

A Formylating Agent by Dehydration of the Natural Product DIMBOA

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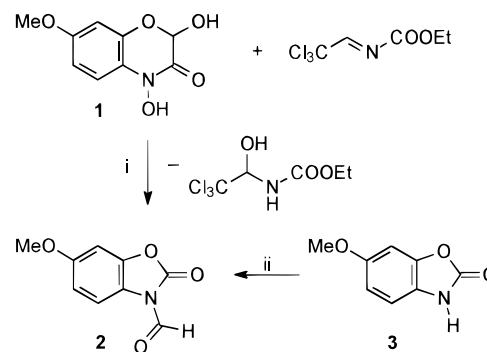
The natural aglucone 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA, **1**) of maize underwent spontaneous dehydration and rearrangement to form 3-formyl-6-methoxybenzoxazolin-2(3*H*)-one (FMBOA, **2**) on reaction with *N*-ethoxycarbonyl-trichloroacetalimine. Compound **2** was proven to be a reactive formyl donor toward *N*-, *O*-, and *S*-nucleophiles, which may be important in case **2** is formed under biological conditions.

Crop plants such as maize,¹ rye,² and wheat,³ exhibit a natural preinfectious resistance system⁴ toward pathogens structurally based upon acetal 2- β -D-glucosides of the (2*R*)-2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one skeleton. After a pest attack, the bioactive aglucones are released by β -glucosidase and act against the pest. They have also been found to occur in phytotoxic root exudates⁵ as a weapon in the fight between different plant species. The best investigated aglucone is 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA, **1**) of maize.⁶ Synthetic approaches to **1** have been reviewed,⁷ and recently a novel DIMBOA synthesis has been reported.⁸ We now report on the dehydration of **1** with formation of 3-formyl-6-methoxybenzoxazolin-2(3*H*)-one (FMBOA, **2**), which is proven to be a very reactive formyl donor toward typical nucleophiles that occur in biomolecules.

DIMBOA is an enzyme inhibitor of, for example, α -chymotrypsin,⁹ aphid cholinesterases,¹⁰ and plasma membrane H^+ -ATPase.¹¹ Several proposals for the molecular mode of action are based on model experiments with **1** and structural analogues. Hence, it was suggested that the inhibition may result from reaction with nucleophilic groups such as $-NH_2$ from a lysine or $-SH$ from a cysteine unit. Hemiacetal **1** undergoes an oxo-cyclo tautomerization. The aldehyde group of the oxo form reacts with the ϵ - NH_2 group of *N*- α -acetyl lysine.¹² The importance of subunits of DIMBOA was studied by removing either the 2-OH group or the 7-MeO group, together with an activation of the analogues, by acetylation at N-OH.¹³ Both model compounds, 4-acetoxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one and 4-acetoxy-2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one, were shown to react with various C-, N-, or S-nucleophiles, regioselectively. Even DNA reacted with the hydroxamic acid N atom.¹⁴ Thus, **1** was regarded as a precursor of a reactive, multi-centered cationic electrophile, generated by N-O bond heterolysis, which is facilitated by the 7-MeO group. Hence, the high activity of **1** results from the combination of both the hemiacetal- and the 7-donor-activated cyclic hydroxamic acid moieties.

We have now found that another very reactive species can be obtained from **1** by formal dehydration. Usually, cyclic hydroxamic acids, including the natural aglucone 2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIBOA), an analogue of **1**, form stable adducts with azavinologous *N*-acyl-trichloroacetaldimines.¹⁵ However, on addition of an equimolar amount of *N*-ethoxycarbonyl-trichloroacetalimine¹⁶ to a solution of natural **1** in THF at 20 °C, **1** showed

Scheme 1. Syntheses of 3-Formyl-6-methoxybenzoxazolin-2(3*H*)-one (**2**)



(i) THF, 20 °C, 1h; (ii) 100% HCOOH+Ac₂O+4-DMAP, THF

an exceptional behavior due to a cascade of spontaneous reactions. Instead of an adduct, the hitherto unknown FMBOA (**2**) was isolated as the product of a formal dehydration and rearrangement (Scheme 1). (2,2,2-Trichloro-1-hydroxy-ethyl)-carbamic acid ethyl ester was unequivocally identified as the second product by comparison of the mp, ¹H NMR, and IR spectra of a sample isolated from the reaction mixture with those of an authentic sample. A ¹H NMR spectrum of the crude reaction product showed it to consist of equivalent amounts of **2** and (2,2,2-trichloro-1-hydroxy-ethyl)-carbamic acid ethyl ester.

After initial addition to the imine the combined action of the strong acceptor unit at N-O together with the donor effect of the MeO group causes a spontaneous heterolysis of the N-O bond in the oxo form. As result of a rearrangement sequence, **2** is formed.¹⁷ The structure of **2** was unequivocally assigned by means of spectroscopic data. ¹H NMR showed deshielding for H-4 and a singlet at δ 9.20 ppm, which did not undergo an H-D exchange with D₂O. This signal corresponds to a carbon signal at 158.4 ppm with $J_{C,H} = 218.6$ Hz both typically for CHO in formamides. The mass spectrum showed a fragment for $[M^+ - 16]$ at m/z 177 besides the base peak m/z 165. An NOE between CHO and H-4 was not observed. This can be explained assuming a partial double bond between C and N in the *N*-formyl unit as is typical for formamides with an orientation of the O atom toward H-4, thus preventing an NOE of CHO. Finally, this conformational feature was exactly revealed by X-ray analysis,¹ which showed four molecules of **2** per unit cell, connected through hydrogen bonds (Figure 1, Tables 1 and 2).

FMBOA (**2**) resembles *N*-formylbenzotriazole, reported as a reactive formyl donor.¹⁸ We have studied the behavior

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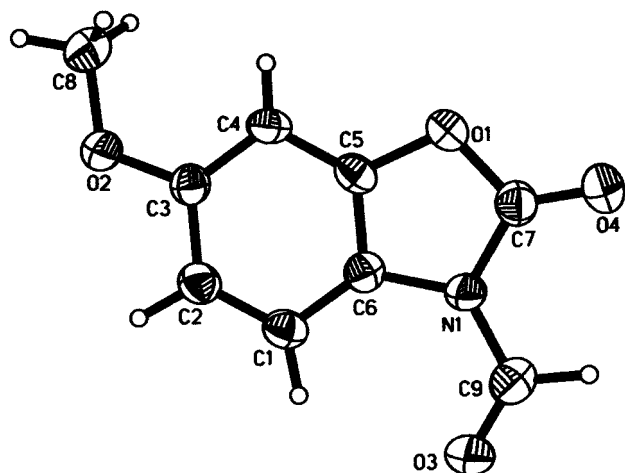


Figure 1. An ORTEP diagram of 3-formyl-6-methoxybenzoxazolin-2(3H)-one (**2**).

Table 1. Crystal Data for **2**

empirical formula	C ₉ H ₇ NO ₄
formula weight	193.16
crystal system, space group	triclinic, <i>P</i> $\bar{1}$
unit cell dimensions	<i>a</i> = 9.357(1) Å α = 107.63(1) $^\circ$ <i>b</i> = 10.522(1) Å β = 113.66(1) $^\circ$ <i>c</i> = 10.567(1) Å γ = 99.76(1) $^\circ$
number of formulas per unit cell	4
calculated density	1.499 g/cm ³
wavelength	0.710 73
goodness-of-fit on <i>F</i> ²	0.955
final <i>R</i> indices [<i>I</i> > 2σ(<i>I</i>)]	<i>R</i> ₁ = 0.0509 <i>wR</i> ₂ = 0.1125
<i>R</i> indices (all data)	<i>R</i> ₁ = 0.0992 <i>wR</i> ₂ = 0.1350

Table 2. Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($\text{\AA}^2 \times 10^3$) for **2**

	<i>x</i>	<i>y</i>	<i>z</i>	<i>U</i> (eq) ^a
O(1)	3919(2)	8058(2)	8486(2)	40(1)
O(2)	9980(2)	9364(2)	11 419(2)	46(1)
O(3)	3567(3)	3840(2)	5299(2)	51(1)
O(4)	1317(2)	6686(2)	6580(2)	50(1)
O(5)	2244(2)	1456(2)	6475(2)	41(1)
O(6)	2436(2)	-3199(2)	3808(2)	42(1)
O(7)	7817(3)	3104(2)	9604(2)	56(1)
O(8)	3277(2)	3738(2)	8215(2)	46(1)
N(1)	3625(3)	5949(2)	6834(2)	34(1)
N(2)	5003(3)	2366(2)	8071(2)	33(1)
C(1)	6750(3)	6219(3)	8106(3)	37(1)
C(2)	8266(4)	7186(3)	9320(3)	39(1)
C(3)	8400(3)	8511(3)	10 301(3)	34(1)
C(4)	6991(3)	8903(3)	10 116(3)	36(1)
C(5)	5493(3)	7905(3)	8909(3)	33(1)
C(6)	5353(3)	6615(3)	7925(3)	32(1)
C(7)	2767(3)	6861(3)	7198(3)	38(1)
C(8)	10 171(5)	10 764(3)	12 369(4)	51(1)
C(9)	2832(4)	4636(3)	5591(3)	44(1)
C(10)	5609(4)	72(3)	6975(3)	34(1)
C(11)	4793(3)	-1300(3)	5847(3)	33(1)
C(12)	3082(3)	-1812(3)	4872(3)	33(1)
C(13)	2091(3)	-955(3)	4984(3)	33(1)
C(14)	2950(3)	405(3)	6128(3)	32(1)
C(15)	4632(3)	930(3)	7091(3)	31(1)
C(16)	3508(3)	2668(3)	7673(3)	39(1)
C(17)	681(4)	-3755(4)	2788(4)	52(1)
C(18)	6527(4)	3369(3)	9245(3)	46(1)

^a *U*(eq) is defined as one-third of the trace of the orthogonalized **U**_{ij} tensor.

of **3** toward model nucleophiles. To obtain **2** in larger quantities, an independent synthesis, starting from 6-methoxy-benzoxazolin-2(3H)-one (MBOA, **3**),¹⁹ was accomplished with in-situ-prepared acetylformylanhydride in THF solution in the presence of 4-DMAP, adapting a literature

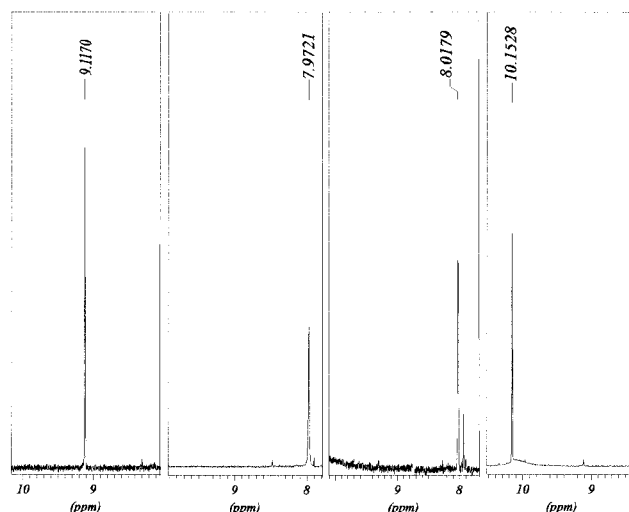


Figure 2. CHO part of the ¹H NMR in THF-*d*₈ of **2** (left), and its reactions with *N,N*-diethylamine (middle left), MeOH (middle right), and methyl thioglycolate (right).

method.²⁰ In NMR tubes 0.1 mmol of *N,N*-diethylamine, MeOH, and methyl thioglycolate dissolved in THF-*d*₈ have been reacted each with 0.1 mmol of **2** at room temperature. Spectra measured after 24 h showed almost complete reactions. The progress of these reactions could be observed using the signal of the CHO proton, which occurs in THF-*d*₈ at δ 9.12 for **2** and undergoes a distinct shift in the products formed, as in *N,N*-diethylformamide (δ 7.97), methyl formate (δ 8.02), and methyl *S*-methoxycarbonyl thioformate (δ 10.15) (Figure 2). A preparative formylation of benzylamine is described in the Experimental Section. Therefore, **2** is a reactive formyl donor toward *N*-, *O*- and *S*-nucleophiles comparable to *N*-formylbenzotriazole.

The dehydration sequence of **1** discussed offers yet another interpretation of the bioactivity mode of **1**. Under natural conditions **1**, after metabolic *N*-OH acylation followed by *N*-O heterolysis may, if a nucleophile is not immediately present, rearrange to **2** as a potent formylating agent toward -NH₂, -OH, or -SH groups. Therefore, some of the biological effects observed could also result from formylations of biomolecules.

Experimental Section

General Experimental Procedures. Melting points were measured on a Boetius micro hot-stage and are corrected. The NMR spectra were recorded with a Varian Gemini 200 (¹H, 199.975 MHz; ¹³C, 50.289 MHz) spectrometer with hexamethyldisiloxane as the internal standard. The MS was recorded on a VG Masslab Manchester VG 12-250 spectrometer (70 eV EI ionization). The elemental analysis was performed on a Heraeus CHN-O-Rapid analyzer. For the X-ray structure determination a SIEMENS CCD diffractometer with a graphite monochromator and Mo K α radiation was used. The intensity data were collected at 223 K; 4524 reflections (were) collected with $2 < \theta < 27^\circ$, of these 3199 were independent (*R*_{int} = 0.032). All 1946 observed reflections [*I*(*hkl*) > 2 σ(*I*)] were used for determination of the unit cell parameters. The structures were solved by direct methods (SHELX-97) and subsequent difference Fourier synthesis and refined by full-matrix least-squares on *F*² (SHELX-97). The H atoms were located by difference Fourier map and refined isotropically. The starting DIMBOA (**1**) was isolated from frozen maize seedlings according to the procedure reported.⁶ *N*-Ethoxycarbonyl-trichloroacetaldehyde was prepared following the literature method.¹⁶ MBOA (**3**) was accessible from 5-methoxy-2-nitrophenol in two steps.¹⁹

3-Formyl-6-methoxybenzoxazolin-2(3H)-one (2) from DIMBOA (1). To a stirred solution of DIMBOA (1) (0.5 mmol, 106 mg) in dry THF (15 mL) was added a solution of *N*-ethoxycarbonyl-trichloroacetaldimine (0.5 mmol, 109 mg) in dry THF (5 mL) in one portion at 20 °C. After 1 h the solvent was removed in vacuo and the oily residue triturated with EtOH (5 mL) to cause crystallization. The crude pale-brown crystals were recrystallized from CHCl₃ to yield 3-formyl-6-methoxybenzoxazolin-2(3H)-one (3) (43 mg, 45%) as off-white crystals: mp 144–146 °C; ¹H NMR (200 MHz, CDCl₃) δ 9.20 (s, 1H, CHO), 7.82 (d, 1H, H-4), 6.83 (d, 1H, H-7), 6.78 (dd, 1H, *J*_{4,5} = 9.0 Hz, *J*_{5,7} = 2.2 Hz, H-5), 3.83 (s, 3H, OCH₃); ¹³C NMR (50 MHz, CDCl₃) δ 158.7 (C-6), 158.4 (CHO), 152.7 (C-2), 143.9 (C-7a), 119.6 (C-4a), 115.4 (C-4), 110.7 (C-5), 98.1 (C-7), 56.4 (OCH₃), EIMS, *m/z* 193 [M]⁺ (23), 177 (11), 165 (100), 150 (70), 136 (12); *anal.* C 56.30%, H 3.97%, N 7.56%, calcd for C₉H₇NO₄, C 55.96%, H 3.65, N 7.25%.

For identification, (2,2,2-trichloro-1-hydroxy-ethyl)-carbamic acid ethyl ester was isolated by column chromatography (Si gel 60 0.040–0.063 mm, Merck, eluent isohexane–CHCl₃ 1:1) from the remaining residue: mp 99–100 °C (lit.¹⁶ mp 103 °C), ¹H NMR (200 MHz, CDCl₃) δ 5.68 (s, 1H, Cl₃C–CH–), 4.20 (q, 2H, *J* = 7.0 Hz, –OCH₂–), 1.28 (t, 3H, *J* = 7.0 Hz, –CH₃), (identical with the spectrum of an authentic sample).

3-Formyl-6-methoxybenzoxazolin-2(3H)-one (2) from MBOA (3). At 0 °C to Ac₂O (25 mmol, 1 mL) 100% HCO₂H (12.5 mmol, 0.5 mL) was added. The mixture was warmed to 50 °C, kept for 15 min at this temperature, and cooled in an ice bath to 0 °C again. A solution of 4-(dimethylamino)pyridine (1 mmol, 122 mg) in dry THF (30 mL) was added. To the rapidly stirred solution, 6-methoxybenzoxazolin-2(3H)-one (3) (10 mmol, 1.65 g) was added and stirred for 1 h at 0 °C and 14 h at 20 °C. The solvent was removed in vacuo, and the solid residue was stirred with EtOH (12 mL) and filtrated. Off-white crystals of 3-formyl-6-methoxybenzoxazolin-2(3H)-one (3) (1.14 g, 59%) remained, identical in their properties with 2 prepared from 1.

Formylation of Benzylamine with 2. To a solution of 2 (386 mg, 2 mmol) in dry THF (30 mL) benzylamine (215 mg, 2 mmol) was added. The solution was refluxed for 30 min. The solvent was removed in vacuo, and from the solid residue *N*-benzyl-formamide was extracted with refluxing *n*-hexane (7 × 25 mL). On cooling to 0 °C the *N*-benzyl-formamide crystallized to form off-white needles (184 mg, 68%), mp 53–

55 °C (lit.²¹ mp 56–58 °C), its NMR identical with that of an authentic sample.

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Supporting Information Available: A scheme showing proposed mechanism of formation of 2 by dehydration of 1, tables of crystal structure information of 2, and a figure showing the unit cell of 2. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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