, Lepidoptera: Pyralidae), are discussed in the light of their relative stability in solution and their reactivities towards thiols. Some preliminary work is presented concerning the inhibition of larval gut proteases by the analogues, and their activities as feeding deterrents.

ACKNOWLEDGEMENTS

I am indebted to my supervisors Dr. Peter Morand and Dr. John T. Arnason for their Zen-like prescriptions of freedom and restraint. The freedom allowed me to see firsthand the work necessary to bring an idea to life and the restraint, of course, kept me from meandering needlessly. Their direction and suggestions kept me confident and perservering. To Dr. Arnason I am additionally thankful for the experience of our trip to Belize. The jungles are awe-inspiring and unique. We must not let them perish.

Dr. Hermann Niemeyer of the Laboratorio de Química Ecológica, Universidad de Chile, Santiago, was of invaluable help in the kinetic analysis of the thiol reaction and the decomposition reaction. He is a renaissance man, more alive than many of us and he has become a close friend. To those in his laboratory whom I had discussions with while visiting, including Francisco 'Pancho' Perez, Hector Bravo, Arturos Givovich and André Quiroz, I also extend thanks. Funds for traveling to the laboratory in Chile were provided by the Dean of Science, University of Ottawa, Dr. B.J.R. Philogène, and are gratefully acknowledged.

Much of this and other work was planned and coordinated with Francesca Campos in the laboratory of Dr. Arnason. It is sometimes difficult to wait for the success of others, but Fran never lost her humour as the analogues accrued at something less than glacial speed. Our work together was always reciprocal, tools and concepts from both disciplines guiding a particular experiment.

The nature of the synthetic work and the large number of reaction trials that had to be run would have readily overburdened a mortal mass spectroscopist, but Dr. Clem Kazakoff is a magician having turned GC/MS into an art form. I thank him for his cheerful cooperation.

Raj Capoor survived a late deluge of samples and he made the NMR study of the thiol reaction possible through his patience and interest.

Two summer students assisted in the preparation of certain materials: Elaine Troughton (MBOA and nitrophenols) and Joel Pelletier (nitrophenols). Collaboration with Elaine is continuing.

My parents, to whom this work is dedicated, have taught me perhaps the most important thing of all; when to speak truthfully of my thoughts and ideas, and when to remain quiet, guarding my loosely held opinions. It is this balance that is so difficult and so human. The Middle Path is not the average.

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Chemical Name Abbreviations

DIMBOA 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one

DIM_2BOA 2,4-dihydroxy-7,8-dimethoxy-1,4-benzoxazin-3-one

DIBOA 2,4-dihydroxy-1,4-benzoxazin-3-one

HMBOA 2-hydroxy-7-methoxy-1,4-benzoxazin-3-one

HBOA 2-hydroxy-1,4-benzoxazin-3-one

MBOA 6-methoxybenzoxazolinone

1 INTRODUCTION

Secondary Chemicals in Crop Protection

The efficiency of modern agriculture demands the large scale monocropping of high yielding plant cultivars, but extensive acreages of genetically uniform plants create an opportunity for the exponential growth of the insect populations which feed on these crops, often with disastrous results. Approximately one-third of the world's human food supply is lost to pests.[1]

Although the use of pesticides has contributed to a several-fold increase in many crop yields in North America since World War II, the continued use of a limited number of pesticides has lead to some serious problems. Since 1950 over 400 species of insects have manifested resistance to insecticides.[1,2] Furthermore, differential toxicity of the insecticide to those species present in the field may leave the targeted pest better off than its natural predators or parasites. This means that another regulator of the pest's population has been lost and crop infestation may worsen.[3] Aside from the effects on the insect in question there are the uncertainties concerning the toxicity of applied pesticides to higher organisms, the persistence of the chemical in the environment and its ultimate biological fate. All of these criteria must be addressed in some fashion before a new pesticide can pass the regulatory process. This usually costs $18-20 million[4,5] and gambles that we will continue to produce new compounds as fast as the pests establish resistant populations.

Most researchers in the area of crop protection agree on the urgency of developing new pest management strategies that reduce our dependence on pesticides and their associated environmental and economic costs. One of the most promising areas of research is the development of plant varieties resistant to pest attack.[5,6] 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[7]. Much recent research[8-16] demonstrates that the presence of secondary chemicals has an equally important role in protecting the plant against pest attack. These 'secondary chemicals' are those naturally occurring compounds which are not directly involved in primary metabolism such as photosynthesis, respiration, *etc.* The core of modern organic chemistry has evolved around the structural identification and synthesis of these 'natural products'. The study of their biology, however, has remained daunting largely because of the vast

structural diversity of compounds (even within a sub-class, such as the indole alkaloids or the diterpenes), and because their biological activity, *if known*, was equally diverse. Coevolutionary theory[17,18] offers a conceptual framework for the development of chemically diverse toxins in response to pest attack. Doubts have been expressed, however, that the presence of many secondary chemicals may not be evolutionarily significant.[19]

Little is known at the molecular level about the modes of action of those chemicals which have tentatively been assigned the role of defense compounds. Without this sort of understanding it is difficult to rationally select resistant varieties of plants.

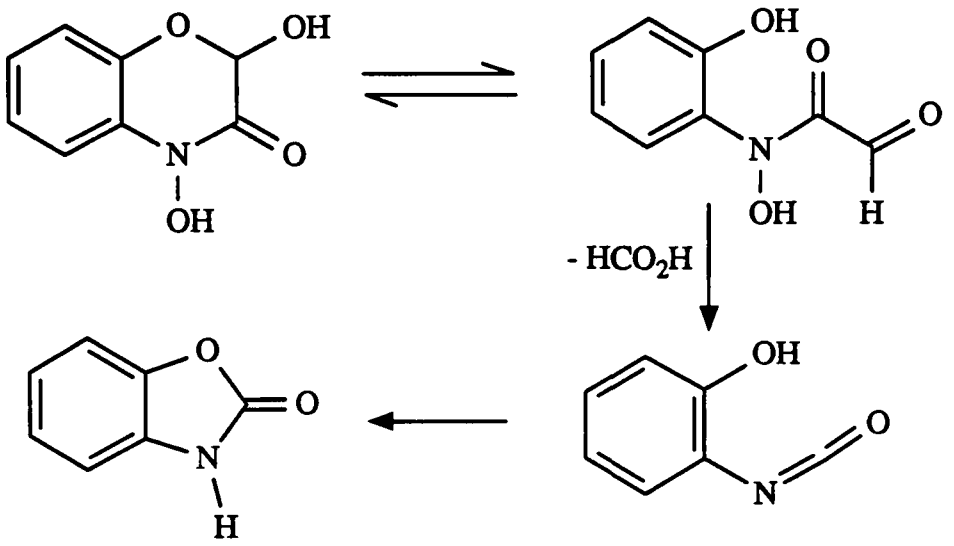
Maize

Maize (corn, *Zea mays*) is the world's third largest crop after rice and wheat. Approximately 100 million hectares are planted worldwide in temperate, sub-tropical and tropical climes, wherever rainfall is adequate.[20] It is the major food crop for 100 million people[21] and the number one feed grain in the world.[22] In Ontario alone the combined farm value of grain and fodder corn in 1985 was nearly $770 million.[23] Still, despite mankind's apparent success at cultivating this plant, disease and insect attack are the major factors limiting increased yields.[24]

The European corn borer (*Ostrinia nubilalis*, Lepidoptera: Pyralidae) has become one of the most damaging pests to maize since its introduction to North America early this century, with losses estimated to exceed $200 million a season in the US,[25,26] and $38 million in Ontario.[27] The young larvae feed primarily on the spirally rolled leaves in the whorl[28] while later instar larvae bore into and weaken the stalk, which may make the plant unrecoverable by mechanical harvesters. In experiments using resistant varieties of maize, most larval mortality was observed to take place in the first few days after the eggs had hatched. Thus, resistance to the seasons first generation of insects is, specifically, resistance to leaf feeding.[28,29] By the time the second generation eggs are hatching, the maize has tassled and resistance is better described as being to sheath-collar feeding[30] and stalk feeding.[31-34]

Chemical Defenses of Maize

Over thirty years ago the first reports of suspected resistance factors in maize were published.[35-40] This early work focused attention on the benzoxazolinones (Scheme 1.1). When it was later shown that these were the degradation products of cyclic hydroxamic acids,[41,42] the latter were then suspected to be the real resistance factors. (See Biological



benzoxazolinone

Scheme 1.1. Mechanism of decomposition of hydroxamic acids (2,4-dihydroxy-1,4-benzoxazin-3-ones) to benzoxazolinones.

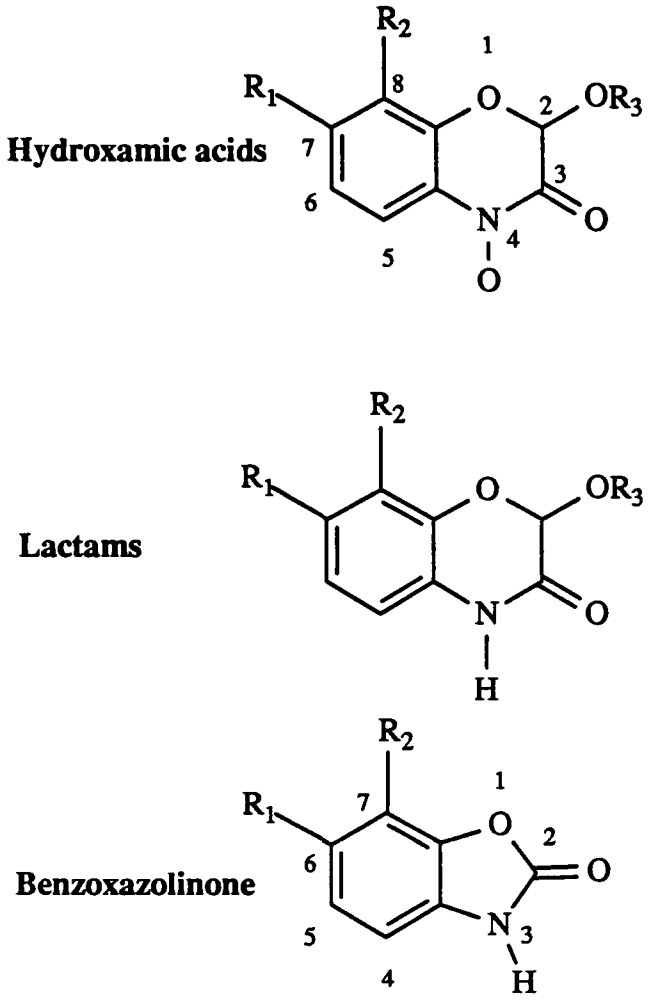
Activity: DIMBOA, below). More recent work on this pH dependent unimolecular decomposition[43-45] has helped enormously in qualifying what species (hydroxamic acid or benzoxazolinone) actually exist in the plant, after isolation, and during feeding trials with artificial diets.

The 1,4-benzoxazin-3-ones occur naturally as the 2-O-β-D-glucosides. These can be isolated if care is taken to deactivate the β-glucosidases that normally hydrolyse this group when the plant tissue is damaged.[46] A number of different hydroxamic acids have been isolated from Graminae, as well as the corresponding lactams and ring-contracted benzoxazolinones. These have been thoroughly described in a recent review.[47] A GC/MS technique for quantitative analysis of these compounds in plant tissues has been developed.[48]

Of the naturally occurring compounds only the most abundant hydroxamic acids, their corresponding lactams and one benzoxazolinone have been investigated in depth in this work. Figure 1.1 illustrates these compounds both as the free aglucone and the glucosides.

Biological Activity: MBOA

MBOA has been shown to inhibit the growth of the European corn borer[39,49] and a number of other insects including the cereal aphid *Metopolophium dirhodum*,[50] the



R_1	R_2	R_3	Abbreviation
H	H	H	DIBOA
H	H	Glc	DIBOA - Glc
MeO	H	H	DIMBOA
MeO	H	Glc	DIMBOA - Glc
MeO	MeO	H	DIM_2BOA
MeO	MeO	Glc	DIM_2BOA - Glc
H	H	H	HBOA
H	H	Glc	HBOA - Glc
MeO	H	H	HMBOA
MeO	H	Glc	HMBOA - Glc
MeO	H		MBOA

Figure 1.1. Compounds of the naturally occurring 1,4-benzoxazin-3-ones and the one benzoxazolinone that were used in this work. Only the aglucones (Glc = glucose) were used. The occurrence of MBOA in undamaged maize tissue is doubtful.[47]

silkworm *Bombyx mori*,[51] the German cockroach *Blatella germanica*, and *Prodemia eridana*.[35]

Fungal pathogens such as *Fusarium nivale*,[52] *Fusarium moniliforme, Gibberella zeae, Pyrrenochaeta terrestris, Diplodia zeae*,[53] *Sclerotina trifoliorum*,[54] and *Penicilium chrysogenum*[39] all have their growth inhibited by MBOA.

The bacteria *Staphylococcus aureus, Pseudomonas fluorescens* and *Escherichia coli* also have their growth inhibited by MBOA.[54] All of the activites listed above occur with concentrations of MBOA at 1-6 mM. The wide range of aerobic organisms affected

by MBOA suggested that it might be interfering with energy metabolism. BOA (the methoxy group of MBOA has been replaced by hydrogen) has since been shown to inhibit electron transfer between the flavin and ubiquinone of Complex 1 and ATP synthesis at the F_1 site of the ATPase complex.[55]

Biological Activity: DIMBOA

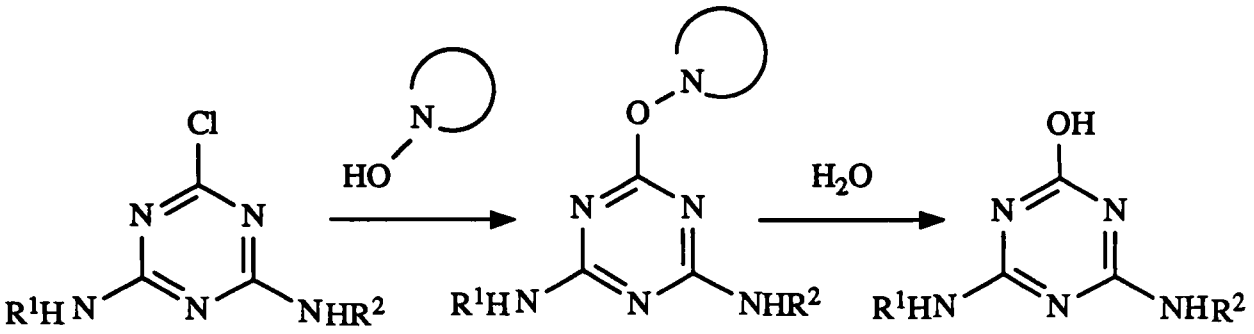
Leaf feeding resistance to European corn borer in *Zea mays* has been shown by a number of authors to be strongly correlated with levels of DIMBOA present in the whorl of the plant.[40,56-60] Inbred lines of maize with high concentrations of DIMBOA (the most abundant hydroxamic acid in maize[47]) are highly resistant to borer attack.[59,61]

There is an abundant literature on the biological activities of DIMBOA. This has recently been thoroughly reviewed[47] and only a brief survey is offered here. DIMBOA exhibited both antibiotic[63] and antifeedant[63,64] effects on cereal aphids raised on treated holidic diets. High DIMBOA levels in inbred maize lines were concluded to be responsible for resistance to the Northern corn leaf blight fungus *Helminthosporium turcicum*,[65] as well as the inhibition of germination of *H. turcicum* spores.[66] Correlations also exist between hydroxamic acid levels and resistance of maize to stalk rot caused by the fungi *Diplodia maydis*[67], *Fusarium moniliforme*, *Gibberella zeae*,[53] and *Cephalosporium maydis*.[68]

The mutagenicities of 4-hydroxy-1,4-benzoxazin-3-ones, including those that occur in maize, have been reported.[69] The small analogue series used suggested that for activity either an aryl ring methoxy group (C-7 of DIMBOA) or a 2-hydroxy group be present. This, as will be seen, was an important conclusion. Recently[70] Japanese scientists have reported the anti-inflammatory activities of some of the compounds listed in Figure 1.1. They also stressed the necessity of a 2-hydroxy group for the greatest activity.

Hydroxamic Acids as Nucleophiles

The 2-chloro-s-triazine derived herbicides atrazine and simazine are important in maize protection largely because of this crop's resistance to their effects. Resistance is accounted for by the nucleophilic substitution of the chlorine on the triazine ring by hydroxamic acids present in the plant.[71] (Scheme 1.2) Lines rich in hydroxamic acids are tolerant to these herbicides whereas lines with low levels are more susceptible.[72,73] The hydrolysis of diazinon is also catalysed in the presence of DIMBOA.[74]



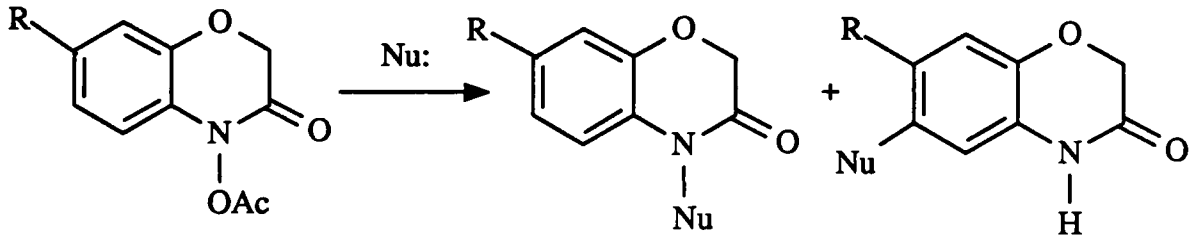
Simazine : $R^1 = R^2 = Et$
Atrazine : $R^1 = Et$, $R^2 = i\text{-Pr}$

Scheme 1.2. Hydrolysis of 2-chloro-s-triazine herbicides enhanced by hydroxamic acid nucleophilic catalysis.

Hydroxamic Acids as Electrophiles

The molecular mechanism by which DIMBOA chemically defends maize is not known. However, several interesting leads have developed over the last few years.

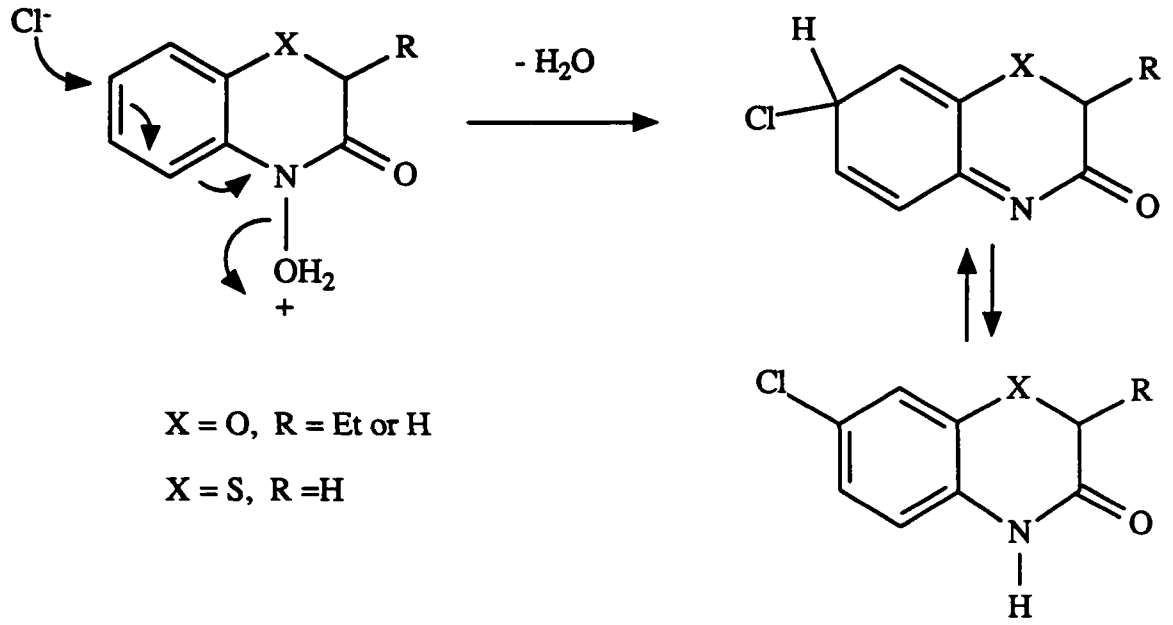
DIMBOA (as a representative member of this class of cyclic hydroxamic acids) shares the *N*-arylhydroxamic acid substructure with a number of compounds (*i.e.* *N*-(2-fluorenyl)acetohydroxamic acid) that have been shown to be the intermediates responsible for the carcinogenicity of many nitroaromatics.[75-79] After biological acylation to *N*-acetoxy amides these materials become potent electrophiles and are thought to react with nucleophiles within DNA. In a more closely related system, it has been shown that 4-acetoxy-2H-1,4-benzoxazin-3-ones (Scheme 1.3) also react with nucleophiles, substitution taking place predominantly on nitrogen.[80-82]



R = H , MeO

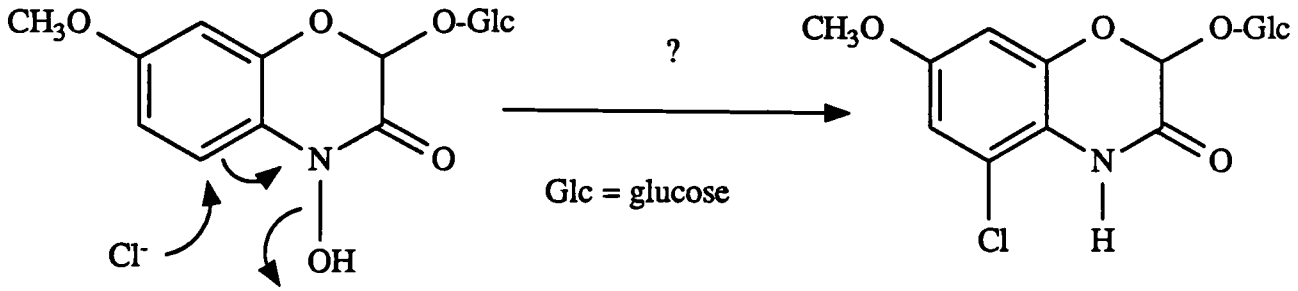
Scheme 1.3. Reaction of 4-acetoxy-2H-1,4-benzoxazin-3-ones with nucleophiles .[50]

Coutts[83] has reported the nucleophilic displacement of the hydroxamic acid hydroxyl group by attack of chloride on the aromatic ring for a number of benzoxazine and benzothiazine derived hydroxamic acids.



Scheme 1.4. Nucleophilic displacement of the hydroxamic acid hydroxyl group by chloride in acidic media.

The isolation of a chlorine containing benzoxazine-3-one from corn has been reported[84] (see Scheme 1.5), but it is difficult from the details provided to conclude whether this is a molecule that actually exists in the plant or whether it is an artefact from isolation, having undergone a similar nucleophilic displacement as described by Coutts.



Scheme 1.5. Possible mechanism of formation of the chlorine containing lactam isolated from corn (*Zea mays*)[84]

DIMBOA also acts as an electrophile during a reaction with thiols.[85,86] It was found that

DIMBOA need not be derivatized (*i.e.* the substituent on nitrogen is hydroxy not acetoxy) and that the reaction takes place in aqueous solution at a basic pH. The net reaction with excess thiol was reduction of the hydroxamic acid moiety to the lactam without incorporation of the thiol in the aromatic ring. Thiols were also observed to react with the aldehyde of the open form of the lactol. Of further significance, insects reared on diets containing DIMBOA were protected from its toxicity when the diet also contained added cysteine.[87]

Enzyme Inhibition

Hydrolytic enzymes including the proteases and esterases depend on a nucleophilic residue in their active sites for catalytic activity, usually a serine or a cysteine.[88] Suspecting that DIMBOA may be reacting in its role as an electrophile, some recent work has focused on the activity of DIMBOA as an enzyme inhibitor. DIMBOA inhibits the activity of papain,[89] a sulfhydryl protease (EC 3.4.2.2) and the loss of activity is concurrent with loss of thiol titre on the enzyme. The activity can be partially recovered following the addition of dithiothreitol and a kinetic model was offered as an explanation of these results.

DIMBOA has also been shown to inhibit mammalian chymotrypsin, a serine protease.[90] Reaction with the enzyme appears to be taking place at the aldehyde of the open form of DIMBOA since phenylglyoxal also inhibits the enzyme but the methyl acetal of DIMBOA does not.

Inhibition of Energy-linked Metabolism

ATP synthesis and ATP-ase activity in isolated spinach chloroplast coupling factor, CF_1, are inhibited by DIMBOA.[91,92] Energy-linked mitochondrial reactions are also inhibited by DIMBOA.[93] Treatment of the isolated CF_1ATP-ase with iodoacetamide prior to adding DIMBOA protected the enzyme activity suggesting that DIMBOA was reacting with thiol groups on the enzyme. The protection was not complete, however, so DIMBOA is likely reacting at other centres on the enzyme as well. The observed inhibition was of two types: (i) an initial reversible uncompetitive inhibition and (ii) a simultaneous progressive irreversible inactivation. The experiments with submitochondrial particles from bovine heart showed a similar reversible inhibition of ATP synthesis, P_i-ATP exchange and ATPase activity, but the ATPase activity was never inhibited irreversibly. These results are consistent with the fact that the CF_1 β-subunit has essential cysteine residues, but the mitochondrial β-subunit does not.[93]

Structure - Activity Series

The great proportion of the work done on DIMBOA strongly suggests that its biological activity is mediated by reaction with biological nucleophiles, but it is difficult to adequately explore the molecular mechanism without the aid of synthetic analogues. When biological activity has been observed to increase or diminish after a molecule has been structurally modified, one can more confidently speak about sites of reactivity or substituents necessary for that reactivity than one could when only the single compound was available. This is especially true of this family of cyclic hydroxamic acids since their reactivity is so diverse. They react with thiols (both a reduction and an addition reaction), with amines,[94] and they undergo a unimolecular decomposition reaction (*i.e.* Figure 1.1). Their biological activity includes action as inhibitors of enzymes and of energy-linked metabolism (see above). Since all these activities can be quantified to varying degrees a wisely chosen series of analogues would offer a wealth of information. The major goal of this work, then, was the synthesis of both the naturally occurring hydroxamic acids of maize and their analogues. The analogues were chosen both to test how certain structural features effect reactivity and to produce a contiguous series of compounds that should participate in linear free energy relationships. This latter consideration was of special importance for the study of the decomposition reaction of the hydroxamic acids to benzoxazolinones.

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2 CHEMICAL SYNTHESES

General Comments

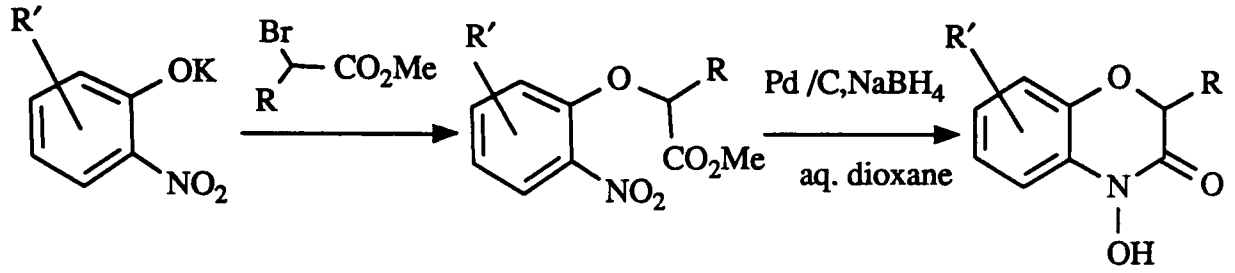
Hydroxamic acids have characteristically been synthesized from substituted hydroxylamines and acylating agents, usually acid chlorides or ethyl esters.[1-3] The preparation of aliphatic *N*-unsubstituted hydroxamic acids can be achieved by the use of *N,N,O*-tris(trimethylsilyl)hydroxylamine[4] which protects against over acylation, and by treatment of primary nitroalkanes with SeO_2 in the presence of triethylamine.[5] As a special case, cyclic hydroxamic acids have been produced by the acid hydrolysis of 2-cyano-1-pyrroline 1-oxides.[6]

Hydroxamic acids have also been produced oxidatively from amines and amides. A recent report[7] describes a tungstate catalysed oxidation of tetrahydroquinolines with H_2O_2 producing hydroxamic acids in 52-84% yields. Sammes[8] has detailed the synthesis of myceliamamide analogues by oxidizing chloropyrazines to the *N*-oxides with H_2O_2 and hydrolyzing the product chloro-*N*-oxides to a tautomeric form of the hydroxamic acid. Sammes has also reported the oxidation of silylated amides with Mo^{VI} peroxides.[9] He was aware of Virtanen's isolation and low yield synthesis of DIMBOA and he purports to have synthesized it (diagramming the wrong structure in his paper), but gives no synthetic details for that compound with a methoxy group on the aryl ring. His yields for the amide to hydroxamic acid conversion were generally in the 40% range. Other workers[10] appear to have improved the procedure by changing to a more powerful silylating system, but they never freed the hydroxamic acid from the molybdenum complexes since they were more interested in these intermediates. In our hands, small amounts of the hydroxamic acid from the oxidation of acetanilide could be detected, but the yields were very poor and in the case of an amide more closely related to our system they were zero.

Preferred Methodology

While reviewing the literature methods for synthesizing hydroxamic acids it became obvious that a large amount of work had been published on this family of compounds by Coutts[11] and it was clear that his method of synthesis was quite general and certainly easier to perform than the oxidations with Mo^{VI} peroxides. Indeed, it was Coutt's methodology that was used in a patent[12] concerning the synthesis of DIMBOA and a small number of analogues. The reaction (Scheme 2.1.) involves the reductive

cyclization of an appropriately substituted methyl (o-nitrophenoxy)-acetate, available from the reaction of a potassium o-nitrophenoxide with an α-halo acetate. The nitrophenols in the present work were synthesized by standard methods and their preparation and purification is detailed in the experimental section.



Scheme 2.1. Reductive cyclisation of (o-nitrophenoxy)-acetates which are available from the coupling of potassium 2-nitrophenoxides with bromoacetates.

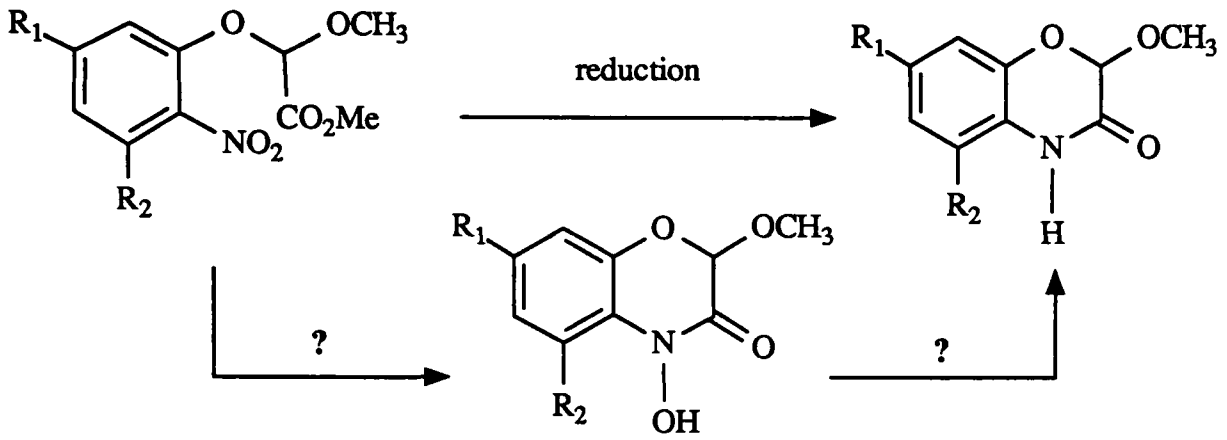
For the synthesis of DIMBOA and analogues the bromoacetate must incorporate the functionality necessary to form the hemiacetal moiety. Thus, for this series of compounds, methyl α-bromo-α-methoxy acetate was used.(R=MeO in Scheme 2.1.) It is easily prepared by brominating methyl methoxyacetate with Br_2 in boiling CCl_4.

Reductive Cyclization[13]

The reductive cyclization proceeded well for most compounds, the isolated yields depending strongly on the substituent para to the nitro group. Electron withdrawing groups, alkyl, and methoxy groups allowed facile reduction to the hydroxamic acid, but stronger electron donors such as dimethylamino and acetamido did not. The reductions of **19, 20, 21** (Scheme 2.2.) were highly coloured, usually oranges and reds. On attempted extraction of the acidified reaction media with EtOAc a deep blue-purple colour developed at the interface which intensified on shaking and aeration, suggesting the presence of an air oxidizable species. The colour could be made to disappear by reduction with dithionite ($Na_2S_2O_4$), but it was extremely difficult to remove from the other organic products. The cyclic amide **27**, a product of the reduction of **21**, was readily isolated as well as a small amount of the dimethylamino compound **25**, but products from the acetamido substituted material were very water soluble and purification and characterisation was 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 minutes or an hour and the isolated yield of hydroxamic acid did not change. It is unclear whether the hydroxamic acids **22**, **23**, and **24**, 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 arylhydroxylamine produced which can cyclise.



19 $R_1 = Me_2N$, $R_2 = H$ → **22** → **25**

20 $R_1 = CH_3CONH$, $R_2 = H$ → **23** → **26**

21 $R_1 = MeO$, $R_2 = CH_3$ → **24** → **27**

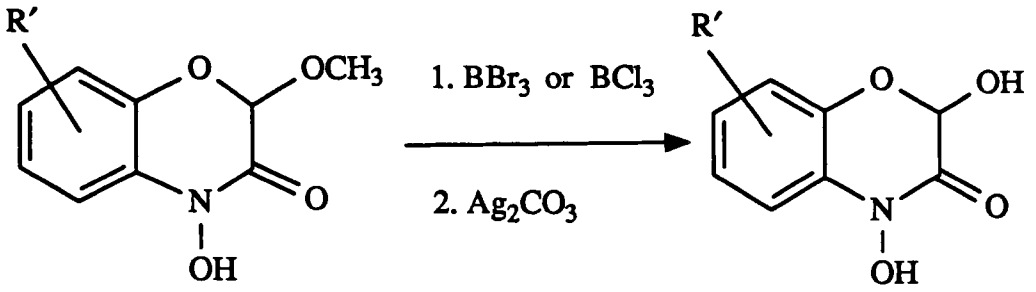
Scheme 2.2. Over-reduction of (o-nitrophenoxy)-acetates with electron rich aromatic rings to lactams.

The preparative reduction of nitroaromatics to hydroxylamines is subject to few generalisations. Neutral reduction with Zn/NH_4Cl is sensitive to substituent type and pattern, temperature and concentration, and often produces coloured by-products. Even advances in this area such as transfer hydrogenation [14] are not successful for nitro-aromatics with electron donating substituents, ie. methoxy groups.[15] It is for these reasons that aryl hydroxylamines were not used as starting materials or reagents, other than to recognize that they necessarily exist as intermediates during the reductive cyclization.

Methyl Acetal Cleavage

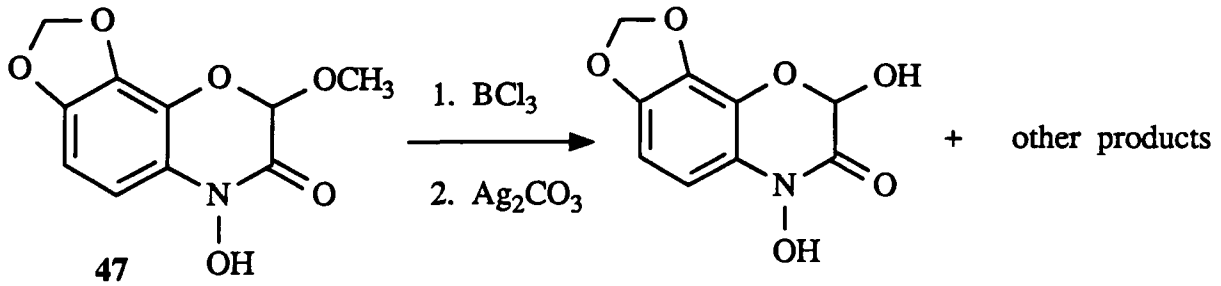
The methyl group can be removed to reveal the hemiacetal by treatment with a boron trihalide, either BCl_3 or BBr_3[16,17]. (Scheme 2.3.)

The boron reagents were generally adequate for this transformation, however, they did have some limitations. When the aryl ring had more than one methoxy group or



Scheme 2.3. Demethylation of 4-hydroxy-2-methoxy-1,4-benzoxazin-3-ones by boron trihalides followed by Ag(I) assisted hydrolysis.

contained a methylenedioxy group the reagents showed poor selectivity. Both the acetal and the aryl substituents were attacked and yields of the desired hydroxamic acid hemiacetal were lower. A hydroxamic acid acetal with a 7,8-methylenedioxy ring (Scheme 2.4) was synthesized, but attempted demethylation with BCl_3 was unsuccessful. At -78°C in CH_2Cl_2 the reaction did not occur and as the reaction mixture was warmed to 0°C, TLC showed the presence of at least three products one of which was the desired compound. Longer reaction times did not favour this product and the resulting mixtures became more difficult to purify. Bromodimethylborane did not react with this compound, even at room temperature after twelve hours. As a result, although the demethylated product could be detected by TLC and GC/MS it was never purified sufficiently to be used in further studies.



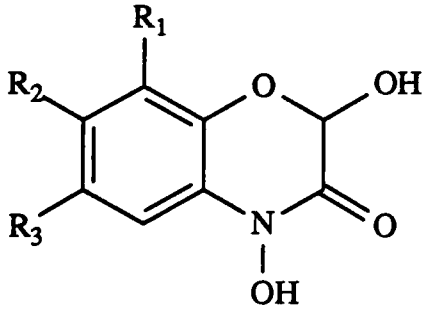
Scheme 2.4. Attempted demethylation of **47**, that analogue bearing a 7,8-methylenedioxy group

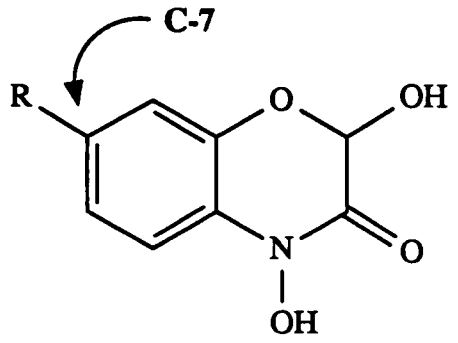
As the electron withdrawing nature of R′ increased (Scheme 2.3.) the reaction proceeded more slowly. As a relative comparison; the methyl acetal of DIMBOA reacts completely with BCl_3 within 2.5 hours from O°C to room temperature, the 7-Cl analogue, even with the more reactive BBr_3, requires three hours at room temperature, and for

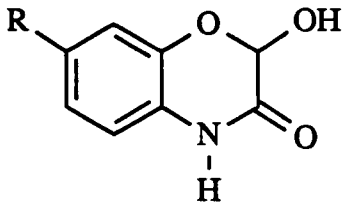
strongly withdrawing substituents such as CN and CF_3 the reaction did not occur after being exposed to a ten mole excess of BBr_3 at 20°C for 16 hours. For compounds **10** and **11a** it was found that the use of Ag_2CO_3 was not absolutely necessary though it was used in the case of **11b**. An aqueous work-up was all that was needed to hydrolyse the unisolated Br-hydroxamic when BBr_3 was used. All of these acetals were resistent to preparative acid hydrolysis. After treatment with a variety of aqueous acids (HCl, HBr, $HClO_4$, H_2SO_4, AcOH, CF_3CO_2H), TLC showed that the starting acetal largely remained. The only change observed was the formation of coloured products both more and less polar on TLC than the hydroxamic acid acetal.

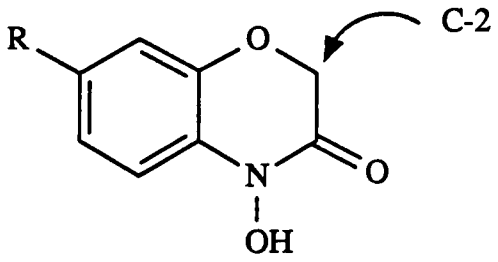
DIBOA Analogues

Those analogues that have been successfully synthesized are shown below. They have been grouped according to general substitution type and pattern. Some miscellaneous compounds are also included. Numbers in brackets represent compounds that have been successfully synthesized previously and reported in the literature.

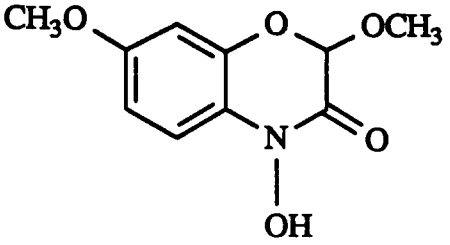
GROUP 1. Electron rich aromatic rings	Cmpd.	Substitution
	(1*)[12]	$R_1R_3 = H$ $R_2 = MeO$
	2*	$R_1R_2 = MeO$ $R_3 = H$
	3	$R_1 = H$ $R_2R_3 = MeO$
	4	$R_1 = H$ $R_2R_3 = OCH_2O$
	5	$R_1 = MeO$ $R_2R_3 = H$

GROUP 2. C-7 Substituted	Cmpd.	Substituent R
	(1 *)[12]	MeO
	6	t - Bu
	(7)[12]	Me
	(8 *)[12]	H
	9	Cl
	10	F
	11	CO_2Me

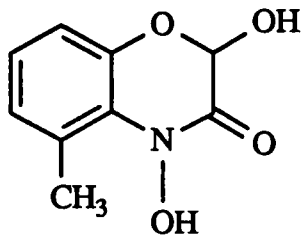
GROUP 3. Amides	Cmpd.	Substituent R
	(12 *)[18]	H
	(13 *)[18]	MeO

GROUP 4. 2-Deoxy	Cmpd.	Substituent R
	(14)[18]	H
	(15)[18]	MeO

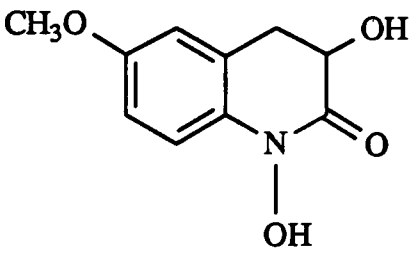
NOTE. An asterisk (*) denotes naturally occurring (as 2-O-β-D-glucoside).



16



17



18

Notice that DIMBOA is a member of both Group 1. and Group 2. since it is oxy-substituted (an electron rich aromatic ring) at C-7. The synthesis of **18**, which lacks a phenolic oxygen, follows a different procedure than the others and this is detailed in the experimental section.

The substituents in this series were chosen to span as large a range of Hammett σ-values as was possible with this synthetic method. Since compounds with substituent constants (σ) less than -0.27 (MeO) could not be synthesized, a few compounds with electron rich aromatic rings were included, though strictly these are not part of the series.

Only methyl and *t*-butyl were chosen as alkyl substituents since including others such as ethyl, propyl, etc. introduces redundancy into the series and overemphasizes this type of substituent.[19,20] A similar rationale exists for the choice of only Cl and F as the halo-substituents. Compounds **16**, **17**, and **18** were included as single tests for those structural elements that might affect reactivity, i.e. lack of a lactol (**16** and **18**), and the substituent or steric effect of a methyl group next to the nitrogen (**17**).

Tritium Labeled DIMBOA and MBOA

Both DIMBOA and MBOA bearing a radioactive label were needed to trace the fate of these compounds in insect tissues and to determine body burdens (defined as the concentration of the compound in the tissues/concentration in the feces). Tritiated MBOA (^{3}H-MBOA) was prepared by Dr. N.H. Werstiuk and G. Timmins, Department of Chemistry, McMaster University, using a modified high-temperature, dilute-acid method[21] directly from cold MBOA using tritiated water. The same technique was used to label the 5-methoxy-2-nitrophenol precursor of DIMBOA. DIMBOA could not be labeled directly because it decomposed under the conditions of the labeling. The synthesis from this 'hot' precursor followed the identical procedures outlined in the experimental section. The incorporation of label was fairly low (MBOA: 2.0 mCi/mmol, DIMBOA: 0.41 mCi/mmol) but sufficient for the studies intended. Experiments with MBOA using D_2O confirmed that deuterium incorporation was at C-5 on the aromatic ring.[22,23] The

nitrophenol presumably tritiates in the same manner, ortho to the methoxy group (C-6, DIMBOA numbering).

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3 REACTION OF CYCLIC HYDROXAMIC ACIDS WITH THIOLS

Kinetic Model

The hydroxamic acid moiety of DIMBOA and a few analogues react with excess thiols in aqueous media to give the corresponding lactams as the main isolable products.[1] The rate of this reaction can be monitored spectrophotometrically and, at wavelengths around 300 nm, the absorbance decays over time in a pseudo-first order manner. The disappearance of the hydroxamic acid is attributable to two reactions: a unimolecular decomposition (Chapter 4), and reduction to the lactam by thiol. The rate expression shown in Equation 3.1 represents the sum of these processes:

$$-d\,[DH_2]_t \,/dt = k_{obs}\,[DH_2] = k_1\,[DH_2] + k^*_2[DH_2]_t\,[RSH]_t \qquad (3.1)$$

where DH_2 represents the hydroxamic acid as a diprotic acid, RSH is the thiol, subscripts 't' indicate total concentrations (formalities), k_1 is the first order rate constant for the pH-dependent unimolecular decomposition and k^*_2 is the apparent second order rate constant for the reaction of the hydroxamic acid with thiols, which are obtainable from the slope of plots k_{obs} vs thiol concentration. The slope of these plots varies with pH. Between pH 5 and 11 the values of k^*_2 for the reaction of DIMBOA with mercaptoethanol describe a bell-shaped curve with a maximum at pH ≈ 8.5.[2] (Figure 1.1)

Of all the possible reacting couples of hydroxamic acid and thiol expected in solution only $[DH_2][RS^-]$ and $[DH^-][RSH]$ will predict a curve of similar shape to that in Figure 3.1. Furthermore, it is reasonable to assume that $[DH^-][RSH]$ is unlikely since scission of the N-O bond would not be facilitated by the oxygen bearing a charge and because RSH is not as good a nucleophile as RS^-. Thus, assuming $[DH_2][RS^-]$ is the reacting couple, it is possible to write the following equations, where the subscripts 'e' indicate effective concentrations, k_2 true pH-independent second order rate constants for the reaction and 'ℓ' molar fractions.

$$k^*_2\,[DH_2]_t\,[RSH]_t = k_2\,[DH_2]_e\,[RS^-]_e \qquad (3.2)$$

$$k_2 = k^*_2 \Big/ \ell_{DH_2}\,\ell_{RS^-} \qquad (3.3)$$

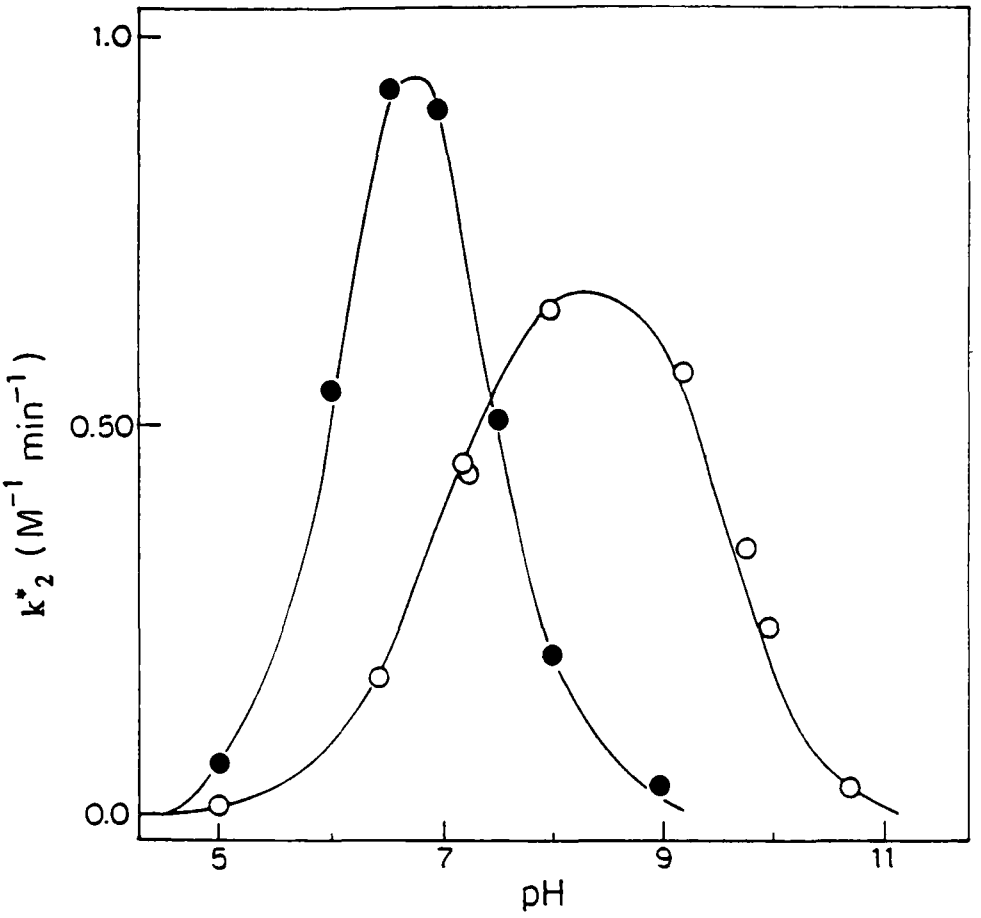


Figure 3.1. pH dependence of the apparent second order rate constant k^*_2 for the reaction of DIMBOA with mercaptoethanol (o) or with cysteine methyl ester (•). Values of k^*_2 were determined from the slopes of graphs of k_{obs} against thiol concentration (see, for example, Figure 3.3) This figure is reproduced from reference 2.

The molar fractions of reactive species (f_{DH_2} and f_{RS^-}) in Equation 3.3 can be expressed in terms of their dissociation constants as shown in Equation 3.4:

$$k_2 = k^*_2 \left(\frac{[H^+]^2 + K_1[H^+] + K_1K_2}{[H^+]^2} \right) \times \left(\frac{[H^+] + K_3}{K_3} \right) \qquad (3.4)$$

where $K_1 = 1.20 \times 10^{-7}$ and $K_2 = 7.94 \times 10^{-11}$ are the dissociation constants of DIMBOA

and K_3 that of the thiol. Values of k_2 were obtained[2] either from experiments at a single pH utilising Equation 3.4, or by least squares fit of curves such as those in Figure 3.1 to Equation 3.4.

The assumption that $[DH_2][RS^-]$ is the reactive couple has been shown to be valid [2] since a plot of k_2s for the reaction of DIMBOA vs pK_a of various thiols was linear. (Figure 3.2)

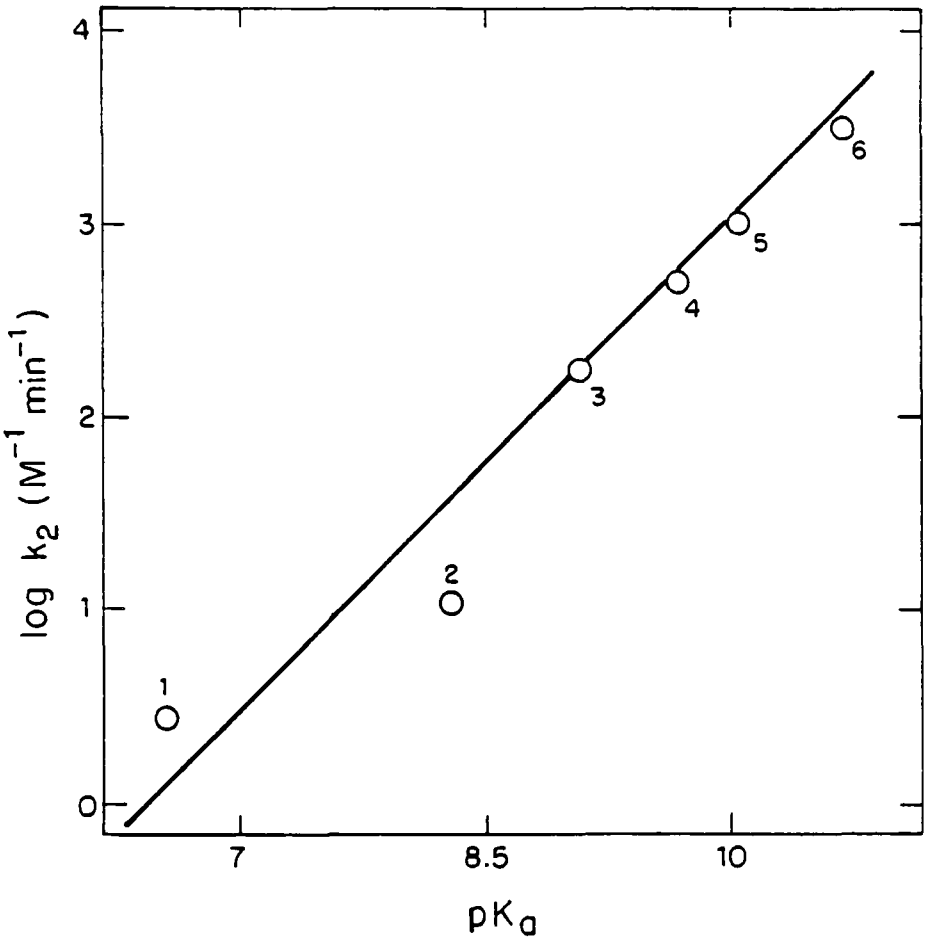


Figure 3.2. Dependence of the logarithm of the true second order rate constant for the reaction of DIMBOA with thiols on the pK_a of the thiol. The true second order rate constant k_2 for each thiol was determined by least-squares fitting of k^*_2 to Equation 3.4. Thiols used were: cysteine methyl ester (1), cysteine (2), dithiothreitol (3), mercaptoethanol (4), thiolactic acid (5) and mercaptoacetic acid (6). This figure reproduced from reference 2.

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,[2] and the absorbance differences

between the hydroxamic acids and the product lactams was sufficient for reproducible results. Equation 3.4 may be simplified by treating the hydroxamic acids as monoprotic acids (*i.e.* by assuming K_2 is very small) since this results in very small differences in k_2 values.

$$k_2 = k^*_2 \left(\frac{[H^+] + K_1}{[H^+]} \right) \times \left(\frac{[H^+] + K_3}{K_3} \right) \qquad (3.5)$$

Values of k^*_2 and k_2 (apparent and true second order rate constants respectively) for DIMBOA and three analogues are listed in Table 3.1

Table 3.1. Values of k^*_2 and k_2 for the reaction of DIMBOA and three analogues with mercaptoethanol.

Compound	k^*_2 ($Lmol^{-1}s^{-1}$)	k^*_2 ($Lmol^{-1}min^{-1}$)	K_2 ($Lmol^{-1}min^{-1}$)
4	$1.49 \pm 0.12 \times 10^{-1}$	9.00 ± 0.72	6720 ± 800
3	$3.30 \pm 0.16 \times 10^{-2}$	1.98 ± 0.10	1480 ± 135
1	$5.23 \pm 0.26 \times 10^{-3}$	0.312 ± 0.016	227 ± 20
2	$2.65 \pm 0.16 \times 10^{-3}$	0.156 ± 0.009	88.5 ± 9

Pseudo-first order rate constants were determined at pH 9.0, ionic strength I = 0.15, at 23 °C ± 0.4 C°, spanning a concentration of thiol from 80 - 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 Equation 3.5.

Similar experiments with other synthetic analogues were unsuccessful. When the substituent on C-7 was H, Me, t-Bu, Cl, F or CO_2Me 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. Figure 3.3 compares the reactivity of DIMBOA, which *is* reduced by mercaptoethanol (ME), with that of the 7-t-Bu analogue **6** which is not reduced at the conditions of these experiments.

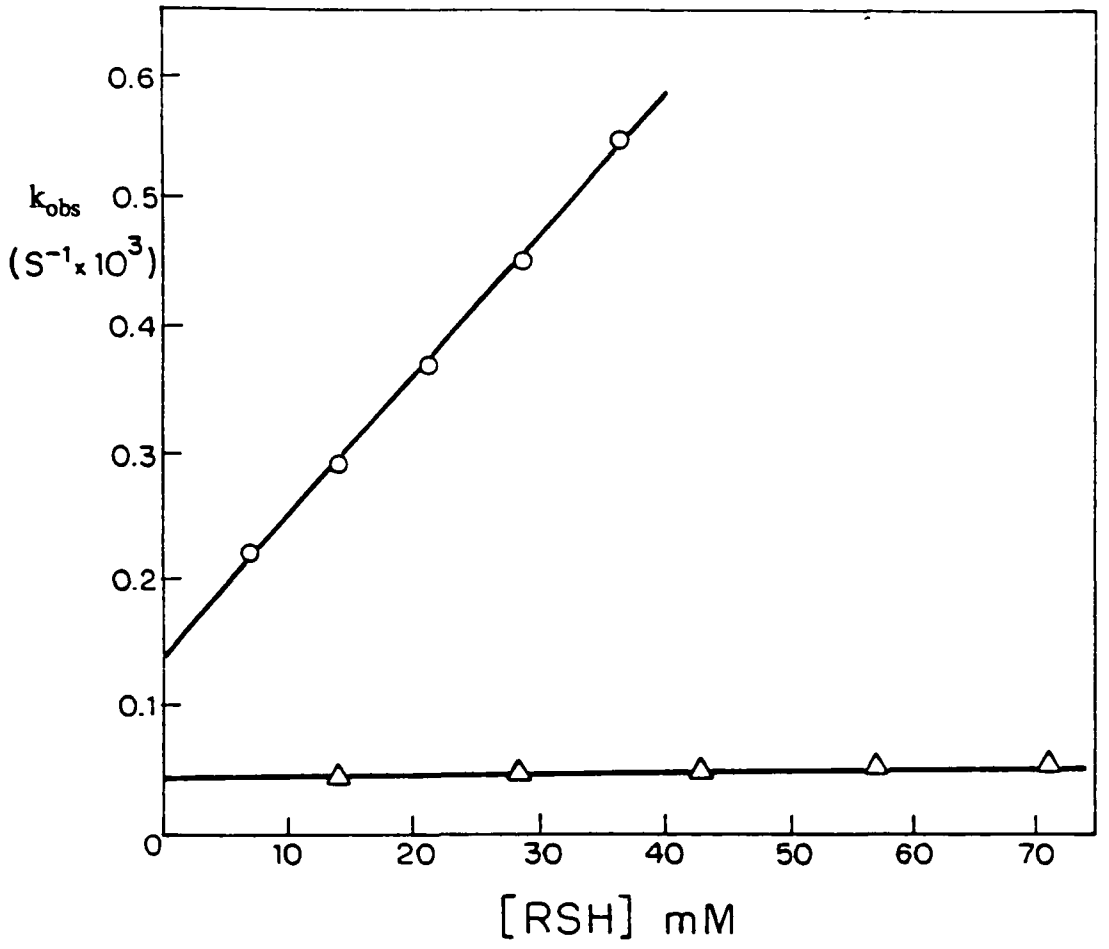
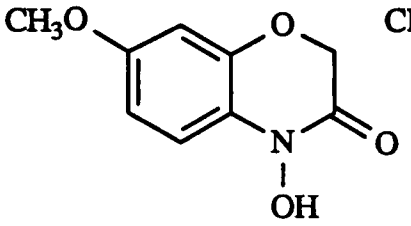
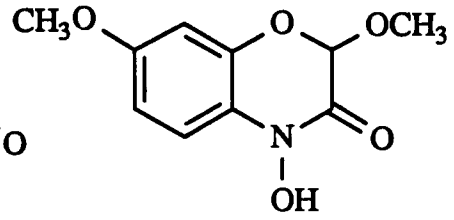


Figure 3.3. Variation of the observed pseudo-first order rate constants for the reaction of DIMBOA (○) and the 7-*t*-Bu analogue **6** (Δ) with mercaptoethanol at pH 9.0 (Tris 0.1 M) and 37±0.5 C°

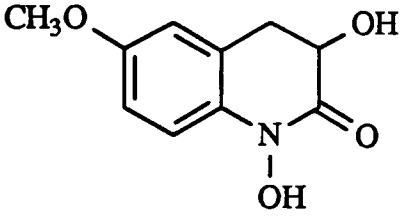
The reactivities of compounds **15**, **16** and **18** were also investigated. At the same conditions as the other kinetic trials their UV spectra did not change and thus rate constants were not calculable.



15



16



18

When small amounts (3 mg) of the analogues were allowed to react with a 40-fold molar excess of mercaptoethanol (ME) at 45°C, pH=9, for 16 hours, GC/MS analysis of the product mixtures showed the presence of lactam in the case of the 7-t-Bu, 7-Me, 7-H (DIBOA), 7-Cl, and 8-MeO analogues (and also, of course, the oxy-substituted analogues **1**, **2**, **3**, and **4** which had measurable rate constants for reaction with ME). It was impossible accurately to quantify these analyses because of broad, poorly integrated peaks at long retention times and problems of analytical reproducibility.

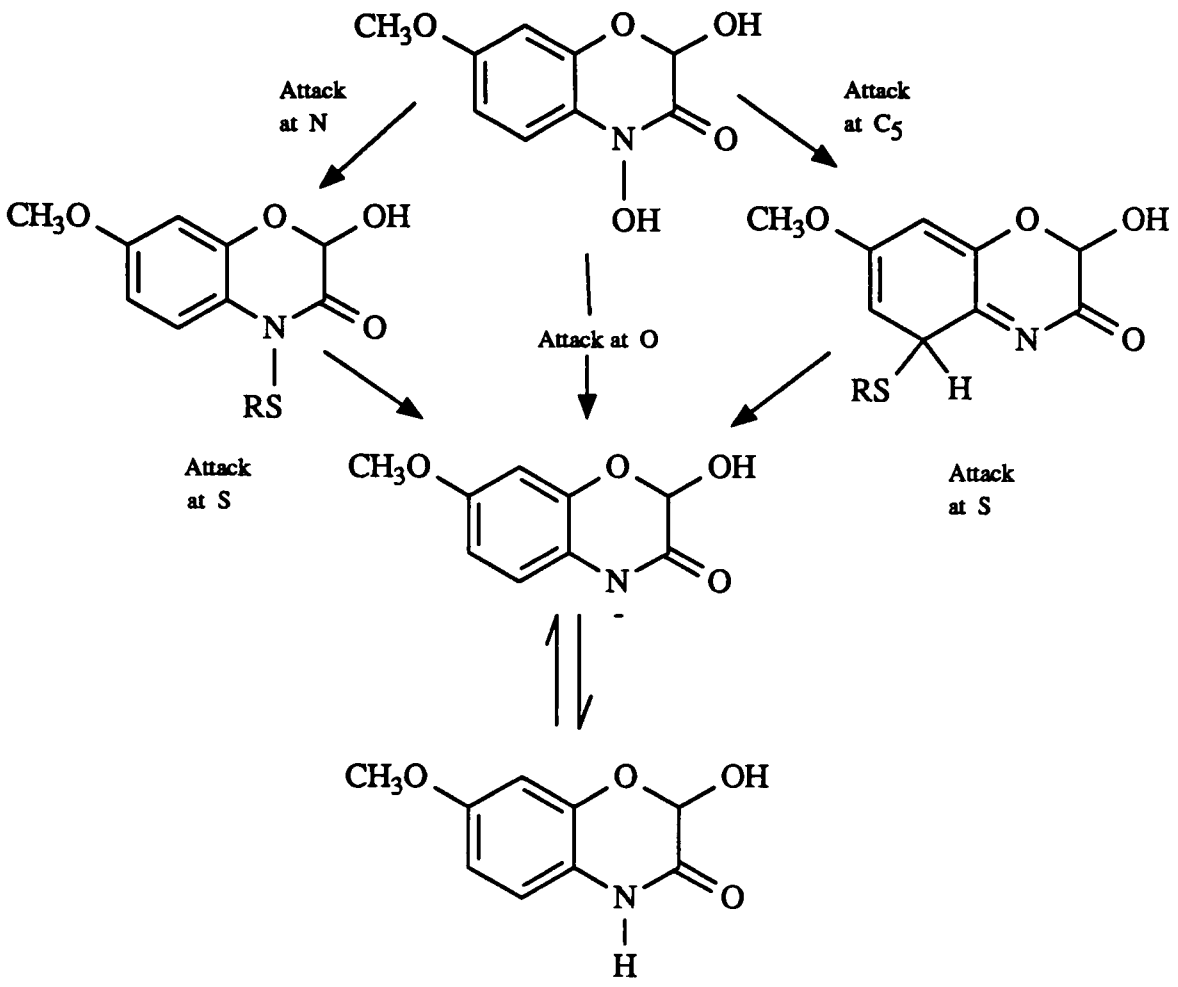
When compounds **15**, **16** and **18** were subjected to the same conditions and analysed by GC/MS, only **15** showed a small amount of the lactam - the starting hydroxamic acid being the largest peak for all three. Also detected in the case of **15** and **16** were peaks whose m/z suggest the addition of a molecule of ME with concomitant loss of water. The products of **15** + ME on a preparative scale (50 mg of **15**) could not be obtained. Only the starting hydroxamic acid was recovered.

The conclusion must be that the analogues of DIMBOA do react with ME in pH=9 buffer, but at a much reduced rate. For the 7-substituted series of Group 2, attempts to force the reaction with more extreme conditions, such as higher temperatures, serves only to accelerate the decomposition of the hydroxamic acids by other means.

^{1}H-NMR in D_2O Buffers

Several mechanisms can be drawn to describe the overall reduction of these hydroxamic acids by thiols (Scheme 3.1). To better understand the nature of the products or intermediates of the reaction with ME, the NMR spectra of selected compounds were recorded in 2 M carbonate buffer (D_2O), 'pD'≈ 9,[3] and during reaction with ME.

The spectrum of HMBOA, **13**, alone in buffer was not complicated.(Figure 3.4a) The sample had been dissolved in the buffer for about fifteen minutes before the first



Scheme 3.1. Possible mechanisms for the reduction of DIMBOA by thiol.

spectrum was run. The spectrum showed aromatic protons (δ 7.5 - 6.2) with the expected splitting pattern, a 1H-singlet at 5.62 and a 3H-singlet at 3.71. A series of low intensity peaks (≈10%) in the aromatic region were also visible. The coupling constants of these peaks were not measurable at the low intensity of the spectrum, but the splitting appeared similar to the higher intensity aromatic signals, though the chemical shifts were quite different. On addition of a 7.5 fold excess of ME and inspection of the spectrum after five minutes (Figure 3.4b), the original higher intensity aromatic signals had almost completely disappeared and the lower intensity ones had increased. In addition, the 1H-singlet at δ 5.62 was absent and a new 1H-singlet at 5.45 had appeared. The 3H-singlet was now slightly changed at δ 3.69. After $2\frac{1}{4}$ hours 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 ME 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 3.5a), exhibiting the expected splitting pattern for the aromatic protons and a 1H-singlet at δ 5.60. A spectrum taken five minutes after addition of the thiol was much more complex. (Figure 3.5b) At least twenty 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 five minutes (δ 5.34), and three others (δ 5.55, 5.50 and 5.48) that increased more slowly over the forty minutes when the last spectrum was taken. (Figure 3.5c-e)

In contrast, a similar experiment with the methyl acetal of DIMBOA, **16**, and a 2.2 molar excess of ME showed no changes from the original spectrum after two hours. (Figure 3.6a-c)

The reaction of the 7-Me analogue, **7**, with ME was also followed by NMR. This compound was chosen for two reasons; it does not exhibit calculable kinetics for reduction by ME and the decay of its UV spectrum in pH 9 buffer is slight, indicating that complicating side reactions are at least slow. Its NMR spectrum in buffer alone is also clean. (Figure 3.7a) After addition of a 7.5 molar excess of ME the spectrum at five minutes had changed only slightly. Some new peaks had appeared in the aromatic region, as well as two others at δ 5.61 and 5.47, but they were less than 5% of the original peaks. After twenty minutes it was clear that the original peaks were decreasing and the others were increasing in intensity. (Figure 3.7b) Another spectrum, taken after 72 hours at room temperature, showed the complete disappearance of the original peaks with the concomitant growth of the others (Figure 3.7c).

This same sort of experiment was performed with the 7-Cl analogue **9**, with parallel results. After two hours (Figure 3.8b) peaks were visible *ca.* δ 5.4 with intensities less than 15%. Spectra after 18 hours showed the almost complete disappearance of the original spectrum (Figure 3.8c).

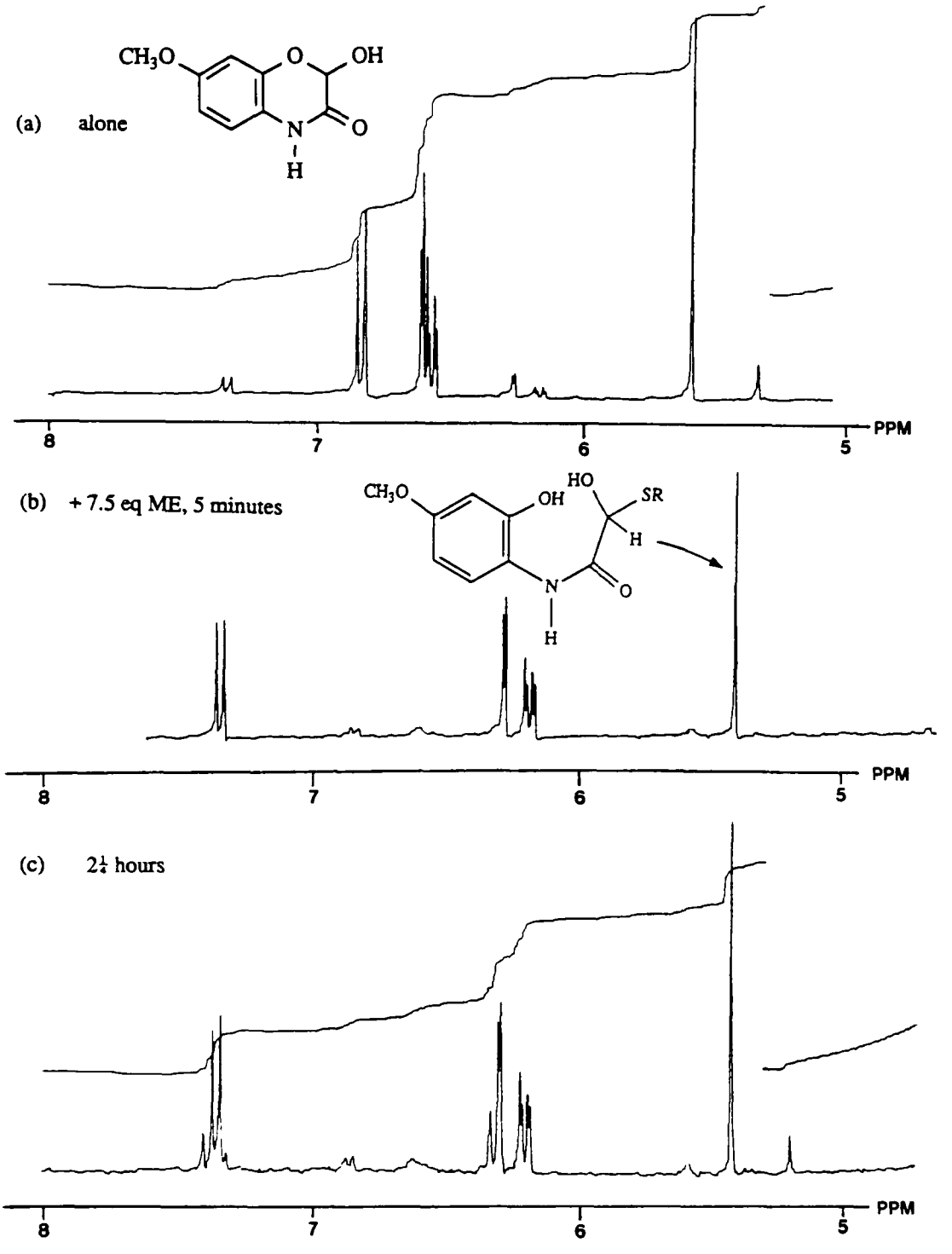


Figure 3.4. NMR spectra of HMBOA (**13**) in D_2O buffer (2 M carbonate, pD 9.0) alone (a) and during reaction with 7.5 molar equivalents of mercaptoethanol (ME), (b) and (c). T=20°C ± 1 C°.

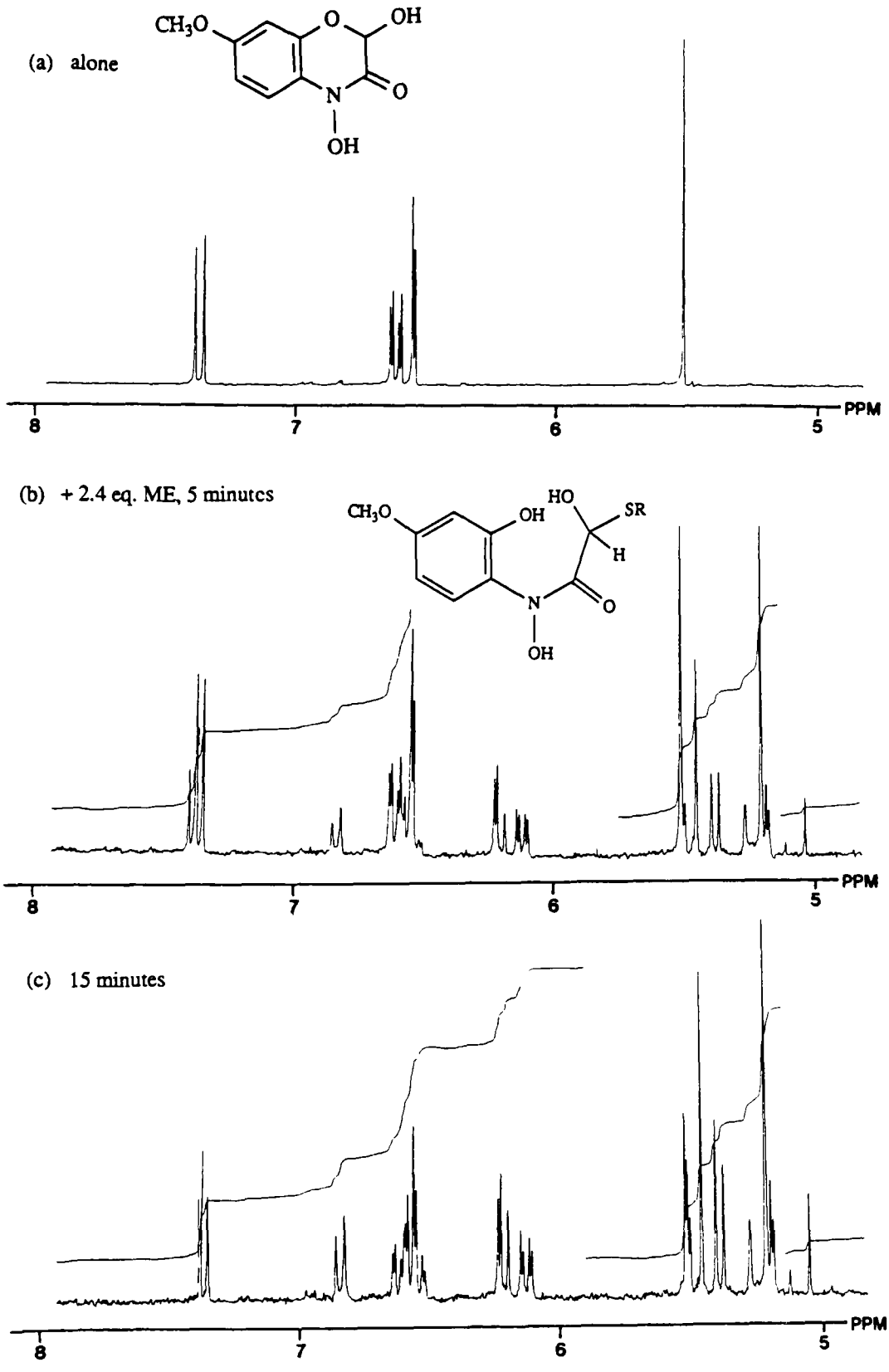
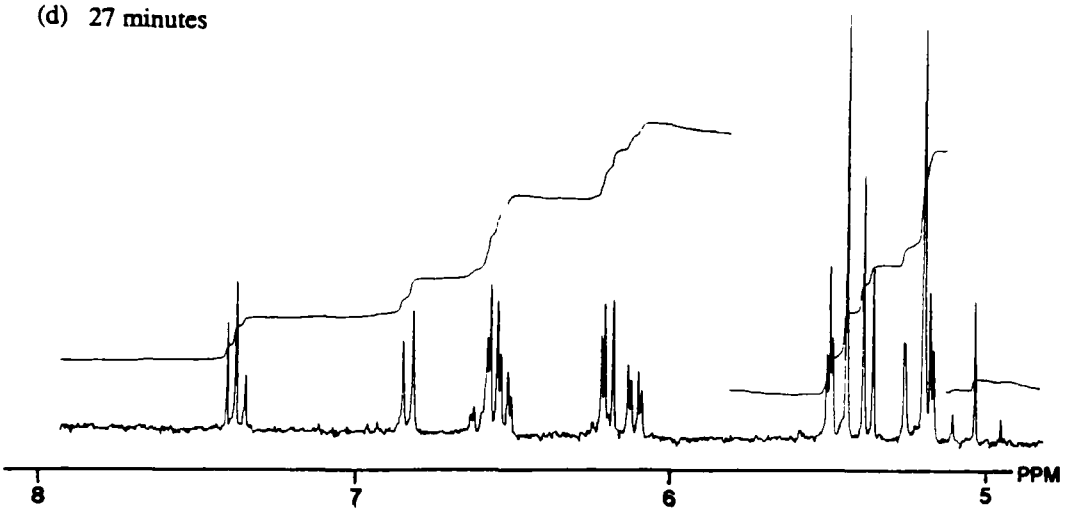
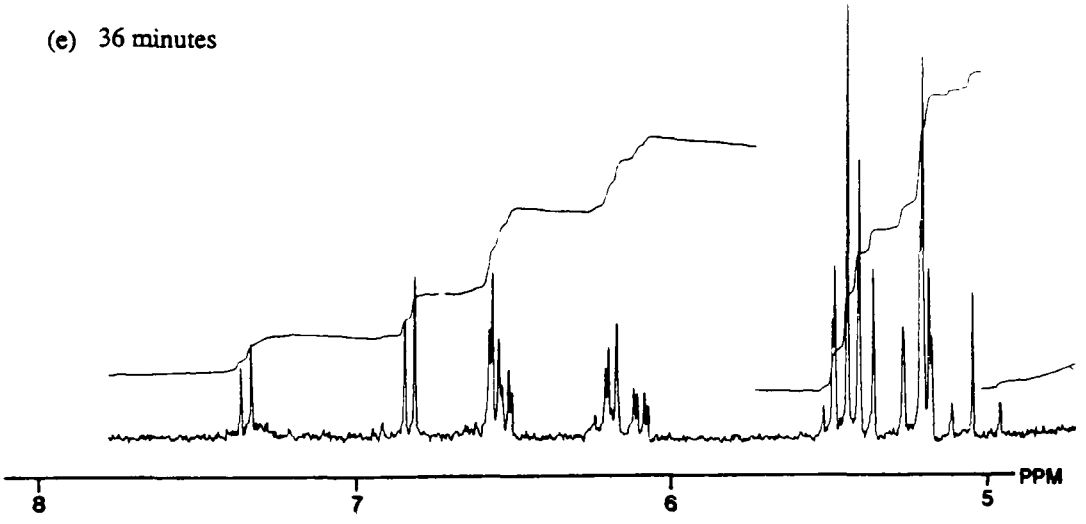


Figure 3.5. NMR spectra of DIMBOA (**1**) in D_2O buffer (2 M carbonate, pD 9.0) alone (a) and during reaction with 2.4 molar equivalents of mercaptoethanol (ME), (b) - (e). T=20°C ±1 C°.

(d) 27 minutes



(e) 36 minutes



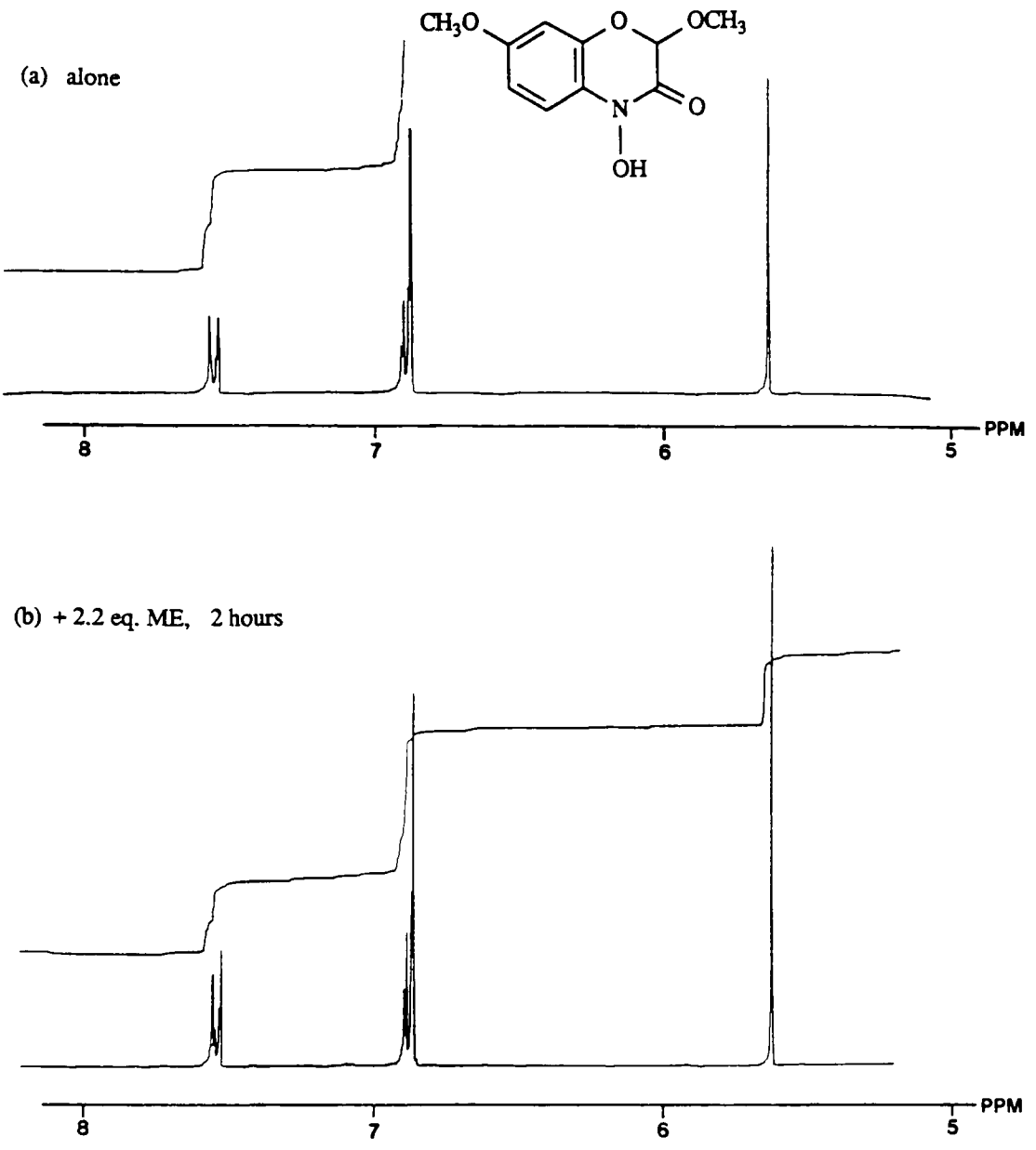


Figure 3.6. NMR spectra of the methyl acetal of DIMBOA (45) in D_2O buffer (2 M carbonate, pD 9.0) alone (a) and in the presence of 2.2 molar equivalents of mercaptoethanol (ME), (b). T=20°C ± 1 C°.

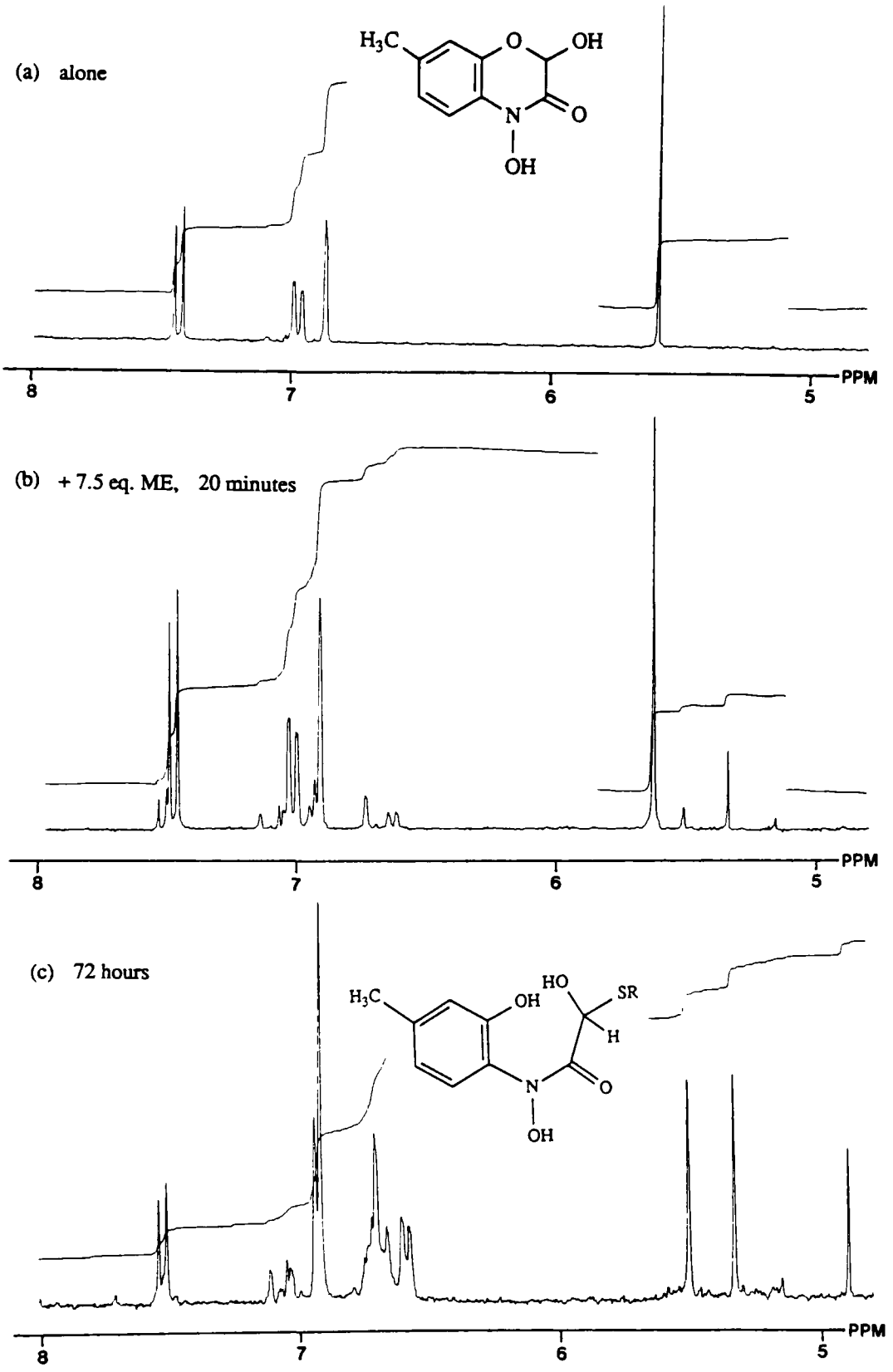


Figure 3.7. NMR spectra of the 7-Me analogue (7) in D_2O buffer (2 M carbonate, pD 9.0) alone (a) and during reaction with 7.5 molar equivalents of mercaptoethanol (ME), (b) and (c). T=20°C ± 1 C°.

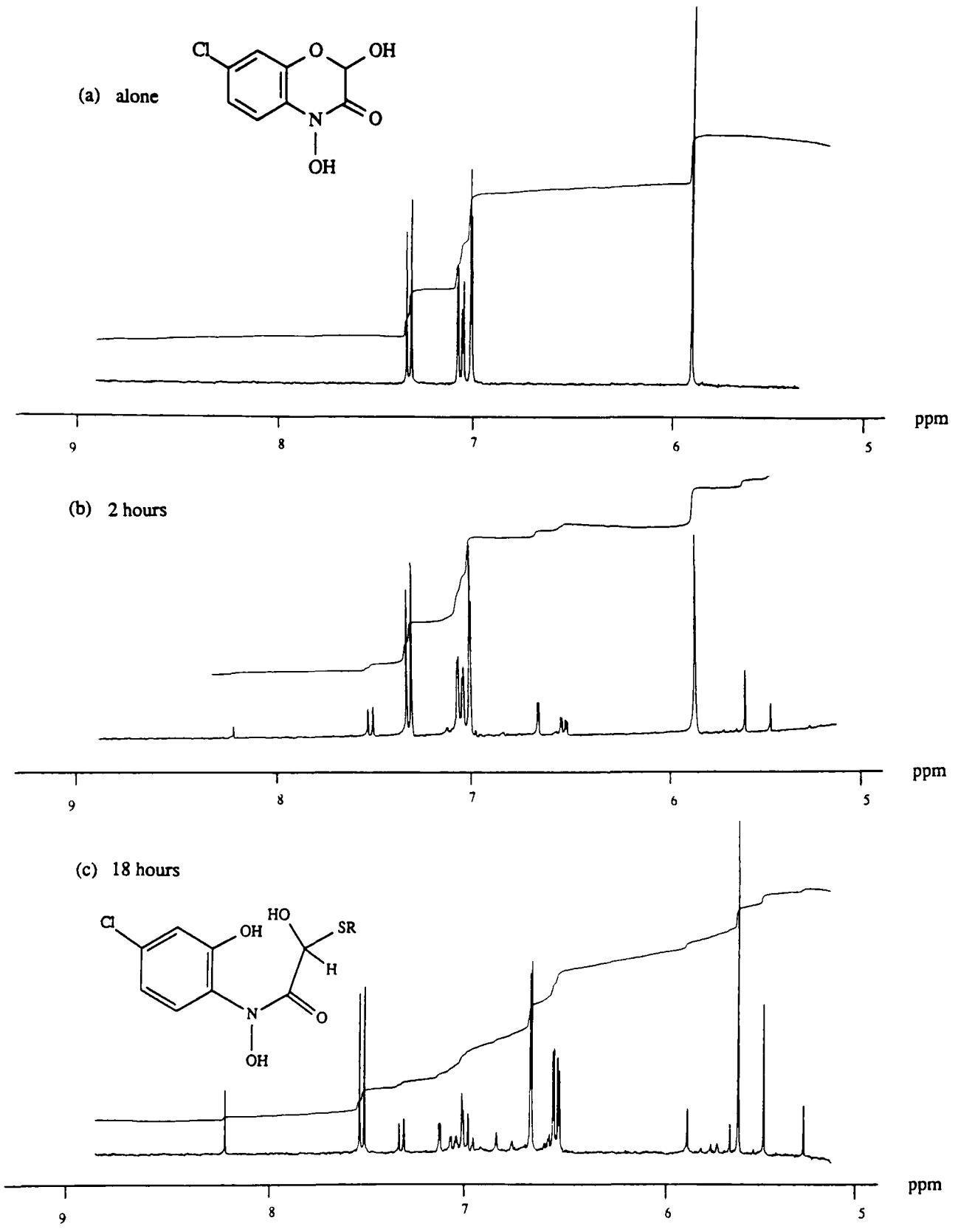
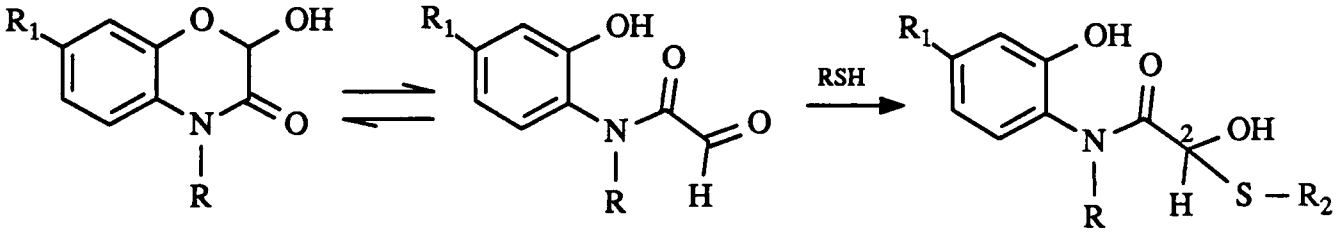


Figure 3.8. NMR spectra of the 7-Cl analogue (**9**) in D_2O buffer (2 M carbonate, pD 9.0) alone (a) and during reaction with 7.5 molar equivalents of mercaptoethanol (ME), (b) and (c). T=20°C ± 1 C°.

Equilibria of Species in Solution

Most cyclic hemiacetals exist in solution predominantly in the closed form,[4] but are usually in equilibrium with the open form. The NMR experiments show that the cyclic form of these hydroxamic acids predominates, whether in acetone-d_6 or basic buffer. This is clear from the 1H-singlet at ≈ δ 5.6, corresponding to the hemiacetal proton. For HMBOA in buffer small peaks were visible in the aromatic region (see Figure 3.5a) whose chemical shifts support the ring opened structure and the presence of a phenol. No aldehyde 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 laactol/aldehyde has been replaced by the less electronegative sulphur. Indeed, the NMR spectrum of HMBOA, **13**, 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 hemi-thioacetal produced by attack of thiolate on the acetal/aldehyde carbon. Also, the pattern of the changes in the chemical shifts of the aromatic protons is consistent with the revealing of a phenolic oxygen, which would occur if theopen form of the ring (the aldehyde) were reacting with thiol, draining the equilibrium. These sort of spectral changes also occurred with the hydroxamic acids investigated. The structuresof the species likely responsible for the changes in the NMR are depicted in Scheme 3.2.



R_1 = MeO, H, Me, Cl; R = OH

R_1 = MeO; R = H

Scheme 3.2. Production of a hemithioacetal at C-2 for selected hydroxamic acids and one lactam (HMBOA).

The result of a reported ESR experiment[2] is consistent with the assumption that the

net reduction of DIMBOA by thiols does not occur by a single electron transfer mechanism. No signal could be detected during the reaction of 0.02 M DIMBOA with 1.0 M mercaptoethanol at pH 8.

The same report suggested that the reduction of these hydroxamic acids by thiols proceeds via attack of thiolate directly at the nitrogen atom.[2] 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 3.9.) The productionof the ion pair is facilitated by the resonance stabilization of the positive charge by the methoxy group through the aromatic ring, and by the strong tendency of oxygen to retain the electron pair that constitutes the N-O bond.[5]

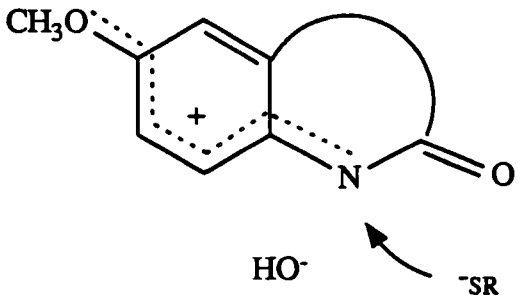


Figure 3.9. Hypothetical ion-pair after scission of the N-O bond. The para-methoxy group stabilizes the species bearing the positive charge. It is this species that is then attacked by thiolate.

The reaction can also be discussed utilising the hard soft acid base (HSAB) principle.[6] As described by Saville[7] one expects a substitution reaction to procede 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 centre at nitrogen. In a reciprocal fashion, the leaving group HO^-, a hard base, attacks the hard acid H^+ in the buffered solvent. Saville's rules as applied to this reaction are illustrated in Figure 3.10. Attack of sulphur on nitrogen is not without precedent. Thiols are known to attack the nitrogen atom of nitrate esters during reactions catalysed by glutathione transferase.[8]

With the ring opening equilibria and the thiol addition products in mind, one can envision the six species in Figure 3.11 occurring in solution over the course of the reaction.

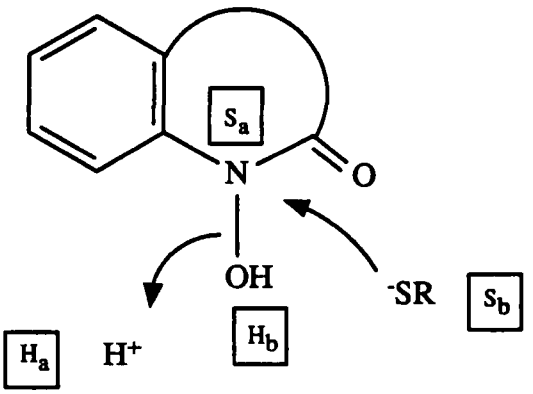


Figure 3.10. Illustration of the attack of thiolate on the hydroxamic acid nitrogen atom following Saville's rules.

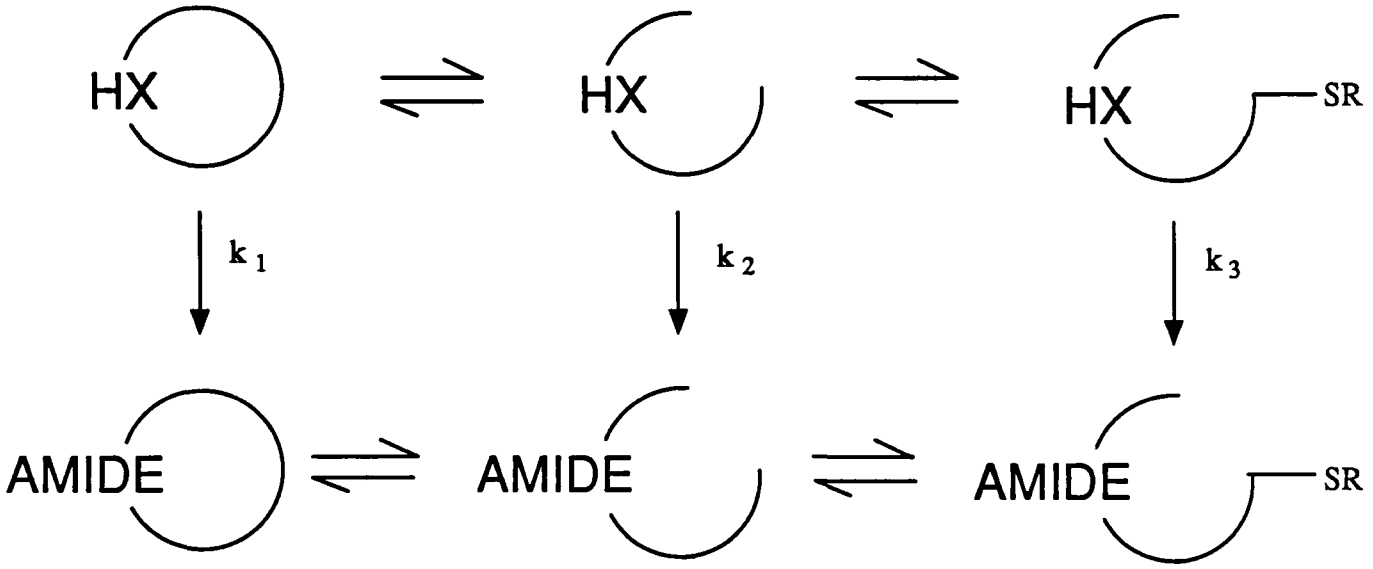


Figure 3.11. Schematic diagram of the equilibrating species and reaction products in solution during the reaction of a cyclic hydroxamic acid with a thiol. The rate constants k_1, k_2, and k_3 represent the overall reduction of each of the hydroxamic acid species that conceivably exist in solution. Closed rings are the lactols, open rings the phenol/aldehyde and species containing -SR are hemithioacetals at C-2.

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 NMR signal appeared in the case of HMBOA, **13**, (Figure 3.4), the eventual product in solution in the presence of excess thiol must be the amide

hemithioacetal. In effect, all equilibria drain towards this product. That the 'free' amides - without the thiohemiacetal moiety - are isolated from reactions is simply a matter of the hemithioacetal's instability on removal from a basic medium. Extraction of neutralised reaction solutions, and chromatography on silica would hydrolyse this group. The thiohemiacetals of both the lactam and the hydroxamic acid *have* been isolated in small amounts (≈3% unoptimized yields[1]) 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 equivalents of ME was followed by NMR no resonances were visible that corresponded to the expected product, HMBOA hemithioacetal. This is expected, since three molecules of ME are needed to fully reduce the hydroxamic acid moiety *and* make the hemithioacetal. However, when the reaction was repeated with eight molar equivalents of ME the spectrum after $2\frac{1}{8}$ hours 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 were 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 ME at those conditions (20-37°C, pH 9, 1-2 hours) where DIMBOA showed measurable kinetics. With more extreme conditions (45°C, 16 hours) **15** is forced to react in the closed form and after extraction from the reaction buffer some of the lactam was detected. The carbocyclic compound **18** also yields some lactam at these more extreme conditions, but considerable (~93% by GC) starting hydroxamic acid remained.

One may have expected **15** and **18** to exhibit greater reactivity since their pK_as are higher and more of the undissociated hydroxamic acid exists in solution, but clearly other factors - lack of an open chain form and, in the case of **18**, a phenolic oxygen - influence reactivity to a greater extent.

Given the rapidity in which the signals in the NMR spectra *ca.* δ 5.6 change upon addition of ME to the buffer solutions of DIMBOA, it is likely that the attack at nitrogen occurs on the already formed hydroxamic acid hemithioacetal.

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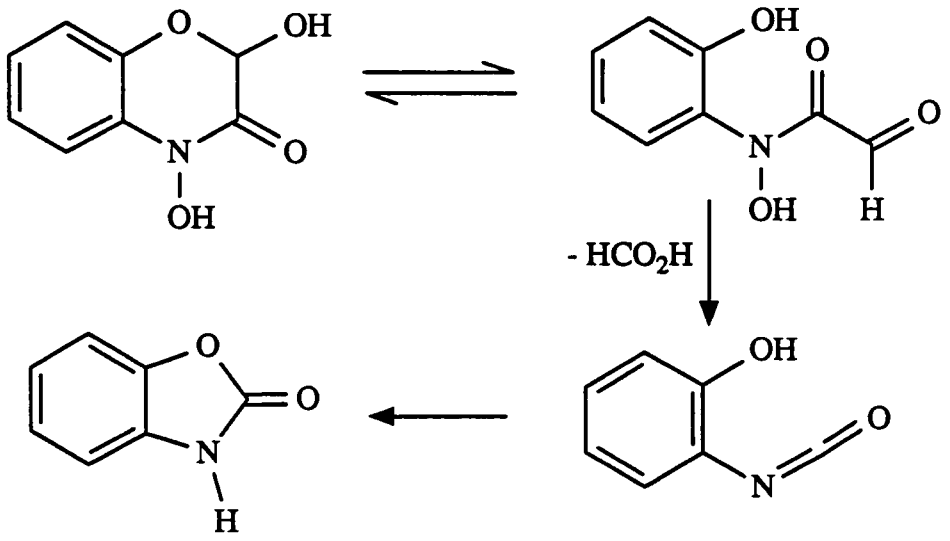
4 TRANSFORMATION OF 2,4-DIHYDROXY-1,4-BENZOXAZIN-3-ONES TO BENZOXAZOLINONES

pH Dependence and Reactive Species

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.[1-6]



benzoxazolinone

Figure 4.1. Mechanism of decomposition of hydroxamic acids (2,4-dihydroxy 1,4-benzoxazin-3-ones) to benzoxazolinones.

For DIMBOA the major product of decomposition is MBOA. The mechanism depicted in Figure 4.1 shows the proposed mode of decomposition for the un-ionized hydroxamic acid as expected in most organic solvents. The pH dependence in aqueous solution of the rate of decomposition describes a bell shaped curve with a maximum at pH 9 (Figure 4.2), and it has been shown that it is the monoanion of DIMBOA that decomposes the fastest, nearly three orders of magnitude faster than the undissociated form.[6]

At pH ~9 (the approximate pH of the larval lepidopteran gut[7]) one would expect all ionized forms of the hydroxamic acid to be present to some degree. They would then have their own pathways for decomposition to products. This is shown in Figure 4.3.

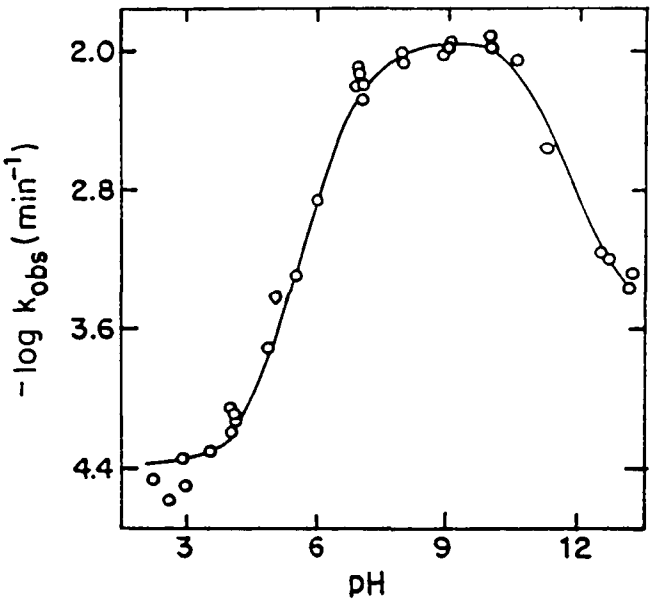


Figure 4.2. Effect of pH on experimental pseudo first-order rate constants for decomposition of DIMBOA at 31 °C. This figure is reproduced from reference 6.

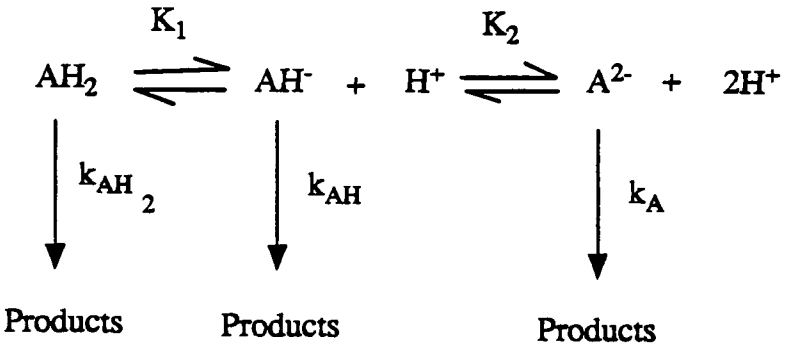


Figure 4.3 Decomposition of 2,4-dihydroxy-1,4-benzoxazin-3-ones and their two conjugate bases in aqueous solution.

Taking into account the possible reactive species shown in Figure 4.3, and their respective rates of decomposition - k_{AH2}, k_{AH}, and k_A, one can write an expression for the observed pseudo-first order rate constant k_{obs} such that:

$$k_{obs} = \frac{k_{AH_2}(H^+)^2 + k_{AH}K_1(H^+) + k_AK_1K_2}{(H^+)^2 + K_1(H^+) + K_1K_2} \qquad \text{Eq. (4.1)}$$

where the constants K_1 and K_2 are the first and second dissociation constants for the hydroxamic acids. In practice, the observed rate constants can be directly equated to the rate constants for the monodissociated hydroxamic acid ($k_{obs} \approx k_{AH}$) since it is clear that this species fully dominates the rate profile (Figure 4.2) and because at the pH of the experiments (*ca.* pH 8.5) nearly every molecule was monodissociated. These measurements were very 'clean'; the UV spectra of the completed reaction matching those of standard benzoxazolinones.[8]

The dissociation constants K_1 and K_2 (pK_a values) were determined spectrophotometrically by recording the differences in absorbance between the mono- and unionized forms at various pH values spanning the pK_a. Complete details of the measurement conditions and data for all analogues are given in the Experimental (Section 7.4 and Table 7.2.) A partial list of pK_a values is included in Table 4.1.

The pK_{a2} values showed greater variation than those for pK_{a1} (1.6 pK_a units versus 0.8). This is reasonable since the measured pK_{a2} is actually for the phenol in the lactol/phenol-aldehyde equilibrium (see Figure 4.1). Here, the substituent on C-7 is closer to the site of ionization than it is for the hydroxamic acid moiety.[9] A measured value for the pK_{a2} 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 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 pK_{a2} value could be obtained for **11a**.

Linear Free Energy Relationships (LFERs)

The compounds in Table 4.1 were chosen (within the bounds of synthetic availability) to offer the widest possible variation in physical parameters, such as pK_a, within a structurally defined set, *i.e.* C-7 substituted. The aim was to similarly affect the

Table 4.1. Sigma (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.

							λ (cm^{-1}) / 10^3		10^3 x k	
Compound	**7-X**	σ_m [a]	σ_p	σ^+	pK_{a1} [b]	pK_{a2}	λ_1 [c]	λ_2	min^{-1}	(pH) [d]
1	MeO	0.10	-0.26	-0.78	6.92	10.1	38.02	34.72	75.9	(8.5)
6	*t*-Bu	-0.10	-0.19	-0.26	6.94	11.00	38.75	35.30	6.61	(9.0)
7	Me	-0.06	-0.17		6.83	10.56	38.46	35.34	8.94	(8.7)
8	H	0	0		6.91	10.55	39.37	35.46	6.01	(8.7)
9	Cl	0.37	0.23	0.11	6.78	10.22	39.22	35.21	17.4	(8.5)
10	F	0.34	0.06		6.63	~10.1	40.16	35.71	-	
11a	CO_2Me	0.37	0.45		6.52	9.90	36.63	33.56	2.25	(8.2)
- [e]	NO_2	0.71	0.78		6.14	9.40	33.33	29.85	3.03	(7.8)

[a] Sigma constants were taken from references 9-11. [b] pK_a's were determined spectrophotometrically. Standard deviations have been included in Table 6.2. 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. [c] UV spectra were recorded in MeOH at concentrations around 7 x 10^{-5} M. [d] Rate 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 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.
[e] This compound was kindly provided by Dr. Hector Bravo, Universidad de Chile, Santiago.

rates of their decomposition. One compound with a NO_2 substituent on C-7 was provided by Dr. Hector Bravo, Universidad de Chile, Santiago.

Ultraviolet Spectra

In MeOH the UV spectra of the compounds in this series tend to show two maxima, one as a shoulder (λ_2). Table 4.1 lists these maxima as well as the sigma constants and the pseudo-first order rate constants for the decomposition of the members of this series.

Plots of the variation in λ_1 (Figure 4.4) or λ_2 (Figure 4.5) with the substituent constant σ_p show clearly that there are two distinct lines for each relation; one for electron releasing substituents and one for electron withdrawing. This can be rationalized by envisioning that conjugation of the nitrogen lone pair is enhanced by both types of substituents: electron-donating towards the carbonyl of the hydroxamic acid, and electron-withdrawing towards the the aromatic ring.

First and Second pK_a's

Figure 4.6 shows that the pK_{a1} values correlate reasonably well with σ_p (r^2=0.86) though the rho value (ρ=0.706) is much higher than that reported for acyclic *N*-phenylhydroxamic acids (ρ=0.1)[12]. It is likely that this is a result of stereochemical differences since the cyclic hydroxamic acids of concern here are much stronger acids ($pK_a \approx 6.8$) than those reported in reference 12 ($pK_a \approx 8.5$).

Figure 4.7 shows that the pK_{a2} values also correlate fairly well with σ_m (r^2=0.85), and the magnitude of the ρ value (ρ=1.62) resembles that for ionization of a phenol where $\rho \approx 2$.[13] This suggests that at high pH these compounds exist in the open-chain form.

Rate Constants

The pseudo-first order rate constants for decomposition (See Table 4.1) 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,[9,11] those substituents that could exhibit resonance interactions with a partial positive charge at the reactive centre, such as MeO, Cl, *and* *t*-Bu, became part of a LFER. The relation, shown in Figure 4.8, is not as strict as those already seen for acidities (r^2=0.67 for the rate constants versus r^2=0.85 for the acidities), but the trend is clear. The presence of the strong electron-donating MeO group greatly enhances the rate of the reaction. Strong electron-withdrawing substituents such as

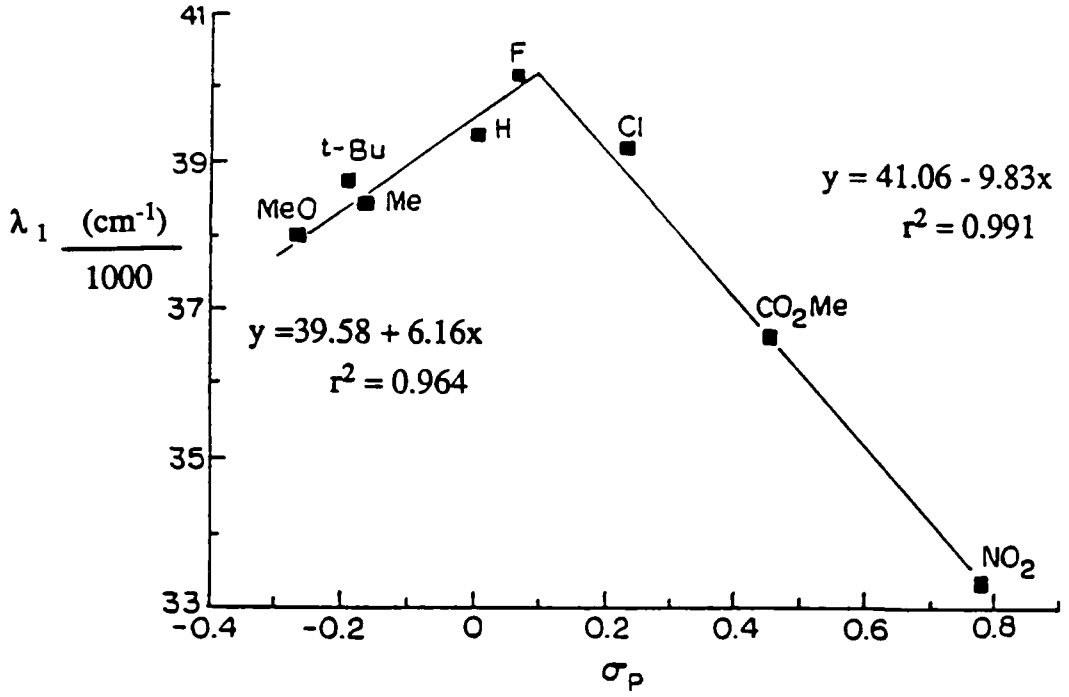


Figure 4.4. Plot of UV-λ_1 versus the substituent constants sigma para (σ_p) for the C-7 substituted series of 2,4-dihydroxy-1,4-benzoxazin-3-ones.

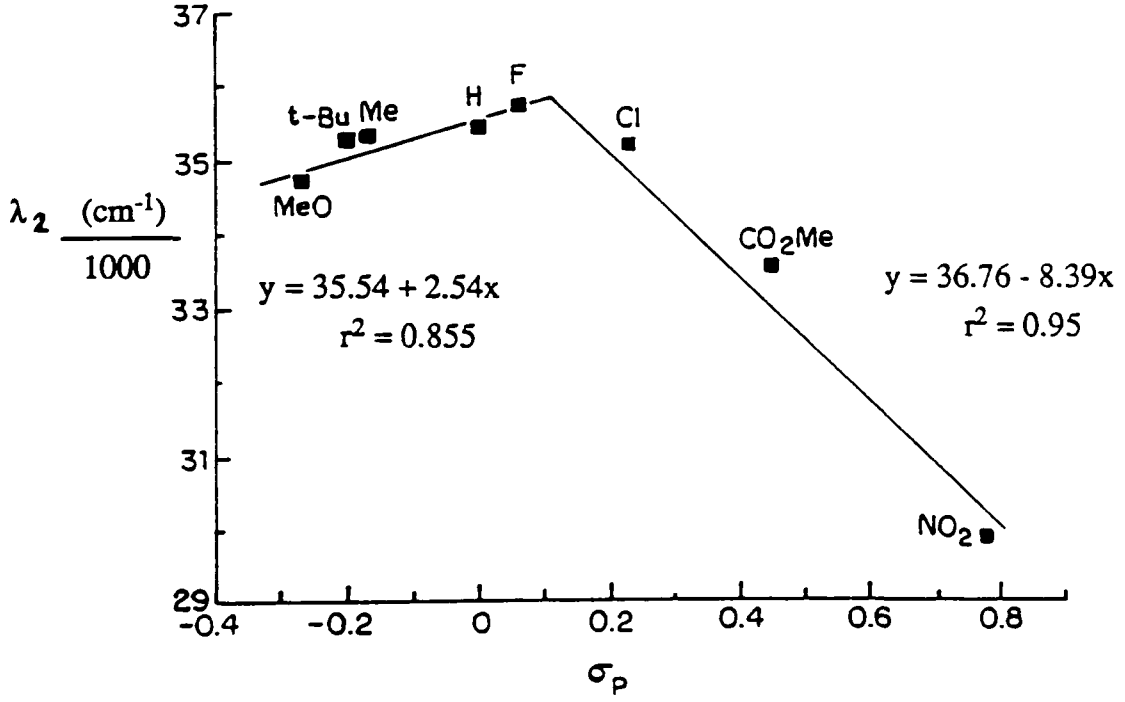


Figure 4.5. Plot of UV-λ_2 (shoulder) versus the substituent constants sigma para (σ_p) for the C-7 substituted series of 2,4-dihydroxy-1,4-benzoxazin-3-ones.

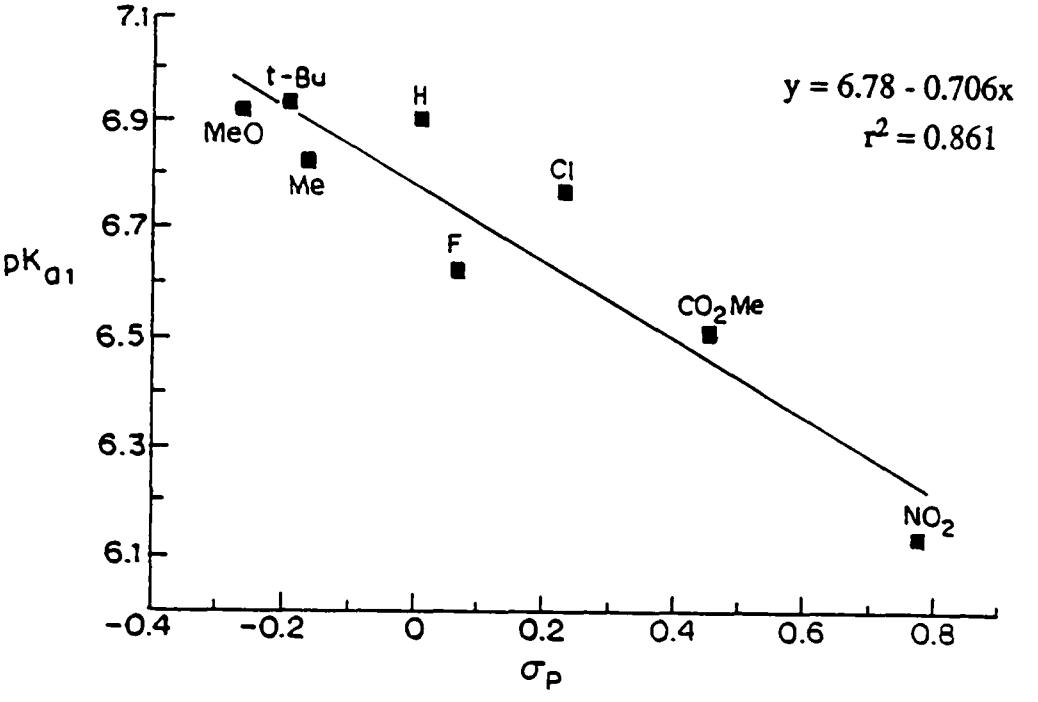


Figure 4.6. Plot of pK_{a1} (hydroxamic acid group) versus the substituent constants sigma para (σ_p) for the C-7 substituted series of 2,4-dihydroxy-1,4-benzoxazin-3-ones.

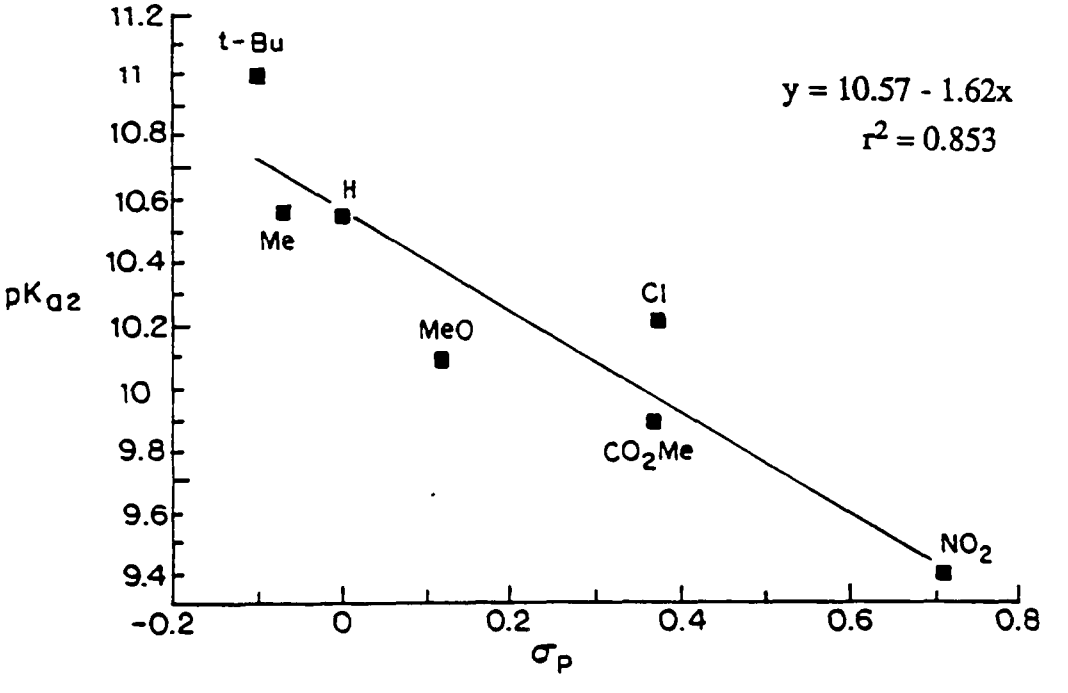


Figure 4.7. Plot of pK_{a2} (phenol) versus the substituent constants sigma para (σ_p) for the C-7 substituted series of 2,4-dihydroxy-1,4-benzoxazin-3-ones.

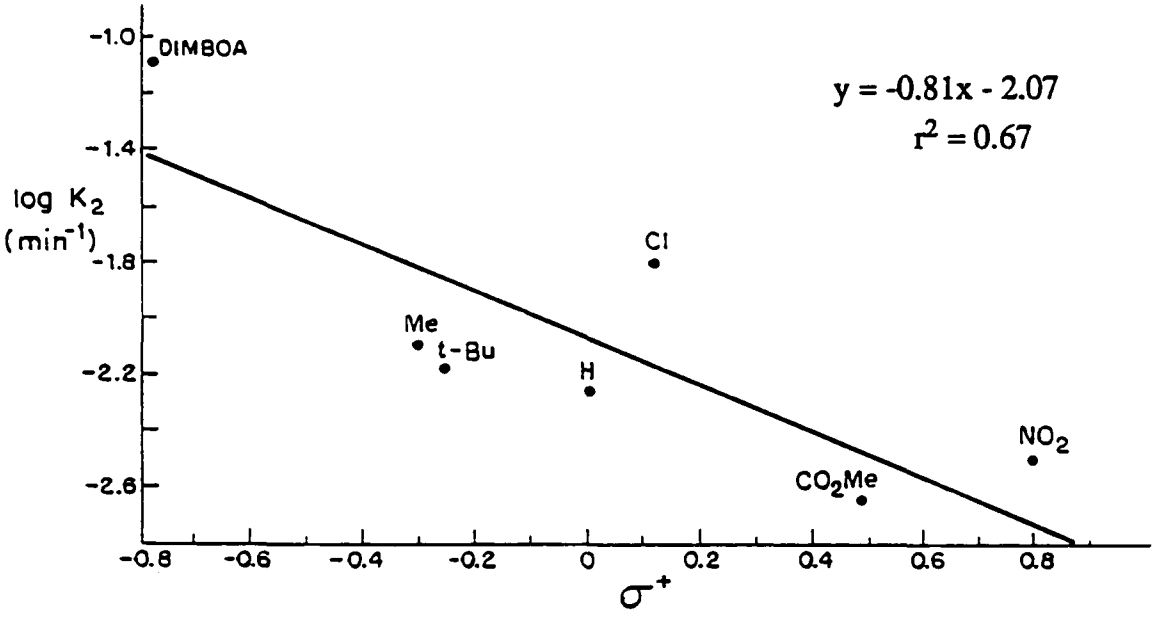
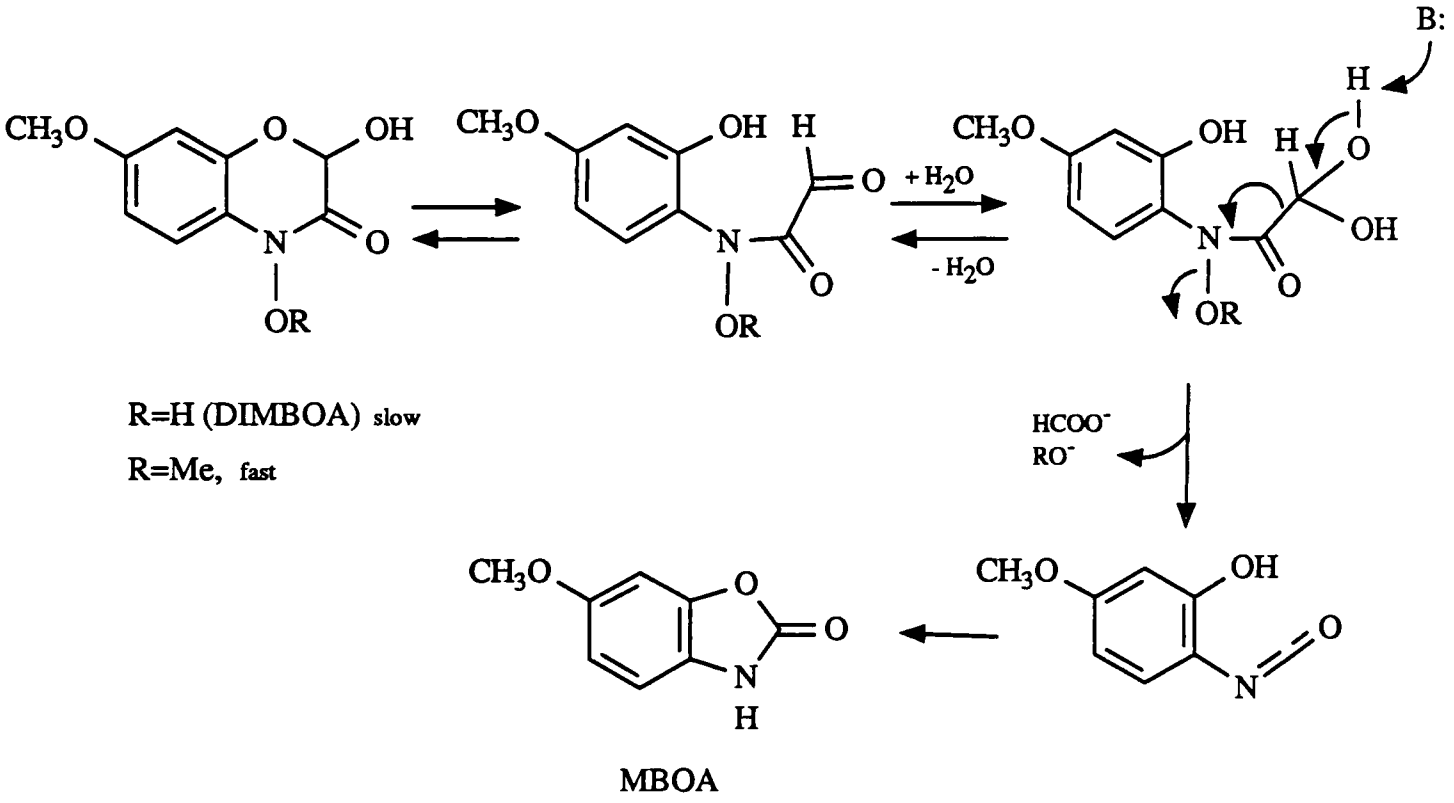


Figure 4.8. Plot of the observed psuedo first-order rate constants for the decomposition of the C-7 substituted series of 2,4-dihydroxy-1,4-benzoxazin-3-ones versus the substituent constants sigma plus (σ^+). The rates were measured at the pH values listed in Table 4.1 at 48°C.

CO_2Me and NO_2 retard the rate considerably. This correlation has ρ=-0.81 meaning that, during the transition state for formation of the isocyanate (see Figure 4.1), electron density at nitrogen decreases with respect to reactants.

Reaction Mechanisms

A number of mechanisms have been used to explain the production of the isocyanate intermediate during the decomposition of cyclic hydroxamic acids. They are based on two opposing assumptions: (i) that the hydroxamic acid hydroxyl group is acting as an internal nucleophile,[3,6] or (ii) that it is a leaving group.[4,14] Evidence for the hydroxamic acid moiety's role as a leaving group in an elimination/fragmentation reaction is based largely on the observation that the N-OMe analogue of DIMBOA decays faster than DIMBOA itself (See Scheme 4.1).

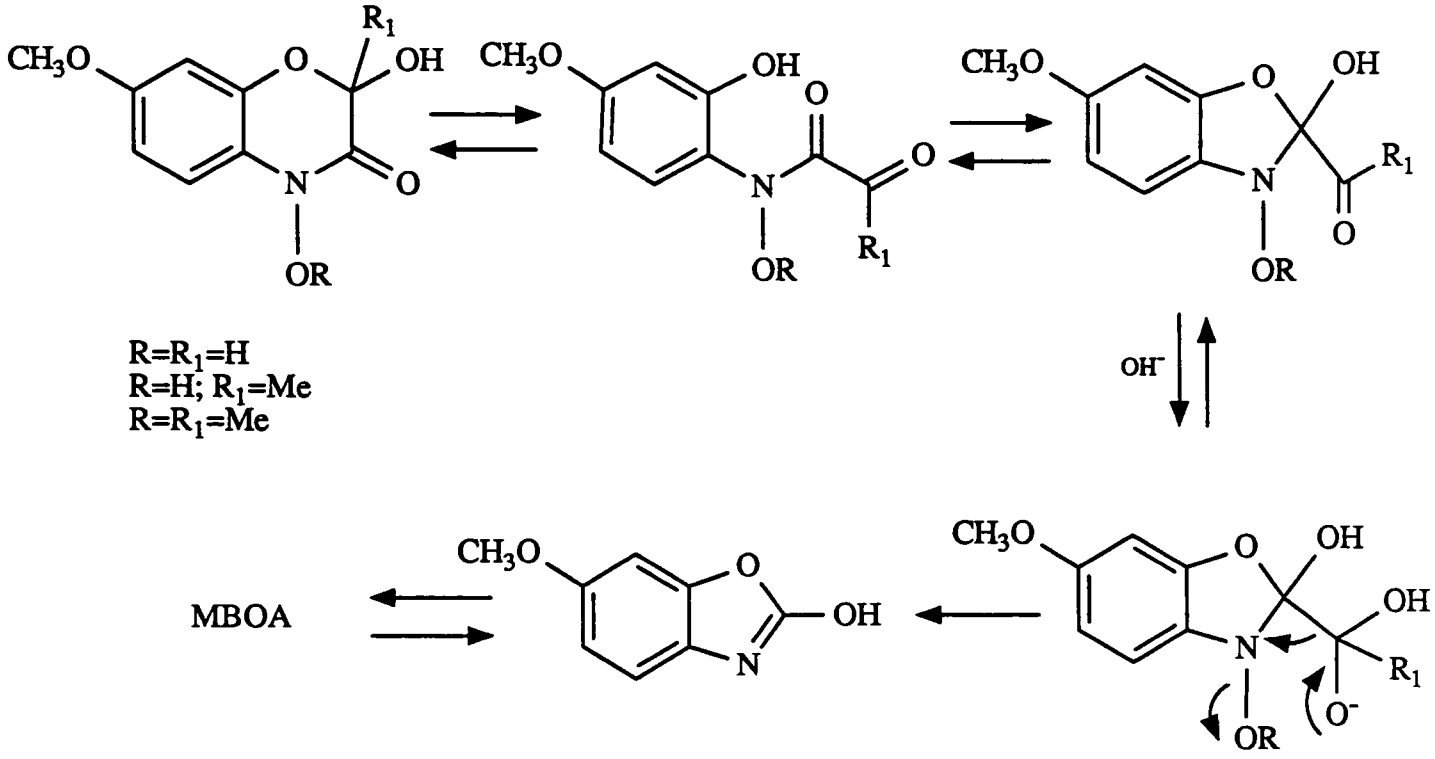


Scheme 4.1. Suggested 1,5-elimination mechanism[14] for the decomposition of DIMBOA to MBOA. The rate enhancement when R=Me was accounted for by accepting MeO^- as a better leaving group the HO^-.

Since the mechanism described is an elimination, it was rationalized[14] that the observed rate enhancement for the compound with the N-OMe group was due to MeO^- being a better leaving group than HO^-. The authors also argued that this mechanism obeys the

observed pH-profile for the reaction. At low pH there is no base present to abstract a proton from the hydrated aldehyde. At high pH the hydroxamic acid exists almost completely as the oxyanion which would be an extremely poor leaving group.

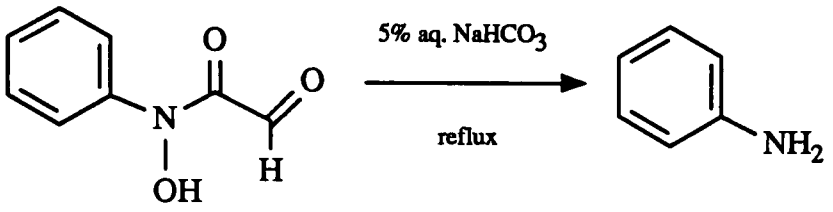
A similar mechanism has been proposed by others[4] except that an explicit role has been given to the phenolic oxygen. (See Scheme 4.2.) In this instance, elimination occurs from a hydrated aldehyde only after attack of the phenol on the carbonyl of the hydroxamic acid.



Scheme 4.2. Decomposition of DIMBOA and two analogues according to the mechanism of Smissman *et al.*[4] Prior to scission of the N-O bond during an elimination/fragmentation reaction the phenol has attacked the carbonyl of the hydroxamic acid.

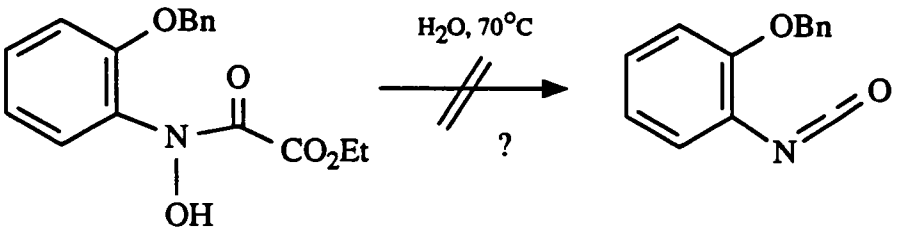
Smissman *et al*[4] purported to find support for this mechanism by showing that precursors which lack the phenol or have it 'blocked' as a benzyl ether do not react normally. The interpretation of this evidence is doubtful however. After boiling *N*-phenylglyoxylohydroxamic acid in 5% aqueous $NaHCO_3$, aniline was recovered in 36% yield. (See Scheme 4.3) This result was attributed to simple hydrolysis products of the hydroxamic acid.[15] It seems equally likely that an isocyanate intermediate was produced, but that it was quickly hydrolysed to the unstable carbamic acid which subsequently decarboxylated to give aniline. Scheme 4.4 shows a benzyl ether protected phenol which

was stable to prolonged heating at 70°C in EtOH or water (of unspecified pH). The assumption that the ester does not change the nature of the reaction and that it is a reasonable replacement for an aldehyde seems questionable.



Scheme 4.3

N-Phenylglyoxylohydroxamic acid

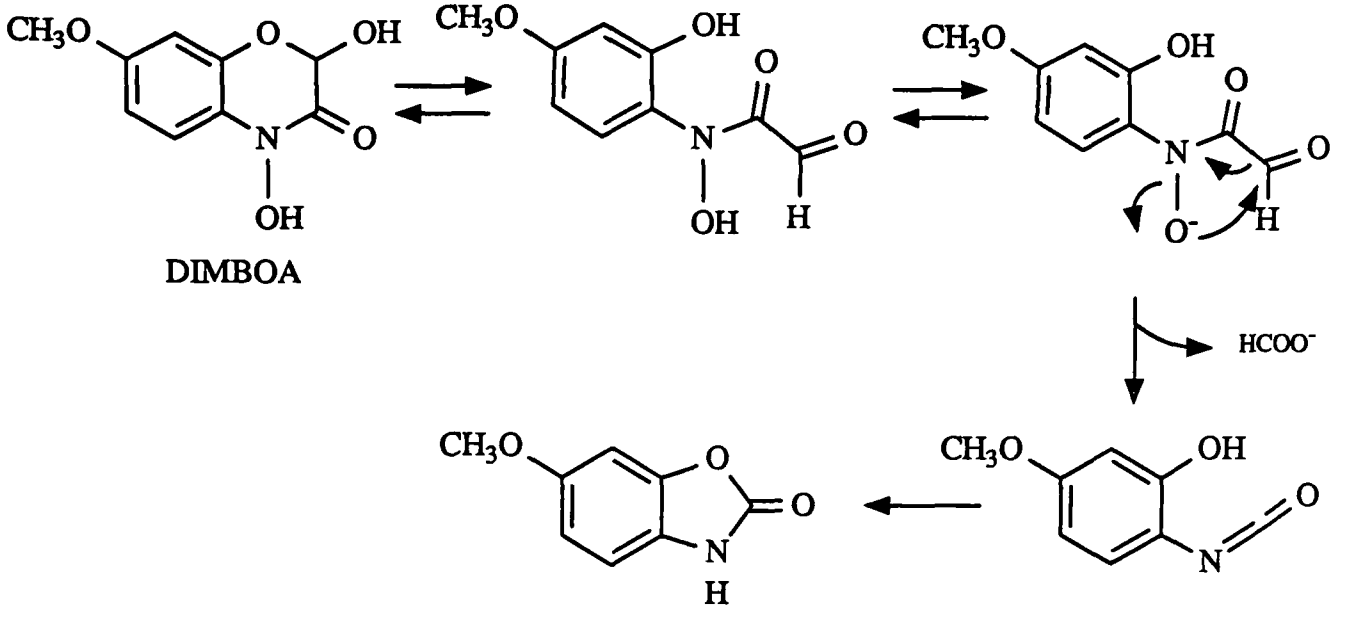


Scheme 4.4

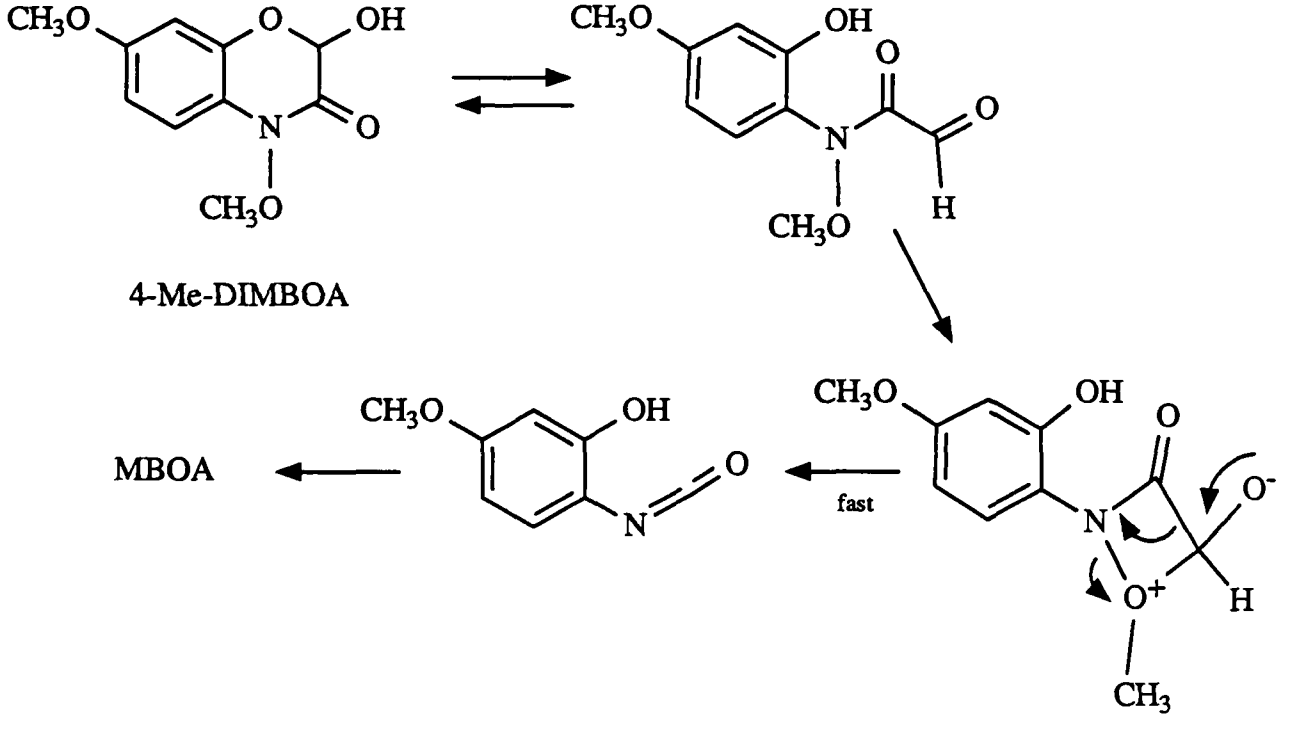
The alternative mechanism, where the hydroxamic acid hydroxyl acts as an internal nucleophile, was first described nearly thirty years ago[3]. More recent work[6] has shown that, in organic solvents, the rates of decomposition of DIMBOA correlate linearly with the solvent donor number.[16] Since the donor number is a measure of a solvent's capacity to act as a Lewis base, it appeared that acidic sites in DIMBOA were interacting with basic sites in the solvent molecules. This was confirmed by IR studies of OH stretching frequencies. These results support the conclusion that the hydroxamic hydroxyl group is acting as a nucleophile. The proposed mechanism is illustrated in Scheme 4.5.

It is still possible to rationalize the rate acceleration brought about by methylation of the hydroxamic hydroxyl group that was mentioned above. It is doubtful that the difference in rate is accounted for by the leaving group abilities of MeO^- and HO^- since, in water, the pK_a's of the two conjugate acids are nearly the same, 15.5 for MeOH and 15.75 for H_20.[17] It is possible, however, to envision a good leaving group being formed subsequent to attack of the hydroxamic ester oxygen on the aldehyde carbonyl, in a similar manner as depicted in Scheme 4.5 for the ionized hydroxamic acid. (See Scheme 4.6.)

Although the oxygen atom in this instance is not as good a nucleophile as in the



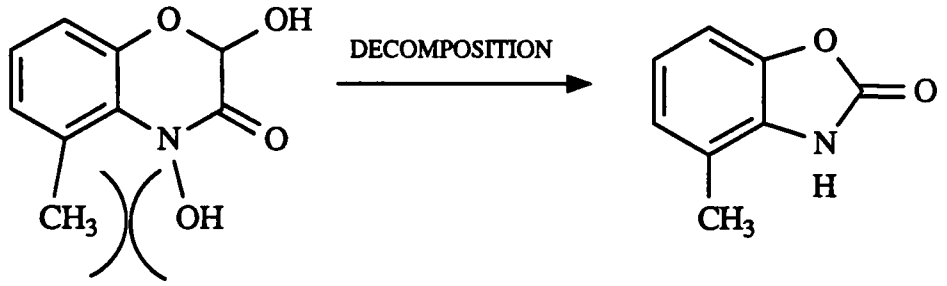
Scheme 4.5 Decomposition of DIMBOA by internal nucleophilic attack of the hydroxamic acid hydroxyl group on the aldehyde carbonyl with fragmentation to the isocycante. For clarity, only one equilibrium of ionization is shown.



Scheme 4.6. Possible mechanism for the decomposition and rate enhancement for the methylated derivative 4-Me-DIMBOA.

ionized hydroxamic acid hydroxyl group, the cyclic oxonium ion, if formed, *is* a much better leaving group. The 4-Me-DIMBOA is a very reactive compound. It decays in ethereal solution when made by methylation of DIMBOA with diazomethane. Attempted trituration of impure material at room temperature gave tars. Aqueous work-up gave only MBOA and tars.

One of the analogues prepared in this work (**17**, 5-Me-DIBOA) had 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 5-Me BOA with scission of the N-OH bond would remove this strain.



5-Me-DIBOA (**17**) 4-Me-BOA

Scheme 4.7. Steric interaction in 5-Me-DIBOA (**17**) that is likely responsible for its accelerated rate of decay (as compared to other cyclic hydroxamic acids) to the benzoxazolinone 4-Me-BOA.

It is worth noting that the methyl acetal of DIMBOA, **16**, 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 enzymes[18] that cleave the sugar moiety from the toxic hydroxamic acid.

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5 BIOLOGICAL ACTIVITY

Measurement of the biological activities of the analogues in this study including nutritional indices, feeding trials, and enzyme assays were performed by Francesca Campos in the laboratory of Dr. J.T. Arnason, Department of Biology, University of Ottawa. Gut enzymes of *Ostrinia nubilalis* were originally characterized and purified by J.G. Houseman *et al*[1], Department of Biology, University of Ottawa. Preliminary studies to determine antifeedant activities were performed jointly (Campos and Atkinson).

Nutritional Indices

Determination of Waldbauer's nutritional indices[2] for DIMBOA and MBOA has indicated that the site of action of these compounds is different. As seen in Table 5.1, for MBOA, the consumption index (CI) and approximate digestibility (AD) were not significantly different from control for all the concentrations used. For DIMBOA the CI increases at 0.2 mg/g diet. The AD of DIMBOA decreased at increased concentrations, as did the efficiency of conversion of ingested food (ECI) since the two are directly related (ECD=ECI/AD). The ECI is an overall measure of an insect's ability to utilize the food which it ingests for growth. For larvae fed DIMBOA the efficiency of conversion of digested food (ECD) remained about the same. The ECI and ECD for MBOA both decrease as the concentration increases. Since the ECD reflects the proportioning of assimilated food between biomass production and respiratory costs[3] MBOA appears to increase the proportion of ingested food that is metabolized for energy. This is consistent with the known ability of BOA (and thus likely MBOA) to inhibit mitochondrial electron transfer and ATP synthesis at the ATPase complex in bovine mitochondria.[4]

These results suggest that DIMBOA acts within the larval gut to lower the efficiency of digestion. MBOA did not lower AD but it did interfere with the utilization of nutrients (for growth) that have already crossed the gut wall. The log P values [log (octan-1-ol/water) partition coefficients] have been determined for MBOA and un-ionized DIMBOA.[5] 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

Table 5.1. Nutritional indices for third instar larvae of the European corn borer fed on diets containing MBOA and DIMBOA.

MBOA

[MBOA] (mg / g)	weight gain (mg, dry weight)	CI	AD (%)	ECI (%)	ECD (%)
0	5.30 [a] (0.8)	6.13 [a] (0.27)	55.3 [a] (1.89)	18.1 [a] (0.78)	33.0 [a] (1.80)
1.0	3.30 [a] (0.3)	5.55 [a] (0.25)	52.5 [a] (2.37)	15.5 [a] (1.07)	30.8 [a] (2.70)
3.0	2.60 [b] (0.3)	6.07 [a] (0.31)	59.6 [a] (3.42)	11.9 [b] (1.03)	21.6 [b] (2.06)

DIMBOA

[DIMBOA] (mg / g)	weight gain (mg, dry weight)	CI	AD (%)	ECI (%)	ECD (%)
0	5.00 [a] (0.30)	10.9 [a] (1.0)	56.5 [a] (2.5)	11.4 [a] (0.6)	21.1 [a] (1.5)
0.05	5.50 [a] (0.40)	10.8 [a] (0.6)	51.0 [ab] (2.5)	12.3 [a] (0.6)	25.9 [a] (1.9)
0.2	3.80 [b] (0.30)	15.0 [b] (1.3)	44.9 [b] (3.2)	8.8 [b] (0.9)	20.9 [a] (1.8)

Within a column, means with the same letter indicate no significant difference ($P \leq 0.05$) in Tukey's Studentized range test. Numbers in brackets are standard deviations. This data taken from the Ph.D. thesis of Francesca Campos, Dept. of Biology, University of Ottawa, 1989.

transported across the gut wall at an appreciable rate, MBOA would more easily cross membrane barriers to other potential sites of interaction.

The CI of DIMBOA rises, apparently, because the larvae sense a lowering in the efficiency of digestion (a lower ECI) and thus consume more diet in an attempt to compensate. DIMBOA does not affect the larvae's ability to utilize nutrients provided by digestion, but, rather, limits the availablity of these nutrients by interfering directly with digestive processes in the gut. The distinction that must be made between DIMBOA and MBOA is that the former acts *within* the gut and the latter appears to act both within the gut and after being absorbed into body tissues.

Association of ^{3}H-DIMBOA with Proteins[5]

If DIMBOA is suspected to react with biological nucleophiles then it must at some time form a covalent bond with those molecules that support a nucleophile in their structure. To test whether this was occurring larvae were fed ^{3}H-DIMBOA then combined with their frass (feces) and homogenized in buffer (Tris, pH 8). The homogenate was then subjected to gel filtration on a Sephadex G-25 column. The radioactive label was observed (by liquid scintillation) to co-elute with fractions that contained protein (as detected by UV absorbance at 280 nm).

Digestive Enzymes and Feeding Trials

With the above results in mind, a likely (and testable) mode of toxicity in the gut is the inhibition of digestive enzymes. At least four serine proteases have been identified in the digestive tract of the European corn borer. They have been characterized by their hydrolysis of synthetic substrates and by their pH optima.[1] During the study of both the natural and synthetic 1,4-benzoxazin-3-ones two inhibitory activities were monitored;[5] tryptic activity as measured by the rate of hydrolysis of the synthetic substrate BAEE (*N*-benzoyl-L-arginine ethyl ester), and chymotryptic activity as measured with the synthetic substrate BTEE (*N*-benzoyl-L-tyrosine ethyl ester). Table 5.2 presents the inhibitory activities as a percentage of controls and compares these results with those from feeding trials. The feeding trials were necessary to assess the relative activity of the analogues by ingestion. Equimolar concentrations in semi-synthetic (meridic) diets were used such that the concentration of DIMBOA (0.25 mg/g diet) resulted in a 50% reduction in growth from the control over the four days of the trial.

Before discussing these results in detail it is helpful to recall the various kinds of reactions that DIMBOA can undergo. DIMBOA reacts with thiols[6] (both an addition

reaction and a nucleophilic substitution with overall reduction) and with amines[7], and the rates for these reactions show maxima at pH 8.5 and pH 10.5 respectively. Similarly, the unimolecular decomposition to produce the benzoxazolinones (BOA's) has a maxima at pH≈9. The larval lepidopteran gut is known to be alkaline[8], ranging generally from pH 8-11. Thus, one must assume that once ingested, DIMBOA (as well as other cyclic hydroxamic acids of the same class) is reactive in all the ways described. Comparison of the biological activities must then take into account the known rates of each reaction or the proclivities of each analogue to react in a certain fashion.

Of the four compounds (**1-4**) that yielded kinetic results for reaction with mercaptoethanol (ME), the two with the largest rate constants (**3** and **4**, see Table 3.1) did not inhibit the growth of larvae to an extent that was significantly different from control. Naturally occurring compounds **1** and **2** (DIMBOA and DIM_2BOA respectively) were less reactive towards reduction by mercaptoethanol (see Chapter 3) but did inhibit growth, DIM_2BOA to a lesser extent than DIMBOA. All of the 7-substituted series except for 7-CO_2Me, **11a**, inhibited the growth of larvae during the feeding trials and all had activites equal to or surpassing (**7**) that of DIMBOA. The remaining compounds (**12-18**, and MBOA) did not significantly reduce growth.

The enzyme assays show little informative variation except for the surprising result that the two amides **12** and **13** are the most potent inhibitors, almost completely inhibiting proteolytic activity.

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 toxicology. 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.[5]

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 BOA's will compete. Since it is the hydroxamic acid parent structure (with both the hydroxamic acid moiety and the lactol) that is necessary for activity in the feeding trial (see Table 5.2), those analogues which unimolecularly decay the slowest should have a

Table 5.2. The effects of various 1,4-benzoxazin-3-ones on growth of the third instar larvae of *Ostrinia nubilalis* and on the activity of two midgut proteases of the larvae, low alkaline trypsin and chymotrypsin.

Compound	Substituent	% growth from control [a]	Activity of low alkaline trypsin [b] (% control)	Activity of chymotrypsin [c] (% control)
1	7-MeO	49.1 *	41.1	40.0
2	7,8-diMeO	78.6 *	44.1	33.1
3	6,7-diMeO	97.1	75.6	83.6
4	6,7-MDO	92.5	84.0	77.7
5	8-MeO	104.8	50.0	69.2
6	7-t-Bu	52.6 *	47.9	54.6
7	7-Me	26.6 *	37.5	37.2
8	7-H	55.5 *	20.7	27.5
9	7-Cl	37.6 *	60.0	45.8
10	7-F	56.7 *	66.9	61.1
11	7-CO_2Me	104.8	79.9	75.2
12	(HBOA)	110.1	0	11.3
13	(HMBOA)	98.8	3.8	0
14	7-H-[2H]	89.0	36.3	50.0
15	7-MeO-[2H]	104.8	50.0	69.2
16	(DIMBOA-Me)	97.1	45.1	31.3
17	5-Me	101.7	62.4	71.2
18	(carbocyclic)	108.2	22.2	42.7
MBOA		104.2	69.1	79.1

[a] 100% growth of early third instar larvae over the four days of the feeding trial was 0.0346 ± 0.002 g / larva. [b] 100% activity was 1.1 µmole BAEE hydrolysed/min/mg protein. [c] 100% activity was 0.5 ± 0.015 µmole BAPNA hydrolysed/min/mg protein. Values followed by an asterisk indicate significant difference from control (t-test, $\alpha = 0.05$). All analogues were tested at the same concentration; 500 µM.

greater opportunity to manifest toxicity. This comparison is easily seen in the data of Table 5.3. The pseudo-first order rate constants 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 pK_a'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 BOA's with a rate constant greater than $\sim 1 \times 10^{-2}$ min^{-1} at pH 9 would not persist long enough in the larval gut to manifest toxicity. Remarkably, the two naturally occurring compounds **1** and **2** (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 ME. Those compounds that react faster with ME (**3** and **4**) decompose too quickly in solution, apparently, to show toxicity in the growth study. Compound **17** decomposes so fast at pH 9 that it was impossible to determine accurate absorbance data for it. The 7-CO_2Me analogue **11a** is an anomaly since it undergoes unimolecular decomposition very slowly and yet showed no activity in the growth study.

It is difficult to understand the variation in the growth inhibition data. Why, for instance, does the 7-Me analogue **7**, which decays very slowly unimoleculary, exhibit twice the growth inhibition of the 7-*t*-Bu analogue with a comparable stability? And what accounts for **7** having twice the activity of DIMBOA? These questions will likely have to be answered by more specific experiments with isolated enzymes or organelles, and by isolation and characterization of the enzyme with the inhibitor covalently bound. Incubation of ^{3}H-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. It has recently been shown[9] that DIMBOA inhibits mammalian chymotrypsin. The tentative conclusion is that it covalently links to the enzyme's active site serine at the aldehyde available from the open chain form of the lactol. This conclusion was reached because phenylglyoxal also inhibits the enzyme. The NMR experiments reported in Chapter 3 support this claim, having shown that the aldehyde of the ring-opened form of these molecules is highly reactive towards a thiol nucleophile. Also, two published reports concerning the mutagenicity[10] and the anti-inflammatory[11] activities of DIMBOA and related compounds have deduced the necessity of the lactol moiety for activity.

Table 5.3. Comparison of rates of reduction by mercaptoethanol, k_2, rate constants for unimolecular decomposition, k_{obs}, and growth inhibition of *Ostrinia nubilalis*, for a series of cyclic hydroxamic acids.

—Compound—			unimolecular	
#	Substituent	k_2 (M^{-1} min^{-1})[a]	k_{obs} (min^{-1}) [b]	% Growth [c]
17	5-Me	-	$> 10^{-1}$	NS
4	6,7-MDO	6720	4.0×10^{-2}	NS
3	6,7-diMeO	1480	2.9×10^{-2}	NS
1	**DIMBOA**	227	**8.6×10^{-3}**	**49**
2	7,8-diMeO	88.5	7.2×10^{-3}	79
10	7-F	no rxn	9.0×10^{-3}	57
9	7-Cl	no rxn	3.0×10^{-3}	38
8	DIBOA	no rxn	$< 10^{-3}$	56
7	7-Me	no rxn	$< 10^{-3}$	27
6	7-t-Bu	no rxn	$< 10^{-3}$	53
11a	7-CO_2Me	no rxn	$< 10^{-4}$	NS

[a] True second order rate constants were calculated from Equation 3.5. (See Chapter 3) from trials at 23 ± 0.3 C°, pH 9.00 (Tris) and ionic strength (I) of 0.15. [b] Psuedo first-order rate constants for unimolecular decomposition were determined at 37 ± 0.3°C, pH 9.00 (Tris-HCl) and ionic strength of 0.15. [c] Growth is expressed as percent of control. NS: not statistically different from control (χ^2 test, $\alpha = 0.10$)

Cyclic Hydroxamic Acids as Antifeedants of European Corn Borer

Another important variable in the study of plant secondary chemicals as compounds for chemical defense is their potential as antifeedants. A growth study necessarily assumes that the insects actually eat the diet, but this may not be so if the compounds under scrutiny are strong antifeedants. Compounds **1-18** were tested in a leaf-disk assay feeding deterrency. In these experiments, 10μL of an acetone solution containing 1.0 mg eq DIMBOA/mL was evaporated onto the surface of a 1 cm corn leaf disk exposed at the bottom of a plastic dish. One third instar larva was placed in the dish and the covered dish kept in an incubator for 48 hours. After this time the degree of consumption was measured (area of disk consumed as a percentage) and means calculated. It had been hoped that a correlation might be evident between the degree of deterency and the rate of reduction by ME. A similar correlation had been made[12] bewteen the antifeedant properties of a series of tenulin analogues (sesquiterpene lactones) and their abiltiy to react with thiols in a Michael addition reaction. Unfortunately, the variation between each disk treated with the same compound was so high as to make a comparison of the means meaningless. A choice test was also performed. Pieces of corn stalk, dipped in equimolar solutions of compounds **1-4** and **8** (including a control), where placed in a petri dish along with one third instar larva and consumption scored after 24 hours. All samples were found to have been eaten to the same degree, no preference being given to any compound.

DIMBOA is known to act as a feeding deterrent in the field[13,14] but in meridic diets it is usual for the antifeedant properties of tested compounds to be attenuated.[15] Still, the above results suggest that these compounds are not potent antifeedants for larvae of the European corn borer, though to be conclusive these experiments should be repeated and attempts made to limit the variations in consumption.

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6 SUMMARY AND CONCLUSION

A variety of naturally occurring and new cyclic hydroxamic acids sharing the 2,4-dihydroxy-1,4-benzoxazin-3-one skeleton have been successfully synthesized by the reductive cyclisation of substituted methyl α-(o-nitrophenoxy)-α-methoxyacetates. This methodology is fairly general, but is limited to those molecules containing a substituent para to the nitro group in the precursor that is less electron donating than methoxy. When the substituent was dimethylamino or acetamido (Me_2N- or CH_3CONH-) no hydroxamic acids were detected or isolated from the highly coloured reaction mixtures. In the case of the Me_2N-substituted precursor the lactam **25** was isolated. When the ring contained multiple oxygen substituents (*i.e.* **2**, **3**, and **4**) hydroxamic acids were still readily obtained, though the yields were slightly lower and the contaminating lactams were produced to a greater extent.

Demethylation of the C-2 methoxy group was possible for most analogues by choosing the appropriate reagent and reaction conditions (BCl_3 or BBr_3, temperature and reaction time), but when strongly electron withdrawing groups were present on C-7 (CF_3, CN) it was not possible to remove the methyl group using any of the methods explored.

The pK_a values of the hydroxamic acid and of the phenol correlate with the σ_p constants in a LFER (ρ=0.706 and 1.62 respectively). One might expect a larger ρ-value for these cyclic hydroxamic acids because of steric interactions that make them stronger acids than the acidities reported in the literature[1] for *N*-arylhydroxamic acids. The phenol acidities showed a greater range of values, likely because of its close proximity to the C-7 substituent. The rate constants of decomposition to benzoxazolinones (a reaction which also demands a 2-hydroxy group) correlate in a LFER with σ^+ (ρ= -0.81), indicating a decrease in electron density at nitrogen during the transition state for the formation of an isocyanate. Electron withdrawing substituents, thus, retard the rate of decomposition. These results are consistent with a mechanism for decomposition in which the hydroxamic hydroxyl group acts as a nucleophile (Scheme 4.5) in a concerted (?) process that eliminates formic acid.

The biological activity of compounds **1** - **18** (page 18-19) have been evaluated as

growth inhibitors of the larvae of the European corn borer (*Ostrinia nubilalis)* in four-day feeding trials. Several have shown activities equal to (**6**, **8**, **10**) or better than (**7**, **9**) DIMBOA, the most abundant hydroxamic acid in maize. It appears that there are at least two modes of reactivity occurring. In the growth studies the amides HBOA and HMBOA (**12** and **13**) were not active, but during *in vitro* testing of inhibitory properties towards two classes of insect proteases these same compounds were the most potent. It is possible that inhibition of gut proteases is not the sole mode of toxicity to corn borer larvae, and yet the nutritional indices for DIMBOA clearly indicate that it disrupts digestion within the gut. It could be that the amides are more easily detoxified by the insect and that *in vitro* measurements of their activity are misleading. On the other hand, the hydroxamic acids may interfere with digestion, not by interacting directly with digestive enzymes, but by inhibiting secretion of the enzymes or by inhibiting those enzymes in the gut wall that maintain the gut pH and ion balance.[2] It is also recognized that hydroxamic acids are excellent nucleophiles when ionized[3,4] since they fall into the general class of nucleophiles governed by the α-effect. Thus, one must not eliminate the possibility that these compounds are biologically activated to produce the real toxin. A greater measured activity may reflect a greater ease of acylation at the hydroxamic hydroxyl to produce a potent electrophile similar to those shown in Figure 1.3.

The biological activites do not correlate with the rates of reduction of the compounds by mercaptoethanol. Only the ring oxygenated analogues **1** - **4** had measurable rates and they did not inhibit growth of the larvae to a degree significantly different from control. It was evident, however, that those compounds (**3**, **4**, **17**) that rapidly degraded to benzoxazolinones were inactive as growth inhibitors, whereas those compounds that remained as hydroxamic acids in solution longer showed greater activites. The additional criteria for activity (other than the hydroxamic acid moiety) was the presence of a 2-hydroxy group, since compounds **12** - **16** and **18** were inactive. A postulated mechanism for the reduction reaction involving direct attack of thiolate on nitrogen is consistent with Saville's rules for the interaction of a soft base (RS^-) with a soft acid (N).

Although only a few compounds reacted with mercaptoethanol such that they were reduced at nitrogen, NMR spectra recorded during the reaction show that addition of thiol to the aldehyde of the open form takes place for both those compounds that are reduceable and for those that are not. It is probable, therefore, that attack of biological nucleophiles at this site is required for activity. Recall that compounds lacking this functionality (**14**, **15**, **16** and **18**) did not inhibit growth in feeding trials. The 2-hydroxy group appears not to be necessary for enzyme inhibition activity since compounds **14**, **15** *and* MBOA inhibit the

enzymes to roughly the same degree. Variation within the enzyme inhibition assays is difficult to explain without taking into consideration the myriad of factors that affect enzyme rates and substrate selectivity[5,6] and this was not a goal of the present work. The results reported herein will allow future studies to be more specific in their questions; about those species or sites within the gut that participate in the pharmacokinetics of cyclic hydroxamic acids and about the molecular mechanism of that interaction.

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EXPERIMENTAL

7.1 MATERIALS AND METHODS

Chemicals

All reagent chemicals were purchased from Aldrich Chemical Compa where indicated. Solvents were glass distilled, initial and final fractions being Methylene chloride and carbon tetrachloride were stored over 4Å molecular s had been activated at 250°C overnight. Absolute MeOH and EtOH were pr distillation of the 99% alcohol over Mg turnings with I_2 following the method Mercaptoethanol was distilled over $LiAlH_4$ and stored under nitrogen. The l periodically checked by GC for the presence of impurities. Microanalyses were by M-H-W Laboratories, Phoenix, Arizona. Melting points were determined on Uni-melt apparatus and are uncorrected.

Chromatography

Thin-layer chromatography (TLC) was performed on aluminum bac plates (Whatman, AL-SIL-G/UV, 250 μm). Visualization of hydroxamic acids respective amides was achieved by dipping the plate in a 5% H_2SO_4 solution and heating on a hot plate. Most of the hydroxamic acids turned a purplish-brov The amides gave less intense colours and were beige to brown.

Column chromatography (gravity flow) for purification of the nitroph performed on Terochem silica (normal type) equivalent to Merck 7734, 60-200 6 x 20 cm column.

Chromatographic purification of hydroxamic acids and of lactams performed using the Chromatotron® (Model 7924T, Harrison Research, I California) with either 1mm or 2mm silica covered rotors. The plates were m Merck Kieselgel 60 PF_{254} silca for preparative TLC.

Gas chromatographic analyses were performed on a Varian 6000, Vi chromatograph, fitted with a 10m length of a vitreous silica capillary colum BP5-0-25, i.d. 0.22 mm). Approximately 0.5 mg of the sample to be anal derivatized with one drop of bis(trimethylsilyl)acetamide (BSA) at room te After 5-10 minutes the material was brought up in ~400 μL of CH_2Cl_2 and 0.5 this solution was injected into the GC with a Hamilton syringe. Most injection with the same temperature program; 150°C for two minutes, then warmed at 10 to 250°C and maintained there for 10-25 min. These conditions gave good sep

all components in under 20 minutes. The carrier gas was helium and the detecor a FID.

Spectrophotometers

60 MHz ^{1}H-NMR spectra were recorded on a Varian EM360A spectrometer (with tetramethylsiane, TMS, as internal standard) and 300 MHz ^{1}H-NMR spectra on a Varian XL-300 instrument. ^{13}C-NMR spectra were obtained on a Varian FT-80 (80 MHz) instrument. 300 MHz ^{1}H-NMR spectra were referenced on the solvent peak - either acetone-d_6 or $CDCl_3$. ^{13}C-NMR spectra were refernced on DMSO-d_6. Proton spectra that were recorded in D_2O buffers were referenced at the HDO signal (1385.7 Hz).

Infrared spectra were recorded on a Perkin-Elmer 783 IR spectrophotometer and ultraviolet (UV) spectra on a Cary (Varian) 2200 UV-VIS spectrophotometer using quartz cuvettes.

Buffers and pH

All buffers (citrate, acetate, TRIS, and carbonate) were made to 0.1 M except phosphate which was made between 0.05 and 0.08 M. KCl was added (if necessary) to make all ionic strengths (I) equal to 0.15. pH measurements were made on a Radiometer, Copenhagen, PHM62 Standard pH meter using a Fisher calomel elctrode (SN8019153). TRIS buffers [2-amino-2-(hydroxymethyl)-1,3-propanediol] were measured at the temperature at which they were intended to be used.

pK_a Measurements

Acid dissociation consants (pK_a's) were determined spectrophotometrically[2] at 37°C ± 0.3 C°, I=0.15. The temperature within the spectrophotometer was maintained by a circulating thermostated water bath (Haake, D1 L). The entire apparatus was always allowed to warm for at least 40 minutes prior to the day's first runs. No fewer than five determinations of pK_a were made for each compound, most having seven. Analytical wavelengths were chosen where the absorbance difference between the species in solution was the largest. Isosbestic points were clearly present in the determination of pK_{a1}, confirming the presence of two species in equilibrium. They were less obvious in the determination of pK_{a2}, because the absorbance differences in this case were much smaller.

Preparation of Hydroxamic Acid Stock Solutions

Stock solutions of the hydroxamic acids were made at concentrations such that 100 µL of stock, diluted in 5.00 mL of buffer gave an absorbance (λ_{max}) 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 x 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 (CH_2Cl_2/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 other hand, the lactams HBOA and HMBOA (**12** and **13**) re-acetalize slowly on standing in MeOH solutions. Consequently, solutions of these compounds were made fresh when needed.

Reaction of Hydroxamic Acids With Mercaptoethanol (ME)

(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 ME (~40 fold molar excess). The solutions in 10 mL vials were well sealed and left in a thermostatted oven at 45°C for 16 hours. After this time some of the solutions had developed faint colours, 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 colours, if present, lightened), and immediately extracted with EtOAc. The organics were dried over anhydrous $MgSO_4$ and the solvent removed *in vacuo*. The oils that remained were derivatised with BSA and analysed by GC/MS immediately. Waiting before injection for any more than an hour gave poor chromatograms with few recognizable peaks. Samples were worked up indiviually to minimize the time spent in the acidified solution. Not surprisingly, much of the starting ME remained. It occasionally interfered with the observation of benzoxazolinone products (BOA's r.t.=4-9 minutes versus 4.8 minutes for ME(TMS_2), but not with observation of the lactams or starting hydroxamic acids (r.t.=6-11 minutes). The temperature program was always 150°C for 2 minutes then increased to 250°C at the rate of 10 C°/min.

(ii) Measurement of the kinetics of reaction with mercaptoethanol (ME)

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°C higher than the thermostatted 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 ME (80-400 fold excess) such that a series of buffer solutions of increasing concentration of ME 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 ME in 10.00 mL 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 ME) was blanked against the ME containing buffer of the appropriate concentration. When all cuvettes had been loaded into the machine (this took ~5 minutes) acquisition of absorbance data was not begun for another 1.5 minutes 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 very close to the wavelengths used in the pK_a determinations. Data was acquired and manipulated by a Varian DS-15 data station and the 2200/2300 Series/DS-15 Kinetics storage and calulation 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 were less than ± 2.5%.

Determination of the Rate Constants for the Decomposition of Hydroxamic Acids to Benzoxazolinones

Two different measurements of the rate constants for the decomposition reaction were made; (i) the pH was held constant and most of the synthetic series was investigated (see Table 5.3), and (ii) the pH was adjusted to equalize the concentrations of the monodissociated hydroxamic acid for only the C-7 substituted series (see Chapter 4). In the first instance the methodology was very similar to that described above for reaction with ME. Buffer of pH 9.00 , either TRIS or carbonate (0.1 M, I=0.15), was warmed to ~45°C (8 C° higher than the trial temperature of 37°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 then placed in the spectrophotometer and the sample 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. Some of the values reported in Table 5.3 have been estimated because of either their rapidity of reaction (compound **17**) or their sluggishness (compounds **6-8,** and **11a**) at these conditions.

Rate constants for the C-7 substituted series were determined at a higher temperature (48°C ± 0.3 C°) so that more reliable values could be obtained for the entire

series, especially those that were less reactive. At this temperature the product spectra for the 7-F compound **10** did not resemble a benzoxazolinone so a rate constant has not been reported. In these trials, stock solutions of hydroxamic acids were made up in acetone. Warmed buffer (0.1 M phosphate or 0.2 M carbonate) of the appropriate pH (see Table 4.1 and text) was added to a vial to which had been added 100 μL of stock and the acetone evaporated under a stream of nitrogen. The vial was capped and shaken briefly to ensure dissolution. Absorbance readings were then acquired at the same analytical wavelengths as used previously and data stored and manipulated as described above.

Feeding trials and enzyme assays

These two measures of the activity of the analogues described herein were performed by Francesca Campos and are reported in detail in her Ph.D. thesis, Dept. of Biology, University of Ottawa, 1989.

References

1. Vogel, A.I. *A Textbook of Practical Organic Chemistry,* 3rd Edition, Longman, London, 1956, p. 167.
2. Albert, A.; Serjeant, E.P. *The Determination of Ionization Constants,* 3rd Edition, Chapman and Hall, London, 1984, Chapter 4.

7.2 SYNTHESIS OF NITROPHENOLS

Most of the o-nitrophenols were made by nitrating 3-substituted phenols in acetic acid, some from published procedures and others by analogous methods. In some instances the starting phenols were not commercially available and were synthesized.

Nitration of very active phenols can be a dirty business. Oxidation and polymerization are frequent problems which can severely limit yields. Protection of the phenol as an ester often helps. Historically, nitric acid has been added to cooled solutions of the phenol, but this can compound polymerization by keeping the concentration of phenol high. Cooling the phenol solution can worsen the situation for reactive aromatics, since when cool there is usually a threshold concentration of HNO_3 below which nitration occurs only slowly if at all. Increasing the concentration of HNO_3 by further addition will finally initiate the reaction, which is exothermic, and it will likely 'run away with itself'.[1] Consequently, in this work, the reactive phenols were added as solutions in acetic acid to rapidly stirring solutions of HNO_3 in acetic acid.

5-Methoxy-2-nitrophenol, 28

Following the method of Kinugawa *et al*[2] which utilizes fuming nitric acid, we witnessed instantaneous oxidation resulting in black-red tars. Using normal (70%) HNO_3 and larger volumes of acetic acid the yields are fair. 3-Methoxyphenol (Fluka) (50g, 0.40 mol) was dissolved in 300 mL of acetic acid and added dropwise over 5 hours to a rapidly stirring solution (magnetic stirrer) of 700 mL AcOH, 150 mL acetone and 28 mL 70% HNO_3 (1.1 eq) which was cooled in an ice bath to 5-10°C. The deep red solution was then poured into a 2-L beaker half-filled with crushed ice and a precipitate soon formed. After filtering, the damp solid was recrystallized from EtOH giving 27.5g (40%) of tan crystals. The material could be chromatographed on silica ($CHCl_3$) to get analytically pure material which was a bright lemon-yellow, but the crude crystals were sufficient for making the potassium phenoxide and carrying into the next step. mp 92-93°C (Lit.[3] 91.5-92.5°C) ^1H NMR (60 MHz,$CDCl_3$) δ 7.85 (d, 1H, $J_{4,5}$ = 10 Hz), 6.4 (m, 2H,), 3.86 (s, 3H). MS(EI) m/z 169(88)M^+, 139(47), 136(8), 111(48), 108(18), 95(13), 93(12) 79(25). IR ($CHCl_3$)

ν_{max} 3260-3080 (br), 2940 (m), 1622 (s), 1595 (s), 1532 (m), 1487 (m), 1445 (m), 1333 (m), 1285 (s), 1257 (s), 1187, 1162, 1153, 1193 cm^{-1}.

2,3-Dimethoxy-6-nitrophenol, 29

A variation of the method of Orphanos and Taurins[4] was used. 2,3-Dimethoxybenzaldehyde (10.0g, 60.2 mmol) in 80 mL of $CHCl_3$ was added dropwise over 20 minutes to a cooled (15°C, cold water) stirring solution of m-CPBA in $CHCl_3$,(17g 85% m-CPBA in 300 mL $CHCl_3$). When the originally exothermic reaction was complete the solution was set to reflux for 3 hours. GC analysis of the reaction mixture at that time revealed the complete disappearance of starting material. On cooling some m-chlorobenzoic acid crystallized and could be filtered off. The remaining solution was washed 2 x 150 ml 10% Na_2CO_3 in which had been dissolved a few grams of sodium sulphite. The organics were dried ($MgSO_4$) and evaporated to 10.1g of an orangey oil. This oil (the crude formate ester) was dissolved in 50 mL of acetic acid and added dropwise over 2.5 hours to a rapidly stirring solution of HNO_3 (4.2 mL) in AcOH (250 mL) at room temperature. Thirty minutes after addition was complete the reaction mixture was poured into ice-water. An oil separated and was extracted with EtOAc. The wet EtOAc solution was then evaporated and the residual oil brought up in 10% NaOH and heated to 80° for one hour. On acidification yellow crystals precipitated and were filtered. Recrystallization from EtOH yielded 5.5g (46% from starting benzaldehyde) of 2,3-dimethoxy-6-nitrophenol. mp 101-102°C (Lit.[5]102-103°C) ^1H NMR ($CDCl_3$) δ 7.90 (d, 1H, *J*=10 Hz), 6.55 (d, 1H,) 3.95 (s, 3H), 3.89 (s, 3H) MS(EI) m/z 199(100)M^+, 182(99), 181(12), 156(17), 139(39), 123(12), 109(19), 96(19). IR ($CHCl_3$) ν_{max} 3320-3060 (br, m), 2950 (m), 2855 (m), 1620 (s), 1591 (m), 1539 (s) 1487 (s), 1460 (s), 1440 (s), 1350 (m), 1337 (m), 1290 (vs), 1100 (s), 1022 (m) cm^{-1}.

4,5-Dimethoxy-2-nitrophenol, 30

This synthesis was completely analogous to that of 2,3-dimethoxy-6-nitrophenol described above. 3,4-Dimethoxybenzaldehyde (15.0g, 90 mmol) gave 16.0g of the uncharacterized formate ester by oxidation with m-CPBA. This oil was nitrated in AcOH at room temperature and when the reaction mixture was poured into ice-water precipitated yellow crystals after about 20 minutes. These were filtered off and dissolved in 300 mL of 10% NaOH and heated at 80°C for 1 hour. On acidification (HCl) a yellow solid precipitated which when filtered and dried yielded 8.30g of the nitrophenol. Another 1.17g could be recovered by extraction (EtOAc) of the aqueous portion after nitration. Total

yield: 9.47g (53%) of bright lemon-yellow microcrystals. mp 142-143.5°C (Lit.[4]145-146°C) ^{1}H-NMR (60 MHz, $CDCl_3$), δ 7.48 (s,1H), 6.53 (s,1H), 3.96 (s, 3H), 3.90 (s, 3H). MS(EI) m/z 199(100)M^+, 184(38), 125(7), 110(9), 95(19), 69(24), 66(12). IR ($CHCl_3$) ν_{max} 2975, 2951, 2845 (all weak), 1634 (s), 1595 (s), 1529 (s), 1508 (s), 1440 (s), 1420 (m), 1320 (m), 1280 (vs), 1178 (s), 1090 (s), 1132 (m), 1102 (s), 880 (m), 860 (m) cm^{-1}.

2,3-Methylenedioxy-6-nitrophenol, 31

(Synthesized as above). 2,3-Methylenedioxybenzaldehyde[6] (18.25g, 0.12 mol) gave 15.25g of crude formate ester. 5.7g of this ester could be nitrated, hydrolysed and recrystallized (EtOH) to yield the nitrophenol, (4.85g, 58% from the aldehyde). mp 167-168°C. ^{1}H-NMR (60 MHz,$CDCl_3$) δ 7.79 (d, 1H, *J*=9Hz), 6.70 (d, 1H,) 6.06 (s, 2H). MS(EI) m/z 183(100)M^+, 167(4), 166(5), 153(10), 137(14), 123(4), 107(19), 95(5), 80(16). IR ($CHCl_3$) ν_{max} 3380-3060 (br, m), 2915 (m), 2800 (w), 1656 (s), 1615 (w), 1530 (s), 1470 (s), 1408 (w), 1340 (vs), 1290 (vs), 1165 (m), 1070 (s), 1035 (s), 940 (m) cm^{-1}.

4,5-Methylenedioxy-2-nitrophenol, 32

This nitrophenol was synthesized in excellent yield following the method described for this compound in reference 5. mp 93-94°C (Lit[4] 95-96°C and 93-94°C) ^{1}H-NMR (60 MHz, $CDCl_3$) δ 7.28 (s, 1H), 6.42 (s, 1H), 5.93 (s, 2H). MS(EI) m/z 183(100)M^+, 153(13), 137(5), 107(26), 95(6), 79(16), 69(28), 53(71). IR ($CHCl_3$) ν_{max} 3210-3060 (br, w), 2920 (w), 1620 (s), 1543 (s), 1508 (m), 1478 (vs), 1447 (s), 1325 (w) 1315 (w), 1272 (s), 1162 (m), 1143 (m), 1040 (s), 940 (s), 870 (s) cm^{-1}.

6-Methoxy-2-nitrophenol (nitroguaiacol), 33

Guaiacol, (20.0g, 0.16 mol), was dissolved in 100 mL of AcOH and added dropwise to a solution of 12 mL 70% HNO_3 in 400 ml of AcOH which had been cooled to 8-10°C in an ice-water bath. Complete addition took about two hours whereupon the reaction mixture was a very dark red. It was stirred for another hour, warmed to room temperature and then poured into ice. No crystals separated so the water was extracted with $CHCl_3$. After washing with brine and drying with Na_2SO_4 the $CHCl_3$ was evaporated leaving a dark oil which slowly crystallized after an hour at room temperature. This material was chromatographed on silica (21cm x 6cm column, essentially a large silica plug) eluting with $CHCl_3$/hexane. Combining the pertinent fractions (those of highest R_f) gave **33** as an orange solid. (10.1g, 37%) mp 54-56°C (Lit.[7] 62°C) ^{1}H-NMR (60 MHz,

$CDCl_3$) δ 7.50 (dd, 1H, $J_{3,4}$= 9Hz, $J_{3,5}$= 2Hz), 6.9 (mult, 2H), 3.83 (s, 3H). MS(EI) m/z 169(100)M^+, 152(54), 122(13), 121(19), 109(24), 108(35), 107(35), 93(18), 79(26). IR ($CHCl_3$) ν_{max} 3340-3120 (br, w), 2978, 2950, 2850 (all weak), 1618 (m), 1549 (s), 1460 (s), 1395 (m), 1331 (s), 1160 (m), 1090 (m), 1057 (m), 839 (m) cm^{-1}.

5-Dimethylamino-2-nitrophenol, 34

3-Dimethylaminophenol (13.5g, 99 mmol) was acetylated as in reference 5 and the resultant dark green oil was nitrated in AcOH at room temperature (as above) and then gently warmed (40°C) for one hour. The dark mixture was poured into water, extracted with $CHCl_3$, evaporated, and then heated to 80°C for 1.5 hours in 10% NaOH. On careful acidification (HCl) a precipitate formed and was filtered off. The yellow-green solid was used without further purification. Yield of dry solid; 8.8g (49% from starting phenol) mp 140-141°C. ^{1}H-NMR (60 MHz,$CDCl_3$) MS(EI) m/z 182(100)M^+, 136(20), 135(12), 124(17), 108(9), 107(7), 106(7), 92(14). IR ($CHCl_3$) ν_{max} 3260-3060 (vw), 1630 (s), 1570 (s), 1550 (m), 1450 (m) 1396 (m), 1325 (s), 1275 (s), 1178 (s), 1102 (w), 1056 (w), 975 (w), 901 (w), 833 (w) cm^{-1}.

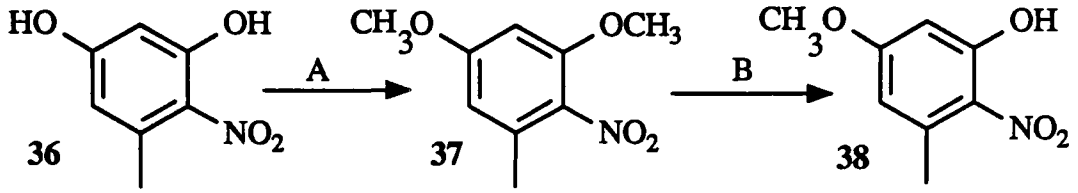
5-Acetamido-2-nitrophenol, 35

This material was synthesised following the procedure in reference 8. mp 215-216°C. (Lit.[8,6] 217-218°C, 216°C) ^{1}H-NMR (60 MHz, acetone-d_6) δ 8.10 (d, 1H, $J_{3,4}$=11Hz), 7.80 (d, 1H, $J_{4,6}$=3Hz), 7.18 (dd, 1H), 3.02 (bs, exchangeable with D_2O), 2.32 (s, 3H). MS(EI) m/z 196(35)M^+, 154(73), 150(13), 124(16), 108(11), 96(8), 80(6), 51(12), 43(100). IR (KBr) ν_{max} 3310 (s), 1688 (s), 1600 (vs), 1555 (s), 1525 (s), 1478 (m), 1395 (m), 1330 (s), 1315 (s), 1282, 1271 (vs), 1252 (s), 1230 (m), 1184 (m), 1154 (m) 1095 (w), 823 (w), 764 (m) cm^{-1}.

5-Methoxy-3-methyl-2-nitrophenol, 38

Orcinol (3,5-dihydroxytoluene) was nitrated according to reference 8. This method involves nitration in ether at 0°C and later bringing the solution to reflux. The reaction procedes very slowly at first and the experimenter should resist the urge to warm prematurely the mixture or reduce the volume of ether. Addition of the nitric acid should be slow until an obvious change in colour (to the yellow-orange of the nitrated product) has taken place, whereupon the rate of addition may be increased. Adding too much HNO_3 before the material has actually begun to react can make the reaction race out of control; warming up, over-oxidizing and releasing gaseous nitrogen oxides.

Attempts were made to monomethylate nitroorcinol directly to the o-nitrophenol **38**, but selectivity was poor and over methylation a problem. However, the net conversion was accomplished in two high yielding steps as shown below.



A: K_2CO_3,acetone,MeI B: BBr_3,CH_2Cl_2

Scheme 6.1.

(A) Nitroorcinol **36**, (2.48g, 13.4 mmol) was dissolved in 80 mL of acetone to which was added 2g of K_2CO_3. While at room temperature 1.8 mL (2 eq) of MeI was added as a solution in 20 mL of acetone. (Reference 9 prepares this material with Me_2SO_4, but it was found that this complicated purification due to the reaction being incomplete.) The stirring mixture was then set to reflux overnight. After cooling, the reaction mixture was poured into ice water and the light brown precipitate was filtered. This material was 96% pure by GC analysis and yielded 2.50g (87%) of **37**. It was used directly in the next step. A sample was chromatographed for analysis. mp 103-103.5°C (Lit.[9] 106-107°C). ^{1}H-NMR (60 MHz, $CDCl_3$) δ 6.17 (s, 2H), 3.66 (s, 6H, overlapping CH_3O signals), 2.18 (s, 3H). MS(EI) m/z 197(100)M^+, 180(96), 167(23), 139(30), 136(19), 124(15), 121(22), 120(15), 109(12), 108(11). IR ($CHCl_3$) ν_{max} 2950 (w), 2850 (w), 1600 (s), 1525 (s), 1461 (m), 1370 (m), 1342 (s), 1171 (s), 1128 (m), 1100 (m), 1053 (w), 845 (m), 837 (m) cm^{-1}.

(B) Two batches of **37** were pooled for this reaction. **37** (5.5g, 27.9 mmol) was dissolved in 100 mL of CH_2Cl_2 and cooled to 0°C. To this was added 1.0 M BBr_3 in CH_2Cl_2 (28 mL, 1 eq) under nitrogen via syringe. The orange solution turned a *deep* orange-red, almost black. After stirring for one hour TLC showed complete disappearance of starting material. 20 mL of THF was then injected into the reaction while cold (some white fumes were visible, but there was no warming) and then 50 mL of H_2O was poured into the open reaction vessel. The mixture was separated in a funnel, the aqueous layer wwas extracted 1 x CH_2Cl_2 and 1 x EtOAc, the organics were washed with brine, dried and evaporated (Na_2SO_4) to give a brown solid. This was recrystallized from EtOH which gave 3.64g in two crops. A further 1.32g could be recovered after column chromatography (SiO_2, $CHCl_3$). Total yield of **38**: 4.96g (97%) mp 99-99.5°C.(Lit.[10] 104-106°C) ^{1}H-NMR (60 MHz, $CDCl_3$) δ 6.40 (broad singlet which on expansion becomes a multiplet, 2H) 3.87 (s,

3H), 2.66 (s, 3H), 1.9 (very broad, 1H). MS(EI) m/z 183(80)M^+, 166(100), 138(19), 136(26), 125(28), 124(10), 123(55), 122(15), 111(28), 110(14), 109(15), 108(11). IR ($CHCl_3$) ν_{max} 3440-3060 (w), 2950 (w), 2860 (w), 1620 (s), 1596(s), 1540 (m), 1471 (m), 1458 (m), 1444 (m), 1425 (w), 1380 (m), 1338 (m), 1296 (s), 1184 (s), 1170 (s), 1065 (w), 1041 (w), 1000 (w), 956 (w), 850 (w) cm^{-1}.

5-*t*-Butyl-2-nitrophenol, 39

3-*t*-Butylphenol (15.0g, 0.10 mol) in 75 mL of AcOH was added dropwise over two hours to a solution of 7.5 mL 70% HNO_3 in 150 mL of AcOH which had been cooled to 10°C. The yellow solution was then allowed to warm to room temperature where it sat over night. Some starting phenol was still visible on TLC so the solution was warmed (40°C) for one hour. The contents were then poured into cold water, extracted with $CHCl_3$, dried (Na_2SO_4), evaporated, and the residual oil chromatographed on a silica column eluting with hexane:$CHCl_3$ (5:1). The first eluting fractions, on NMR analysis, proved to be the desired material. 4.50g were collected initially and on re-chromatographing the later eluting isomers another 1.50g could be recovered. Total yield of **39**: 6.00g (31%) of a bright yellow mobile oil. ^{1}H-NMR ($CDCl_3$) δ 8.00 (d, 1H, $J_{3,4}$=9.0 Hz), 7.12 (d, 1H, $J_{6,3}$=2.0 Hz), 7.01 (dd, 1H). 1.52 (s, 9H). MS(EI) m/z 195(31)M^+, 180(100), 152(12), 134(13), 122(6), 105(4). IR ($CHCl_3$) ν_{max} 3400-3080 (br,m), 2975 (s), 2918 (w), 2880 (w), 1624 (s), 1591 (s), 1525 (m), 1485 (s), 1463 (m), 1440 (s), 1372 (s), 1328 (vs), 1280 (s), 1180 (s), 1072 (m), 946 (s), 881 (w), 850 (w), 828 (w) cm^{-1}.

5-Chloro-2-nitrophenol, 40

An aromatic nucleophilic displacement reaction was used to synthesize this compound. 2,4-Dichloronitrobenzene was dissolved in a dioxane/NaOH mixture and the solution refluxed in the presence of a phase transfer catalyst.[11] This method allowed easy preparation of 40g quantities of **40** in 90% yields. mp 39.5-40°C (Lit.[3] 39-40°C) ^{1}H-NMR ($CDCl_3$) δ 8.30 (d, 1H, $J_{3,4}$=9.2Hz), 7.17 (d, 1H, $J_{6,4}$=2.2Hz), 6.95 (dd, 1H). MS(EI) m/z 175(24) and 173(72)M^+, 145(13), 143(38), 117(8), 115(26), 101(8), 99(26). IR ($CHCl_3$) ν_{max} 3460-3080 (br, s), 1619 (s), 1586 (s), 1529 (s), 1478 (s), 1461 (s), 1320 (s), 1300 (s), 1183 (m), 1159 (m), 1098 (m), 1079 (w), 1080 (w), 1042 (w), 978 (w), 928 (s), 870 (m), 847 (m) cm^{-1}.

5-Fluoro-2-nitrophenol, 41

3-Fluorophenol (12.0g, 0.107 mol) in 20mL of AcOH was added over 15 minutes to a solution of 10 mL 70% HNO_3 in 200 mL AcOH at room temperature. The solution was then warmed to 50°C in a water bath and stirred for four hours. At this time, GC analysis showed the final disappearance of the starting phenol. The reaction mixture was poured into water and extracted with $CHCl_3$, washed with water and then brine, dried ($MgSO_4$) and evaporated to give 13.2g of a orange oil. This was chromatographed on silica, eluting with 1:1 hexane:CH_2Cl_2. An NMR analysis of the purified fractions showed that the first isomer to elute was the one desired. The fractions were evaporated to give pure 5-fluoro-2-nitrophenol (5.8g, 34%) as a bright yellow oil that smelled like wood smoke. (Lit.[3] mp 29-30°C) ^{1}H-NMR ($CDCl_3$) δ 8.10 (dd, 1H, $J_{3,4}$=9.5Hz, $J_{H4,F}$=7.0Hz), 6.83 (dd, 1H, $J_{6,4}$=2.5Hz, $J_{H6,F}$=7Hz), 6.73 and 6.69 (2 x q, 1H,) MS(EI) m/z 157(100)M^+, 127(20), 126(10), 111(5), 110(4), 99(16), 83(32), 82(22), 81(11). IR ($CHCl_3$ ν_{max} 2965 (s), 2935 (vs), 2860 (s), 1752 (m), 1625 (m), 1598 (m), 1530 (m), 1495 (w), 1370 (m), 1355 (m), 1285 (m), 1263 (m), 1090 (s), 1020 (m) cm^{-1}.

5-Carbomethoxy-2-nitrophenol, 42a (methyl 3-hydroxy-4-nitrobenzoate)

Commercial 3-hydroxy-4-nitrobenzoic acid (5.00g, 27.3 mmol) was esterified by refluxing in a solution made up of 100 mL CH_2Cl_2, 100 mL absolute methanol, and 5 mL concentrated H_2SO_4 overnight with a drying tube attached to the condenser. When cool the contents were poured into water, separated, and the aqueous layer extracted again with CH_2Cl_2. The organics were then evaporated and the residual solid recrystallized from EtOH to give **42a** (5.38g, 93%) of the title nitrophenol. mp 89-90°C (Lit.[3] 90.5-91°C). ^{1}H-NMR (60 MHZ, $CDCl_3$) δ 7.94 (d, 1H, $J_{3,4}$=9Hz), 7.60 (d, 1H, $J_{6,4}$=2HZ), 7.40 (dd, 1H), 3.80 (s, 3H) MS(EI) m/z 197(67)M^+, 166(100), 136(13), 120(18), 119(17), 108(11), 91(9), 63(17). IR ($CHCl_3$) ν_{max} 3700 (m), 3410-3140 (br,m), 2960 (w), 1732 (s), 1634 (m), 1595 (s), 1538 (m), 1485 (s), 1444 (s), 1372 (w), 1326 (vs), 1288 (vs), 1110 (m), 1079 (m), 997 (m), 985 (m), 919 (w), 901 (w), 848 (m) cm^{-1}.

5-Carboethoxy-2-nitrophenol, 42b (ethyl 3-hydroxy-4-nitrobenzoate)

In a similar manner as for **42a**, 3-hydroxy-4-nitrobenzoic acid (7.0g, 38.2 mmol) was dissolved in 200 mL of $CHCl_3$ and 80 mL of absolute ethanol containing 8 mL of concentrated H_2SO_4 and refluxed for 24 hours. After cooling, the solution was washed with water and then brine, dried, and evaporated to a light orange solid. This material was found to be 98% pure by GC analysis. Yield of **42b**: 7.8g (97%). mp 84-85°C (Lit.[3] 84-85°C) ^{1}H-NMR (60 MHz, $CDCl_3$) δ 7.95 (d, 1H, $J_{3,4}$=9 Hz), 7.65 (d, 1H, $J_{6,4}$=2.5 Hz),

7.48 (dd, 1H, $J_{4,6}$=2.5 Hz, $J_{4,3}$=9 Hz), 4.33 (q, 2H), 1.41 (t, 3H). IR ($CHCl_3$) ν_{max} 3280 (br), 3000 (w), 1730 (vs), 1633 (s), 1595 (s), 1540 (m), 1449 (m), 1376 (m), 1325 (vs), 1287 (vs), 1110 (m), 1000 (m), 1023 (m), 961, 905, 850 cm^{-1}.

5-Trifluoromethyl-2-nitrophenol, 43

3-Trifluoromethylphenol (10.0g, 61.7 mmol) in 25 mL of AcOH was added dropwise over 45 minutes to 35 mL of 70% HNO_3 cooled to 3°C. During the addition the ice bath was allowed to melt and to warm to room temperature. As the reaction proceeded a brown gas (NO_2) was clearly visible over the solution. After 30 minutes at room temperature the flask contents were poured into ice-water. The resulting orange-red oil was extracted from the water with EtOAc. This oil was chromatographed on silica, eluting with hexane-CH_2Cl_2 (2:1). The first fractions collected were shown by NMR and TLC characteristics to be the desired isomer. The yield of pure **43** was 2.60g (20%) as a thick orange oil. 4.60g of other isomers and/or products were rinsed from the column with CH_2Cl_2, but these were not characterized. ^{1}H-NMR (60 MHz, $CDCl_3$) δ 8.25 (d, 1H, *J*=9Hz), 7.42 (d, 1H, *J*=1.5Hz), 7.23 (dd, 1H) MS(EI) m/z 207(100)M^+, 188(16), 177(17), 161(28), 149(23), 141(11), 132(22), 113(25), 101(16), 96(15). IR ($CHCl_3$) ν_{max} 3780 (br, m), 1638 (m), 1599 (s), 1545 (s), 1490 (s), 1450 (m), 1377 (m), 1338 (vs), 1308 (s), 1182 (s), 1150 (vs), 1162 (m), 1128 (w), 890 (m), 849 (m), 831 (m) cm^{-1}.

5-Cyano-2-nitrophenol, 44

3-Cyanophenol (8.0g, 67.1 mmol) in 40 mL of AcOH was added dropwise over 15 minutes to 35 mL of 70% HNO_3 cooled to 10°C in an ice water bath. After one third of the phenol had been added, the cooling bath was removed and the flask contents were allowed to warm to room temperature, where addition was completed. A slight evolution of a brown gas (NO_2?) was visible. After 20 minutes at room temperature, the reaction mixture was poured into ice water and the resulting crystals filtered off . TLC showed that these were not pure so this material and that recovered by extraction (EtOAc) of the aqueous phase were combined and evaporated onto about 30g of silica. This was poured onto the top of a prepared silica column (21 x 6 cm) and eluted with hexane:CH_2Cl_2 3:1, 2:1, 1:1, 1:3, and finally just CH_2Cl_2 with 1% acetone. Again, the first fractions off the column contained the desired material as determined by NMR. On evaporation they yielded **44** (2.29g, 20%) as a light yellow solid. mp 118-119.5°C. (Lit.[12] 120°C) ^{1}H-NMR ($CDCl_3$) δ 8.22 (d, 1H, $J_{3,4}$=8.8Hz), 7.49 (d, 1H, $J_{4,6}$=1.8Hz) 7.26 (dd, 1H) MS(EI) m/z 164(100)M^+, 134(36), 118(7), 117(7), 106(31), 90(16), 89(10), 64(14), 63(52), 62(23). IR ($CHCl_3$) ν_{max}

3270 (br, w), 2245 (w), 1629 (s), 1590 (vs), 1547 (w), 1482 (s), 1447 (w), 1329 (vs), 1274 (s), 1078 (w), 967 (m), 885 (m), 849 (m) cm^{-1}.

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7.3 SYNTHESIS AND REDUCTIVE CYCLIZATION OF METHYL α-(o-NITROPHENOXY)-α-METHOXYACETATES

All compounds 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 hours as observed by the disappearance of the brightly coloured nitrophenoxides. Yields were dependent on the completeness of removal of HBr from the solutions of MBMA. A representative procedure for the bromination of methyl methoxy acetate and subsequent coupling with potassium 5-methoxy-2-nitro phenolate is given below.

Bromination of Methyl Methoxyacetate

A modification of the method of Bendich *et al*[1] was used. Methyl α-bromo-α-methoxyacetate was made fresh 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. Usually, no more than 2 mmol (~ 2g) of the acetate was brominated at one time, but the technique was adequate for scales as large as 20g.

In a 500 mL three-necked round-bottomed flask equipped with an efficient condenser, stirring bar, and a dropping funnel, methyl methoxyacetate (11.3g, 10.8 mmol) was set to reflux in 200 mL of dry CCl_4. Over 4 hours, Br_2(17.3g,10.8 mmol) in 25 mL of CCl_4 was added dropwise to the solution. Throughout the reaction a slow stream of nitrogen was flushed through the flask from a needle piercing a rubber septum fitted to the flask. A white mist could be seen blowing from the top of the condenser, and this was scrubbed by fitting a tube to the top of the condenser and immersing one end in a small volume of 1N NaOH. 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 minutes this could be completed by briefly evaporating a small portion of the solution at reduced pressure. (Addition of portions of carbonate as a buffer was not effective.)

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. For those nitrophenols that were not completely soluble in ether, up to one third volume could be EtOH. The KOH/EtOH solution was added until obvious precipitation of the insoluble salt stopped. The solutions were chilled in a freezer or ice bath for 30 minutes and the colourful (bright yellows to reds) potassium phenoxides filtered with suction. They were rinsed with a 3:1 solution of Et_2O:EtOH (v/v) and dried under vacuum. Yields were all >95%.

Typical Coupling Procedure: α-bromo-α-methoxyacetate + potassium 5-methoxy-2-nitrophenoxide

Potassium 5-methoxy-2-nitrophenoxide (20.7g, 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 (11.3g, 0.11 mol), after removal of HBr, was added all at once and the solution stirred for two hours. The bright orange colour of the nitrophenoxide gradually disappeared and a sludgey precipitate of KBr was visible.

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 Et_2O from forming two layers. The organics were then washed 3 x with 10% $NaCO_3$ to remove any starting nitrophenol, once with H_2O, once with brine, and finally dried over anhydrous Na_2SO_4 or $MgSO_4$. Evaporation of the solvent gave an orangey oil (24.7g, 92%) that slowly crystallised on standing. GC analysis of this and all other o-nitrophenoxyacetates produced by this method showed purities >98%.

TLC sometimes showed the presence of starting nitrophenol, but they were not visible on GC traces when compared with standards. Acidification of the carbonate extractions allowed recovery of the starting nitrophenol, (usually <10 mol%) either as precipitated crystals or extractable oils. Yields reported in Table 1 for this coupling are corrected for recovered starting material. These products were not characterized, but were immediately subjected to the reductive cyclization conditions decribed below.

General Procedure for Reductive Cyclization

The Pd/C-$NaBH_4$ reductive cyclization method described by Coutts[2] for similar

α-(o-nitrophenoxy)esters was used with slight modifications. The original method had the reaction performed under a nitrogen atmosphere, but this was not observed to enhance yields nor did it appear to have any other distinct advantages, so this detail was not included. For each gram of nitroester to be cyclized 0.5g of $NaBH_4$ and 0.05g 10% Pd/C was used. Variations in these amounts did not drastically change the nature or the yields of the reaction, though it is wise to keep the $NaBH_4$ at this ratio since the excess must later be destroyed. A large excess causes a great deal of foaming and wastes a valuable reagent. It was also observed that using more dilute conditions lessened the extent of colour formation. 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 colour formation. This was especially important for reactions on a scale larger than a few grams.

An α-methoxy-α-(o-nitrophenoxy)acetate (2g, 6-9 mmol) was dissolved in ~10 mL of dioxane and added dropwise to a rapidly stirring suspension of $NaBH_4$ (50% of nitroester by weight) and 10% Pd on charcoal (50 mg for every gram of nitro ester) in 80 mL of 1:1 water/dioxane. This suspension was made by dissolving the $NaBH_4$ in 35 mL of distilled water and diluting this with 40 mL of dioxane. The Pd/C was first suspended in 5 mL of distilled water and then *carefully* added dropwise to the borohydride solution without getting any on the sides of the flask as this can ignite the hydrogen gas evolved! The solution foamed slightly on addition of the catalyst. Addition of a drop of the α-methoxy-α-(o-nitrophenoxy)acetate tinted the reaction mixture with a yellow to red colour. More was not added until this colour disappeared, which usually took about 15 seconds, longer as addition of the nitroester proceeded. After complete addition of the nitroester the mixture was stirred for another 15-30 minutes. It was then filtered and the filtrate acidified with 1N HCl until no more foaming was evident and the pH as judged by pH paper was ~4. The solution was immediately extracted 3 x with EtOAc, the organics washed once with brine, dried with Na_2SO_4, (treated with activiated charcoal to decolourize if necessary) and evaporated. Purification of the products so obtained varied for the particular compound and this is detailed below.

Purification of Hydroxamics with Fe(III)-Sephadex

The ability of hydroxamic acids to form chelates with metal ions is the basis for their purification by ion exchange chromatography.[3] This is an excellent method for separating the oft-found lactam contaminant from hydroxamic acid samples. It suffers, however, from low yields of recovered hydroxamic acid. DIMBOA could be purified in this manner with total mass recoveries, averaging about 60% even though the original

material was known to be contaminated by only 4-6% of the lactam as measured by GC. The reductive cyclization reaction produced larger amounts of contaminating lactams with those compounds (**16, 45-48**) that had electron rich aromatic rings. Despite GC chromatograms that showed these crude products of cyclization to be >80% pure, total mass yields of lactams and hydroxamic acids isolated by this procedure averaged 50% (including purifications of **49** and the 7-chloro derivative **53**). Apparently it is difficult to remove *all* of the hydroxamic acid from the gel, since it is unlikely that this much degradation has taken place in these near neutral solutions over the times necessary (2-3 hours) to run a column.

This was a relatively high price to pay for pure material when often only a few hundred milligrams of a compound were available. Consequently, this technique was only used when samples could not be recrystallized or triturated, or when colours could not be removed by chromatography on silica.

Synthesis of Hydroxamic Acid Methyl Acetals 45-60

2,7-Dimethoxy-4-hydroxy-2H-1,4-benzoxazin-3-one (DIMBOA methyl acetal) 16

Methyl α-(5-methoxy-2-nitrophenoxy)-α-methoxyacetate (9.48g, 34.9 mmol) was cyclized following the method in section **7.3**. Dilution of the dried EtOAc extracts with a one half volume of hexane provided, after cooling in a refrigerator overnight, 3.27g of slightly pink crystals. Trituration of the oil left after evaporation of the mother liquor with hexane:chloroform (4:1) gave two more lots of crystals; 0.820g and 0.670g. Total yield 4.76g (60%) mp 150-152°C decomposition. (Lit.[4] 148-150°C) ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.26 (d, 1H, $J_{5,6}$= 9.3 Hz), 6.72 (multiplet, 2H), 5.39 (s, 1H), 3.78 (s, 3H, Ar-OCH_3), 3.50 (s, 3H, 2-OCH_3). 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} 3090 (br), 2980, 2900, 2840, 2800 (broad, overlapped) 1675 (br, s), 1600 (m), 1503 (s), 1469 (w), 1485 (w), 1440 (s), 1360 (m), 1343 (m), 1370 (m), 1284 (m), 1196 (m), 1165 (s), 1140 (m), 1115 (w), 1075 (s), 1025 (s), 982, 970, 850, 805, 800 cm^{-1}.

4-Hydroxy-2,7,8-trimethoxy-2H-1,4-benzoxazin-3-one, 45 (DIM_2BOA methyl acetal)

From the reaction of potassium 2,3-dimethoxy-6-nitrophenoxide (2.33g, 9.83 mmol) with 1.1 equivalents of MBMA, 0.71g (48%) of crude methyl

α-(2,3-dimethoxy-6-nitrophenoxy)-α-methoxyacetate was isolated. The yield was based on the recovery of 0.98g of the starting nitrophenol **29**. This 0.71g was cyclized using the usual conditions. Evaporation of the EtOAc extracts of the acidified reaction mixture gave a purplish solid wich was triturated with hexane:chloroform (3:1) to yield 0.57g (95%) of the title compound. mp 143-146°C (decomposition) ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.02 (d, 1H, $J_{5,6}$=10.1 Hz), 6.79 (d, 1H,), 5.44 (s, 1H), 3.84 (s, 3H), 3.83 (s, 3H), 3.53 (s,3H), 2.85 (s, 1H, 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} 3420 (br), 3190 (br), 2940 (w), 1664 (s), 1605 (w), 1500 (s), 1422 (w), 1315 (m), 1278 (m), 1232 (w), 1209 (w), 1108 (m), 1078 (s), 1018 (m), 1001 (m), 973, 872, 806 cm^{-1}. HRMS (using m/z 239 as refernce mass) calcd for $C_{11}H_{13}NO_5$ 239.0792, found 239.0780.

4-Hydroxy-2,6,7-trimethoxy-2H-1,4-benzoxazin-3-one, 46

Potassium 4,5-dimethoxy-2-nitrophenoxide (2.00g, 8.4 mmol) gave 2.50g (99%) of crude methyl α-(4,5-dimethoxy-2-nitrophenoxy)-α-methoxyacetate after reaction with excess MBMA. 2.30g of this dark yellow brown oil was reductively cyclized to yield 2.05g (105% theoretical) of a dark brown tar that could not be successfully triturated with hexane:EtOAc:$CHCl_3$ mixtures. The material was purified on Fe^{3+}-Sephadex to give 0.585g (30%) of the title compound as a greyish powder. mp 96-97°C ^{1}H-NMR (300 MHz, acetone-d_6) δ 6.98 (s, 1H), 6.79 (s, 1H), 5.34 (s, 1H), 3.81 (s, 3H), 3.79 (s, 3H), 3.48 (s, 3H), 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} 3450, 2940 (br), 1698 (s), 1507 (s), 1460 (m), 1447 (m), 1302 (m), 1250 (m), 1205 (s), 1155 (s), 1073 (m), 1033 (s), 1008 (m), 858, 798 cm^{-1}. Anal. Calcd for $C_{11}H_{13}NO_6$: C, 51.77; H, 5.13; N, 5.49. Found: C, 51.96; H, 5.34; N, 5.48.

4-Hydroxy-2-methoxy-7,8-methylenedioxy-2H-1,4-benzoxazin-3-one, 47

Potassium 2,3-methylenedioxy-6-nitrophenoxide (2.26g, 10.2 mmol) gave methyl α-(2,3-methylenedioxy-6-nitrophenoxy)-α-methoxyacetate (crude) as a thick orange oil (2.70g, 101%). 168mg of the starting nitrophenol **31** was recovered. Reductive cyclization of 2.50g of this nitro ester gave 1.43g (68%) of the title compound as a beige powder after trituration with hexane:$CHCl_3$ (4:1) and a few drops of acetone. mp 169-172 °C, decomposition. ^{1}H-NMR (300 MHz, acetone-d_6) δ 6.83 (d, 1H, $J_{5,6}$=8.5 Hz), 6.64 (d, 1H), 6.08 (d, 1H, J_{gem}=1.0 Hz), 6.02 (d, 1H, J_{gem}=1.0 Hz), 5.45 (s, 1H), 3.52 (s, 3H), 2.84 (s, OH). MS(EI) m/z 239(26)M^+, 223(96), 192(12), 179(32), 164(26), 163(100), 162(44),

151(12.5), 106(12). IR (KBr) ν_{max} 3145, 2920 (br), 1675 (vs), 1660, 1642 (s), 1480 (s), 1350 (m), 1275 (m), 1225 (m), 1138 (m), 1102 (m), 1061 (s), 1008 (m), 985, 981, 930, 890, 800 cm^{-1}. Anal. Calcd for $C_{10}H_9NO_6$: C, 50.22; H, 3.79; N, 5.86. Found: C, 50.01; H, 4.00; N, 5.82.

4-Hydroxy-2-methoxy-6,7-methylenedioxy-2H-1,4-benzoxazin-3-one, 48

Pottassium 4,5-methylenedioxy-2-nitrophenol (2.54g, 11.5 mmol) gave, after coupling with MBMA, methyl-α(4,5-methylenedioxy2-nitrophenoxy)-α-methoxyacetate (2.79g, 90%) as a thick orange oil. Reductive cyclization was compounded by colour formation after acidification of the reaction mixture. Evaporation of the EtOAc extracts gave a dark purple tar which could be triturated with hexane:EtOAc yielding 1.74g of a light grey-violet solid. GC analysis of this material showed the presence of ~9% of the corresponding lactam (77% yield of hydroxamic acid). Purification on Fe^{3+}-Sephadex gave 0.574g(33%) of the title compound. mp ~150°C, slow decomposition) ^{1}H-NMR (300 MHz, acetone-d_6) δ 6.91 (s, 1H), 6.72 (s, 1H), 6.01 (d, 1H, J_{gem}=1.Hz), 5.97 (d, 1H, J_{gem}=1Hz), 5.34 (s, 1H), 3.48 (s, 3H), 2.81 (s, 1H, 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} 3440, 3130, 2920 (br), 1668 (s), 1510 (m), 1491 (s), 1293 (m), 1243 (w), 1198 (w), 1171(m), 1118 (m), 1074 (m), 1040 (m), 1010 (m), 970, 940, 861, 808 cm^{-1}. Anal. Calcd for $C_{10}H_9NO_6$: C, 50.22; H, 3.79; N, 5.86. Found: C, 50.17; H, 4.00; N, 5.91.

2,8-Dimethoxy-4-hydroxy-2H-1,4-benzoxazin-3-one, 49

Potassium 6-methoxy-2-nitrophenoxide (2.62g, 12.6 mmol) after reaction with MBMA yielded methyl α-(6-methoxy-2-nitrophenoxy)-α-methoxyacetate (2.25g, 65%). No attempt was made to recover starting nitrophenol from this reaction. Reductive cyclization yielded 1.80g of an orange oil that only partially crystallized after storing in a refrigerator for one week. This material was found by GC to be only 75% pure with over 20% being the lactam. Purification of 0.500g of this material gave 0.212g of **49** representing a 42% yield from the nitroester. (Despite the degree of impurities, the crude material was carried into the reaction with BCl_3 because the demethylated product was more easily triturated to a solid.) Pure **49** had mp 139-141°C, decomposition. ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.15 (m, 2H), 6.82 (dd, 1H, $J_{7,6}$=8.2 Hz, $J_{7,5}$=1.5 Hz), 5.42 (s, 1H), 3.88 (s, 3H), 3.48 (s, 3H). MS(EI) m/z 225(3)M^+, 209(95), 178(17), 166(11), 149(100), 134(20), 107(13), 94(10). IR (KBr) ν_{max} 3170 (br), 3010, 2950, 2840 (m), 1670

(s), 1612 (m), 1597 (m), 1508 (s), 1495 (s), 1465 (m), 1425 (m), 1311 (m), 1268 (m), 1212 (m), 1170 (m), 1151 (m), 1063 (s), 1030 (s), 969, 869, 770, 729 cm^{-1}. Anal. Calcd for $C_{10}H_{11}NO_5$: C, 53.33; H, 4.92; N, 6.22. Found: C, 53.08; H, 5.00; N, 6.15.

7-t-Butyl-4-hydroxy-2-methoxy-2H-1,4-benzoxazin-3-one, 50

Potassium 5-t-butyl-2-nitrophenoxide (6.17g, 26.5 mmol) after reaction with MBMA gave of methyl α-(5-t-butyl-2-nitrophenoxy)-α-methoxyacetate (7.15g, 91%) as a yellow oil. No attempt was made to recover starting nitrophenol. Reductive cyclization yielded 4.61g of a purple solid which was chromatographed on silica with CH_2Cl_2 and increasing the amount of MeOH slowly. Fractions were monitored by TLC, visualizing with UV and charring with 5% H_2SO_4 in MeOH. The hydroxamic acid charred pink-brown. The relevant fractions were combined and recrystallized from Et_2O:hexane giving four crops of light beige crystals totaling 2.58g (56%) mp 141-143°C, (decomposition). ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.28 (d, 1H, $J_{5,6}$=8.4 Hz), 7.16 (m, 2H), 5.40 (s, 1H), 3.49 (s, 3H), 2.86 (s, 1H, OH), 1.30 (s, 9H). 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} 3170 (br), 2960 (br), 1685 (s), 1665 (s), 1517 (m), 1408 (m), 1301 (m), 1112 (m), 1068 (m), 1030, 1022 (m) 984, 967, 825, 793 cm^{-1}. Anal. Calcd for $C_{13}H_{16}NO_4$: C, 62.14; H, 6.42; N, 5.57. Found: C, 62.24; H, 6.50; N, 5.57.

4-Hydroxy-2-methoxy-7-methyl-2H-1,4-benzoxazin-3-one, 51

Potassium 5-methyl-2-nitrophenoxide (5.81g, 30.4 mmol, 5-methyl-2-nitrophenol was purchased from Aldrich) gave after reaction with MBMA, methyl α-(5-methyl-2-nitrophenoxy)-α-methoxyacetate (3.48g, 13.6 mmol) as a dark yellow oil. Adjusting for the 2.37g of starting phenol recovered, the yield was 91%. This material was reductively cyclized to yield, after extraction from the acidified reaction mixture, 2.53g of a greenish solid. Recrystallization from hexane:acetone gave, in multiple crops, a total of 2.85g (76%) of the title compound. mp 168-171°C, (decomposition). ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.23 (d, 1H, $J_{5,6}$=8.7 Hz), 6.94 (m, 2H), 5.38 (s, 1H), 3.48 (s, 3H), 2.83 (br s, OH), 2.29 (s, 3H). MS(EI) m/z 209(5), 193(18), 164(3), 149(9), 134(15), 133(12), 104(4). IR (KBr) ν_{max} 3060, 2850 (br), 1672 (vs), 1510 (s), 1469, 1418 (br), 1352 (w), 1301 (m), 1245 (w), 1200 (w), 1149 (m), 1071 (s), 1039 (s), 990, 982, 925, 873, 821 cm^{-1}. HRMS Calcd for $C_{10}H_{11}NO_4$ 209.0687, found 209.0687.

4-Hydroxy-2-methoxy-2H-1,4-benzoxazin-3-one, 52 (DIBOA methyl acetal)

Potassium 2-nitrophenoxide (4.00g, 22 mmol, o-nitrophenol purchased from Aldrich) after reaction with freshly prepared MBMA gave methyl α-(2-nitrophenoxy)-α-methoxyacetate (5.50g, 99%) as an orange oil that slowly (30 minutes to one hour) crystallized at room temperature. Reductive cyclization yielded 2.02g of a brown stiff oil that crystallized on standing over 24 hours. Trituration with hexane:$CHCl_3$ removed most of the colour leaving 1.99g (83%) of dark beige crystals. mp 136-138°C (Lit.[4] 121-123°C, impure) ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.37 (dd, 1H, $J_{5,6}$=7.9 Hz, $J_{5,7}$=1.7 Hz), 7.13 (m, 3H), 5.42 (s, 1H), 3.49 (s, 3H), 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} 3080, 2890, 2780 (broad, overlapped), 1678 (s), 1610 (m), 1500 (s), 1431 (s), 1353 (m), 1300 (m), 1282 (m), 1222 (m), 1199 (w), 1118 (m), 1079 (s), 1049 (s), 995, 992, 845, 775. cm^{-1}. Anal. Calcd for $C_9H_9NO_4$: C, 55.39; H, 4.65; N, 7.18. Found: C, 55.18; H, 4.84; N, 7.05.

7-Chloro-4-hydroxy-2-methoxy-2H-1,4-benzoxazin-3-one, 53

Potassium 5-chloro-2-nitrophenoxide (13.29g, 0.063 mol) after reaction with MBMA gave methyl α-(5-chloro-2-nitrophenoxy)-α-methoxyacetate (16.48g, 95%). The spontaneous crystallization of the oil remaining after evaporation of the organic extracts was quite exothermic, warming the flask to ~40°C. Reductive cyclization of 8.00g (29.1 mmol) of this material and extraction of the acidified reaction mixture gave a dark brown tar, which could be triturated with hexane:$CHCl_3$:EtOAc to give 4.41g (66%) of a light brown powder and a further 1.35g (20%) of a crude oil which was nearly 85% pure (GC) but which was not used in any further steps. For the first crop: mp 163-165°C, (decomposition). ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.37 (d, 1H, $J_{5,6}$=9.3 Hz), 7.17 (m, 2H), 5.46 (s, 1H), 3.52 (s, 3H), 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} 3440 (br, w), 3060,2840 (br), 1675 (s), 1392 (s), 1440 (m), 1408 (m), 1393 (m), 1120 (m), 1084 (m), 1070 (m), 1026 (s), 984, 975, 862, 820 cm^{-1}. HRMS calcd for $C_9H_8ClNO_4$ 229.0140, found 229.0126.

7-Fluoro-4-hydroxy-2-methoxy-2H-1,4-benzoxazin-3-one, 54

Potassium 5-fluoro-2-nitrophenoxide (1.83g, 9.38 mmol) gave, after reaction with MBMA, methyl α-(5-fluoro-2-nitrophenoxy)-α-methoxyacetate (1.92g, 80%). No attempt was made to recover starting nitrophenol. Reductive cyclization produced 1.47g (93%) of **54** as a beige-brown solid. mp 162-165°C, (decomposition). ^{1}H-NMR (300 MHz,

acetone-d_6) δ 7.37 (dd, 1H, $J_{5,6}$=8.8 Hz, J_{mF}=5.4 Hz), 6.95 (m, 2H), 5.46 (s, 1H), 3.52 (s, 3H), 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} 3090, 2885 (br), 1672 (s), 1604 (m), 1509 (s), 1426 (m), 1350 (m), 1300 (m), 1198 (m), 1151 (m), 1120 (s), 1070 (s), 1028 (s), 985, 975, 852, 822, 623 cm^{-1}. Anal. Calcd for $C_9H_8FNO_4$: 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.

7-Carbomethoxy-4-hydroxy-2-methoxy-2H-1,4-benzoxazin-3-one, 55a

Potassium 5-carbomethoxy-2-nitrophenoxide (2.75g, 11.9 mmol) gave methyl α-(5-carbomethoxy-2-nitrophenoxy)-α-methoxyacetate (1.94g, 75%, based on 0.610g of starting nitrophenol recovered) as a deep orange oil. Reductive cyclization under the usual conditions gave 1.39g of a dark orange oil that only partially crystallized on standing for one week at 6°C. Trituration with hexane:$CHCl_3$ was unsuccessful. GC showed the material to be only 77% pure. Chromatography on silica (2mm Chromatatron® plate, CH_2Cl_2) gave 0.900g (54%) of **55a** as a light orange oil which slowly crystallized on standing at room temperature for a day. mp 125-131°C, (slow decomposition over this range). ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.81 (dd, 1H, $J_{6,5}$=8.4 Hz, $J_{6,8}$=1.8 Hz), 7.67 (d, 1H, $J_{8,6}$=1.8 Hz), 7.48 (d, 1H, $J_{5,6}$=8.4 Hz), 5.51 (s, 1H), 3.87 (s, 3H), 3.54 (s, 3H), 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} 3160, 2960 (br), 1725 (s), 1672 (s), 1612 (m), 1599 (m), 1510 (m), 1435 (m), 1411 (m), 1289 (s), 1217 (m), 1101 (m), 1174 (m), 1130 (s), 980, 968, 889, 840, 811, 762 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-Carboethoxy-4-hydroxy-2-methoxy-2H-1,4-benzoxazin-3-one, 55b

Potassium 5-carboethoxy-2-nitrophenoxide (2.00g, 8.0 mmol) subjected to the usual coupling procedure gave methyl α-(5-carboethoxy-2-nitrophenoxy)-α-methoxyacetate (2.24g, 89%) as a clear orange oil. Reductive cyclization yielded 1.78g (93%) of an orange toffee-like tar that solidified only after five hours on a mechanical pump with gentle warming (~40°C) and standing at room temperature for a day. Trituration was unsuccessful.

This amorphous solid, one spot by TLC (10:1 $CHCl_3$:MeOH), >97% by GC, 'melted' over 80-90°C. ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.81 (dd, 1H, $J_{6,5}$=8.4Hz, $J_{6,8}$=1.8Hz), 7.68 (d, 1H, $J_{8,6}$=1.8Hz), 7.47 (d, 1H,), 5.51 (s, 1H), 4.31 (q, 2H), 3.54 (s, 3H), 2.88 (br s, OH),

1.35 (t, 3H). 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} 3230 (br, s), 3000, 2950 (m), 1720 (s), 1678 (s), 1618 (m), 1600 (m), 1518 (s), 1480 (m), 1437 (m), 1408 (m), 1372 (m), 1292 (s), 1250 (m), 1108 (m), 1075 (m), 1032 (s), 985, 938, 902, 846, 838, 770 cm^{-1}.

4-Hydroxy-2-methoxy-7-trifluoromethyl-2H-1,4-benzoxazin-3-one, 56

Potassium 5-trifluoromethyl-2-nitrophenoxide (2.20g, 9.7 mmol), after reaction with MBMA gave methyl α-(5-trifluoromethyl-2-nitrophenoxy)-α-methoxyacetate (1.77g, 70% based on 189mg starting nitrophenol recovered), as a green-yellow oil. Reductive cyclization of 1.63g (5.6 mmol) of this nitroester gave 1.46g of an orange oil that foamed under vacuum (~1 torr) and could not be triturated. GC showed this to be 87% pure. Chromatography of 600mg of this crude hydroxamic acid on silica (Chromatotron®, 2mm) eluting with hexane:chloroform (1:1) and increasing the proportion of chloroform, gave 470 mg of slightly impure material. Fractions from chromatography were decolourized with charcoal, but on standing overnight the same orange-brown colours returned. On evaporation a purple oil was obtained which solidified on standing for a few hours. On first melt this material liquified 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. ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.51 (m, 3H), 5.54 (s, 1H), 3.55 (s, 3H), 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} 3400, 3140, 2955 (broad and overlapped), 1705 and 1690 (s), 1630 (m), 1520 (m), 1447 (m), 1405 (m), 1333 (s), 1268 (m), 1170 (m), 1150 (m), 1129 (s), 1081 (s), 1039 (s), 990, 976, 848, 830, 815, 751, 723 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, 57

Potassium 5-cyano-2-nitrophenoxide (2.06g, 11.2 mmol) gave, after coupling with MBMA, methyl α-(5-cyano-2-nitrophenoxy)-α-methoxyacetate (2.46g, 89%) as a thick lemon-yellow oil, which crystallized on standing for 4-5 days at room temperature. Reductive cyclization of 2.34g (9.43 mmol) of this nitroester gave 1.94g (93%) of the title compound as an oil which foams under vacuum (~1 torr). This could be triturated with hexane:$CHCl_3$ to give a tan solid. mp 155-158°C, (decomposition). ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.56 (m, 3H), 5.55 (s, 1H), 3.55 (s, 3H). 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}

3140 (br), 2960 (w), 2858 (w), 2240 (m), 1700 (s), 1672 (m), 1607 (m), 1507 (m), 1502 (m), 1463 (m), 1388 (br), 1309 (m), 1277 (w), 1239 (w), 1203 (w), 1107 (w), 1074 (s), 1030 (s), 979 (m), 927, 896, 841, 806 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.

4-Hydroxy-2-methoxy-5-methyl-2H-1,4-benzoxazin-3-one, 58

Potassium 3-methyl-2-nitrophenoxide (2.17g, 11.4 mmol) gave methyl α-(3-methyl-2-nitrophenoxy)-α-methoxyacetate (2.65g, 91%). No starting nitrophenol was recovered on acidification of the carbonate extracts. Reductive cyclization of 2.60g of this nitroester gave 2.01g (94%) of the title compound as a light violet powder after trituration of the crude solid with hexane:chloroform. mp 92-95°C (decomposition). ^{1}H-NMR (300MHz, acetone-d_6) δ 6.96 (m, 3H), 5.40 (s, 1H), 3.46 (s, 3H), 2.87 (br s, OH), 2.55 (s, 3H). 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} 3420, 3150 (broad and overlapped), 1670 (s), 1470 (br, m), 1400 (m), 1277 (m), 1192 (m), 1116 (m), 1089 (m), 1035 (s), 979, 804, 778, 720 cm^{-1}. Anal. calcd for $C_{10}H_{11}NO_4$: C, 57.41; H, 5.30; N, 6.70. Found: C, 57.26; H, 5.57; N, 6.59.

7-Dimethylamino-2-methoxy-2H-1,4-benzoxazin-3-one, 25

Potassium 5-dimethylamino-2-nitrophenoxide (2.22g, 10.1 mmol) gave, after reaction with MBMA, methyl α-(5-dimethylamino-2-nitrophenoxy)α-methoxyacetate (2.69g, 94%) as a yellowy-green oil which slowly crystallized at room temperature. Numerous attempts at reductive cyclization were hampered by the production of colours and tars. An attempt at partial reduction using H_2/Pt poisoned with DMSO[5] did not produce a hydroxamic acid, but gave the best isolated yield of the title lactam.

100mg of the nitroester (0.35 mmol) was dissolved in 20 mL of EtOAc. To this was added 0.5 μL of DMSO and 10mg of Pt/C (10%). Hydrogenation at atmospheric pressure was very slow (2 mL H_2/45 minutes). After uptake of 2 equivalents of H_2 (17 mL) the solution was filtered. On aeration the filtrate turned green. Chromatography (Chromatotron®, 1mm, silica) eluting with CH_2Cl_2 and adding increasing amounts of MeOH gave 56.5mg (72%) of the title compound as a pinkish solid. (The Chromatotron plate remained blue after elution of **25**, even when washed with MeOH and 2% AcOH.) mp 174-175°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.28 (br s, NH), 6.72 (d, 1H, $J_{5,6}$=8.6 Hz), 6.46 (d, 1H, $J_{8,6}$=1.5 Hz), 6.37 (dd, 1H, $J_{6,5}$=8.6 Hz, $J_{6,8}$=1.5 Hz), 5.25 (s, 1H), 3.54 (s, 3H), 2.90 (s, 6H). 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} 3460 (br, w), 3200-2800 (broad and overlapped, w), 1690 (s), 1637 (m), 1600 (m), 1528 (s), 1440 (br, m), 1270 (m), 1230 (m), 1192 (m), 1135 (m), 1089 (s), 1029 (s), 988, 962, 825, 789 cm^{-1}. Anal. calcd for $C_{11}H_{14}N_2O_3$: 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, 27

Potassium 5-methoxy-3-methyl-2-nitrophenoxide (2.15g, 9.73 mmol) gave, after reaction with MBMA following the usual procedure, methyl α-(5-methoxy-3-methyl-2-nitrophenoxy)-α-methoxyacetate (2.72g, 98% based on 68 mg starting nitrophenol recovered) as a yellow-orange oil. On acidification of the filtrate from the reductive cyclization reaction, the light yellow solution darkened considerably. On extraction of this aqueous solution with EtOAc the colours intensified. The dark purple colour could be removed with addition of small amounts (20mg) of dithionite, but on further shaking or on standing overnight the colours returned. On standing, both the aqueous and organic layers became coloured. Evaporation of the dried EtOAc extracts gave a dark oil (1.66g) which could not be induced to crystalize nor would it solidify on trituration with hexane:$CHCl_3$ or hexane:acetone mixtures. The oil tested positive for the presence of a hydroxamic acid by giving a blue-purple colour with Fe^{3+}, (5.0g $FeCl_3$-$6H_2O$, 100 mL 95% EtOH, 1 mL concentrated HCl) but attempted purification of 598mg of the crude oil on 4g of Fe^{3+}-Sephadex gave only 12mg of an oily mixture of compounds in the hydroxamic acid fraction. EtOAc extraction of the column rinses, and trituration (hexane-$CHCl_3$) of the oil remaining on evaporation gave 90mg of beige crystals in a first crop and 143mg of slightly impure darker beige crystals in a second crop. Total 233mg (31% from nitroester) mp 215-217°C ^{1}H-NMR (300 MHz, acetone-d_6) δ 6.54 (m, 2H), 5.36 (s, 1H), 3.76 (s, 3H), 3.45 (s, 3H), 2.51 (s, 3H). 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} 3480 (br), 3220 (br), 1703 (s), 1632 (w), 1511 (s), 1370 (m), 1344 (m), 1301 (m), 1240 (m), 1200 (m), 1146 (s), 1102 (m), 1068 (s), 1059 (s), 1014 (m), 988, 970, 950, 810 cm^{-1}. Anal. calcd for $C_{11}H_{13}NO_4$: C, 59.19; H, 5.87; N, 6.27. Found: C, 59.04; H, 5.84; N, 6.49.

2-Methoxy-2H-1,4-benzoxazin-3-one, 59 (HBOA-Me acetal)

Methyl α-(2-nitrophenoxy)-α-methoxyacetate (3.00g, 12.4 mmol, see synthesis of **52** and general coupling procedure in section **7.3**) in 40 mL of EtOAc was combined with 350mg of 10% Pd/C and placed on a hydrogenation apparatus. The reaction vessel

was shaken mechanically. Uptake of H_2 was fairly slow (4 hours for the first 390 mL of H_2). More catalyst (100mg) was added after this time and shaking continued until 900 mL of H_2 had been used. A $FeCl_3$ test at this point gave a strong positive (blue-purple colour) for the presence of a hydroxamic acid. The reaction mixture was filtered of catalyst, the solvent was evaporated and the residual semi-solid purified on Fe^{3+}-Sephadex. 0.215g of the hydroxamic acid **16** was recovered as well as 1.15g (52%) of the title lactam **59**, which crystallized from the 25% aqueous MeOH used to rinse the gel of non-hydroxamic acids. mp 168-171°C. ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.05 (m, 4H), 5.24 (s, 1H), 3.49 (s, 3H). 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} 3400 (br), 3200, 3150, 3090, 2990, 2916, 2860 (all medium), 1704 (s), 1613 (m), 1501 (s), 1437 & 1423 (m), 1348 (w), 1312 (w), 1281 (m), 1259 (w), 1218 (m), 1190 (m), 1092 (s), 1037 (s), 1020 (s), 983, 960, 819, 780, 755, 733, 703 cm^{-1}.

2,7-Dimethoxy-2H-1,4-benzoxazin-3-one, 60 (HMBOA-Me acetal)

Methyl α-(5-methoxy-2-nitrophenoxy)-α-methylacetate (3.00g, 11.1 mmol, see preparation of **16** in general coupling procedure, Section **7.3**) was disolved in 15 mL of THF and added to 4.0g of NaH_2PO_2 hydrate[6] disolved in 50 mL of H_2O. To this was added 100 mg of 10% Pd/C and the mixture was stirred at room temperature overnight. The mixture was filtered and extracted with CH_2Cl_2. On standing 62 mg of DIMBOA-Me , **16**, crystallized from solution. The oil remaining after evaporation of the dried extracts was column chromatographed on silica (CH_2Cl_2 with increasing proportions of acetone up to 4%) yielded 0.510g of the starting nitroester, 0.620g of the title compound and 0.440g of a mixture of **16** and **60**. This mixture was purified on Fe^{3+}-Sephadex yielding another 0.193g of **60**. Total yield of **60**: 0.813g (42%) mp 149-150°C. ^{1}H-NMR (300 MHz, acetone-d_6) δ 9.62 (weak and broad, NH), 6.93 (d, 1H, $J_{5,6}$=8.7 Hz), 6.69 (d, 1H, $J_{8,6}$=2.6 Hz), 6.62 (dd, 1H, $J_{6,8}$=2.6 Hz, $J_{6,5}$=8.7 Hz), 5.21 (s, 1H), 3.76 (s, 3H), 3.49 (s, 3H). 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} 3190, 3160, 3000, 2970, 2945, 2900 (broad and overlapped), 1692 (vs), 1611 (m), 1513 (s), 1466 (m), 1444(m), 1428 (m), 1416 (m), 1369 (m), 1344 (m), 1276 (m), 1248 (m), 1200 (m), 1162 (m), 1129 (m), 1085 (s), 1021 (s), 980, 959, 932, 840 cm^{-1}.

6-Methoxybenzoxazolinone, MBOA

MBOA was syntheszed by the method of Kubo and Kamikawa[7]. Methyl 4-methoxysalicylate (10.1 g, 55.5 mmol) was dissolved in 25 mL of MeOH and to this was

added 16 mL of 95% hydrazine. The mixture was then set to reflux for 8 hours. When cooled, the product 4-methoxysalicylhydrazide crystallized and was filtered and washed with cold methanol. Yield was 8.48 g (84%), mp 172.5-173.5°C. MS(EI) m/z (%) 182(29)M^+, 151(100), 108(21), 95(24), 63(10), 53(10), 52(14), 51(12).

In a 500 mL round-bottom flask, 5.3 g (29.1 mmol) of the hydrazide was dissolved in 100 mL of glacial acetic acid. To this was added 100 mL of ether giving two phases. The stirred mixture was cooled in an ice-bath to 10-12°C, and 5.3 g of $NaNO_2$ in 20 mL of water was added dropwise over 10 min. After 30 minutes, the reaction mixture was diluted with water and extracted with ether. Drying and evaporation of ethereals gave a peach coloured solid (4-methoxysalicylazide, 5.3 g, 94%). MS(EI) m/z (%) 193(100)M^+, 165(12), 151(68), 150(97), 122(53), 109(33), 108(24), 107(20), 106(62).

The crude azide was immediately added to 500 mL of dry xylene and refluxed for two hours, wherupon TLC showed no azide remained. On cooling, a solid precipitated. This was collected and washed with hexane yielding 4.6 g crude MBOA. Silca gel flash chromatography ($CHCl_3$ with gradually increasing MeOH content up to a few percent) provided varying amounts of pure MBOA with each reaction. mp 152.5-153.5°C (Lit.[8] 154°C and 168-170°C[7]) ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.01 (d, 1H, $J_{4,5}$= 9 Hz), 6.88 (d, 1H, $J_{7,5}$= 2.2 Hz), 6.71 (dd, 1H, $J_{5,4}$= 8.9 Hz, $J_{5,7}$= 2.2 Hz), 3.78 (s, 3H). ^{13}C-NMR (80 MHz, acetone-d_6) 156.6, 155.1, 145.3, 124.3, 110.3, 109.6, 97.5, 55.9. IR ($CHCl_3$) 1750 cm^{-1} (C=O).

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7.4 DEMETHYLATION OF HYDROXAMIC ACID METHYL ACETALS

General Comments

The use of boron trichloride to cleave acetals of this type has been described in the patent of Jernow and Rosen[1] for the synthesis of DIMBOA, DIBOA, some alkyl, alkoxy and one chloro analogue. It is not explicitly clear in the patent whether the 7-Cl analogue ever had the 2-methoxy group demethylated since no experimental details were given. All included examples used BCl_3 and no indication was given that reaction times longer than a few hours at 0°C were necessary. Experience gained in the present work suggests that using BCl_3 would require prolonged reaction times at room temperature to effect the demethylation of a 7-chloro-2-methoxy analogue. Changing to the more reactive BBr_3 allows the conversion to be achieved even more easily.

Temperatures, reaction times and reagents used were particular to each analogue synthesized and are detailed below under the individual compound names.

All reactions were performed in CH_2Cl_2 that had been glass-distilled and stored over molecular sieves. The boron reagents were purchased as 1.0 M solutions in CH_2Cl_2 (Aldrich) and were handled by appropriate syringe techniques. Reactions were run under a slight positive pressure of nitrogen.

Removal of these reagents upon completion of the reactions could be achieved by bubbling with nitrogen or evaporation under reduced pressure (aspirator), but this was more easily accomplished by adding excess THF to the reaction solution and then careful addition of water. On addition of the THF some misting was visible, but soon disappeared. Most of the compounds were immune to the acidic, biphasic conditions so produced, but most were buffered by addition of a small volume of 10% carbonate solution. Only in the larger scale reactions (*e.g.* >15 mmol starting methyl acetal) was there an obvious exothermic reaction and then only of a few degrees. This method drastically shortened times for removal of these reagents, from 3-4 hours for the larger scale reactions to only the few minutes necessary to mix and separate the aqueous layer in a separatory funnel.

2,4-Dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one, 1 (DIMBOA)

In a three-necked flask fitted with a nitrogen line, bubbler and rubber septa, **16**

(5.31g, 23.6 mmol) was partially dissolved in 150 mL of CH_2Cl_2 and cooled to -50°C in a dry-ice/acetone bath. 70.9 mL of 1M BCl_3 in CH_2Cl_2 (3 molar equivalents) was added from the bottle of reagent with a double ended syringe and a gentle pressure of nitrogen. The reagent bottle had been cooled to ~0°C before addition. After the addition of approximately one equivalent of BCl_3 the reaction mixture darkened to a purple-black and all of **16** appeared to go into solution. The flask was removed from the bath and allowed to warm to room temperature (1.5 hours) where the solution stirred for a further hour. 25 mL of THF was added via pipette, then 40 mL of water all at once. The mixture was rapidly stirred for a minute then poured into a separatory funnel. More water was added to establish two distinct layers. The CH_2Cl_2 layer was removed and the aqueous 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 40 mL THF and this solution added over 10 minutes to a rapidly stirring suspension of 13g Ag_2CO_3 in 2:1 H_2O:THF. The green colour of the silver salt changes to a grey-brown over the 30 minutes this mixture was allowed to stir. It was then filtered, the solids washed with THF and extracted with EtOAc until the aqueous showed no colour with $FeCl_3$. The organics were washed with brine, dried and evaporated to ~50 mL. Addition of hexane and cooling in a refrigerator for one day gave 1.9g of a tan solid. Concentration of the mother liquor gave another 0.43g of a beige solid. The residual oil on evaporation could be triturated with hexane:acetone to give 1.15g of beige solid. Total yield: 3.48g (70%). mp 163-164.5 (decomposition). (Lit.[1] 162-163°C) ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.25 (d, 1H, $J_{5,6}$=8.8Hz), 6.68 (dd, 1H, $J_{6,5}$=8.8Hz, $J_{6,8}$=2.6 Hz), 6.61 (s, 1H, $J_{8,6}$=2.6 Hz), 5.72 (s, 1H), 3.57 (s, 3H), 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 (ϵ=10,300) IR (KBr) ν_{max} 3345, 3140 and 2940 (broad and overlapped), 1662 (vs), 1605 (m), 1519 (s), 1469 (m), 1453 (m), 1400 (w), 1318 (m), 1282 (m), 1197 (w), 1163 (s), 1151 (m), 1098 (m), 1059 (m), 1031 (s), 982, 911, 850, 811, 799, 746, 730 cm^{-1}. Anal. Calcd for $C_9H_9NO_5$: C, 51.19; H, 4.30; N, 6.63. Found: C, 51.34; H, 4.63; N, 6.60.

2,4-Dihydroxy-7,8-dimethoxy-2H-1,4-benzoxazin-3-one, 2

45 (0.480g, 1.88 mmol) was dissolved in 15 mL CH_2Cl_2 and cooled to -50°C in an acetone/dry ice bath. 5.7 mL of 1M BCl_3 in CH_2Cl_2 (3 eq.) was added under N_2. The reaction vessel was removed from the cooling bath and allowed to warm to room temperature where stirring was continued for 2 hours. 10 mL of THF and 2 mL of 10% Na_2CO_3 were added to decompose the BCl_3. The aqueous layer was separated and the

organics washed once with water then evaporated to a few millilitres volume. This was dissolved in 6 mL of THF and added dropwise to a stirring suspension of 1.2g Ag_2CO_3. The solids were filtered off after 15 minutes, extracted 3 x with EtOAc, washed once with brine, dried, and decolourized with activated charcoal. The organics were then evaporated to an orange-brown oil that foamed under vacuum (~1 torr). Trituration of this with hexane:$CHCl_3$ gave 175 mg of pinkish-brown solid, but TLC (CH_2Cl_2:MeOH, 10:1) showed this to be impure. The solid so obtained and the tar remaining from trituration, which was hydroxamic acid positive ($FeCl_3$ test), were combined and purified on Fe^{3+}-Sephadex (prepared from 1.7g of Sephadex-SP). 290 mg of impure material gave 140 mg (31%) of pure **2**. mp 145-146°C (decomposition to red tar with bubbling). ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.01 (d, 1H, $J_{5,6}$=9.0 Hz), 6.76 (d, 1H, $J_{6,5}$=9.0 Hz), 5.77 (s, 1H), 3.81 (s, 3H), 3.78 (s, 3H). 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 (ε=11,300). IR (KBr) ν_{max} 3430 (s), 3200-2800 (br), 1660 (s), 1604 (w), 1500 (s), 1484 (m), 1400 (m), 1318 (m), 1290 (s), 1272 (s), 1231 (m), 1208 (m), 1174 (m), 1110 (s), 1073 (m), 1046 (m), 1024 (m), 999 (m), 870, 807, 800, 760, 739, 692 cm^{-1}.

2,4-Dihydroxy-6,7-dimethoxy-2H-1,4-benzoxazin-3-one, 3

46 (0.850g, 3.33 mmol) was suspended in 40 mL of CH_2Cl_2 and cooled under nitrogen to -45°C in a dry ice/acetone bath. To the stirring mixture was added 10.0 mL of 1M BCl_3 in CH_2Cl_2 (3 eq) whereapon **46** appeared to go into solution. The reaction mixture turned a deep brown-black. Addition of dry ice to the cooling bath was stopped and the reaction mixture allowed to warm to 0-5°C where it was kept for ~2 hours, at which time TLC of a worked-up aliquot showed the complete absence of starting material. The TLC showed only one spot, which was more polar than **46** (10:1 CH_2Cl_2:MeOH). The reaction was quenched with 10 mL THF and 20 mL water, separated in a separatory funnel, the wet organics evaporated to a few millilitres, redissolved in ~10 mL of THF and added dropwise to a stirring suspension of 2g Ag_2CO_3. After 20 minutes, the mixture was filtered, the solids washed with THF, the filtrate diluted with 50 mL water and then extracted 3 x EtOAc. After drying and evaporation the resultant oil, which was almost black, was purified on a Fe^{3+}-Sephadex column (prepared from 3g Sephadex-SP) yielding 178 mg (22%) of **3** as a beige solid. mp 154-155°C, (decomposition to red-black tar, bubbling). ^{1}H-NMR (300 MHz, acetone-d_6) δ 6.96 (s, 1H), 6.68 (s, 1H), 5.67 (s, 1H), 3.80 (s, 3H), 3.77 (s, 3H) 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

(ϵ=8,100), 264 nm (ϵ=5,100). IR (KBr) ν_{max} 3430 (s), 3000-2700 (br), 1664 (s), 1515 (s), 1460 (m), 1447 (m), 1400 (w), 1302 (m), 1248 (m), 1210 (m), 1197 (s), 1183 (m), 1154 (s), 1070 (w), 1030 (m), 1022 (m), 954, 853, 818, 801 cm^{-1}.

2,4-Dihydroxy-6,7-methylenedioxy-2H-1,4-benzoxazin-3-one, 4

48 (0.500g, 2.09 mmol) was suspended in CH_2Cl_2 and cooled under nitrogen to -50°C in a dry-ice/acetone bath. To this was added, via syringe, 6.2 mL of 1M BCl_3 (3 eq) whereapon the solution darkened. The flask was then allowed to warm, while still in the cooling bath, to -20°C. After ~30 minutes at this temperature TLC (10:1 CH_2Cl_2:MeOH) of an aliquot that had been worked-up through the silver assisted hydrolysis showed that most of the starting **48** had disappeared. However, some material was observed to have remained at the origin (cleavage of the methylenedioxy ring?) and the reaction was stopped at this time by addition of 20 mL of THF and 2 mL of 10% Na_2CO_3 solution. The mixture was poured into a separatory funnel and the layers were separated. The aqueous was extracted with two portions of EtOAc. The combined organics were then evaporated to an oil, redissolved in THF and added dropwise to a stirring suspension of 1.1g Ag_2CO_3. After 15 minutes, when the green salt had turned brownish-grey, the mixture was filtered, extracted 3 x with EtOAc, washed once with brine, dried and evaporated to give 303 mg brown solid. This was purified on Fe^{3+}-Sephadex (prepared from 3g Sephadex-SP) and yielded 177 mg (38%) of **4** as a beige solid. mp 152-153°C (decomposition to red-black tar, bubbling). ^{1}H-NMR (300 MHz, acetone-d_6) δ 6.90 (s, 1H), 6.62 (s, 1H), 5.99 (d, 1H, J_{gem}=1.0 Hz), 5.95 (d, 1H, J_{gem}=1.0 Hz), 5.66 (s, 1H). 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 (ϵ=8,600), 264 nm (ϵ=4,000). IR (KBr) ν_{max} 3200, 3000, 2820, 2740 (broad and overlapped), 1680 (s), 1500 (s), 1395 (m), 1294 (s), 1242 (m), 1167 (s), 1124 (m), 1099 (m), 1043 (s), 1007 (s), 960 (w), 913 (m), 909 (m), 851, 812, 754 cm^{-1}.

2,4-Dihydroxy-8-methoxy-2H-1,4-benzoxazin-3-one, 5

49 (0.610g, 2.71 mmol) was dissolved in 30 mL CH_2Cl_2 and cooled under nitrogen to -45°C in a dry-ice/acetone bath. On addition of 8.1 mL (3 eq.) of 1M BCl_3 the solution darkened to a deep purple colour. The flask was removed from the cooling bath and allowed to warm to room temperature over 2.5 hours. At these conditions stirring was continued for ~45 minutes. The reaction was quenched with ~8 mL of THF, a few millilitres of water and 2 mL of 10% Na_2CO_3 solution. The two phases were separated

and the organics evaporated down to a damp oil which was redissolved in THF. This solution was added dropwise to a stirring suspension of 1.5g Ag_2CO_3. The grey-brown suspension was filtered after 15 minutes and extracted 3 x with EtOAc. Drying with (Na_2SO_4) and evaporation of the extracts gave 330 mg of a tan solid. This was purified on Fe^{3+}-Sephadex (prepared from 2.4g Sephadex-SP) yielding 187 mg of **5** as a light beige solid. mp 160-162°C (decomposition to red-black tar, bubbling). ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.01 (m, 2H), 6.78 (dd, 1H, $J_{7,6}$=7.7 Hz, $J_{7,5}$=1.9 Hz), 5.75 (s, 1H), 3.83 (s, 3H). GC/MS (TMS deriv.) m/z 355(52)M^+, 340(80), 327(22), 267(13), 238(100), 222(21), 207(61), 194(16), 191(25), 166(33). UV λ_{max} (MeOH) 257 nm (ϵ=8,200). IR (KBr) ν_{max} 3465 (s, br), 3240 (br), 2410 (br), 1679 (s), 1616 (m), 1599 (w), 1503 (s), 1479 (m), 1395 (m), 1331 (w), 1316 (w), 1270 (m), 1208 (m), 1180 (w), 1148 (m), 1092 (m), 1058 (m), 1028 (s), 1000 (w), 867, 820, 770, 740, 707 cm^{-1}.

7-t-Butyl-2,4-dihydroxy-2H-1,4-benzoxazin-3-one, 6

50 (0.500g, 2.0 mmol) was dissolved in CH_2Cl_2 and cooled under nitrogen to -60°C in a dry-ice/acetone bath. On addition of 6.0 mL (3 eq.) of 1M BCl_3 the reaction solution darkened only slightly. The flask was allowed to warm to room temperature over 2.5 hours where it remained for a further hour. The reaction was then quenched with ~15 mL THF and 2 mL of 10% Na_2CO_3 solution. The phases were separated in a separatory funnel, and the wet organics were evaporated to an oil. This was then redissolved in THF and added dropwise to a stirring suspension of 1.1g of Ag_2CO_3. After 15 minutes the grey-brown mixture was filtered and extracted 3 x with EtOAc. Dried over anhydrous $MgSO_4$ and evaporated to 505 mg crude orange oil which foamed under vacuum (~1 torr). This could be triturated with hexane:EtOAc and yielded 404 mg (86%) of **6** as a beige solid. mp 135-138°C (decomposition to red-black tar, bubbling). ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.26 (d, 1H, $J_{5,6}$=8.4 Hz), 7.14 (dd, 1H, $J_{6,5}$=8.4 Hz, $J_{6,8}$=2.1 Hz), 7.04 (d, 1H, $J_{8,6}$=2.1 Hz), 5.73 (s, 1H) 1.29 (s, 9H). 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 (ϵ=5,500), 258 nm (ϵ=7,100). IR (KBr) ν_{max} 3300 (br), 2960 (m), 2910 (m), 2870 (m), 1694 (s), 1670 (s), 1650 (s), 1600 (w), 1510 (m), 1399 (br, m), 1363 (m), 1302 (m), 1259 (m), 1207 (m), 1201 (w), 1154 (w), 1108 (m), 1060 (s), 1042 (s), 980, 910, 879, 827, 803, 707 cm^{-1}.

2,4-Dihydroxy-7-methyl-2H-1,4-benzoxazin-3-one, 7

51 (0.500g, 2.4 mmol) was dissolved in 40 mL of CH_2Cl_2 and cooled under

nitrogen to -60°C in a dry-ice/acetone bath. 1M BCl_3 in CH_2Cl_2 (7.2 ml, 3 eq.) was added via syringe and the flask was then allowed to warm to room temperature over 2.5 hours where stirring was continued for one hour. The reaction was quenched with ~8 mL of THF and 2 mL 10% Na_2CO_3 solution. The phases were separated in a separatory funnel and the aqueous extracted once with EtOAc. The wet organics were combined and evaporated to an oil. This was redissolved in THF and added dropwise to a stirring suspension of 1.3g of Ag_2CO_3. The mixture was filtered after 15 minutes, extracted 3 x with EtOAc, washed once with brine, dried, and evaporated to give 449 mg (96%) of **7** as a beige solid. mp 163-164°C (decomposition to a red-black tar, bubbling). ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.22 (d, 1H, $J_{5,6}$=8.2 Hz), 6.92 (m, 1H), 6.84 (d, 1H, $J_{8,6}$=1 Hz), 5.71 (s, 1H), 2.28 (s, 3H). 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 (ε=11,900). IR (KBr) ν_{max} 3460 (br), 1648 (s), 1620 (m), 1604 (m), 1522 (m), 1404 (w), 1305 (m), 1292 (m), 1240 (w), 1160 (m), 1150 (w), 1108 (w), 1054 (s), 1030 (s), 923, 814 cm^{-1}.

2,4-Dihydroxy-2H-1,4-benzoxazin-3-one, 8 (DIBOA)

52 (0.738g, 3.78 mmol) was dissolved in 50 mL CH_2Cl_2 and cooled to -30°C while under nitrogen in a dry-ice/acetone bath. 1M BCl_3 in CH_2Cl_2 (11.2 mL, 3 eq) was added via syringe and in ~5 minutes the solution had turned an emerald green colour. The flask was removed form the cooling bath and allowed to warm to room temperature where stirring was continued for 2 hours. (Total reaction time: 4 hours) The reaction was quenched with 20 mL THF and ~50 mL of water. After separtation of the phases, the organics were evaporated to an oil, redissolved in THF and added dropwise to a stirring suspension of 2g of Ag_2CO_3. After 30 minutes the mixture was filtered, the solids washed with THF, and the aqueous filtrate extracted 3 x EtOAc. The extracts were dried (anhydrous Na_2SO_4) and evaporated to 0.450g of a grey solid. Most of the colour could be removed by trituration with hexane and a few drops of acetone to give 0.424g (62%) of **8**, DIBOA. mp 155-157°C (Lit.[2] 155-56°C); (decomposition to red-black tar, bubbling). ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.36 (m, 1H), 7.06 (m, 3H), 5.74 (s, 1H) 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 (ε=7,800), 282 nm (ε=5,100). IR (KBr) ν_{max} 3270 (br), 2425 (br), 1666 (s), 1655 (s), 1601 (m), 1490 (m), 1401 (w), 1302 (w), 1279 (m), 1220 (m), 1096 (m), 1060 (s), 1043 (s), 982 (m), 831 (m), 790 (m), 756 (s), 690 (w) cm^{-1}.

7-Chloro-2,4-dihydroxy-2H-1,4-benzoxazin-3-one, 9

53 (1.50g, 6.53 mmol) was dissolved in 80 mL of CH_2Cl_2 and the resultant solution was cooled in an ice/water bath to 0-5°C while under nitrogen. 1M BBr_3 in CH_2Cl_2 (19.7 mL, 3 eq.) was added via syringe and the reaction mixture was stirred for 3 hours at this temperature. The cold reaction was quenched with 30 mL of THF and 50 mL of water. The phases were separated in a separatory funnel. (The addition of ~50 mL of extra CH_2Cl_2 was necessary to achieve good phase separation.) The organics were then evaporated to an oil, redissolved in THF and added over 10 minutes to a stirring suspension of 3.6g of Ag_2CO_3. After 30 minutes the grey-green mixture was filtered, the filtrate diluted with one-half volume of water and extracted 3 x EtOAc. After drying ($MgSO_4$), the extracts were evaporated to a dark purple semi-solid. This was purified on Fe^{3+}-Sephadex (prepared from 11g of Sephadex-SP). Extraction of the hydroxamic acid fractions with EtOAc, drying and evaporation, yielded a greyish semisolid which was triturated with hexane:$CHCl_3$ (1:1) to give 680 mg (48%) of the title compound, mp 173-174°C (decomposition to red-black tar, bubbling). ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.35 (d, 1H, $J_{5,6}$=8.6 Hz), 7.14 (dd, 1H, $J_{6,5}$=8.6 Hz, $J_{6,8}$=2.2 Hz), 7.07 (d, 1H, $J_{8,6}$=2.3 Hz), 5.78 (s, 1H). 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 (ε=5,900), 255 nm (ε=9,600). IR (KBr) ν_{max} 3170 (br, s), 1675 (s), 1600 (m), 1520 (s), 1487 (m), 1390 (m), 1291 (s), 1220 (m), 1105 (m), 1081 (m), 1040 (s), 984 (m), 940 (w), 885 (m), 867 (m), 815 (s) cm^{-1}.

2,4-Dihydroxy-7-fluoro-2H-1,4-benzoxazine-3-one, 10

54 (100 mg, 0.47 mmol) was partially dissolved in CH_2Cl_2 and cooled to -45°C while under nitrogen in a dry-ice/acetone bath. On addition of 1M BBr_3 in CH_2Cl_2 (1.4 mL, 3 eq.) the material appeared to finally dissolve completely and the solution turned purple. The flask was then allowed to warm to 10°C over about two hours, whereapon the reaction mixture was quenched with 4 mL of THF and 1 ml of 10% Na_2CO_3 solution. The contents of the flask were poured into a separatory funnel with 20 ml of water and 45 mL of EtOAc was added to make two distinct layers. TLC at this time appeared to show conversion to the desired compound **10** without the use of Ag_2CO_3. The organics were dried ($MgSO_4$) and evaporated to a brown oil. This would not solidify on trituration with hexane:$CHCl_3$. While working with the material it had darkened considerably.

Chromatographed on silica (1mm Chromatotron® plate) using CH_2Cl_2 and increasing the proportion of MeOH up to 5%. The fractions that appeared to show product by TLC were combined and evaporated to a light brown oil. This was dissolved in Et_2O (2 mL) and diluted with hexane (2 mL) whereapon crystals formed. These were filtered and yielded 23.4 mg (25%) of pure **10**. mp 145-146°C (decomposition to black tar with bubbling). ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.35 (m, 1H), 6.88 (m, 2H), 5.77 (s, 1H). 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 (ε=4,700), 249 nm (ε=6,500). IR (KBr) ν_{max} 3400-3200, 2860 (br), 1659 (s), 1615 (m), 1504 (s), 1412 (w), 1291 (w), 1271 (m), 155 (m), 1121 (m), 1102 (w), 1060 (m), 1039 (m), 984, 854, 818 cm^{-1}.

7-Carbomethoxy-2,4-dihydroxy-2H-1,4-benzoxazin-3-one, 11a

55a (0.600g, 2.37 mmol) was dissolved in CH_2Cl_2 and cooled to -60°C while under nitrogen in a dry-ice/acetone bath. To this was added 1M BBr_3 in CH_2Cl_2 (7.1 mL, 3 eq.). No colour change from the light orange of **55a** was evident. The flask contents were then allowed to warm slowly. At -15°C, after about 1.5 hours, an aliquot was worked up and appeared to show partial conversion to product. The reaction vessel was removed from the cooling bath and warmed to room temperature where stirring was continued for one hour. 10 mL of THF was then added (the solution fumed briefly), followed by ~6 mL of 10% Na_2CO_3 solution, and 40 mL of water. The mixture was then poured into a separatory funnel and enough EtOAc added to 'float' the CH_2Cl_2 layer. The organics were evaporated without drying, and the resultant dark oil was redissolved in 10 mL THF. This solution was added dropwise to a stirring suspension of 1.8g of Ag_2CO_3. The green colour of the silver salt did not change to the normal grey. After 30 minutes the suspension was filtered and the filtrate extracted 3 x with EtOAc, dried over anhydrous Na_2SO_4, decolourized with activated charcoal, and evaporated to ~1 mL of mobile oil. This represented more than 100% theoretical yield. The volume could not be reduced to any extent by gentle warming on a mechanical pump (1 torr). This residue was then purified on Fe^{3+}-Sephadex (prepared from 3.6g Sephadex-SP). Extraction of the relevant fractions with EtOAc after release from the ferric chelate with EDTA, drying and evaporation gave 40.0 mg (7%) of the title compound. mp 186-187°C (decomposition to red-black tar with bubbling). ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.78 (dd, 1H, $J_{6,5}$=8.4 Hz, $J_{6,8}$=1.7 Hz), 7.59 (d, 1H, $J_{8,6}$=1.7 Hz), 7.46 (d, 1H, $J_{5,6}$=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 (ε=8,000), 273 nm (ε=9,600). IR (KBr) ν_{max}

3260 (br), 1685 (shoulder), 1673 (s), 1607 (m), 1593 (m), 1511 (m), 1443 (m), 1393 (m), 1320 and 1301 (m, overlapped), 1256 (m), 1113 (m), 1102 (m), 1023 (m), 987, 970, 900, 888, 838, 811, 770 cm^{-1}.

7-Carboethoxy-2,4-dihydroxy-2H-1,4-benzoxazin-3-one, 11b

55b (0.647g, 2.42 mmol) was dissolved in 25 mL of CH_2Cl_2 and kept under nitrogen. 1M BCl_3 in CH_2Cl_2 (12.0 mL, 5 eq.) was added at room temperature and the solution was stirred for 48 hours. After quenching with 10 mL of THF and 15 mL of water the layers were separated and the aqueous layer was extracted once with EtOAc. TLC of the dried organics at this point showed partial conversion to the expected product. The organic solution was evaporated to an oil, redissolved in THF and added dropwise to a stirring suspension of 2g Ag_2CO_3. After 15 minutes most of the green silver salt appeared to remain. The mixture was filtered and the filtrate was extracted 3 x EtOAc. These extracts were washed with brine, dried (anhydrous $MgSO_4$) and evaporated to an orange oil. Trituration of this material with hexane:acetone at 0-5°C gave 183 mg of a pale beige solid. Concentration of the mother liquor and a second trituration gave another 100 mg of material. Both lots were pure by GC analysis. Total yield of **11b**: 283 mg (46%). mp 163-165°C (decomposition to red-black tar with bubbling). ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.79 (dd, 1H, $J_{6,5}$=8.4 Hz, $J_{6,8}$=1.8 Hz), 7.60 (d, 1H, $J_{8,6}$=1.8 Hz), 7.46 (d, 1H, $J_{5,6}$=8.4 Hz), 5.82 (s, 1H), 4.32 (q, 2H), 1.35 (t, 3H). 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} 3360 (br), 3120 (br), 2900 (br), 2700 (br), 1690 (s), 1615 (m), 1600 (m), 1500 (w), 1442 (m), 1395 (m), 1295 (s), 1254 (m), 1220 (w), 1188 (w), 1108 (m), 1065 (m), 1040 (m), 983, 929, 835, 810, 765 cm^{-1}.

2,4-Dihydroxy-5-methyl-2H-1,4-benzoxazin-3-one, 17

58 (0.620g, 2.96 mmol) was dissolved in 45 mL of CH_2Cl_2 and cooled while under nitrogen in a dry-ice/acetone bath to -55°C. 1M BCl_3 (8.9 mL, 3 eq.) was added via syringe. The flask contents were allowed to warm to 15°C over 2.5 hours. It was stirred at 15-20°C for a further 30 minutes then quenched with 10 mL of THF and ~10 mL of water. The layers were separated, the aqueous portion extracted once with EtOAc, and the combined organics evaporated to a wet oil. This was redissolved in THF and added dropwise to a stirring suspension of 1.6g Ag_2CO_3. After 15 minutes the green salt had darkened to grey-brown. The mixture was filtered, the aqueous filtrate was extracted 3 x

with EtOAc, washed with brine, dried over anhydrous $MgSO_4$ and evaporated to a yellow-brown oil. TLC showed that a small amount of the starting material **58** was still present. The oil only partially solidifed on standing for two days. Trituration with hexane:CH_2Cl_2 (1:1) gave 207 mg of **17** as a pale beige solid. mp 138-141°C (decomposition to red-black tar with bubbling). ^{1}H-NMR (300 MHz, acetone-d_6) δ 9.55 (br s, <1H, aldehyde?), 6.92 (m, 3H) 5.70 (s, 1H), 2.55 (s, 3H) 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=2,600), 257 nm (ε=6,000). IR (KBr) ν_{max} 3270, 3120, 2980, 2715 (broad and overlapped), 1690 (vs), 1670 (s), 1609 (w), 1592 (w), 1509 (w), 1473 (m), 1450 (m), 1434 (m), 1390 (m), 1282 (m), 1246 (m), 1161 (w), 1089 (m), 1025 (s), 980, 939, 810, 776, 763, 719 cm^{-1}.

2-Hydroxy-2H-1,4-benzoxazin-3-one, 12 (HBOA)

61 (0.500g, 2.79 mmol) was dissolved in 30 mL CH_2Cl_2 under nitrogen and the solution cooled in an ice/water bath to 0-5°C. 1M BCl_3 (8.3 mL, 3 eq.) was added and the solution darkened. Stirring was continued at 0-5°C for one hour then warmed to room temperature for 1.5 hours. The reaction mixture was then quenched with ~10 mL of THF, 10 mL of water, stirred briefly, and then the layers were separated in a separatory funnel. The wet organics were evaporated to an oil, redissolved in THF and added to a stirring suspension of 1.5g of Ag_2CO_3. After 15 minutes this mixture was filtered and the aqueous filtrate extracted with EtOAc. After drying (anhydrous $MgSO_4$) and decolourizing with activated charcoal, the extracts were concentrated and hexane added until the solution was turbid. Two crops of crystals were collected, (223 mg and 104 mg respectively). Total yield of **12**: 327 mg (71%). mp 208-208.5°C (Lit.[3] 201-203°C) ^{1}H-NMR (300 MHz, acetone-d_6) δ 9.65 (br s, <1H, NH), 6.94 (m, 4H), 5.58 (s, 1H). 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=4,300), 250 nm (ε=8,900). IR (KBr) ν_{max} 3220 (br), 3135, 3050, 2985, 2915 (m, overlapped), 1698 (s), 1686 (s), 1610 (m), 1502 (s), 1406 (m), 1280 (m), 1202 (m), 1080 (s), 1039 (s), 1020 (s), 940, 833, 779, 750 cm^{-1}.

2-Hydroxy-7-methoxy-2H-1,4-benzoxazin-3-one, 13 (HMBOA)

62 (0.510g, 2.44 mmol) was dissolved in 40 mL of CH_2Cl_2 and, while under nitrogen, cooled in an ice/water bath. 1M BCl_3 in CH_2Cl_2 (8.5 mL, 3 eq.) was added via syringe, whereapon the solution darkened to a red-black. Stirring was continued at 0-5°C for one hour then the flask was allowed to warm to room temperature where stirring was

continued for two more hours. The reaction mixture was quenched with 10 mL THF and 20 mL of water, the layers separated and the organics evaporated to an oil. This was redissolved in THF and added dropwise to a stirring suspension of 1.4g Ag_2CO_3. After 15 minutes this grey-brown suspension was filtered and the filtrate extracted with EtOAc. Evaporation of the dried extracts gave 0.510g of a semi-solid. This could be triturated with hexane:$CHCl_3$ and yielded 0.429g (90%) of the title compound. mp 198-199.5°C. (Lit.[4] 196-198°C, MeOH) ^{1}H-NMR (300 MHz, acetone-d_6) 9.5 (br s, NH), 6.92 (dd, 1H, $J_{6,5}$=7.1 Hz, $J_{6,8}$=1.8 Hz), 6.58 (m, 2H) 5.55 (s, 1H), 3.74 (s, 3H). 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} 3200, 3070, 2960 (broad and overlapped), 1693 (s), 1610 (w), 1514(s), 1465 (m), 1451 (m), 1428 (w), 1414 (w), 1310 (w), 1276 (m), 1240 (m), 1200 (m), 1192 (m), 1161 (s), 1130 (m), 1108 (m), 1087 (s), 1023 (s), 981, 959, 932, 840 cm^{-1}.

Synthesis of 2-deoxy compounds 14 & 15

4-Hydroxy-2H-1,4-benzoxazin-3-one, 14

o-Nitrophenol (10.0g, 72 mmol) was dissolved in 150 mL warm ethanol and one equivalent of KOH in 95% ethanol was added with stirring, giving a red orange suspension of the nitrophenoxide. To this was added ethyl bromoacetate (8.0 mL, 12.00g, 1 eq.) and the mixture was set to reflux for 20 hours. TLC of the cooled reaction mixture showed that starting nitrophenol still remained, but it was worked up anyway. The contents of the reaction vessel were poured into 300 mL of water and extracted 3 x chloroform.The combined organics were then washed 3 x 10% Na_2CO_3 to get rid of o-nitrophenol, once with brine, dried over anhydrous Na_2SO_4 and evaporated to an orange oil. This material recrystallized rather poorly from MeOH giving crude ethyl α-(2-nitrophenoxy)-acetate (10.55g, 65%) as an oily orange solid.

A portion of this nitroester (5.38g, 24.0 mmol) was reductively cyclized following the general procedure outlined in section **7.3**. Acidification of the filtrate, after removal of the catalyst, spontaneoulsy gave a mass of fluffy, fibrous crystals. Filtering and cooling of the mother liquer allowed the collection of three crops: 1.13g, 0.433g and 0.320g. Extraction of the filtrate with EtOAc and purification on a silica column (CH_2Cl_2 with 1% MeOH) gave another 0.246g of the title compound. Total yield of **14**: 2.13g (54%) mp 162-165°C (slow decomposition). (Lit.[2] 167-169°C.) ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.32 (dd, 1H,

$J_{5,6}$=7.8 Hz, $J_{5,7}$=1.6 Hz), 7.05 (m, 3H), 4.69 (s, 2H), 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 (ϵ=4,400), 255 nm (ϵ=5,600). IR (KBr, cm^{-1}) 3180 and 2840 (broad and overlapped), 1680 (s), 1646 (s), 1608 (m), 1499 (s), 1460 (m), 1439 (m), 1400 (m), 1380 (m), 1305 (w), 1280 (m), 1230 (m), 1129 (w), 1088 (w), 1058 (m), 930, 825, 745 cm^{-1}.

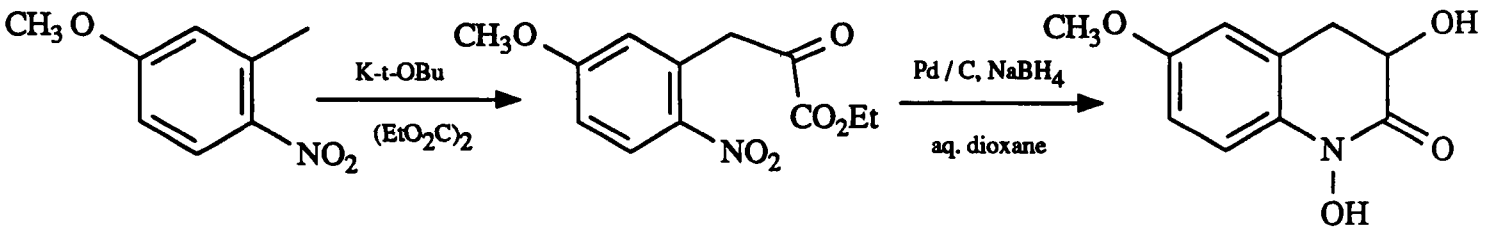
4-Hydroxy-7-methoxy-2H-1,4-benzoxazin-3-one, 15

5-methoxy-2-nitrophenol, **28**, (2.53g, 15.0 mmol) was dissolved in 80 mL acetone to which had been added 6g of anhydrous K_2CO_3. Methyl bromoacetate (1.43 mL, 15.0 mmol) was added and the mixture refluxed overnight. After cooling, the solids were removed by filtration and the acetone solution poured into 200 mL of water. This was then extracted with chloroform and the combined organics were extracted with 10% Na_2CO_3 to remove starting nitrophenol. After drying over anhydrous Na_2SO_4 and evaporating an oil was isolated which slowly crystallized on standing. This could be recrystallized from EtOH and gave 2.31g (64%) of a yellowish solid.

Reductive cyclization of this nitroester (1.15g, 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 **15** (0.589g, 64%) as as pinkish solid. mp 121-123°C (decomposition to dark tar); (Lit.[2] 125-127°C ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.21 (d, 1H, $J_{5,6}$=8.8 Hz), 6.63 (dd, 1H, $J_{6,5}$=8.8Hz, $J_{6,8}$=2.6 Hz), 6.55 (d, 1H, $J_{8,6}$=2.6 Hz), 4.67 (s, 2H), 3.76 (s, 3H). 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) (ϵ=6,200), 268 nm (ϵ=8,100). IR (KBr) ν_{max} 3190, 2870, 2690 (broad and overlapped), 1665 (s), 1620 (m), 1601 (m), 1513 (s), 1502 (s), 1472 (m), 1441 (m), 1430 (m), 1418 (m), 1366 (w), 1357 (m), 1308 (m), 1275 (m), 1239 (m), 1207 (m), 1200 (m), 1164 (s), 1129 (m), 1086 (w), 1035 (m), 928, 835, 820, 800, 768, 640 cm^{-1}.

Synthesis of carbocyclic analogue 18
(1,3-dihydroxy-6-methoxy-1,2,3,4-tetrahydroquinolin-2-one)

Following the procedure of Allen *et al*[5] diethyl oxalate (9.49g, 65 mmol) was added to a suspension of potassium *t*-butoxide (6.90g, 61.1 mmol) in 250 mL of benzene. This gave a lemon-yellow solution. A solution of 5-methoxy-2-nitrotoluene (10.00g, 59.9 mmol) in 50 mL of benzene was added dropwise over 20 minutes from a dropping funnel



18

Scheme 6.2.

fitted to the flask, then the dark red solution was set to reflux overnight. GC analysis of an acidified aliquot of the reaction mixture showed that the reaction had progressed to only ~50% completion. An extra 1.5g of K-*t*-OBu and 4 mL of diethyl oxalate was added, but after three more hours of reflux the degree of completion was not enhanced. At this time a purple-black solid had precipitated from the reaction solution. This was filtered off without protection from the atmosphere and washed with benzene. This material was soluble in water. After acidification (HCl), and extraction from the aqueous phase with EtOAc, drying and evaporation yielded ethyl 3-(5-methoxy-2-nitrophenyl)-pyruvate (6.90g, 43%) as a stiff oil which was nearly pure (>99% by GC).

This nitroester (5.6g, 21.0 mmol) was then reductively cyclized by the standard procedure. Addition of a drop of the nitroester to the stirring reduction mixture gave a deep-red colour that was allowed to slowly disappear before more material was added. The filtered and acidified (HCl) solution was extracted with EtOAc, treated with activated charcoal to remove most of the colour, and evaporated to a red-brown oil. This was disolved in $CHCl_3$:EtOAc and diluted with hexane to give several crops of brown crystals of total mass 2.26g. This was then purified on Fe^{3+}-Sephadex (prepared from 30g Sephadex-SP) and yielded 1.10g (25%) of pure **18**. mp 159-160°C. ^{1}H-NMR (300 MHz, acetone-d_6) δ 7.22 (d, 1H, $J_{5,6}$=9.7 Hz), 6.85 (m, 2H), 4.28 (ABX quartet, 1H, $J_{2,1}$=15.2 Hz and 6.2 Hz), 3.77 (s, 3H), 3.11 (q, 1H), 2.85 (q, 1H, revealed by D_2O 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=12,600). IR (KBr) ν_{max} 3435 (m), 3350, 3200, 2920 (broad and overlapped), 1670 (s), 1647 (s), 1590 (m), 1494 (s), 1450 (m), 1432 (m), 1382 (w), 1308 (m), 1260 (s), 1241 (s), 1160 (m), 1140 (m), 1115 (m), 1071 (m), 1053 (m), 1045 (m), 999 (w), 939 (m), 890, 804, 675 cm^{-1}.

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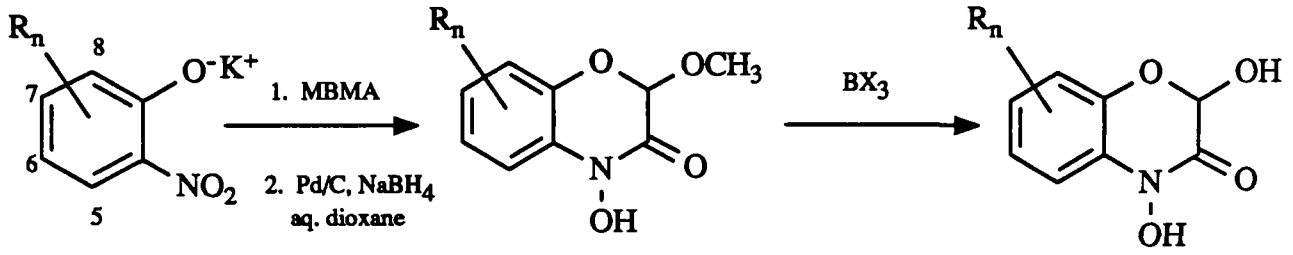
Table 7.1

[a] The numbering system used is that if DIMBOA and related benzoxazinones. [b] These numbers are the yields for the two reactions. The first is the yield of the coupling of the potassium nitrophenoxide with methyl α-bromo-α-methoxyacetate (MBMA) and the second is the yield for reductive cyclization. [c] 'Seph" denotes that the product had to be purified by Fe^{3+}-Sephadex affinity chromatography. For details see section 7.4. [d] MDO describes the methylenedioxy group. [e] Purification of the products of this reaction was not achieved. [f] These nitrophenols were commercially available. During this work **41** also became commercially available. [g] The demethylation of these compounds was not attempted.

TABLE 7.1. Yields for the coupling, reductive cyclization and demethylation reactions .



R_n [a]	Nitrophenoxide		Hydroxamic acid (Me-acetal)		Hydroxamic acid (hemiacetal)
7-MeO	**28**	92 x 70 [b]	**16**	68	**1**
7,8-diMeO	**29**	48 x 90	**45**	31 (Seph) [c]	**2**
6,7-diMeO	**30**	99 x 30 (Seph)	**46**	22 (Seph)	**3**
7,8-MDO [d]	**31**	99 x 68	**47**	- [e]	-
6,7-MDO	**32**	90 x 33 (Seph)	**48**	38	**4**
8-MeO	**33**	65 x 42 (Seph)	**49**	33 (Seph)	**5**
7-t-Bu	**39**	91 x 56	**50**	86	**6**
7-Me	- [f]	91 x 76	**51**	96	**7**
7-H	- [f]	99 x 83	**52**	62	**8**
7-Cl	**40**	95 x 66	**53**	48 (Seph)	**9**
7-F	**41**	80 x 93	**54**	25	**10**
7-CO_2Me	**42a**	75 x 54	**55a**	7 (BBr_3, Seph)	**11a**
7-CO_2Et	**b**	89 x 93	**b**	30 (BCl_3)	**b**
7-CF_3	**43**	70 x 99	**56**	no rxn	-
7-CN	**44**	89 x 93	**57**	no rxn	-
5-Me	- [f]	91 x 94	**58**	36	**17**
7-Me_2N	**34**	94 x 72	**25**	[g]	-
7-MeO-5-Me	**38**	98 x 30 (Seph)	**27**	[g]	-
HBOA	- [f]	92 x 52	**59**	71	**12**
HMBOA	**28**	92 x 59	**60**	90	**13**

Table 7.2. Ultraviolet spectral data for compounds **1** - **18** and their measured pK_a's.

		UV Maxima					
Compound	Substituent	pH = 9 (Tris)	ϵ (x 10^{-3})	pH=13 (NaOH)	ϵ (x 10^{-3})	pK_{A1}	pK_{A2}
1	7-MeO	293	10.4	298	11.0	6.92 ± 0.02	10.10 ± 0.07
2	7,8-diMeO	286	10.3	NM		7.03 ± 0.06	NM
3	6,7-diMeO	310	10.7	NM		6.91 ± 0.04	NM
4	6,7-MDO	317	10.1	NM		6.91 ± 0.03	NM
5	8-MeO	284	8.6	NM		6.88 ± 0.03	NM
6	7-t-Bu	296	10.2	298	9.5	6.94 ± 0.03	11.00 ± 0.07
7	7-Me	296	10.1	298	9.8	6.83 ± 0.03	10.56 ± 0.11
8	7-H	296	10.0	298	9.9	6.91 ± 0.04	10.55 ± 0.12
9	7-Cl	302	13.3	304	12.6	6.78 ± 0.02	10.22 ± 0.10
10	7-F	286	7.3	-	≈5.7 [b]	6.63 ± 0.02	-
11	7-CO_2Me	324	11.1	334	7.3 [b]	6.52 ± 0.02	9.90 ± 0.07
12	(HBOA)	251 282 sh	7.4 3.5	NM		NA	
13	(HMBOA)	260 286 sh	8.9 5.5	NM		NA	
14	7-H-[2H]	296	8.4	NA		7.40 ± 0.02	NA
15	7-MeO-[2H]	298	9.7	NA		7.23 ± 0.03	NA
16	(DIMBOA-Me)	295	12.0	NA		6.74 ± 0.03	NA
17	5-Me	273 [b]	-			-	
18	(carbocyclic)	288	12.4 [c]	NM		7.96 ± 0.10	NM

[a] Due to problems of reproducibility at pH 12.5, the UV spectra of doubly ionized **10** and **11** were recorded at pH 11.4. [b] **17** decayed rapidly in solution, consequently reliable absorbance data were unobtainable. [c] The UV monoanion of **18** was recorded at pH 10.2.
NM = not measured
NA = not applicable

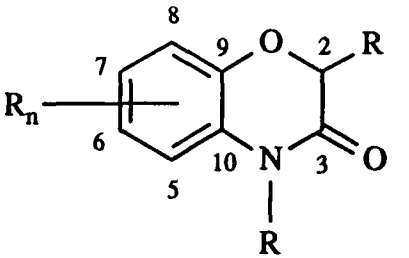


TABLE 6.3. ^{13}C NMR chemical shifts (δ) of compounds **1** - **18** in DMSO-d_6 .

CARBON	COMPOUND																	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18** [a]
1																		33.6
2	92.4	92.2	92.2	92.1	91.6	92.1	92.0	92.0	92.2	92.3	91.8	90.3	90.5	67.7	68.1	98.2	92.5	55.2
3	156.7	157.0	156.8	157.0	157.3	157.2	157.2	157.4	157.2	159.7	157.8	162.5	161.9	159.8	159.1	156.1	159.0	165.1
5	113.6	106.0	98.4	99.7	105.3	113.9*	112.7	112.9	114.1	113.7	112.9	115.6	120.0	115.7	123.1	113.8	126.4*	113.4*
6	107.6	107.2	144.8	143.1	129.5*	119.0	122.7	123.6	122.4	108.8	124.1*	123.1	108.1	122.3	107.5	108.1	126.1*	111.9
7	155.9	149.3	143.9	142.4	107.7	146.7	133.2	122.4	127.1*	156.7	124.7*	122.4	155.5	123.4	155.9	155.0	124.1	154.8
8	103.4	138.3	102.7	94.9	148.7	112.5*	117.4	117.0	117.1	105.0	117.5	117.4	103.6	112.8	102.5	103.5	115.2	113.8*
9	141.5	134.9	133.8	134.8	121.9	140.2	140.4	140.5	141.4	141.5	140.2	140.6	141.0	143.3	144.5	140.9	142.1	122.6
10	122.4	123.7	121.1	122.6	129.4*	126.2	126.3	128.6	127.9*	125.5	132.7	126.6	116.0	129.3	113.8	122.3	127.4	132.7
7-MeO	55.5	56.2	56.1*										55.6		55.6	55.9*		66.4
		60.7 (8-MeO)	56.0* (6-MeO)	101.4 (MDO)[d]	55.8 (8-MeO)	34.1[b] 31.1	20.4 (7-Me)				165.2[c] 52.1					55.4* (2-MeO)	20.6 (5-Me)	

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.
[a] The numbering of the hydroxamic acid parent structure has been used for the tetrahydroquinolin-2-one analogue **18** (carbocyclic). [b] Resonance of the quaternary carbon in the *t*-butyl group was 34.1 ppm and of the methyl groups 31.1 ppm. [c] Resonance of the carbonyl in the 7-CO_2Me group was 165.2 ppm and of the associated methoxy 52.1 ppm. [d] MDO refers to the methylenedioxy group in compound **4**.

CLAIMS TO ORIGINAL RESEARCH

A.

The following compounds were synthesized as analogues of the naturally occurring DIBOA and are newly reported:

1. 4-Hydroxy-2-methoxy-7,8-methylenedioxy-1,4-benzoxazin-3-one (**47**)
2. 4-Hydroxy-2-methoxy-7-trifluoromethyl-1,4-benzoxazin-3-one (**56**)
3. 7-Cyano-4-hydroxy-2-methoxy-1,4-benzoxazin-3-one (**57**)
4. 7-Dimethylamino-2-methoxy-1,4-benzoxazin-3-one (**25**)
5. 2,7-Dimethoxy-5-methyl-1,4-benzoxazin-3-one (**27**)
6. 2,4-Dihydroxy-7,8-dimethoxy-1,4-benzoxazin-3-one (**2**)
7. 2,4-Dihydroxy-6,7-dimethoxy-1,4-benzoxazin-3-one (**3**)
8. 2,4-Dihydroxy-6,7-methylenedioxy-1,4-benzoxazin-3-one (**4**)
9. 2,4-Dihydroxy-8-methoxy-1,4-benzoxazin-3-one (**5**)
10. 7-*t*-Butyl-2,4-dihydroxy-1,4-benzoxazin-3-one (**6**)
11. 2,4-Dihydroxy-7-methyl-1,4-benzoxazin-3-one (**7**)
12. 7-Chloro-2,4-dihydroxy-1,4-benzoxazin-3-one (**9**)
13. 2,4-Dihydroxy-7-fluoro-1,4-benzoxazin-3-one (**10**)
14. 7-Carbomethoxy-2,4-dihydroxy-1,4-benzoxazin-3-one (**11a**)
15. 7-Carboethoxy-2,4-dihydroxy-1,4-benzoxazin-3-one (**11b**)
16. 2,4-Dihydroxy-5-methyl-1,4-benzoxazin-3-one (**17**)
17. 1,3-Dihydroxy-6-methoxy-1,2,3,4-tetrahydro-quinolin-2-one (**18**) (carbocyclic)

B.

Linear free energy relationships were developed for the C-7 substituted series and have been used to show that during the transition state for decomposition of these cyclic hydroxamic acids to bexnzoxazolinones electron density decreases at nitrogen. No other reactivity series for these materials has been reported.

C.

The known reactivity of DIMBOA towards thiols has been extended by measuring the rate constants for the reaction with three other analogues **2** (naturally occurring) **3** and

4.

D.

An ^{1}H-NMR study showed that prior to reduction by thiols the reactive aldehyde from the open form of the lactol is attacked by thio(ate), and that this also occurs for those compounds which are not reduced at the nitrogen atom but at different rates. The importance of the open form to the reduction reaction and the possibility of the aldehyde mitigating biological activity now has firm support.

E.

The analogues have been tested for toxicity against the European corn borer (*Ostrinia nubilalis*) in feeding trials and several compounds have shown activity comparable to that of DIMBOA. Their biological activities has been discussed in the light of their reactivity towards thiols, their facility at undergoing the decomposition to BOAs, and their activity as proteolytic enzyme inhibitors during *in vitro* tests.

PUBLICATIONS FROM THIS WORK

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