
3. Decomposition of 2,4-Dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-(4H)-one, a Hydroxamic Acid From Gramineae

H.M. Niemeyer, H.R. Bravo, G.F. Peña and L.J. Corcuera

Abstract. In aqueous solutions, an asymmetric bell-shaped curve was obtained when the logarithms of the observed decomposition rate constants of the title compound (DIMBOA) were plotted against pH. This suggested that DIMBOA as well as its mono- and dianions are reactive species. Microscopic rate constants for decomposition of each of these species were determined: $k_{AH_2} = 4.4 \times 10^{-5} \text{ min}^{-1}$, $k_{AH} = 1.1 \times 10^{-2} \text{ min}^{-1}$ and $k_A = 3.2 \times 10^{-4} \text{ min}^{-1}$ with $pK_1 = 6.9$ and $pK_2 = 10.9$ at 31°C. In organic solvents, the logarithms of the observed decomposition rate constants as well as the OH stretching frequencies correlated linearly with solvent donor number. These results suggested the involvement of hydrogen-bonded hydroxamic hydroxyl oxygen as a nucleophile in the rate-determining step of the reaction.

Introduction

Extracts of certain Gramineae such as wheat, maize, and rye, contain cyclic hydroxamic acids (1) that have been suggested as playing a role in cereal resistance to insects, fungi, and bacteria [7]. These acids are unstable in aqueous solutions. The rate of the reaction is pH-dependent [1] and the main product is the corresponding benzoxazolinone (2 fig. 3.1) [5,6]. The yield of this compound is also pH and temperature dependent [8]. The reaction liberates formic acid from C-2 of the parent hydroxamic acid [2]. Two mechanisms have been proposed for the decomposition of 2,4-dihydroxy-1,4-benzoxazin-3-one (1a fig. 3.2). *Virtanen et al.* [1] proposed a fast opening of the hemiacetal and a rate-determining release of C-2 as formic acid with the formation of an iso-

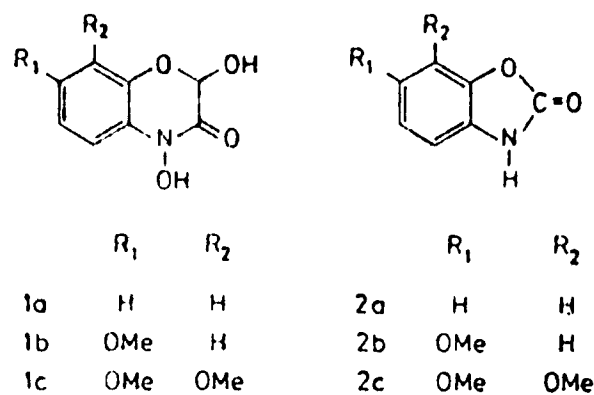


Fig. 3.1. Representative examples of cyclic hydroxamic acids as found in extracts of certain *Gramineae* such as wheat, maize and rye.

cyanate. This intermediate would further rearrange to a benzoxazinone (2a) (Scheme 1 fig. 3.2). On the basis of product and rate studies on analogs of the naturally-occurring hydroxamic acids, *Swissman et al.* [4] criticized the postulation of the isocyanate intermediate. They suggested a fast formation of a five-membered cyclic hemiketal followed by a rate-determining attack of hydroxyl anion (or water) on the carbonyl function originally at position 3. The intermediate formed would rearrange with loss of formic acid to the less stable tautomer of the benzoxazinone, which would rapidly tautomerize (Scheme 2, fig. 3.2).

Despite these studies, the role of the hydroxamic hydroxyl group has not yet been fully clarified. In this paper we examine the behavior of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-(4H)-one (DIMBOA, 1b), the main hydroxamic acid isolated from maize extracts [9], over a wide pH range and in nonaqueous solvents, probing into the role of the hydroxamic hydroxyl oxygen in the decomposition reaction.

Material and Methods

DIMBOA was isolated as described [8]. The kinetics of the decomposition of DIMBOA were followed by treating 0.3 ml aliquots of the reaction mixture with 2.7 ml of FeCl₃ reagent (50 g FeCl₃·6H₂O, 500 ml 95% ethanol, and 5 ml 14 M HCl). The DIMBOA in the aliquot forms a blue complex whose absorbance at 590 nm was measured [9]. The absorbance at 590 nm of a 0.3-

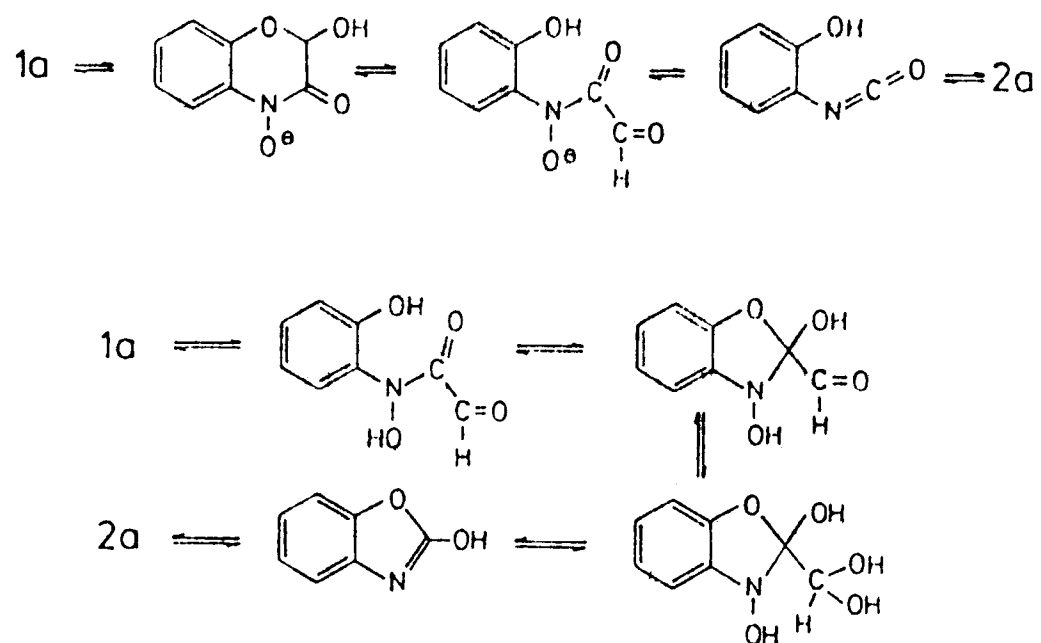


Fig. 3.2. Scheme 1 (top) and Scheme 2 (bottom). Mechanisms of proposed decomposition of cyclic hydroxamic acids in aqueous solution, depending on pH.

ml aliquot of the reaction mixture added to 2.7 ml of the solvent of the FeCl_3 reagent was subtracted for every point. Reactions followed first-order kinetics for at least 2 half-lives.

Solvents of analytic grade were distilled and stored over molecular sieves.

Results

Decomposition of DIMBOA in Aqueous Solutions. DIMBOA was decomposed in aqueous solutions buffered between pH 2.0 and 13.3, at 31°C. The pseudo-first order rate constants increased with increasing pH until around pH 9 and then decreased with further increase in pH (fig. 3.4). These results suggested that DIMBOA behaved in water as a diprotic acid (AH_2) and further that DIMBOA and its two conjugated bases were capable of independently decomposing according to Scheme 3 (fig. 3.3). The observed pseudo-first order rate constant, k_{obs} , may be written as

$$k_{\text{obs}} = \frac{k_{\text{A112}}(\text{H}^+)^2 + k_{\text{A11}}K_1(\text{H}^+) + k_{\text{A}}K_1K_2}{(\text{H}^+)^2 + K_1(\text{H}^+) + K_1K_2}$$

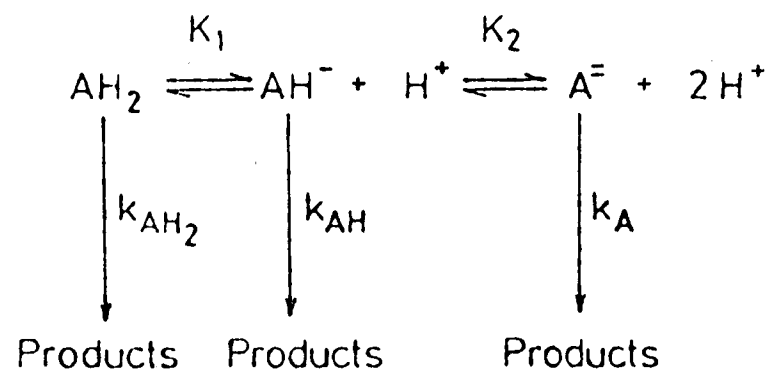


Fig. 3.3. Scheme 3. Decomposition of DIMBOA and its two conjugate bases in water.

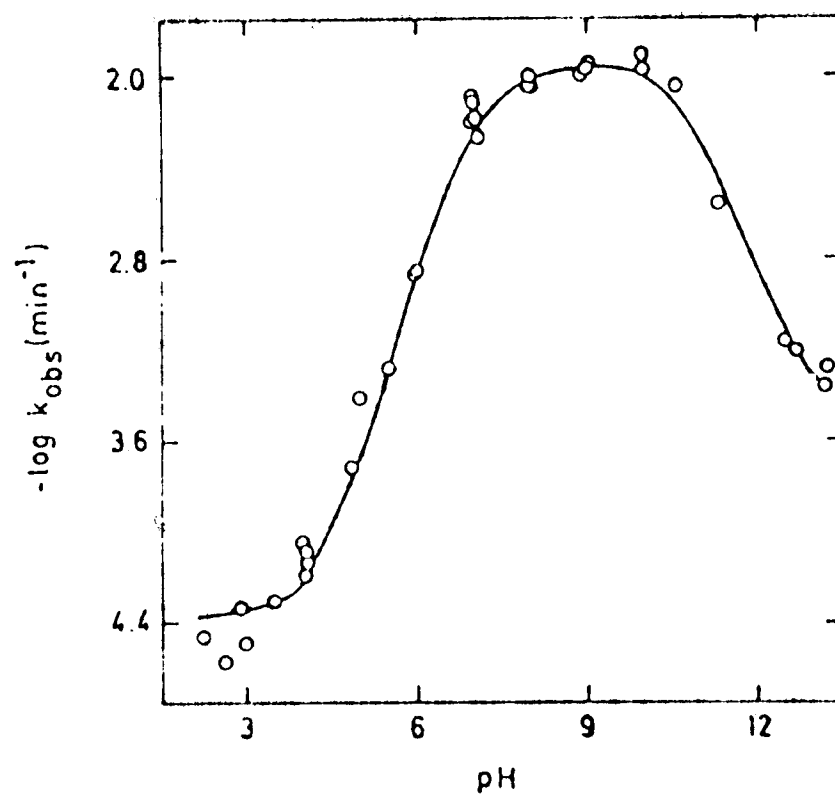


Fig. 3.4. Effect of pH on experimental pseudo first-order rate constants for decomposition of DIMBOA at 31°C. Buffers used were 60 mM citrate (pH 2.2–5.0), 60 mM acetate (pH 4.1 and 5.5), 60 mM phthalate (pH 4.1), 60 mM phosphate (pH 6.0–8.0), 60 mM borate (pH 8.9–10.0), 60 mM carbonate (pH 10.0–11.0), and NaOH (pH 12.5–13.2). DIMBOA was 5.9 mM. The solid line represents the least squares fit to Eq. 3.1).

A least-squares fit of the data in figure 3.1 to equation 3.1, gives $k_{A_{II2}} = 4.4 \times 10^{-5} \text{ min}^{-1}$, $k_{A_{II}} = 1.1 \times 10^{-2} \text{ min}^{-1}$, $k_A = 3.2 \times 10^{-4} \text{ min}^{-1}$, $pK_1 = 6.9$ and $pK_2 = 10.9$. Independent pK values were obtained by measuring the absorbance at 290 nm of buffered solutions of DIMBOA. Results at 31°C were $pK_1 = 6.88 \pm 0.03$ and $pK_2 = 10.5 \pm 0.1$.

Buffer concentration (pH 10.9) had no effect on the decomposition rate.

Decomposition of DIMBOA in Nonaqueous Solvents. The logarithms of the experimental pseudo-first order rate constants for decomposition of DIMBOA in non-aqueous solvents correlated linearly with solvent donor number (3), a measure of its capacity to act as a Lewis base (fig. 3.5). This correlation suggested the interaction of acidic centers responsible for the reactivity of DIMBOA with basic sites in the solvent molecules. This interaction was probed with IR studies of OH stretching frequencies. Two bands are present. The high energy one varies from 3630 to 3507 cm^{-1} in going from benzonitrile to hexamethyl phosphoric triamide. The low energy one varies from 3550 to 3454

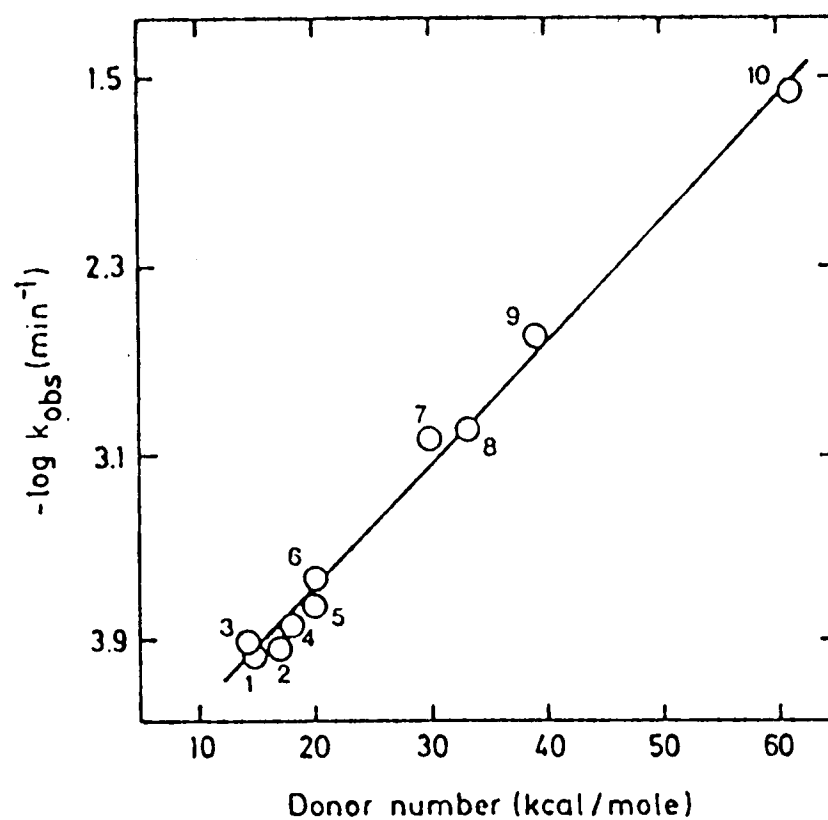


Fig. 3.5. Effect of solvent on the decomposition rate of DIMBOA at 53°C. Solvents employed are: dioxane (1), acetone (2), acetonitrile (3), water, $k_{A_{II2}}$ (4), ethanol (5), tetrahydrofuran (6), dimethylsulphoxide (7), pyridine (8), hexamethylphosphoric triamide (9), and triethylamine (10).

cm^{-1} . Both series correlate linearly with solvent donor number. These values show that both hydroxyl groups in DIMBOA hydrogen-bond to solvent molecules and that hydrogen bonds are stronger in solvents with higher donor number.

The value of k_{AH_2} in H_2O^* falls on the line of the other solvents (fig. 3.5). The similarity between aqueous and nonaqueous decomposition of DIMBOA was further substantiated by product studies. In both cases the main product was MBOA (2b). However, the yields varied with the nature of the solvent, being higher in solvents with higher donor numbers.

Discussion

The insensitivity of the decomposition rate of DIMBOA to buffer concentration points to proton transfers being fast equilibria. The conversion of DIMBOA to its monoanion increases its decomposition rate by nearly 3 orders of magnitude (fig. 3.4) suggesting involvement of the hydroxamic hydroxyl oxygen in the rate-determining step. The lower reactivity of the dianion might be due to an interaction of the phenolic oxygen with the hydroxamic carbonyl which would render the approach by the hydroxamic hydroxyl oxygen difficult or to small effects on the equilibria between open and closed forms (phenol-aldehyde/hemiacetal). Since the microscopic rate constant for the aqueous decomposition of nondissociated DIMBOA, k_{AH_2} , falls on the line obtained with the nonaqueous solvents (fig. 3.5), it is likely that a single decomposition mechanism for undissociated DIMBOA occurs independent of the nature of the solvent. The fact that MBOA appears to be the main product in every solvent studied supports this proposition. IR studies of the OH stretching frequencies of DIMBOA show interactions between hydroxyl hydrogens of DIMBOA and basic sites in solvent molecules. Thus, the increased reactivity of DIMBOA in more basic solvents may be due to an increase in nucleophilicity of the oxygen atoms due to hydrogen bonding. Therefore, a mechanism that assigns to the hydroxamic hydroxyl group a role only as a leaving group (Scheme 2, fig. 3.2), is not supported by the evidence presented. A more likely mechanism would assign this group a role as a nucleophile. The nucleophilic attack may occur on the aldehyde carbonyl to form an isocyanate intermediate (fig. 3.1). The study of pH-effects on the decomposition of derivatives of DIMBOA at the hydroxyl groups would help to further elucidate this problem.

* Determined at pH 3, where 97.5% of k_{obs} corresponds to k_{AH_2} .

Acknowledgments

This work was supported by grants Q622-802 and A254-802 from Universidad de Chile, UNDP/UNESCO RLA 78/024 and by the International Foundation for Science grant 484.

References

- 1 Brendenberg, J.B.; Honkanen, E., and Virtanen, A.I.: The kinetics and mechanisms of the decomposition of 2,4-dihydroxy-1,4-benzoxazin-3-one. *Acta Chem. Scand.* *16*: 135-141 (1962).
- 2 Honkanen, E. and Virtanen, A.I.: On the reaction of 2,4-dihydroxy-1,4-benzoxazin-3-one to 2(3)-benzoxazolinone. *Acta Chem. Scand.* *15*: 221-222 (1961).
- 3 Reichardt, CHR.: *Solvent effects in Organic Chemistry* (Verlag Chemie, Weinheim, New York 1979).
- 4 Smissman, E.E.; Corbett, M.D.; Jenny, N.A., and Kristiansen, O.: Mechanisms of the transformation of 2,4-dihydroxy-1,4-benzoxazin-3-ones and 2-hydroxy-2-methyl-4-methoxy-1,4-benzoxazin-3-one to 2-benzoxazolinone. *J. Org. Chem.* *37*: 1700-1701 (1972).
- 5 Wahlroos, O. and Virtanen, A.I.: The precursors of 6-methoxybenzoxazolinone in maize and wheat plants, their isolation and some of their properties. *Acta Chem. Scand.* *13*: 1906-1908 (1959).
- 6 Wahlroos, O. and Virtanen, A.I.: On the formation of 6-methoxybenzoxazolinone in maize and wheat plants. *Suomen Kemistilehti B32*: 139-140 (1959).
- 7 Willard, J.I. and Virtanen, D.: Benzoxazinones: cyclic hydroxamic acids found in plants. *Residue Rev.* *64*: 64-76 (1976).
- 8 Woodward, M.D.; Corcuera, L.J.; Helgeson, J.P., and Upper, C.D.: Decomposition of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4)-one in aqueous solutions. *Plant Physiol.* *61*: 796-802 (1978).
- 9 Woodward, M.D.; Corcuera, L.J.; Helgeson, J.P.; Kelman, A., and Upper, C.D.: Quantitation of 1,4-benzoxazin-3-ones in maize by gas-liquid chromatography. *Plant Physiol.* *63*: 14-19 (1979).