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-trichloroacetaldimine. 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 preinfectional resistance system[4] 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 exudates[5] 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 −NH₂ 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-NH₂ 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 azavinylogous *N*-acyl-trichloroacetaldimines.[15] However, on addition of an equimolar amount of *N*-ethoxycarbonyl-trichloroacetaldimine[16] 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**)
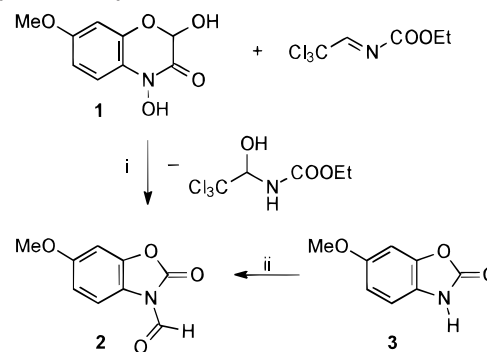
(i) THF, 20 °C, 1h; (ii) 100% HCOOH+Ac₂O+4-DMAP, THF

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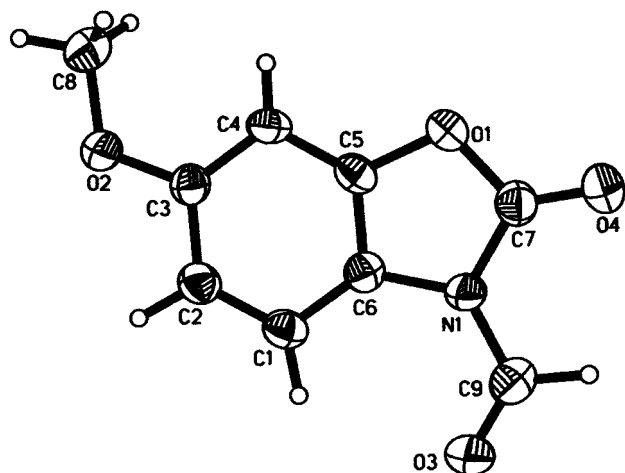
Figure 1. An ORTEP diagram of 3-formyl-6-methoxybenzoxazolin-2(3*H*)-one (**2**).

Table 1. Crystal Data for **2**

empirical formula	$C_9H_7NO_4$
formula weight	193.16
crystal system, space group	triclinic, $P\bar{1}$
unit cell dimensions	$a = 9.357(1)$ Å $\alpha = 107.63(1)°$
	$b = 10.522(1)$ Å $\beta = 113.66(1)°$
	$c = 10.567(1)$ Å $\gamma = 99.76(1)°$
number of formulas per unit cell	4
calculated density	1.499 g/cm^3
wavelength	0.710 73
goodness-of-fit on F^2	0.955
final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0509$ w$R_2 = 0.1125$
R indices (all data)	$R_1 = 0.0992$ w$R_2 = 0.1350$

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

	x	y	z	U(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 $\mathbf{U}_{ij}$ tensor.

of **3** toward model nucleophiles. To obtain **2** in larger quantities, an independent synthesis, starting from 6-meth-oxy-benzoxazolin-2(3*H*)-one (MBOA, **3**),[19] was accomplished with in-situ-prepared acetylformylanhydride in THF solution in the presence of 4-DMAP, adapting a literature
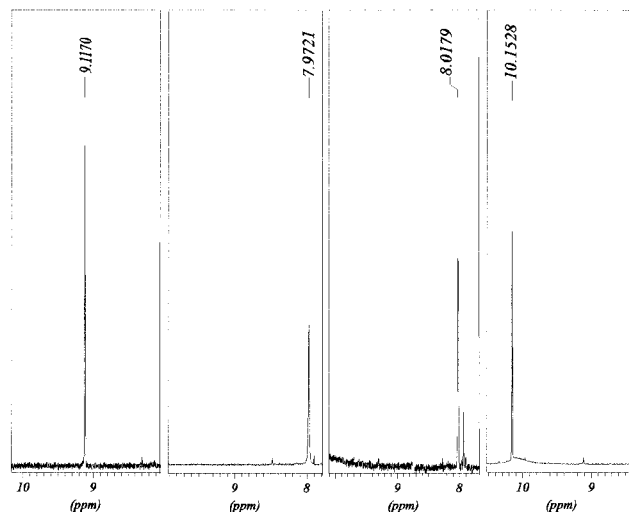
Figure 2. CHO part of the ^{1}H NMR in THF-d_8 of **2** (left), and its reactions with *N,N*-diethylamine (middle left), MeOH (middle right), and methyl thioglycolate (right).

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 C*H*O 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 formiate (δ 8.02), and methyl *S*-methoxycarbonyl thioformiate (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 −NH$_2$, −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 (^{1}H, 199.975 MHz; ^{13}C, 50.289 MHz) spectrometer with hexameth-yldisiloxane 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 graph-ite monochromator and Mo Kα radiation was used. The intensity data were collected at 223 K; 4524 reflections (were) collected with $2 < \theta < 27°$, 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*-Ethoxycar-bonyl-trichloroacetaldimine was prepared following the litera-ture 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 $CHCl_3$ to yield 3-formyl-6-methoxybenzoxazolin-2(3*H*)-one (**3**) (43 mg, 45%) as off-white crystals: mp 144−146 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.20 (s, 1H, C*H*O), 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, OCH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 158.7 (C-6), 158.4 (*C*HO), 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 (O*C*H$_3$), 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 $C_9H_7NO_4$, 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−$CHCl_3$ 1:1) from the remaining residue: mp 99−100 °C (lit.[16] mp 103 °C), ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.68 (s, 1H, Cl_3C−C*H*−), 4.20 (q, 2H, *J* = 7.0 Hz, −OC*H*$_2$−), 1.28 (t, 3H, *J* = 7.0 Hz, −C*H*$_3$), (identical with the spectrum of an authentic sample).

3-Formyl-6-methoxybenzoxazolin-2(3H)-one (2) from MBOA (3). At 0 °C to Ac_2O (25 mmol, 1 mL) 100% HCO_2H (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(3*H*)-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(3*H*)-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] mp56−58 °C), its NMR identical with that of an authentic sample.

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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