JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Isolation and Synthesis of Allelochemicals from Gramineae: Benzoxazinones and Related Compounds

FRANCISCO A. MACÍAS,* DAVID MARÍN, ALBERTO OLIVEROS-BASTIDAS,† DAVID CHINCHILLA, ANA M. SIMONET, AND JOSÉ M. G. MOLINILLO

Grupo de Alelopatía, Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, C/República Saharaui s/n, 11510 Puerto Real (Cádiz), Spain

Compounds with a (2*H*)-1,4-benzoxazin-3(4*H*)-one skeleton have attracted the attention of phytochemistry researchers since 2,4-dihydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA) and 2,4-dihydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIMBOA) were isolated from plants belonging to the Poaceae family. These compounds exhibit interesting biological properties, such as phytotoxic, antimicrobial, antifeedant, antifungal, and insecticidal properties. These chemicals, in addition to a wide variety of related compounds involved in their metabolism, detoxification mechanisms, and degradation on crop soils and other systems, have high interest and in some cases potential agronomic utility. This paper presents a complete review of the methods employed for their synthetic obtention in addition to some of the authors' own contributions to their chemistry. The degradation and phytotoxicity experiments carried out in ongoing research into the potential agronomic utility of these compounds required large amounts of them, which were obtained from natural sources. This paper presents a modified methodology to access DIMBOA from *Zea mays* cv. Apache and to obtain 2-*O*-β-D-glucopyranosyl-2,4-dihydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA-Glc) and DIBOA from *Secale cereale* L. New synthetic methodologies were employed for the obtention of the lactams 2-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one and 2-hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one and the malonamic acids *N*-(2-hydroxyphenyl)malonamic acid and *N*-(2-hydroxy-7-methoxyphenyl)-malonamic acid. The aminophenoxazines 2-amino-7-methoxyphenoxazin-3-one and 2-acetamido-7-methoxyphenoxazin-3-one have been synthesized in the authors' laboratory by novel procedures. All of the methodologies employed allowed the desired compounds to be obtained in high yield and in an easy-to-scale manner.

KEYWORDS: Allelochemicals; DIMBOA; DIBOA; soil degradation products; wheat; isolation; synthesis; preparation

INTRODUCTION

The isolation, structural elucidation, and synthesis of natural products constitute the main origin of new molecules with biological activity. Biological effects of natural products are very diverse. Pharmacological properties and the possibility to control the development of natural or cultivated ecosystems have acquired special relevancy in recent years (*1*).

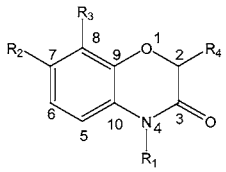
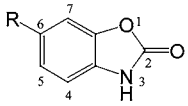
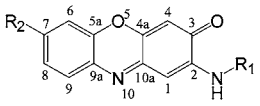
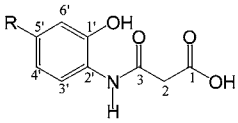
During the past 30 years, large efforts have been dedicated to the discovery of new allelochemicals (natural plant toxins) with potential application in weed management (*2*). The biocides developed from allelochemicals have important advantages with respect to traditional herbicides, fungicides, or insecticides: they have new modes of action, high biodegradability, and also low environmental impact (*3*).

Benzohydroxamic acids containing a 1,4-benzoxazin moiety have attracted the attention of phytochemistry researchers since the first isolation of 2,4-dihydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIMBOA) (*4*) and 2,4-dihydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA) (*5*) (**Table 1A**). These compounds are present in the seedlings of several Poaceae as 2-*O*-β-D-glucosides (*6*−*8*). The main hydroxamic acid in maize is 2-*O*-β-D-glucopyranosyl-4-hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIMBOA-Glc, **Table 1A**) with lesser amounts of 2-*O*-β-D-glucopyranosyl-4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA-Glc, **Table 1**) and 2-*O*-β-D-glucopyranosyloxy-4-hydroxy-7,8-dimethoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIM_2BOA-Glc, **Table 1**) (*9*). DIMBOA-Glc is also the major cyclic hydroxamic acid in wheat, whereas DIBOA-Glc is found in rye (*10*).

* Author to whom correspondence should be addressed (telephone +34-956.016.370; fax +34-956.016.295; e-mail famacias@uca.es).
† Permanent address: Laboratorio de Química Ecológica, Departamento de Química, Facultad de Ciencias, Universidad de Los Andes, Núcleo Universitario Pedro Rincón Gutiérrez, La Hechicera, Mérida 5101-A, Venezuela.

10.1021/jf050896x CCC: $33.50
Published on Web 01/24/2006

Table 1. Benzoxazinone Allelochemical Structures

A Benzoxazinones	B Benzoxazolinones	C Aminophenoxazines	D Malonamic Acids
			
R_1=OH, R_2=R_3=H, R_4=OGlc	R=H	R_1=H, R_2=H	R=H
DIBOA-Glc	**BOA**	**APO**	**HPMA**
R_1=OH, R_2=OCH_3, R_3=H, R_4=OGlc	R=OCH_3	R_1=OAc, R_2=H	R=OCH_3
DIMBOA-Glc	**MBOA**	**AAPO**	**HMPMA**
R_1=OH, R_2=R_3= OCH_3, R_4=OGlc		R_1=H, R_2=OCH_3	
DIM_2BOA-Glc		**AMPO**	
R_1=R_2=R_3=H, R_4=OGlc		R_1=OAc, R_2=OCH_3	
Blepharin		**AAMPO**	
R_1=R4=OH, R_2=OCH_3, R_3=OH			
DIMBOA			
R_1=R_4=OH; R_2=R_3=H			
DIBOA			
R_1=R_3=H, R_2=OCH_3, R_4=OH			
HMBOA			
R_1=R_2=R_3=H, R_4=OH			
HBOA			

There are interesting works that deal with the biological activities of benzohydroxamic acids (*11*−*14*) together with some of their degradation derivatives (*15*, *16*) in different environments and also synthetic analogues (*17*) investigated to discover structure−activity relationships.

There are a wide variety of metabolites related to 1,4-benzoxazin-3-ones, which have also attracted interest in phytochemical and allelopathic studies. The benzoxazinone lactams were first described by Niemeyer et al. (*18*). The most interesting compounds with this base structure are 2-hydroxy-(2*H*)-1,4-benzoxazin-3(*4H*)-one (HBOA) and 2-hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (HMBOA) (**Table 1A**). They have been proposed as biosynthetic precursors as well as degradation products from benzoxazinones (*19*−*21*). The degradability of benzoxazinones in different environments (*21*−*26*) moved the attention to their degradation products, some of them being more stable than the original plant metabolites.

The degradation products 2-benzoxazolinone (BOA) and 6-methoxy-2-benzoxazolinone (MBOA) (**Table 1B**) are produced by means of spontaneous degradation in aqueous solutions (*21*−*23*, *27*) as well as biological processes (*21*, *23*, *24*). There are interesting works about their bioactivity (*11*, *14*−*17*) and mode of action (*28*). Benzoxazolinones, when degraded to the corresponding 2-aminophenols, followed by further dimerization, yield aminophenoxazines (**Table 1C**). They have been found to occur in different crop soils (*21*, *23*, *25*, *26*) as well as by means of microbial transformations (*29*).

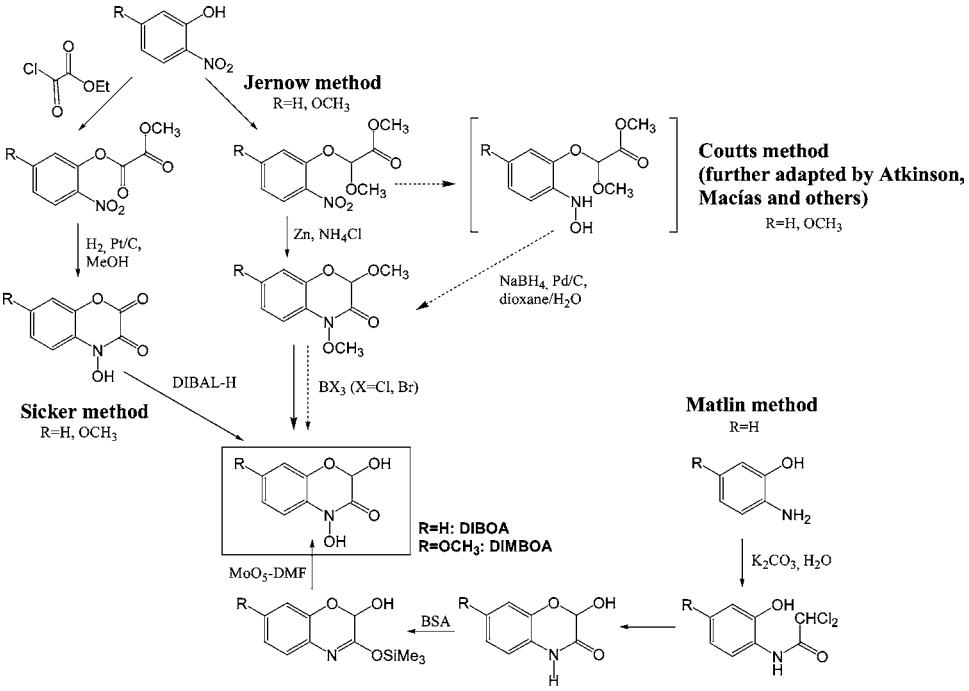
The malonamic acids *N*-(2-hydroxyphenyl)malonamic acid (HPMA) and *N*-(2-hydroxy-4-methoxyphenyl)malonamic acid (HMPMA) (**Table 1D**) have been proposed as fungal detoxification metabolites from benzoxazolinones BOA and MBOA, respectively (*29*−*31*). Their detoxification role has been confirmed (*17*).

Natural Sources of DIMBOA, DIBOA, and Their Glucosides. Most of the methods for benzoxazinone glycosides isolation employed HPLC procedures (*6*, *7*, *32*−*38*), which have analytical value being unsuitable for large-scale isolation, according to Larsen and Christensen (*39*). They developed a convenient high-scale purification procedure for DIMBOA-Glc and DIMBOA from maize. We made some modifications (*21*, *23*) to this process to obtain DIMBOA from maize, and novel extraction methods were developed to produce DIBOA-Glc and DIBOA from rye. The results of these procedures are commented on below. The sources of the aglycones DIMBOA and DIBOA are similar to those of their glucoside precursors (*9*, *10*, *23*, *33*, *40*−*44*).

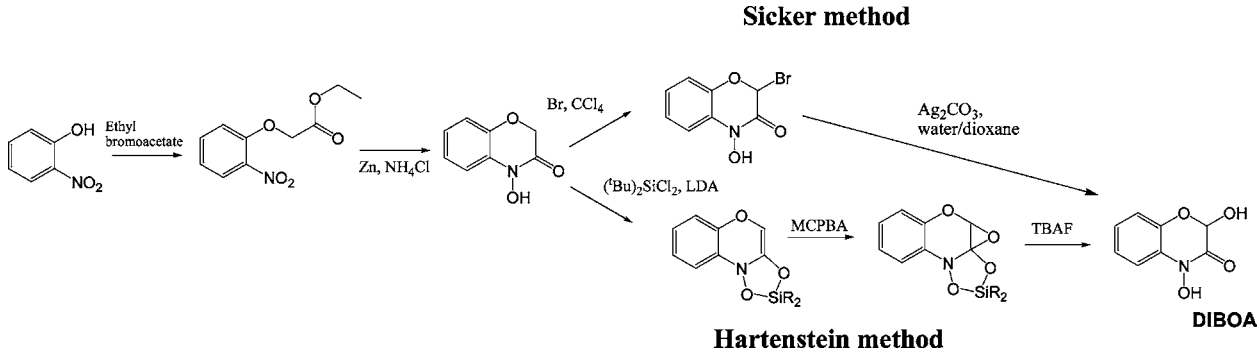
Naturally Occurring Benzoxazolinones, Aminophenoxazines, and Malonamic Acids. BOA and MBOA (**Table 1B**) have been isolated from plants of agronomic (*6*, *45*−*47*) and nonagronomic interest (*37*, *48*).

Aminophenoxazines 2-aminophenoxazin-3-one (APO), 2-amino-7-methoxyphenoxazin-3-one (AMPO), and their acetamide derivatives (**Table 1C**) have been described as microbial degradation products of the benzoxazolinones BOA and MBOA (*30*, *49*). From an agronomic point of view, the production of these metabolites in crop soils by root colonizing bacteria (*21*, *23*, *25*, *26*) has attracted attention to the elucidation of their phytotoxicity and ecological role (*17*).

Scheme 1. Overview of Benzoxazinone Synthetic Methods Based on Oxidation of 1,4-Benzoxazine Heterocycle in C-2



Scheme 2. Overview of Benzoxazinone Synthetic Methods Based on Cyclization of Functionalized Side Chains



The malonamic acids HPMA and HMPMA (**Table 1D**) have been described as detoxification products produced by both pathogenic (*31*) and nonpathogenic fungi (*50*) associated with crop plants. Zikmundová et al. isolated HPMA from *Aphelandra tetragona* (*29*).

Synthesis of Compounds with a (2*H*)-1,4-Benzoxazin-3(4*H*)-one Skeleton. The synthetic methods employed to access (2*H*)-1,4-benzoxazin-3(4*H*)-ones start with a functionalized phenol, to which a suitable side chain is added (*51*). The first successful method for the synthetic obtention of benzoxazinones (*52*) employed several potassium 2-nitrophenoxides as starting materials (**Scheme 1**, Jernow method). As an alternative, the patent also suggested using a reductive cyclization method developed by Coutts (*53*) (**Scheme 1**, Coutts method). The reductive conditions are generated through sodium borohydride and palladium over charcoal, suspended in aqueous dioxane. This convenient method has become general for the obtention of these compounds.

The need for a lactol deprotection step was avoided by three alternative methods, which have the oxidation of the heterocycle after its formation in common. Matlin et al. (*54*) (**Scheme 1**, Matlin method), Sicker and Hartenstein (*55*) (**Scheme 1**, Sicker method), Sicker (*56*) (**Scheme 2**, Sicker method), and again Hartenstein and Sicker (*57*) (**Scheme 2**, Hartenstein method) proposed different methodologies for this purpose. HBOA and HMBOA (**Table 1A**) were obtained in our laboratory with novel methods (*17*) according to this strategy.

The need for large amounts of the benzoxazinone glycosides also forced some researchers to develop interesting methods for their synthetic obtention (*58, 59*).

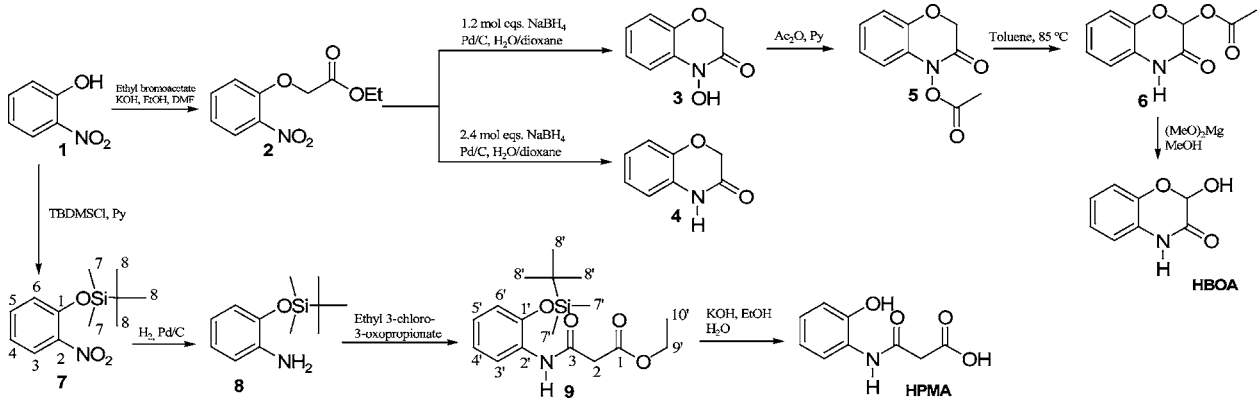
Synthesis of Degradation Derivatives from Benzoxazinones. Some efforts have been made in the benzoxazolinone (**Table 1B**) derivatives synthetic obtention in the search for antimicrobial agents (*60*−*63*).

The synthesis of a wide variety of compounds with an aminophenoxazine skeleton has been extensively developed more for their pharmaceutical interest (*64*−*66*) than for their agronomical use (*24*). All of the synthetic methodologies employed have the dimerization of two phenol units to access the tricyclic structure in common (*67*−*69*), mainly dealing with the synthesis of 2-aminophenoxazin-3-one (APO, **Table 1C**).

Taking into consideration the structural analogy of this compound to 2-amino-7-methoxyphenoxazin-3-one (AMPO) (**Table 1C**), there have not been a large number of efforts toward its synthesis. In fact, it has been obtained just as a byproduct in the search for organometallic reagents (*69, 70*).

The detoxification metabolites HPMA and HMPMA (**Table 1D**) were synthesized by direct acylation of aminophenols (*31*).

Scheme 3. Summary of Reactions and Conditions Employed To Obtain 2-Hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (HBOA) and *N*-(2-Hydroxyphenyl)malonamic Acid (HPMA)



The low stability showed by these chemicals in a wide variety of conditions forced us to look for the alternative method described below.

MATERIALS AND METHODS

General Methods. The purity of the isolated and synthetic compounds was determined by ^{1}H NMR and HPLC analyses and was found to be >98%. ^{1}H and ^{13}C NMR spectra were recorded using MeOH-d_4, $CDCl_3$, and D_2O as solvents in a Varian INOVA spectrometer at 399.99 and 100.577 MHz, respectively. The resonance of residual methanol for ^{1}H was set to δ 3.30, that of residual chloroform to δ 7.25, and that of residual water to δ 4.73. The solvent peak for ^{13}C was set to δ 49.00 (methanol) or δ 77.00 (chloroform) and used as internal reference. When D_2O is used, 10 μL of MeOH-d_4 is added for referencing ^{13}C NMR spectra. An HPLC-PDA detector (diode array UV−vis system), equipped with a Phenomenex SYNERGI 4 micron Fusion RP-80 (250 × 460 mm) column, was used for acquiring UV−vis spectra. Mass spectra were recorded by means of a Varian 1200L quadrupole MS/MS detector. FTIR spectra were obtained by means of a Spectrum BX Perkin-Elmer FTIR system. Frequency values are given in cm^{-1}. Polarimetry data were acquired on a Perkin-Elmer model 341 polarimeter. α values are given in degrees.

Isolation of Benzoxazinones DIMBOA, DIBOA-Glc, and DIBOA. DIMBOA (**Table 1A**) was isolated from maize (*Z. mays* L. cv. Apache) seedlings according to the method reported by Larsen and Christensen (*39*), in which several modifications were included. Seven-day-old maize shoots were frozen and then crushed and homogenized in water (1 kg of plant/L of water). The resulting mixture was filtered and the remaining solid discarded. After this, the liquid phase was allowed to stand for 1 h at room temperature to allow enzymes to degrade the DIMBOA glucoside. Then, 100 g of Amberlite XAD-7 (purchased from Sigma Aldrich Co., used as received) was added and stirred for 1 h at room temperature. Then, the mixture was filtered through cheesecloth, the Amberlite washed with water, and the aqueous phase discarded. The Amberlite was further washed with acetone (2 × 250 mL), yielding an aqueous acetone solution, from which acetone was selectively evaporated at reduced pressure. The remaining aqueous phase was extracted with ethyl acetate (four times). The last extraction was aided by using an ultrasound bath. The organic layers obtained were combined, dried over anhydrous sodium sulfate, filtered, and distilled in vacuo. The remaining solid was recrystallized from hexane (−20 °C, 12 h) to afford pure DIMBOA.

DIBOA-Glc (**Table 1A**) was isolated from 7-day-old rye seedlings by means of the following procedure: the frozen shoots (1 kg) were crushed and homogenized in an acetone/methanol mixture (1:1, 1 L). The obtained mixture was filtered, and the filtrate was then extracted with *n*-hexane and ethyl acetate. These organic phases were discarded, and the aqueous phase was re-extracted with *n*-butanol. The *n*-butanol layers were combined, filtered, and concentrated under reduced pressure. The obtained residue was chromatographed (MPLC, RP C-18, H_2O/methanol 7:3, 1% AcOH) to obtain pure DIBOA-Glc.

To obtain DIBOA (**Table 1A**), the ethyl acetate extracts discarded in the isolation of DIBOA-Glc were combined, dried on anhydrous Na_2SO_4, concentrated in vacuo, and purified by column chromatography (normal phase) by using chloroform/*n*-hexane increasing polarity solutions (0−30% $CHCl_3$). Fractions containing DIBOA were combined and dried by distillation at reduced pressure. Recrystallization from ethyl acetate/hexane afforded pure DIBOA.

Synthesis of Benzoxazinones and Their Degradation Products. *Synthesis of 2-Hydroxy-(2H)-1,4-benzoxazin-3(4H)-one (HBOA).* Reactions and conditions are summarized in **Scheme 3**. Analytical data for the synthetic intermediates matched exactly with those previously reported by Atkinson et al. (*27*) (**2**−**5**) and Hashimoto et al. (*71*) (**6**).

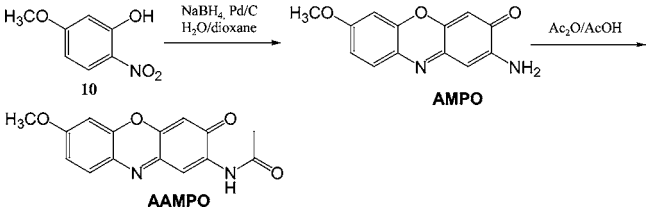
Ethyl 2-(2′-Nitrophenoxy)acetate (2). One gram of 2-nitrophenol (**1**) (purchased from Sigma Aldrich Co., used as received) was dissolved in a solution of 0.1 M KOH in absolute ethanol. After 1 h, the solvent was removed at reduced pressure. The resulting alkoxide was redissolved in *N*,*N*-DMF (50 mL), and 1.2 mol equiv of ethyl 2-bromoacetate was added. The reaction mixture was stirred under argon for 24 h. After this time, 50 mL of ethyl acetate was added, and the resulting organic solutions were washed with five portions of distilled water. The organic layers were combined and dried over anhydrous sodium sulfate, and the solvent was distilled at reduced pressure. Reaction crude was chromatographed (CC, ethyl acetate/hexane, 20:80) to obtain ethyl 2-(2′-nitrophenoxy)acetate (**2**) in quantitative yield.

Obtention of 4-Hydroxy-(2H)-1,4-benzoxazin-3(4H)-one (3) and (2H)-1,4-Benzoxazin-3(4H)-one (4). One hundred milligrams of Pd/C (10%) was suspended in an aqueous solution of 1,4-dioxane (1:1) (50 mL). After this, sodium borohydride (650 mg) was added and the solution was vigorously stirred. To this stirred suspension was added dropwise a solution of **5** in 1,4-dioxane (0.5 g/mL). The reaction evolution was controlled by TLC. Once the reaction was completed, the suspension was filtered through Celite to remove the catalyst, and the filtrate was treated with 10% HCl until pH 2 was reached. This solution was further extracted with ethyl acetate (three times). The organic layers were combined and dried over anhydrous sodium sulfate, and the solvent was distilled at reduced pressure. The obtained residue was chromatographied (CC, ethyl acetate/hexane, 30%) to obtain 4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (**3**) in 70% yield. When a double proportion of sodium borohydride and Pd/C was used, compound **4** was obtained in the same yield.

4-Acetoxy-(2H)-1,4-benzoxazin-3(4H)-one (5). Two hundred and fifty milligrams of **4** was dissolved in dry pyridine (10 mL), and 2 mL of acetic anhydride was added with stirring under argon. After 12 h, ethyl acetate (10 mL) was added to the reaction mixture, and the organic solution was washed with five portions of aqueous HCl (10%). The organic layers were combined and dried over anhydrous sodium sulfate, and the solvent was distilled at reduced pressure. No further purification was needed to isolate 4-acetoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (**5**) in quantitative yield.

2-Acetoxy-(2H)-1,4-benzoxazin-3(4H)-one (6). Compound **5** (100 mg) was dissolved in dry toluene (10 mL) and heated (95 °C, 24 h).

Scheme 4. Summary of Reactions and Conditions Employed To Obtain 2-Amino-7-methoxyphenoxazin-3-one (AMPO) and 2-Acetamido-7-methoxyphenoxazin-3-one (AAMPO)



After this, the solvent was evaporated in vacuo, and the residue was chromatographed (CC, ethyl acetate/hexane mixtures of increasing polarity) to obtain compound **6** in 70% yield.

2-Hydroxy-(2H)-1,4-benzoxazin-3(4H)-one (HBOA). One hundred milligrams of **6** was dissolved in methanol and stirred under argon atmosphere. After this, 360 μL of a magnesium methoxide solution in dry methanol (7.4% w/w) was added through a syringe. Once the reaction was completed (45 min), the solution was treated with diluted HCl (5%) to reach pH 4. After this, ethyl acetate was added and the resulting solution was washed with brine (one time) and distilled water (two times). The organic layers were combined, dried, filtered, and concentrated at reduced pressure. The obtained solid residue was chomatographied to obtain HBOA in quantitative yield.

Synthesis of 2-Hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (HMBOA). One hundred and fifty milligrams of 2,4-dihydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIMBOA (**Table 1A**), isolated in our laboratory (see above for details), was suspended in dry THF (20 mL). This solution was vigorously stirred under argon atmosphere. After this, 1.2 mol equiv of samarium diiodide (0.1 M in dry THF, 8 mL) was added. Once the reaction was completed, a solution of sodium thiosulfate (1%, 20 mL) was added, and the resulting solution was extracted with ethyl acetate (three times). The organic layers were combined, dried, filtered, and concentrated at reduced pressure to obtain a brown oil, which was chromatographed (CC, ethyl acetate/hexane, 40:60) to obtain HMBOA (**Table 1A**) in 70% yield.

Synthesis of Degradation Products 2-Amino-7-methoxyphenoxazin-3-one (AMPO) and 2-Acetamido-7-methoxyphenoxazin-3-one (AAMPO). Synthetic methodology for aminophenoxazines obtention is summarized in **Scheme 4**.

*2-Amino-7-methoxyphenoxazin-3-one (AMPO, **Table 1C**).* Sodium borohydride (185 mg) and 5-methoxy-2-nitrophenol (**12**) (250 mg) were added to a stirred suspension of 10% Pd/C (18 mg) in aqueous dioxane (1:1, 15 mL). After 48 h, the reaction mixture was filtered, and the solid obtained was washed with cold water and dried at 80 °C to yield pure AMPO.

*2-Acetamido-7-methoxyphenoxazin-3-one (AAMPO, **Table 1C**).* Fifty milliliters of acetic anhydride was added to a solution of AMPO in glacial acetic acid (100 mL, 0.05 g/mL), and the resulting suspension was stirred for 48 h at room temperature. After this time, the suspension was filtered to obtain an orangish residue, which was washed with cold water to yield AAMPO.

Synthesis of *N*-(2-Hydroxyphenyl)malonamic acid (HPMA). Reactions and conditions employed for the synthesis of this compound are summarized in **Scheme 3**. The structure of the synthetic intermediates was confirmed by ^{1}H NMR.

O-tert-Butyldimethylsilyl-2-nitrophenol (7). tert-Butyldimethylsilyl chloride (1.2 mol equiv) was added to a stirred solution of 500 mg of 2-nitrophenol (**1**) (purchased from Sigma Aldrich Co., used as received) in dry pyridine (20 mL). The flask was set to argon atmosphere and stirred. After 24 h, ethyl acetate (50 mL) was added to the reaction mixture, and the obtained solution was washed with three portions (50 mL) of aqueous HCl (10%). The organic layers were combined, dried over anhydrous sodium sulfate, filtered, and concentrated at reduced pressure. The obtained residue was chromatographed (10% ethyl acetate/hexane) to obtain compound **7** in quantitative yield.

O-tert-Butyldimethylsilyl-2-aminophenol (8). Two hundred and fifty milligrams of compound **7** was dissolved in ethyl acetate (20 mL). To this solution was added 15 mg of Pd/C (10%), and the mixture was vigorously stirred under 1 atm of H_2. Once the reaction was completed (8 h), the catalyst was removed by filtration through Celite, and the remaining solution was concentrated under reduced pressure to obtain **8** in quantitative yield and without further purification.

N-(2′-tert-Butyldimethylsilyloxyphenyl)malonamic Acid Ethyl Ester (9). One hundred milligrams of compound **8** was dissolved in dry toluene and treated with 1.2 mol equiv of ethyl 3-bromo-3-oxopropionate. After 24 h, the solvent was removed by distillation at reduced pressure, and the remaining residue was chromatographed (CC, ethyl acetate/hexane 60%) to obtain compound **9** with 70% yield.

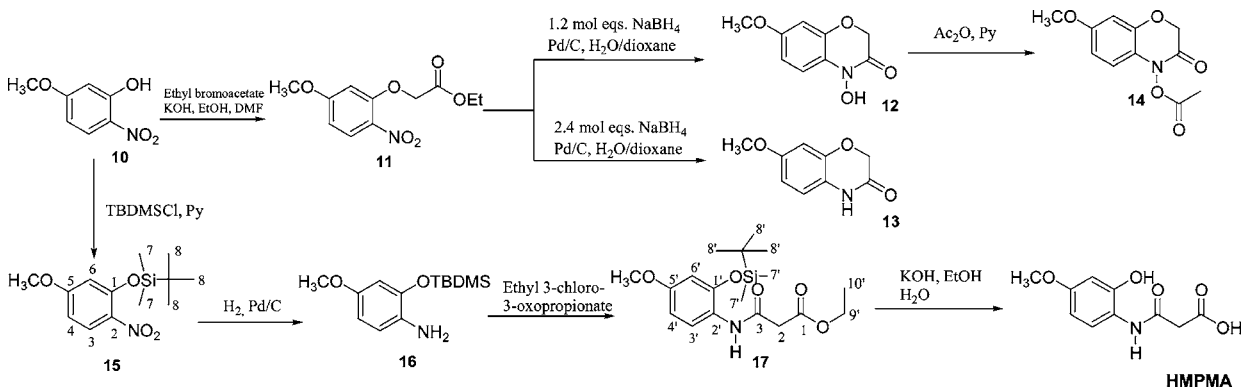
N-(2-Hydroxyphenyl)malonamic Acid (HPMA). One hundred milligrams of compound **9** was dissolved in methanol/water (30:70, 10 mL) and heated at 60 °C for 6 h. After this, methanol was removed by distillation at reduced pressure, and the remaining aqueous solution was acidified to pH 4 by means of the addition of aqueous HCl (10%). This solution was extracted with three portions of ethyl acetate (60 mL), and after the combination, drying, and evaporation of the solvent in the organic layers, the residue was chromatographed (CC) with ethyl acetate/hexane mixtures of increasing polarity to obtain HMPA in quantitative yield.

The analogues ethyl 7-methoxy-2-nitrophenylacetate (**11**), 4-hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (**12**), 7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (**13**), 4-acetoxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (**14**), and the malonamic acid HMPMA (**Scheme 5**) were obtained according to methods analogous to those for compounds **2**, **3**, **4**, **5**, and HPMA (**Scheme 3**), respectively.

RESULTS AND DISCUSSION

Isolation of Benzoxazinones DIBOA, DIBOA-Glc, and DIMBOA. The novel procedures for the preparative isolation of DIBOA-Glc and DIBOA were successful. They afforded the

Scheme 5. Summary of Reactions and Conditions Employed To Obtain DIMBOA Analogues 2-Hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (**12**), 7-Methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (**13**), 2-Acetoxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (**14**), and *N*-(2-Hydroxy-4-methoxyphenyl)malonamic Acid (HMPMA)



desired compounds at the required scales and permitted their bioactivity evaluation (*17*) and the description of their degradation behavior in wheat crop soil (*23*). Recent works that include DIBOA preparative isolation (*74*) deal with lower DIBOA yields of ~0.04% w/w from fresh plants (not reported by the authors, estimated by us), by means of more complicated methodologies in which three preparative TLC methods are used. In addition to this, the authors used oven-dried plant tissue. The temperature employed (60 °C) could accelerate spontaneous degradation of DIBOA.

*2,4-Dihydroxy-(2H)-1,4-benzoxazin-3(4H)-one (DIBOA, **Table 1A**):* λ_{max} (nm) 253, 281; FTIR (cm^{-1}) 3300, 3234, 1678, 1495, 1037; MS (ESI), *m*/*z* found for $C_8H_7NO_4$ 182 (100%, $[M + 1]^+$); ^1H NMR (MeOH-d_4, 400 MHz) δ 5.73 [s, 1H (H-2)], 7.05 [m, 3H (H-6, H-7, H-8)], 7.36 [d, 1H, $J = 7.3$ Hz (H-5)]; ^{13}C NMR (MeOH-d_4, 100 MHz) δ 93.5 (C-2), 114.3 (C-5), 118.3 (C-8), 123.7 (C-7), 125.5 (C-6), 129.3 (C-10), 142.3 (C-9), 159.9 (C-3); yield, 0.1% w/w from the fresh plant.

*2R-2-O-β-D-Glucopyranosyl-4-hydroxy-(2H)-1,4-benzoxazin-3(4H)-one (DIBOA-Glc, **Table 1A**):* λ_{max} (nm) 255, 278; FTIR (cm^{-1}) 3200, 1739, 1263, 1077, 1030; $[\alpha]^{30}$ 69°; MS (ESI), *m*/*z* found for $C_{13}H_{16}NO_9$ 366 (100%, $[M + Na]^+$); ^1H NMR (D_2O, 400 MHz) δ 3.10 [dd, 1H, $J = 9.3$ Hz, 8.0 (H-2′)], 3.21 [dd, 1H, $J = 9.3$, 9.0 Hz (H-4′)], 3.34 [ddd, 1H, $J = 9.5$, 5.4, 2.2 Hz (H-5′)], 3.37 [dd, 1H, $J = 9.3$, 9.0 Hz (H-3′)], 3.56 [dd, 1H, $J = 12.2$, 5.4 Hz (H-6′b)], 3.74 [dd, 1H, $J = 12.2$, 2.2 Hz (H-6′a)], 4.71 [d, 1H, $J = 7.8$ Hz (H-1′)], 5.84 [s, 1H (H-2)], 7.02 [dd, 1H, $J = 7.6$, 1.7 Hz (H-8)], 7.07 [m, 2H (H-6, H-7)], 7.37 [br d, 1H, $J = 7.6$ Hz (H-5)]; ^{13}C NMR (MeOH-d_4, 100 MHz) δ 60.9 (C-6′), 69.5 (C-4′), 73.1 (C-2′), 75.8 (C-5′), 76.7 (C-3′), 97.2 (C-2), 102.3 (C-1′), 114.1 (C-5), 118.0 (C-8), 124.3 (C-7), 126.1 (C-6), 118.3 (C-8), 127.3 (C-10), 140.5 (C-9), 129.3 (C-10), 157.7 (C-3); yield, 0.0088% w/w from the fresh plant.

The DIMBOA yield we have obtained is similar to that obtained by Larsen et al. (0.136 ± 0.12% w/w from fresh plants) (*39*). The additional ethyl acetate extraction provoked higher yields than the unmodified Larsen protocol when applied in our rye variety and culture conditions.

*2,4-Dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one (DIMBOA, **Table 1A**):* λ_{max} (nm) 262; FTIR (cm^{-1}) 3273, 1681, 1505, 1442, 1025; MS (ESI), *m*/*z* found for $C_9H_9NO_5$ 212 (100%, $[M + 1]^+$); ^1H NMR (MeOH-d_4, 400 MHz) δ 3.76 [s, 3H ($-OCH_3$)], 5.67 [s, 1H (H-2)], 6.62 [d, 1H, $J = 2.6$ Hz (H-8)], 6.67 [dd, 1H, $J = 8.8$, 2.6 Hz (H-6)], 7.26 [d, 1H, $J = 8.8$ Hz (H-5)]; ^{13}C NMR (MeOH-d_4, 100 MHz) δ 56.1 ($-OCH_3$), 94.0 (C-2), 104.7 (C-8), 108.9 (C-6), 115.2 (C-5), 123.3 (C-10), 143.6 (C-9), 158.7 (C-7), 159.4 (C-3); yield, 0.12% w/w from the fresh plant.

Synthesis of 2-Hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (HBOA). Among the different possibilities of obtaining benzoxazinones with the hydroxyl moiety at C-2 mentioned above, and after several tests, we decided to take advantage of the [3.3]-acyl sigmatropic rearrangement described by Hashimoto et al. (*71*) (**Scheme 3**), which was employed to obtain some non-natural derivatives useful for bioactivity and chemical reactivity studies. The obtained acyl group (**5**) was further rearranged to position C-2 by heating in dry toluene (85 °C, 24 h). The authors employed benzene, which is highly toxic and has a lower boiling point (80 °C). The higher temperature increased the reaction yield in a significant manner (from 30 to 70%). After this, a transesterification with magnesium methoxide in methanol at room temperature yielded 99% of HBOA. This method was chosen for its simplicity, selectivity, and mild conditions (*72*). Analytical data for **2**−**4** matched exactly those previously reported by Atkinson et al. (*27*). Compounds **5** and **6** had the same spectral data as those previously reported by Hashimoto et al. (*71*).

Atkinson et al. (*27*) prepared a very wide variety of benzoxazinones analogues, which included methoxy, dimethoxy, halo, and cyanide derivatives of 2,4-dihydroxybenzoxazinones, lactams, and some 2-deoxy derivatives for the evaluation of their reactivity and decomposition kinetics. The interest in those 2-deoxy compounds has been recently discussed by us (*17*), and the methodology employed by Atkinson et al. was modified with the purpose of yield optimization in the 2-deoxy derivatives obtention. The nucleophilic substitution used potassium nitrophenoxides solved in *N*,*N*-DMF instead of acetone or THF. Under these conditions, the reaction proceeds with excellent yield at room temperature. This modification increases the yield for 2-deoxy compounds from the original 40 to 70%.

The starting material for this reaction, 4-acetoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (**Schemes 3** and **5**), was obtained by the usual pyridine/Ac_2O acetylation procedure, which gave quantitative yield, instead of the DCM/Ac_2O/Na_2CO_3 system employed by the authors (40% yield). The magnesium methoxide procedure for acyl group cleavage was simple and yielded the desired HBOA with mild conditions. HBOA was obtained in a 48% overall yield after five reaction steps.

*Analytical data for 2-hydroxy-(2H)-1,4-benzoxazin-3(4H)-one (HBOA, **Table 1A**):* λ_{max} (nm) 208.5, 247.6; FTIR (cm^{-1}) 3182, 3026, 1696, 1500, 1016; MS (ESI), *m*/*z* found for $C_8H_7NO_3$ 166 (100%, $[M + 1]^+$); ^1H NMR (MeOH-d_4, 400 MHz) δ 5.53 [s, 1H (H-2)], 6.95 [m, 4H (H-5, H-6, H-7, H-8)]; ^{13}C NMR (MeOH-d_4, 100 MHz) δ 92.0 (C-2), 116.8 (C-8), 118.7 (C-5), 123.7 (C-7), 124.9 (C-6), 127.4 (C-10), 142.3 (C-9), 165.2 (C-3).

Synthesis of 2-Hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (HMBOA). Samarium diiodide has been described as a useful reagent for the cleavage of the N−O bond, and it has been used for the reduction of hydroxylamines, oximes, and hydroxamic acids (*73*). This reagent was employed to access the benzoxazinone lactam moiety for the first time and allowed us the direct obtention of HMBOA (**Table 1A**) from readily available DIMBOA (**Table 1A**) in an easy way.

This methodology can be general in the obtention of lactams from benzohydroxamic acids, facilitating the synthetic access to those compounds. HMBOA was obtained in 70% yield.

*Analytical data for 2-hydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one (HMBOA, **Table 1A**):* λ_{max} (nm) 204.7, 256.3; FTIR (cm^{-1}) 3181, 1686, 1513, 1157, 1030; MS (ESI), *m*/*z* found for $C_9H_9NO_4$ 196 (100%, $[M + 1]^+$); ^1H NMR (MeOH-d_4, 400 MHz) δ 3.74 [s, 3H ($-OCH_3$)], 5.56 [d, 1H, $J = 6.0$ Hz (H-2)], 6.59 [d, 1H, $J = 2.0$ Hz (H-8)], 6.59 [dd, 1H, $J = 9.0$, 2.0 Hz (H-6)], 6.84 [d, 1H, $J = 6.0$ Hz ($-OH$)], 6.92 [d, 1H, $J = 9.0$ Hz (H-5)], 9.58 [s, 1H ($-NH$)]; ^{13}C NMR (MeOH-d_4, 100 MHz) δ 55.8 ($-OCH_3$), 92.0 (C-2), 104.5 (C-8), 108.7 (C-6), 116.7 (C-5), 121.2 (C-10), 157.0 (C-9), 162.7 (C-7), 170.9 (C-3).

Synthesis of Aminophenoxazines. 2-Amino-7-methoxyphenoxazin-3-one (AMPO, **Table 1C**) was obtained in a single reaction step in 70% yield. The described method is a very simple way of obtaining this chemical at a large scale and avoiding the inconveniences associated with the use of organometallics.

The easy access to AMPO allowed the obtention of its acetate derivative 2-acetamido-7-methoxyphenoxazin-3-one (AAMPO, **Scheme 4**) in high yield and in an easy-to-scale manner. The low solubility of AMPO in the common solvents used for

acylation forced us to use acetic acid, in which AAMPO precipitated once the reaction was completed. Overall yield from 5-methoxy-2-nitrophenol was 56%. It is interesting to note the high reactivity difference between the amino group of aminophenoxazines APO and AAMPO (**Table 1C**). Acetylation of APO (**Table 1C**) can be made in good yields by means of the usual pyridine procedure (*25*), yielding 2-acetamidophenoxazin-3-one (**AAPO**, **Table 1C**), whereas base-catalyzed acylation did not proceed successfully with AMPO.

*2-Amino-7-methoxyphenoxazin-3-one (AMPO, **Table 1C**):* λ_{max} (nm) 235.0; FTIR (cm^{-1}) 3450, 3299, 1577, 847; MS (ESI), *m/z* found for $C_{13}H_{10}N_2O_3$ 243 (100%, $[M + 1]^+$); ^1H NMR (MeOH-d_4, 400 MHz) δ 3.85 [s, 3H ($-OCH_3$)], 6.33 [s, 1H (H-4)], 6.35 [s, 1H (H-1)], 7.01 [dd, 1H, $J = 7.0$, 2.7 Hz (H-8)], 7.11 [d, 1H, $J = 2.7$ Hz (H-6)], 7.64 [d, 1H, $J = 9.0$ Hz (H-9); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 56.1 ($-OCH_3$), 98.8 (C-1), 100.1 (C-6), 103.3 (C-4), 113.5 (C-8), 128.4 (C-9a), 129.0 (C-9), 143.3 (C-5a), 145.7 (C-2), 146.6 (C-10a), 148.6 (C-4a), 160.0 (C-7), 179.9 (C-2).

*2-Acetamido-7-methoxyphenoxazin-3-one (AAMPO, **Table 1C**):* λ_{max} (nm) 235.4; FTIR (cm^{-1}) 3298, 2924, 1526, 1257, 805; MS (ESI), *m/z* found for $C_{15}H_{12}N_2O_4$ 285 (100%, $[M + 1]^+$); ^1H NMR ($CDCl_3$, 400 MHz) δ 2.20 [s, 3H ($-OCCH_3$)], δ 3.87 [s, 3H ($-OCH_3$)], 6.38 [s, 1H (H-4)], 6.83 [d, 1H, $J = 3.0$ Hz (H-6)], 6.94, [dd, 1H, $J = 9.0$, 3.0 Hz (H-8)], 7.72 [d, 1H, $J = 9.0$ Hz (H-9)], 8.36 [s, 1H (H-1)]; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.3 ($-OCCH_3$), 56.1 ($-OCH_3$), 91.5 (C-4), 99.8 (C-6), 103.8 (C-8), 114.7 (C-9), 126.9 (C-9a), 131.2 (C-1), 140.5 (C-5a), 144.4 (C-4a), 147.3 (C-2), 149.5 (C-10a), 159.4 (C-7), 179.3 (C-2).

Synthesis of *N*-(2-Hydroxyphenyl)malonamic Acid (HPMA, Table 1D). To avoid the inconvenience of the coexistence of amino and hydroxyl moieties (dimerization of nitrophenols to give aminophenoxazines), a procedure in which aromatic nitrogen could be functionalized with the hydroxyl moiety protected was optimized. 2-Nitrophenol was protected by generating its *tert*-butyldimethylsilyl ether. After this, the nitro group was reduced to amino by catalytic hydrogenation. This 2-amino-*tert*-butyldimethylsilyloxybenzene has potential utility in the obtention of heterocycle ring-opened derivatives of benzoxazinones, which have been proposed as metabolic intermediates of natural benzoxazinones with an important role in their biological activity (*27*). Once the amino group was obtained, the side chain was introduced by amidation with ethyl 3-chloro-3-oxopropionate. The final alkaline hydrolysis afforded the final desired product by cleaving the silyl ether and the ethyl ester simultaneously. The structures of the synthetic intermediates were confirmed by ^1H NMR.

*O-tert-Butyldimethylsilyl-2-nitrophenol (**7**):* ^1H NMR ($CDCl_3$, 400 MHz) δ 7.77 [1H, dd, $J = 1.6$, 8.1 Hz (H-3)], 7.41 [m, 2H (H-4, H-5)], 6.99 [m, 1H (H-6)], 0.99 [s, 9H (H-8)], 0.24 [s, 6H (H-7)].

*O-tert-Butyldimethylsilyl-2-aminophenol (**8**):* ^1H NMR ($CDCl_3$, 400 MHz) δ 7.78 [1H, dd, $J = 1.6$, 8.0 Hz (H-6)], 7.42 [1H, ddd, $J = 1.6$, 8.0, 8.0 Hz (H-4)], 6.99 [m, 2H (H-3, H-5)], 0.99 [s, 9H (H-8)], 0.25 [s, 6H (H-7)].

*N-(2′-tert-Butyldimethylsilyloxyphenyl)malonamic acid ethyl ester (**9**):* ^1H NMR ($CDCl_3$, 400 MHz) δ 8.28 [m, 1H (H-6′)], 6.90 [m, 2H (H-4′, H-5′)], 6.84 [m, 1H (H-3′)], 4.19 [2H, q, $J = 7.7$ Hz (H-9′)], 3.43 [2H, s, (H-2)], 1.21 [3H, t, $J = 7.7$ Hz (H-10′)], 0.99 [9H, s, (H-8′)], 0.27 [s, 6H (H-7′)].

*N-(2-Hydroxyphenyl)malonamic acid (HPMA, **Table 1D**):* λ_{max} (nm) = 208.9, 243.5, 283.7; FTIR (cm^{-1}) 3292, 2924, 1723, 1542, 1254; MS (ESI), *m/z* found for $C_9H_9NO_4$ 150 (67%, $[M - CO_2 - 1]^+$); ^1H NMR (MeOH-d_4, 400 MHz) δ 3.85 [s, 2H (H-2)], 6.90 [ddd, 1H, $J = 8.0$, 7.0, 1.0 Hz (H-4′)], 6.98 [dd, 1H, $J = 8.0$, 1.0 Hz (H-6′)], 7.06 [ddd, 1H, $J = 8.0$, 7.0, 1.0 Hz (H-5′)], 8.08 [dd, 1H, $J = 8.0$, 1.0 Hz (H-3′)]; ^{13}C NMR (MeOH-d_4, 100 MHz) δ 55.4 (C-2), 110.8 (C-6′), 120.4 (C-4′), 121.3 (C-5′), 124.9 (C-3′), 127.2 (C-1′), 149.8 (C-2′), 165.6 (C-3), 170.6 (C-1).

Synthesis of *N*-(2-Hydroxy-4-methoxyphenyl)malonamic Acid (HMPMA, Table 1D). Reactions and conditions employed are summarized in **Scheme 5**. The same synthetic methodology as the employed for HMPA obtention was used for the preparation of its analogue, HMPMA, from 5-methoxy-2-nitrophenol (purchased from Sigma Aldrich Co., used as received) (**10**). The structures of the synthetic intermediates were confirmed by ^1H NMR.

*O-tert-Butyldimethylsilyl-5-methoxy-2-nitrophenol (**15**):* ^1H NMR ($CDCl_3$, 400 MHz) δ 7.90 [1H, d, $J = 9.0$ Hz (H-3)], 6.52 [1H, dd, $J = 9.0$, 2.6 Hz (H-4)], 6.40 [1H, d, $J = 2.6$ Hz (H-6)], 3.82 [s, 3H (CH_3O)], 1.00 [s, 9H (H-8)], 0.24 [s, 6H (H-7)].

*O-tert-Butyldimethylsilyl-2-amino-5-methoxyphenol (**16**):* ^1H NMR ($CDCl_3$, 400 MHz) δ 6.63 [1H, d, $J = 8.3$ Hz (H-3)], 6.39 [1H, dd, $J = 8.3$, 2.6 Hz (H-4)], 6.36 [1H, d, $J = 2.6$ Hz (H-6)], 3.70 [s, 3H (CH_3O)], 1.02 [s, 9H (H-8)], 0.25 [s, 6H (H-7)].

*N-(2′-tert-Butyldimethylsilyloxy-4′-methoxyphenyl)malonamic acid ethyl ester (**17**):* ^1H NMR ($CDCl_3$, 400 MHz) δ 8.07 [1H, d, $J = 8.9$ Hz (H-6′)], 6.39 [2H, m, (H-3′, H-5′)], 4.15 [2H, q, $J = 7.2$ Hz (H-9′)], 3.66 [3H, s, (CH_3O)], 3.37 [2H, s, (H-2)], 1.20 [3H, t, $J = 7.2$ Hz (H-10′)], 1.16 [9H, s, (H-8′)], 0.23 [s, 6H (H-7′)].

*N-(2-Hydroxy-7-methoxyphenyl)malonamic acid (HMPMA, **Table 1D**):* λ_{max} (nm) 208.1, 249.8, 287.4; FTIR (cm^{-1}) 3215, 3081, 1698, 1433, 1202; MS (ESI), *m/z* found for $C_{10}H_{11}NO_5$ 226 (100%, $[M + 1]^+$); ^1H NMR (MeOH-d_4, 400 MHz) δ 3.30 [s, 2H (H-2)], 3.52 [s, 3H ($-OCH_3$)], 6.19 [dd, 1H, $J = 2.0$, 9.0 Hz (H-5′)], 6.25 [d, 1H, $J = 2.0$ Hz (H-3′)], 7.38 [d, 1H, $J = 9.0$ Hz (H-6′)]; ^{13}C NMR (MeOH-d_4, 100 MHz) δ 41.9 (C-2), 54.8 ($-OCH_3$), 102.0 (C-3′), 104.6 (C-5′), 119.0 (C-1′), 123.9 (C-6′), 150.1 (C-2′), 158.5 (C-4′), 166.3 (C-3), 170.8 (C-1).

The synthetic procedures described above yielded HMPA and HMPMA in a 68% overall yield after four reactions. After two reaction steps, Friebe et al. reported yields of 66% for HPMA and 45% for HMPMA (*31*). In addition to the yield increase at HMPMA obtention, the methods described here avoided the undesired dimerizations and provided useful intermediates for further research on benzoxazinone bioactivity and modes of action.

Synthesis of DIMBOA Analogues Ethyl 5-Methoxy-2-nitrophenylacetate (11), 4-Hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (12), 7-Methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (13), and 4-Acetoxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (14). To obtain these compounds, the same methodology as the one employed for the obtention of DIBOA analogues **2**, **3**, **4** and **5**, respectively, was used (see above). Analytical data for **11**−**13** matched exactly those previously reported by Atkinson et al. (*27*). Compound **14** had the same spectral data as those previously reported by Hashimoto et al. (*71*).

Reactions and conditions are summarized in **Scheme 5**. The starting material employed was 5-methoxy-2-nitrophenol (**10**) (purchased from Sigma Aldrich Co., used as received). The synthesis of these compounds was made to obtain HMBOA in

the same way as HBOA, but the acyl [3.3]-sigmatropic rearrangement was not successful when applied on 7-methoxybenzoxazinones. These analogues were included in the SAR study for benzoxazinones mentioned above (*17*).

Efficient isolation and synthetic methodologies exist for the preparation of benzoxazinones and their main degradation products. The synthetic problem of obtaining the heterocycle with a wide functionalization pattern is solved, although some modifications can be done on both isolation and synthesis procedures in the search for higher yields and more simple procedures. The methodologies optimized and designed toward the isolation and synthesis of benzoxazinones, aminophenoxazines, and malonamic acids described here constitute a clear example. These methods have allowed a wide scope of research on their chemical and biological properties and will be useful for further research into the characterization of their bioactivity, modes of action, and ecological role and in the development of new molecules with agronomical interest.

The isolation procedures designed for the obtention of DIBOA and its glycoside, in addition to the improved DIMBOA isolation protocol, are simple and produce sufficient yields to access those compounds in sufficient amounts for their bioactivity evaluation and the description of their degradation dynamics (*17*, *21*, *23*). On the other hand, the synthetic methodologies employed to access lactams HBOA and HMBOA have revealed some aspects about the chemical reactivity of hydroxamic acids. The synthetic route to malonamic acids HPMA and HMPMA, with improved yields, permitted a detailed bioactivity evaluation of them (*17*) and will provide access to a wide variety of aminophenol derivatives soon. The high-scale access to aminophenoxazines AMPO and AAMPO, by a novel reductive method first described here, has also permitted investigation of their role in allelochemical interactions in wheat crops (*17*, *21*), the detection of aminophenoxazines as soil transformation products (*75*, *76*), and research on the ecotoxicological effects of the aminophenoxazines (*77*−*80*).

ACKNOWLEDGMENT

We acknowledge the collaboration of Dr. Raúl F. Velasco (Universidad de Cádiz) for the optimization of benzoxazinone synthetic methods.

LITERATURE CITED

(1) Duke, S. O. Naturally occurring chemical compounds as herbicides. *Rev. Weed Sci.* **1986**, *2*, 15−44.

(2) Macías, F. A. In *Allelopathy: Organisms, Processes, and Applications*; Inderjit, Dakshini, K. M. M., Einhellig, F. A., Eds.; ACS Symposium Series 582; American Chemical Society: Washington, DC, 1995.

(3) Rice, E. L. *Biological Control of Weeds and Plant Diseases*; University of Oklahoma Press: Norman, OK, 1995.

(4) Hamilton, R. H.; Bandurski, R. S.; Reusch, W. H. Isolation and characterization of a cyclic hydroxamate from *Zea mays*. *Cereal Chem.* **1962**, *39*, 107−113.

(5) Virtanen, A. I.; Hietala, P. K. The structures of the precursors of benzoxazolinone in rye plants. II. *Suom. Kemistil. B* **1959**, *32B*, 252.

(6) Wahlroos, Ö.; Virtanen, I. The precursors of 6-methoxybenzoxazolinone in maize and wheat plants, their isolation and some of their properties. *Acta Chem. Scand.* **1959**, *13*, 1906−1908.

(7) Hofman, J.; Hofmanova, O. 1,4-Benzoxazine derivatives in plants. *Eur. J. Biochem.* **1969**, *8*, 109−112.

(8) Hofman, J.; Hofmanova, O. 1,4-Benzoxazine derivatives in plants: absence of 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one from uninjured *Zea mays* plants. *Phytochemistry* **1971**, *10*, 1441−1444.

(9) Tipton, C. L.; Klun, J. A.; Husted, R. R.; Pierson, M. D. Cyclic hydroxamic acids and related compounds from maize. Isolation and characterization. *Biochemistry* **1967**, *6*, 2866−2870.

(10) Virtanen, A. I.; Hietala, P. K. Precursors of benzoxazolinone in rye plants. *Acta Chem. Scand.* **1960**, *14*, 499−501.

(11) Honkanen, E.; Virtanen, A. I. Synthesis of some 1,4-benzoxazine derivatives and their antimicrobial activity. *Acta Chem. Scand.* **1960**, *14*, 1214−1217.

(12) Escobar, C. A.; Sicker, D.; Niemeyer, H. M. Evaluation of DIMBOA analogs as antifeedants and antibiotics towards the aphid *Sitobion avenae* in artificial diets. *J. Chem. Ecol.* **1999**, *25*, 1543−1554.

(13) Schulz, M.; Friebe, A.; Kueck, P.; Seipel, M.; Schnabl, H. Allelopathic effects of living quack grass (*Agropyron repens* L.). Identification of inhibitory allelochemicals exuded from rhizome borne roots. *Angew. Bot.* **1994**, *68*, 195−200.

(14) Wolf, R. B.; Spencer, G. F.; Plattner, R. D. Benzoxazolinone, 2,4-dihydroxy-1,4-benzoxazin-3-one, and its glucoside from *Acanthus mollis* seeds inhibit velvetleaf germination and growth. *J. Nat. Prod.* **1985**, *48*, 59−63.

(15) Bravo, R.; Lazo, W. Antialgal and antifungal activity of natural hydroxamic acids and related compounds. *J. Agric. Food Chem.* **1996**, *44*, 1569−1571.

(16) Sahi, S. V.; Chilton, M.; Chilton, W. S. Corn metabolites affect growth and virulence of *Agrobacterium tumefaciens*. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3879−3883.

(17) Macías, F. A.; Marín, D.; Oliveros-Bastidas, A.; Castellano, D.; Simonet, A. M.; Molinillo, J. M. G. Structure−activity relationships (SAR) studies of benzoxazinones, their degradation products and analogues. Phytotoxicity on standard target species (STS). *J. Agric. Food Chem.* **2005**, *53*, 538−548.

(18) Niemeyer, H. M. Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones), defense chemicals in the Gramineae. *Phytochemistry* **1988**, *27*, 3349−3358.

(19) Peng, S.; Chilton, W. S. Biosynthesis of DIMBOA in maize using deuterium oxide as a tracer. *Phytochemistry* **1994**, *37*, 167−171.

(20) Kumar, P.; Moreland, D. E.; Chilton, W. S. 2*H*-1,4-Benzoxazin-3(4*H*)-one, an intermediate in the biosynthesis of cyclic hydroxamic acids in maize. *Phytochemistry* **1994**, *36*, 893−898.

(21) Macías, F. A.; Oliveros-Bastidas, A.; Marín, D.; Castellano, D.; Simonet, A. M.; Molinillo, J. M. G. Degradation studies on benzoxazinoids. Soil degradation dynamics of 2,4-dihydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIMBOA) and its degradation products, phytotoxic allelochemicals from Gramineae. *J. Agric. Food Chem.* **2004**, *52*, 6402−6413.

(22) Woodward, M. D.; Corcuera, L. J.; Helgeson, J. P.; Upper, C. D. Decomposition of 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one in aqueous solutions. *Plant Physiol.* **1978**, *61*, 796−802.

(23) Macías, F. A.; Oliveros-Bastidas, A.; Marín, D.; Castellano, D.; Simonet, A. M.; Molinillo, J. M. G. Degradation studies on benzoxazinoids. Soil degradation dynamics of (2*R*)-2-*O*-*β*-D-glucopyranosyl-2,4-dihydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA-Glc) and its degradation products, phytotoxic allelochemicals from Gramineae. *J. Agric. Food Chem.* **2005**, *53*, 554−561.

(24) Fomsgaard, I. S.; Mortensen, A. G.; Carlsen, S. C. K. Microbial transformation products of benzoxazolinone and benzoxazinone allelochemicals—a review. *Chemosphere* **2004**, *54*, 1025−1038.

(25) Gagliardo, R. W.; Chilton, W. S. Soil transformation of 2(3*H*)-benzoxazolone of rye into phytotoxic 2-amino-3*H*-phenoxazin-3-one. *J. Chem. Ecol.* **1992**, *18*, 1683−1691.

(26) Kumar, P.; Gagliardo, R. W.; Chilton, W. S. Soil transformation of wheat and corn metabolites MBOA and DIM_2BOA into aminophenoxazinones. *J. Chem. Ecol.* **1993**, *19*, 2453−2461.

(27) Atkinson, J.; Morand, P.; Arnason, J. T.; Niemeyer, H. M.; Bravo, H. R. Analogues of the cyclic hydroxamic acid 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3-one(DIMBOA): decomposition to benzoxazolinones and reaction with β-mercaptoethanol. *J. Org. Chem.* **1991**, *56*, 1788−1800.

(28) Sánchez-Moreiras, A. M.; Coba de la Peña, T.; Martínez, A.; González, L.; Pellisier, F.; Reigosa, M. J. Mode of action of the hydroxamic acid BOA and other related compounds. In *Allelopathy*; Macías, F. A., Galindo, J. C. G., Molinillo, J. M. G., Cutler, H. G., Eds.; CRC Press: Boca Raton, FL, 2004; pp 239−252.

(29) Zikmundova, M.; Drandarov, K.; Bigler, L.; Hesse, M.; Werner, C. Biotransformation of 2-benzoxazolinone and 2-hydroxy-1,4-benzoxazin-3-one by endophytic fungi isolated from *Aphelandra tetragona*. *Appl. Environ. Microbiol.* **2002**, *68*, 4863−4870.

(30) Friebe, A.; Wieland, I.; Schulz, M. Tolerance of *Avena sativa* to the allelochemical benzoxazolinone. Degradation of BOA by root colonizing bacteria. *Angew. Bot.* **1996**, *70*, 150−154.

(31) Friebe, A.; Vilich, V.; Hennig, L.; Kluge, M.; Sicker, D. Detoxification of benzoxazolinone allelochemicals from wheat by *Gaeuannomyces graminis* var. tritici, *G. graminis* var. graminis, *G. graminis* var. avenae, and *Fusarium culmorum*. *Appl. Environ. Microbiol.* **1998**, *64*, 2386−2391.

(32) Hartenstein, H.; Sicker, D. (2*R*)-2 -D-Glucopyranosyloxy-4-hydroxy-2*H*-1,4-benzox-azin-3(4*H*)-one from *Secale cereale*. *Phytochemistry* **1994**, *35*, 827−828.

(33) Copaja, S. V.; Barria, B. N.; Niemeyer, H. M. Hydroxamic acid content in perennial Triticeae. *Phytochemistry* **1991**, *30*, 1531−1534.

(34) Kanchanapoom, T.; Kasai, R.; Picheansoonthon, C.; Yamasaki, K. Megastigmane, aliphatic alcohol and benzoxazinoid glycosides from *Acanthus ebracteatus*. *Phytochemistry* **2001**, *58*, 811−817.

(35) Wolf, R. B.; Spencer, G. F.; Plattner, R. D. DIBOA-Glc in *Acanthus mollis*. *J. Nat. Prod.* **1985**, *48*, 59−62.

(36) Özden, S.; Özden, T.; Attila, J.; Kücükislamoglu, M.; Okatan, A. Isolation and identification via high performance liquid chromatography and thin-layer chromatography of benzoxazolinone precursors from *Consolida orientalis* flowers. *J. Chromatogr.* **1992**, *609*, 402−406.

(37) Nagao, T.; Otsuka, H.; Kohda, H.; Sato, T.; Yamasaki, K. Benzoxazinones from *Coix lachryma jobi* Var. Ma-Yuen. *Phytochemistry* **1985**, *24*, 2959−2962.

(38) Hartenstein, H.; Klein, J.; Sicker, D. Efficient isolation procedure for (2*R*)-2-β-D-glucopyranosyloxy-4-hydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one from maize. *Ind. J. Heterocycl. Chem.* **1993**, *2*, 151−153.

(39) Larsen, E.; Christensen, L. P. Simple method for large scale isolation of the cyclic arylhydroxamic acid DIMBOA from maize (*Zea mays* L.). *J. Agric. Food Chem.* **2000**, *48*, 2556−2558.

(40) Hartenstein, H.; Lippmann, T.; Sicker, D. An efficient procedure for the isolation of pure 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA) from maize. *Ind. J. Heterocycl. Chem.* **1992**, *2*, 75−76.

(41) Grambow, H. J.; Lückge, A.; Klausener, E.; Müller, Z. Occurrence of 2-(2-hydroxy-4,7-dimethoxy-2*H*-1,4-benzoxazin-3-one)-β-D-glucopyranoside in *Triticum aestivum* leaves and its conversion into 6-methoxybenzoxazolinone. *Z. Naturforsch.* **1986**, *41C*, 684−690.

(42) Friebe, A.; Schulz, M.; Kück, P.; Schnabl, H. Phytotoxins from shoot extracts of *Agropyron repens* seedlings. *Phytochemistry* **1995**, *38*, 1157−1159.

(43) Schulz, M.; Friebe, A.; Kück, P.; Seipel, M.; Schnabl, H. Allelopathic effects of living quackgrass (*Agropyron repens* L.). Identification of inhibitory allelochemicals exuded from rhizome borne roots. *Angew. Bot.* **1994**, *68*, 195−200.

(44) Pethö, M. Occurrence of cyclic hydroxamic acids in the tissues of barnyard grass (*Echinochloa crus-galli*/L./P.B.) and their possible role in allelopathy. *Acta Agron. Hung.* **1993**, *42*, 197−202.

(45) Kosemura, S.; Emori, H.; Yamamura, S.; Anai, T.; Aizawa, H. Isolation and characterization of 4-chloro-6,7-dimethoxybenzoxazolin-2-one, a new auxin-inhibiting benzoxazolinone from *Zea mays*. *Chem. Lett.* **1995**, 1053−1054.

(46) Wahlroos, Ö.; Virtanen, A. I. On the formation of 6-methoxybenzoxazolinone in maize and wheat plants. *Suom. Kemistil. B* **1959**, *32*, 139−140.

(47) Virtanen, A. I.; Hietala, P. K. 2(3*H*)-Benzoxazolinone, an antifusarium factor in rye seedlings. *Acta Chem. Scand.* **1955**, *9*, 1543−1545.

(48) Werner, C.; Hedberg, C.; Lorenzi-Riatsch, A.; Hesse, M. Accumulation and metabolism of the spermine alkaloid aphelandrine in roots of *Aphelandra tetragona*. *Phytochemistry* **1993**, *33*, 1033−1036.

(49) Gerber, N. N.; LeChevalier, M. P. Phenazines and phenoxazinones from *Waksmania aerata* sp. nov. and *Pseudomonas iodina*. *Biochemistry* **1964**, *3*, 598−602.

(50) Yue, Q.; Bacon, C.; Richardson, M. Biotransformation of 2-benzoxazolinone and 6-methoxybenzoxazolinone by *Fusarium monoliforme*. *Phytochemistry* **1998**, *48*, 451−454.

(51) Honkanen, E.; Virtanen, A. I. Synthesis of precursor II of benzoxazolinone formed in rye plants, and the enzymic hydrolysis of precursor I, the glucoside. *Acta Chem. Scand.* **1960**, *14*, 504−507.

(52) Jernow, J. L.; Rosen, P. 2*H*-1,4-Benzoxazin-3(4*H*)-ones. U.S. Patent 3,862,180, 1975.

(53) Coutts, R. T.; Hindmarsh, K. W. 4-Benzoxazine hydroxamic acids and related compounds. *Can. J. Pharm. Sci.* **1966**, *1*, 11−17.

(54) Matlin, S. A.; Sammes, P. G.; Upton, R. M. The oxidation of trimethylsylylated amines to hydroxamic acids. *J. Chem. Soc., Perkin Trans. 1* **1979**, *10*, 2481−2487.

(55) Sicker, D.; Hartenstein, H. A new general approach to the 2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one skeleton via diisobutylaluminum hydride reduction of 2,3-dioxo-1,4-benzoxazines. *Synthesis* **1993**, 771−772.

(56) Sicker, D.; Prätorius, B.; Mann, G.; Meyer, L. A convenient synthesis of 2,4-dihydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one. *Synthesis* **1989**, 211−212.

(57) Hartenstein, H.; Sicker, D. α-Hydroxylation of cyclic hydroxamic acids by peroxide oxidation: a novel approach to allelochemicals from Gramineae. *Tetrahedron Lett.* **1994**, *35*, 4335−4338.

(58) Tietze, L. F.; Beller, M.; Terfort, A.; Dölle, A. First synthesis and structural determination of blepharin and 1-epiblepharin. *Synthesis* **1991**, 1118−1120.

(59) Kluge, M.; Sicker, D. Double diastereoselective glucosidation of cyclic hemiacetals: synthesis of the 1,4-benzoxazinone acetal blucosides GDIBOA and GDIMBOA from Gramineae. *Tetrahedron* **1996**, *52*, 10389−10398.

(60) Sicker, D. A facile synthesis of 6-methoxy-2-oxo-2,3-dihydrobenzoxazole. *Synthesis* **1989**, 875−876.

(61) Kluge, M.; Sicker, D. Synthesis of 4-acetylbenzoxazolin-2(3*H*)-one reported from *Zea mays*. *J. Nat. Prod.* **1998**, *61*, 821−822.

(62) Köksal, M.; Gökhan, N.; Erdogan, H.; Özalp, M.; Ekizoglu, M. Synthesis of 3-(2-substituted benzoylmethyl)-2-benzoxazolinones and screening antimicrobial activities. *Il Farmaco* **2002**, *57*, 535−538.

(63) Bonte, J. P.; Lesieur, D.; Lespagnol, C.; Cazin, M.; Brunet, C.; Cazin, J. C. Acyl 6-benzoxazolinones. *Eur. J. Med. Chem. Chim. Ther.* **1974**, *9*, 491−497.

(64) Tomoda, A.; Yamaguchi, T.; Sato, K.; Iwata, A. Antiviral agents containing aminophenoxazines. Jpn. Patent 2004143101, 2004.

(65) Tomota, T.; Yoneyama, Y.; Yajima, K.; Koshimura, S. Biosynthesis of water-soluble 2-aminophenoxazin-3-one derivatives as antitumor agents. Jpn. Patent 02193984, 1990.

(66) Ikekawa, T.; Uehara, N.; Okuda, T. Photochemistry of antibiotics. I. Oxidative coupling of o-aminophenol by photoirradiation. *Chem. Pharm. Bull.* **1968**, *16*, 1705−1708.

(67) Kehrmann, F. Ueber Oxydationsproducte von o-aminophenolen. *Chem. Ber.* **1906**, *39*, 134−138.

(68) Szeverenyi, Z.; Milaeva, E. R.; Simandi, L. I. Kinetics of the oxidation of 2-aminophenol by dioxygen in the presence of tetrakis[(3,5-di-*tert*-butyl-4-hydroxyphenyl) dodeca chloro ftalocyaninato]cobalt(II). *J. Mol. Catal.* **1991**, *67*, 251−258.

(69) Buckley, R. G.; Charalambous, J.; Henrick, K. 2-Amino-7-methoxy-3*H*-phenoxazin-3-one. *Acta Crystallogr., Sect. B* **1982**, *B38*, 289−291.

(70) Charalambous, J.; Kensett, M. J.; Jenkins, J. M. Deoxygenation of 2-nitrosophenols and of their metal complexes with triphenylphosphine. Synthesis of phenazines, dihydrophenazines, triphenyl(*o*-hydroxyphenylimino)phosphoranes and their metal complexes. *J. Chem. Soc., Chem. Commun.* **1977**, *12*, 400−401.

(71) Hashimoto, Y.; Takayoshi, I.; Shudo, K.; Okamoto, T. Rearrangement of 4-acetoxy-2*H*-1,4-benzoxazin-3(4*H*)-one. *Chem. Pharm. Bull.* **1983**, *31*, 3891−3896.

(72) Xu, Y.; Bizuneh, A.; Walker, C. A reagent for selective deprotection of alkyl acetates. *J. Org. Chem.* **1996**, *61*, 9086−9089.

(73) Keck, G. E.; Wager, T. T.; McHardy, S. F. Reductive cleavage of N−O bonds in hydroxylamines and hydroxamic acid derivatives using samarium diiodide. *Tetrahedron* **1999**, *55*, 11755−11772.

(74) Burgos, N. R.; Talbert, R. E.; Kim, K. S.; Kuk, Y. I. Growth inhibition and root ultrastructure of cucumber seedlings exposed to allelochemicals from rye (*Secale cereale*). *J. Chem. Ecol.* **2004**, *30*, 671−689.

(75) Etzerodt, T.; Nielsen, S. T.; Mortensen, A. G.; Christophersen, C.; Fomsgaard, I. S. Elucidating the transformation pattern of the cereal allelochemical 6-methoxy-2-benzoxazolinone (MBOA) and the trideuteriomethoxy analogue [D_3]-MBOA in soil. *J. Agric. Food Chem.* **2006**, *54*, 1075−1085.

(76) Fomsgaard, I. S.; Mortensen, A. G.; Coja, T.; Idinger, J.; Blümel, S. Transformation of benzoxazinones and derivatives and microbial activity in the test environment of soil ecotoxicological tests on *Poecilus cupreus* and *Folsomia candida*. *J. Agric. Food Chem.* **2006**, *54*, 1086−1092.

(77) Coja, T.; Idinger, J.; Blümel, S. Effects of the benzoxazolinone BOA, selected degradation products and structure related pesticides on soil organisms. *Ecotoxicol. Environ. Saf.* **2005**, in press.

(78) Idinger, J.; Coja, T.; Blümel, S. Effects of the benzoxazoid DIMBOA, selected degradation products and structure related pesticides on soil organisms. *Ecotoxicol. Environ. Saf.* **2005**, in press.

(79) Coja, T.; Idinger, J.; Blümel, S. Influence of the soil composition on the effects of benzoxazinoid allelochemicals on two soil nontarget organisms. *J. Agric. Food Chem*. **2006**, *54*, 1093−1098.

(80) Fritz, J.; Braun, R. Ecotoxicological effects of benzoxazinoids on aquatic nontarget organisms. *J. Agric. Food Chem.* **2006**, *54*, 1105−1110.

Received for review April 19, 2005. Revised manuscript received May 31, 2005. Accepted December 6, 2005. This work was financially supported by the program "Quality of Life and Management of Living Resources (1998 to 2002)" of the European Union, FATEALLCHEM Contract QLRT-2000-01967. Fellowships from the Universidad de los Andes-Venezuela (A.O.B.) and from the European Commission (European Union) and Junta de Andalucía-Spain (D.M.) are gratefully acknowledged.

JF050896X