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REACTION OF A CYCLIC HYDROXAMIC ACID FROM GRAMINEAE WITH THIOLS

HERMANN M. NIEMEYER*, LUIS J. CORCUERA and FRANCISCO J. PÉREZ

Facultad de Ciencias Básicas y Farmacéuticas, Universidad de Chile, Casilla 653, Santiago, Chile

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Key Word Index—*Zea mays*; Gramineae; 1, 4-benzoxazin-3-ones; thiols.

Abstract—An insect inhibitor isolated from maize extracts, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), reacted with cysteine, mercaptoethanol, ethanethiol and dithiothreitol. Second order rate constants were 0.54, 0.73 and 1.96 l/mol min for the reaction with cysteine, mercaptoethanol and dithiothreitol, respectively. Products isolated from the reaction of DIMBOA with ethanethiol corresponded to reduction of the hydroxamic acid to amide and/or hemiacetal–hemithioacetal exchange. The reactivity of DIMBOA with sulfhydryl groups of compounds such as cysteine, may be a basis for its inhibitory action on enzymes and its toxicity.

INTRODUCTION

Cyclic hydroxamic acids isolated from wheat, rye and maize, protect plants against insects [1–4]. The main hydroxamic acid found in maize extracts is 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA, **1**) [5]. DIMBOA decreases the survival and reproductive rate of aphids on artificial diets [2], delays development of the European corn borer [3] and inhibits bacterial growth in culture [6]. DIMBOA also inhibits energy transfer reactions in chloroplasts and mitochondria [7].

Although the inhibitory properties of DIMBOA on several organisms have been studied, little is known of its mode of action at the molecular level. We recently reported that addition of cysteine increased decomposition of DIMBOA in insect diets, thus affecting its inhibitory properties [8]. In this paper we describe the reactions of DIMBOA with cysteine and other thiols.

RESULTS

Kinetics of the reaction of DIMBOA with thiols

DIMBOA was allowed to react separately with cysteine, alanine, dithiothreitol (DTT) and mercaptoethanol (ME). Alanine does not affect the rate of disappearance of DIMBOA (Fig. 1). First order plots for the reactions of DIMBOA with cysteine, ME and DTT were linear for at least two half-lives, with initial rate constants proportional to thiol concentrations (Fig. 1). Second order rate constants were 0.54, 0.73 and 1.96 l/mol min, respectively. The logarithms of these constants correlated with reduction potentials of thiols (Fig. 2).

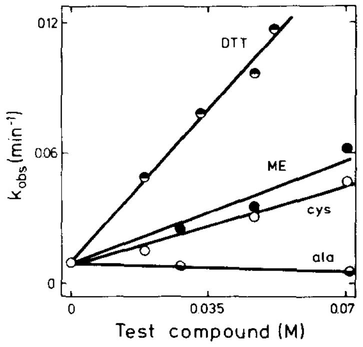
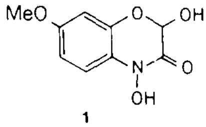


Fig. 1. Observed pseudo-first order rate constants for the disappearance of hydroxamic acids from solutions of DIMBOA (4.7 mM) and added amino acids or thiols (DTT, dithiothreitol; ME, mercaptoethanol). Reactions were performed at 31° in 50 mM KH_2PO_4, pH 8.0, and followed for at least two half-lives. The intercept corresponds to the rate constant for spontaneous decomposition of DIMBOA.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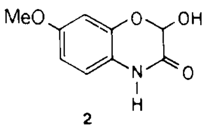
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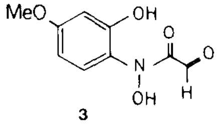
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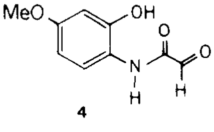
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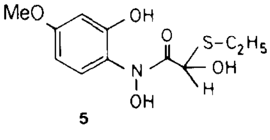
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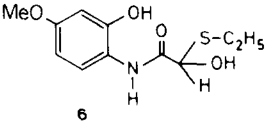
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*To whom correspondence should be addressed.

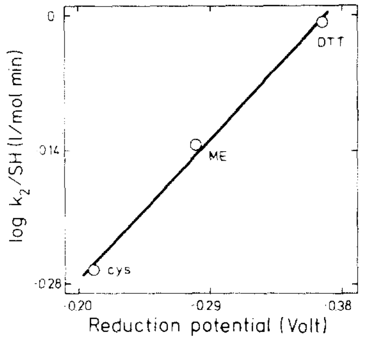

Fig. 2. Second order rate constants for reaction of DIMBOA with thiols of varying reduction potentials (cys, cysteine; ME, mercaptoethanol; DTT, dithiothreitol). Reactions were carried out at 31° in 50 mM, pH 8.0 KH_2PO_4 buffer. Values for reduction potentials were taken from refs. [15] (cys and DTT) and [16] (ME).

Products of the reaction

Ethanethiol was chosen for product studies. Its volatility precluded kinetic studies under the same conditions used with the other thiols. Three compounds (**2**, **5** and **6**) were isolated from the reaction mixture. These compounds had UV spectra similar to that of DIMBOA, suggesting the presence of the same chromophore. UV and IR spectra obtained for lactam **2** were similar to those reported [9]. Mass spectral analysis supported the structures presented. Molecular ions and fragments were as expected for these compounds. With the exception of **5**, base peaks occurred at m/z 166 in amides and 165 in hydroxamic acids. This is the mass of 2-hydroxy-4-methoxyphenylisocyanate [10].

DISCUSSION

The first step for addition of thiols to DIMBOA is its opening to produce the α-ketoaldehyde **3**, a reactive electrophile. The observed rate constant for an analogous reaction, the dehydration of methylglyoxal, is 0.6 min^{-1} at pH 6.6 and 20° [11]. The reaction, being acid–base catalysed, would be expected to be faster under our reaction conditions (pH 8.0 and 31°).

Rate constants for the addition of thiols to aldehydes show a minimum as a function of pH which depends on the pK_a of the thiol [12]. For DTT and cysteine (pK_a 9.1 and 8.3), the minimum is expected between pH 1.5 and 2.5 [12]. Thus, at pH 8.0 the predominant reaction would be that of thiolate with aldehyde. A rate constant of the order of 10^7 l/mol min is predicted for acetaldehyde [12], and α-ketoaldehydes are expected to react still faster.

After these fast carbonyl reactions, the total concentration of hydroxamic acids would remain constant. The decrease in hydroxamic acid concentration measured with ferric chloride reagent would correspond to the reduction reactions. This proposition is substantiated by the correlation between reduction potentials of thiols and the logarithms of the observed second order rate constants (Fig. 2).

The reactions of DIMBOA with thiols may be the basis for the toxicity and inhibitory activity of DIMBOA on many organisms. For example, DIMBOA inhibits ATPase activity of purified CF_1 from chloroplasts [7]. DIMBOA may decrease the activity of this enzyme by reacting with sulfhydryl groups of cysteine residues important in enzyme catalysis. However, this possible mechanism of action remains to be demonstrated.

EXPERIMENTAL

Kinetics. DIMBOA was isolated as described [13] from Et_2O extracts of 6-day-old seedlings of *Zea mays* L. cv. LH Rinconada grown in a greenhouse at 25°. Rates of disappearance of hydroxamic acids from solns of DIMBOA were followed by withdrawing aliquots, adding them to $FeCl_3$ reagent (50 g $FeCl_3 \cdot 6H_2O$, 500 ml 95% EtOH and 5 ml 14 M HCl) and measuring the A_{590} of the resulting blue complex.

Reactions of DIMBOA with ethanethiol. DIMBOA (200 mg) was dissolved in KH_2PO_4 buffer (pH 8.0, 50 mM) and allowed to react with a 15-fold excess of ethanethiol in a sealed ampoule for 24 hr at 45°. The resulting soln was extracted with Et_2O. The Et_2O soln was dried over Na_2SO_4, filtered and taken to dryness. TLC on Si gel of the brown residue with $CHCl_3$–Me_2CO (5:1) afforded compounds **2** (R_f 0.16) and **5** (R_f 0.59) and a mixture (R_f 0.4) which by TLC on Si gel (prepared in pH 8 KH_2PO_4 buffer [14]) with $CHCl_3$–Me_2CO (5:1) afforded compound **6** (R_f 0.42). Yields were less than 5% for **2** and 3% for **5** and **6** and were not optimized. **2**: UV λ_{max}^{MeOH} nm: 256, 287 sh; IR ν_{max}^{KBr} cm^{-1}: 3200, 2900, 1722, 1660, 1500, 1450, 1270, 1130, 1080, 1020, 830; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1700, 1600; EIMS (probe) 70 eV, m/z (rel. int.): 195 $[M]^+$ (42), 166 (100), 150 (14), 138 (6), 124 (25), 110 (8). **5**: UV λ_{max}^{MeOH} nm: 254, 288 sh; IR ν_{max}^{KBr} cm^{-1}: 3150, 2910, 1722, 1660, 1600, 1410, 1370, 1220, 1170, 1130, 1020, 980; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1725, 1660, 1600; EIMS (probe) 70 eV, m/z (rel. int.): 273 $[M]^+$ (3), 256 (28), 255 (100), 226 (13), 217 (52), 195 (25), 193 (17), 170 (26), 165 (42), 153 (52), 142 (30), 138 (53), 137 (63), 122 (25), 110 (47). **6**: UV λ_{max}^{MeOH} nm: 251, 292 sh; IR ν_{max}^{KBr} cm^{-1}: 3200, 2920, 1750, 1710, 1670, 1640, 1590, 1430, 1380, 1230, 1170, 1000, 830; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735, 1670, 1600; EIMS (probe) 70 eV, m/z (rel. int.): 257 $[M]^+$ (8), 224 (5), 220 (5), 196 (5), 166 (100), 151 (47), 150 (52), 137 (8), 123 (18), 107 (38).

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