Quantitation of *N*-(2-Hydroxy-4-methoxyphenyl)glyoxylohydroxamic Acid, a Reactive Intermediate in Reactions of 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one

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Extracts of various Gramineae contain hydroxamic acids such 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), which are involved in the resistance of the plants to pests and pathogens.[1,2] It was suggested that the toxicity of DIMBOA is partly due to reactions of its open-chain isomer, *N*-(2-hydroxy-4-methoxyphenyl)glyoxylohydroxamic acid (1).[3,4] On the basis of rate and product studies,[5-7] this intermediate has been invoked in the decomposition reaction of DIMBOA. Since 1 has electroactive groups different from those of DIMBOA, we attempted its quantitation by polarographic reduction of

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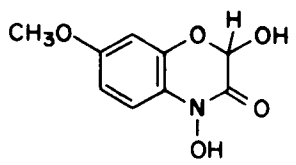
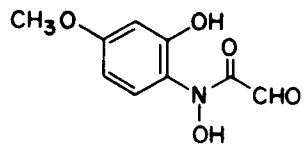


DIMBOA



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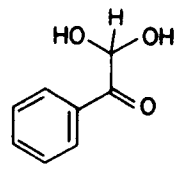
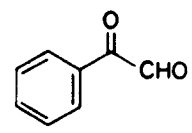


PG



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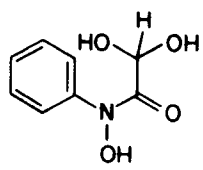

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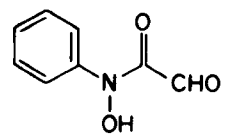

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solutions of DIMBOA. We describe herein the quantitation of 1 in aprotic solvents and discuss its reactivity and the mechanism of decomposition of DIMBOA.

Experimental Section

DIMBOA was isolated as described[8] from ethereal extracts of 6-day old seedlings of *Zea mays* L. cv T129s grown in a greenhouse at 25 ± 5 °C. Phenylglyoxal monohydrate (Aldrich) was used without further purification. Solvents were purified and dried by described methods[9] and were stored under nitrogen.

Polarograms were taken with a PAR Model 174A polarograph on DIMBOA solutions thermostated at 28 °C. The cell, designed for measurements under inert atmosphere, contained a dropping mercury electrode (DME) as working electrode, a platinum auxiliary electrode, and a standard calomel electrode (SCE) as reference electrode. Dry argon was bubbled through the solutions for 10 min before each experiment. Runs were carried out with the solvents in the absence of DIMBOA to test for oxygen leaks. No leaks were detected even after successive potential scans for more than 5 h. The supporting electrolyte was 0.1 M tetraethylammonium perchlorate.

Synthesis of *N*-Phenyl-α,α-dichloroacetohydroxamic Acid. To a solution of 0.035 mol of freshly prepared phenylhydroxylamine in 45 mL of anhydrous diisopropyl ether was added an excess of sodium bicarbonate. To this suspension was added, under constant stirring, 0.018 mol of dichloroacetyl chloride dissolved in 5 mL of diisopropyl ether, with the temperature kept between 0 and 5 °C. After 30 min the mixture was filtered and the filtrate concentrated to turbidity. Petroleum ether was added to complete precipitation. The residue was filtered and recrystallized from benzene–petroleum ether (yield, 93%): mp (uncorrected) 91 °C; UV λ_{max}^{EtOH} 263 nm; IR ν_{max}^{KBr} (cm^{-1}) 3200, 1635; EIMS (probe, 70 eV), *m/z* (relative intensity) 220 (5, $[M]^+$), 136 (32, $[M - CHCl_2]^+$), 108 (100, $[136 - CO]^+$); ^{1}NMR (60 MHz, CD_3COCD_3) δ 7.1 (1 H, s, $CHCl_2$), 7.2–7.9 (5 H, m, Ar); positive $FeCl_3$ test. Anal. Calcd for $C_8H_7NO_2Cl_2$: C, 43.67; H, 3.21; Cl, 32.22. Found: C, 43.89; H, 2.97; Cl, 32.61.

Synthesis of *N*-Phenylglyoxylohydroxamic Acid Monohydrate (PGH). This compound was obtained by hydrolysis of *N*-phenyl-α,α-dichloroacetohydroxamic acid in 1.5 N NaOH at room temperature for 2 h. The resulting solution was extracted with diethyl ether and then acidified to pH 3 with concentrated HCl. The acidified aqueous solution was extracted with diethyl ether. The organic phase was dried with Na_2SO_4 and evaporated under vacuum. The orange oil left was treated with benzene, upon which white crystals were obtained. These crystals were further washed with benzene. The yield was less than 10%: mp (uncorrected) 155–157 °C; UV λ_{max}^{EtOH} 253 nm, $\lambda_{max}^{H_2O}$ 244 nm (ε 14 800); IR ν_{max}^{KBr} (cm^{-1}) 3360, 1665; EIMS (probe, 70 eV), *m/z* (relative intensity) 183 (31, $[M]^+$), 165 (100, $[M - H_2O]^+$), 136 (50, $[165 - CHO]^+$), 108 (32, $[136 - CO]^+$); ^{1}H NMR (60 MHz, CD_3COCD_3) δ 6.9–7.9 (m, C*H*$(OH)_2$ and Ar); positive $FeCl_3$ test. Anal. Calcd for $C_8H_9NO_4$: C, 52.46; H, 4.95. Found: C, 52.24; H, 5.17.

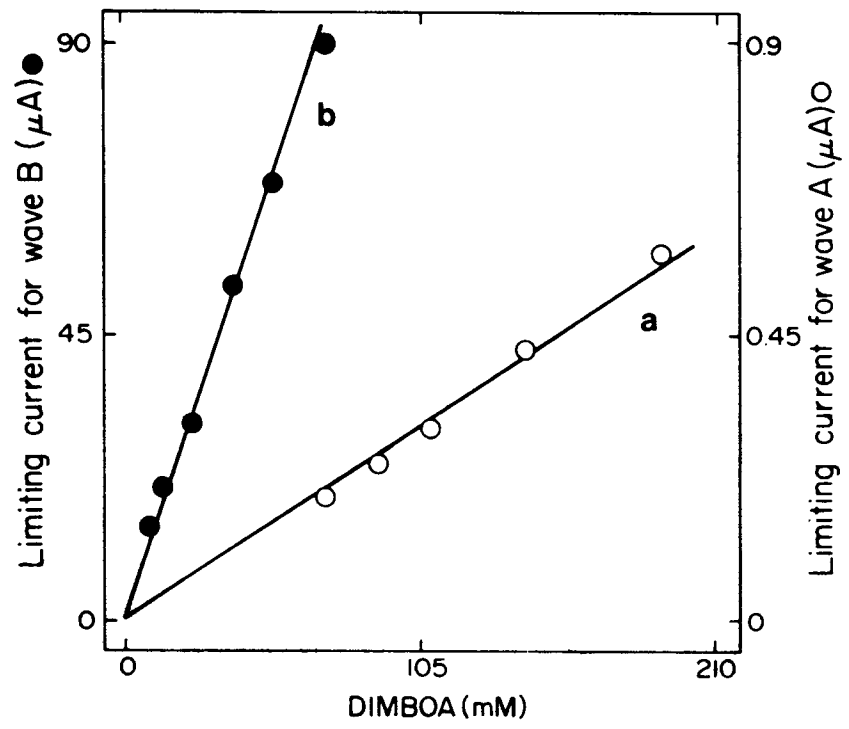


Figure 1. Variation of the limiting current with the concentration of DIMBOA for the reduction waves A (a) and B (b) in dimethylformamide.

Table I. Half-Wave Potentials for the Polarographic Reduction of DIMBOA and Model Compounds

compd[a]	solvent	$-E_{1/2}$/V vs. SCE wave A	$-E_{1/2}$/V vs. SCE wave B
DIMBOA	dimethylformamide	0.63	1.72
	dimethyl sulfoxide	0.62	1.95
	pyridine	0.65	1.73
PG	dimethylformamide	0.62	0.95
PGH	dimethylformamide	0.66	2.06

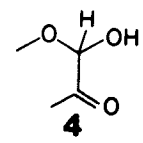
[a] Concentrations employed were: DIMBOA = 1.1×10^{-3} M; PG = 1.1×10^{-3} M; PGH = 1.4×10^{-3} M.

Results

Two reduction waves appeared in the polarographic reduction of solutions of DIMBOA in dimethylformamide. The limiting current for wave B was proportional to the square root of the height of the mercury reservoir, indicating diffusion control, and proportional to the concentration of DIMBOA (Figure 1b). Wave A was detected at DIMBOA concentrations greater than those used to detect wave B. Its limiting current was independent of the height of the mercury head, indicating kinetic control. The limiting current of wave A was over 100 times smaller than that of wave B and increased linearly with DIMBOA concentration (Figure 1a).

The polarographic behavior of solutions of DIMBOA in the other solvents employed was qualitatively similar.

The reduction of two model compounds was also studied: phenylglyoxal monohydrate (PG) and *N*-phenylglyoxylohydroxamic acid monohydrate (PGH). These compounds share with DIMBOA the structural feature depicted in **4**. The polarograms of solutions of these com-



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pounds were similar to those of DIMBOA, showing a diffusion-controlled wave of type B and a wave with kinetic character of type A. These similarities suggest that electroactive groups are present in or are produced from the

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Table II. Thermodynamic and Kinetic Constants for the Decomposition of DIMBOA at 28 °C[a]

solvent	$10^4 k_{obsd}$,[b] min^{-1}	$10^3 K$	k_2, min^{-1}
dimethylformamide	1.72	2.1	0.082
dimethyl sulfoxide	0.81	0.73	0.111
pyridine	0.52	0.27	0.194

[a] See eq 1 in the text. [b] Extrapoled to 28 °C from Arrhenius plots with data from ref 7.

partial structure 4. Half-wave potentials for the polarographic waves described are collected in Table I.

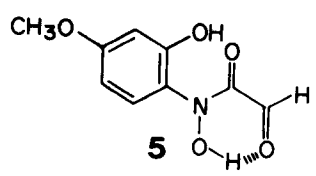
Discussion

Identification of Species Being Reduced. The polarographic reduction of DIMBOA solutions showed a kinetic wave A and a diffusional wave B. The half-wave potential of wave B was similar to that for the corresponding wave in PGH and fell within the range observed for the reduction of the hydroxamic acid moiety.[10] Hence, wave B may be attributed to the hydroxamic function in DIMBOA.

Waves showing kinetic control, such as wave A, have been reported in the polarographic reduction of sugars[11-14] and aldose oximes[15] and have been attributed to hemiacetal opening prior to electron transfer. On the other hand, similar waves have been reported in the polarographic reduction of PG[16] and of other aldehydes,[17] in aqueous solutions. They have been attributed to a dehydration process formally similar to hemiacetal opening (PG to 2). The waves of type A observed in the polarographic reduction of solutions of DIMBOA, PG, and PGH in nonaqueous solvents may thus be identified with compounds 1, 2, and 3, respectively.

Determination of Equilibrium Constant DIMBOA ⇌ 1. At sufficiently high concentrations of DIMBOA, small amounts of 1 could be detected directly. If the diffusion coefficients of cyclic and open-chain species, DIMBOA, and 1 are assumed to be equal, the equilibrium constants for hemiacetal opening can be determined as the ratio of the slopes of lines such as those shown in Figure 1 for dimethylformamide as solvent. Table II collects the values of such equilibrium constants in various solvents.

Studies of the hydroxyl stretching frequencies of DIMBOA in aprotic solvents have shown that the solvents interact with DIMBOA by sharing electron pairs with the hydroxyl groups at positions 2 and 4.[7] One of these interactions is expected to be absent in compound 1 since in it a strong intramolecular hydrogen bond would be formed (5). Hence, these solvents are expected to preferentially stabilize DIMBOA over compound 1. This



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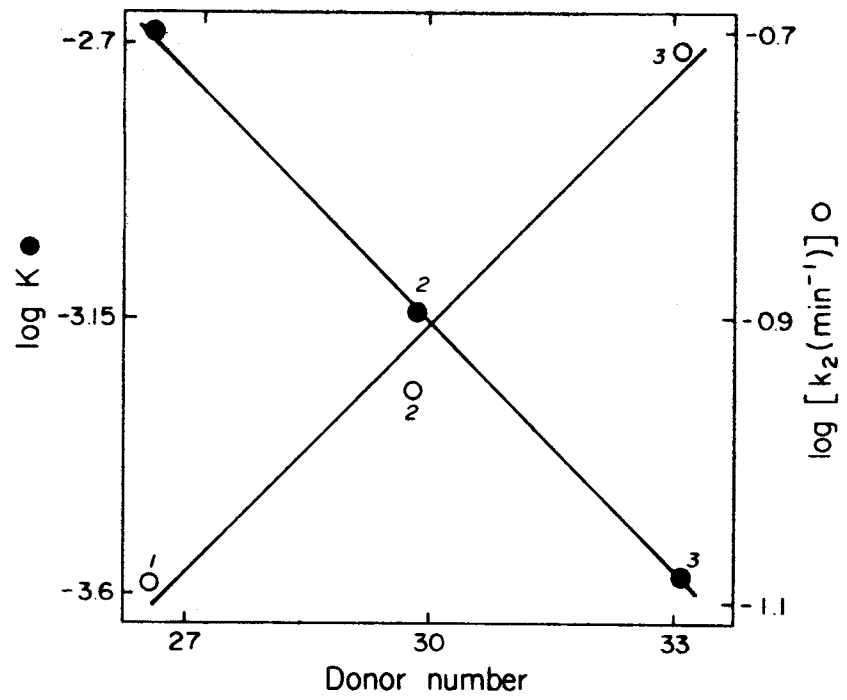
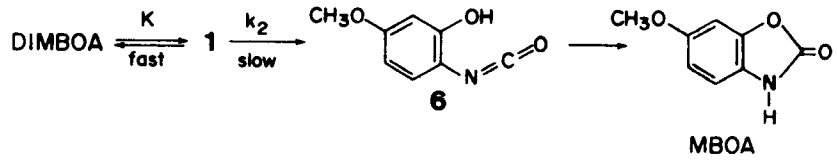


Figure 2. Effect of solvent donor number on the equilibrium constant for the process DIMBOA ⇌ 1 (○) and on the rate constant of formation of isocyanate 6 (Scheme I) (●). Solvents employed were dimethylformamide (1), dimethyl sulfoxide (2), and pyridine (3).

Scheme I



consideration is indeed reflected in the linear solvent energy relationship obtained with log K by using the donor number of the solvent, a measure of its ability to donate an electron pair (Figure 2).[18]

Mechanism of Decomposition of DIMBOA. DIMBOA decomposes to give 6-methoxybenzoxazolin-2-one (MBOA) as the main product both in protic[5,6,19] and aprotic[7] solvents. The comparable effects of basicity of aqueous solutions and donor ability of aprotic solvents on decomposition rate[5,20] and yield of MBOA[19] suggest that similar mechanisms are operating in both types of solvents. Evidence presented for the decomposition of DIMBOA in aprotic solvents gave support to the mechanism depicted in Scheme I.[7] In solvents of low donor number (lower than ca. 23), the opening of the hemiacetal is the rate-limiting step.[7] In solvents of higher donor number, such as dimethylformamide, dimethyl sulfoxide, and pyridine, the rate-limiting step is the formation of isocyanate 6. Under these conditions, the experimental rate constant for decomposition is equal to the product of the equilibrium constant for hemiacetal opening times the first-order rate constant for the formation of 6 (eq 1). The values for K

$$k_{obsd} = Kk_2 \quad (1)$$

determined in high donor number solvents allow the calculation of k_2 (Table II). The logarithms of these values correlated linearly with solvent donor number (Figure 2). Since the proposed formation of isocyanate 6 requires the nucleophilic attack of the hydroxamic hydroxyl group on the aldehyde function in 1, the rate enhancement observed in solvents of high donor number may be taken as further evidence for the mechanism of Scheme I.

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Registry No. **1**, 103150-46-5; **6**, 103150-45-4; DIMBOA, 69884-05-5; PG, 1075-06-5; PGH, 103150-44-3; MBOA, 532-91-2; PhN(OH)C(O)$CHCl_2$, 34282-44-5; PhNHOH, 100-65-2; $CHCl_2C$-(O)Cl, 79-36-7.