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REACTION OF DIMBOA WITH AMINES

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Key Word Index—DIMBOA; hydroxamic acids; Gramineae; plant defence; anti-inflammatory activity.

Abstract—DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), a hydroxamic acid from cereals, reacted with primary amines to give an addition compound with two imino groups. Kinetic studies with DIMBOA and with the DIMBOA analogues 2,7-dimethoxy-1,4-benzoxazin-3-one and 2-hydroxy-7-methoxy-1,4-benzoxazin-3-one indicated that the reaction occurred through the intermediacy of the aldol tautomer of DIMBOA. The data is discussed in relation to the biological activity of DIMBOA.

INTRODUCTION

2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA, **1**), the main hydroxamic acid isolated from extracts of wheat and maize [1], plays an important role in the resistance of the plant against several insect pests [2–4]. In addition, it is toxic towards bacteria [5] and fungi [6]. These effects may be rationalized by the inhibition by DIMBOA of enzymes involved in widespread metabolic processes [7, 8]. The enzymic inhibitions are partially attributed to the reaction of DIMBOA with enzyme nucleophilic sites such as cysteine residues [7]. Model studies with thiols in aqueous solution [9, 10] and with sulphydryl enzymes [11] support this proposition. Another common nucleophile present in enzymes is the ε-amino group of lysine residues.

DIMBOA and the aldol **2**, in equilibrium with DIMBOA in solution [12], possess reactive carbonyl groups which may react with nucleophiles such as amines. In this paper, we describe the reaction of DIMBOA and some analogues of it with butylamine and *N*-α-acetyl lysine, models for lysine residues in enzymes.

RESULTS

Spectroscopic description of the reaction

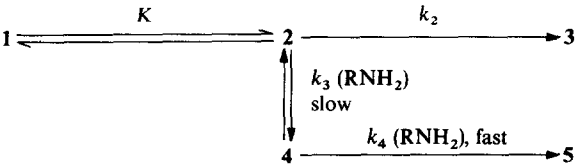
The reaction of DIMBOA with amines was monitored spectrophotometrically in the 240–400 nm region. As the reaction proceeded the band at 290 nm corresponding to the DIMBOA chromophore decreased, a band at 340 nm corresponding to a new chromophore increased, and an isosbestic point was obtained at 317 nm. When the reaction was allowed to go to completeness and treated with sodium borohydride the band at 340 nm disappeared, reflecting the disappearance of the imino chromophore.

Product studies

Butylamine was chosen for product studies, since it is simple structurally, non-volatile, and of adequate solubility. Two compounds were isolated from the reaction mixture of DIMBOA with butylamine (1:100) in ethanol: MBOA (**3**) (54%), a decomposition product of DIMBOA [13], and compound **5**, which was characterized by UV, IR, ^{1}H NMR and mass spectrometry.

Kinetic analysis

As **5**, isolated from the reaction of DIMBOA with butylamine, contains two imino groups, it was of interest to analyse the order of the reaction with respect to butylamine. DIMBOA disappeared in the presence of an excess of butylamine in ethanol with pseudo-first order kinetics. The dependence of the observed rate constant with butylamine concentration was linear. The reaction may be represented by the scheme shown below, which gives the required first order rate law (eqns 1 and 2, where DH_2 represents DIMBOA as a diprotic acid).



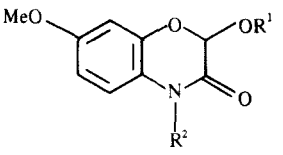
$$-\frac{d(DH_2)}{d(t)} = k_{obs}(DH_2) = k_2K(DH_2) + k_3K(DH_2)(RNH_2) \quad (1)$$

$$k_{obs} = k_2K + k_3K(RNH_2) \quad (2)$$

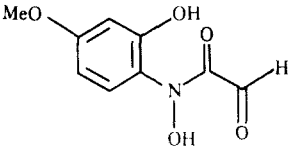
Effect of pH on the reaction of DIMBOA with N-α-*acetyl-lysine*

These studies were carried out with *N*-α-acetyl-lysine, a model for the ε-amino group of lysine residues in enzymes. The kinetics of the reaction of DIMBOA with an excess of *N*-α-acetyl-lysine were pseudo-first order between pH 7 and 12. The dependence of the observed rate constants with *N*-α-acetyl-lysine concentration were linear throughout the amine concentration range at every

pH studied (Fig. 1). From the slopes of such lines, apparent second order-rate constants (k_3K) were calculated. Since DIMBOA is a diprotic acid ($DH_2 \rightleftharpoons DH^- + H^+ \rightleftharpoons D^{2-} + 2H^+$), the second order rate constant k_3K can be dissected according to eqn (3). This equation may be expressed in terms of the dissociation constants of DIMBOA (K_1 and K_2), amine (K_3), and (H^+) [eqn (4)]. The experimental points were fitted to eqn 4 (Fig. 2).



	R^1	R^2
1	H	OH
6	Me	OH
7	OH	H



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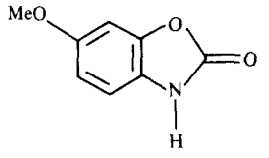

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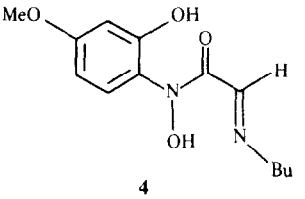

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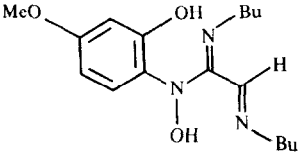

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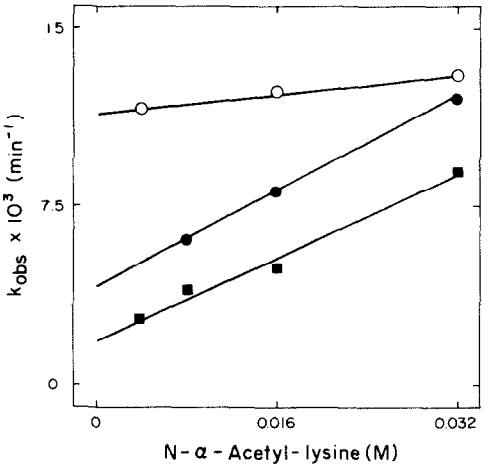

Fig. 1. Variation of the observed first-order rates constants for the reaction of 8×10^{-5} M DIMBOA with *N*-α-acetyl-lysine, as a function of *N*-α-acetyl-lysine concentration, at pH 8.2 (O), 10.5 (●) and 11.5 (■). Second order-rate constants k_3K were calculated for every pH from the slope of such lines.

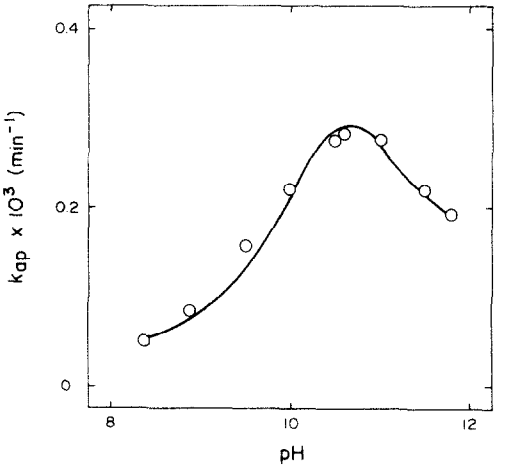

Fig. 2. Dependence on pH of the experimental second order rate constants for the reaction of DIMBOA with *N*-α-acetyl-lysine. The curve represents the least squares fit of the experimental points to eqn (4), where $K_1 = 1.25 \times 10^{-7}$ M and $K_2 = 1.26 \times 10^{-11}$ M [20] and $K_3 = 2.95 \times 10^{-11}$ M [21].

From the fit, the pH-independent second order rate constants for the reaction of each

$$k_{ap} = k_3 K = k_a(DH_2)(RNH_2) + k_b(DH^-)(RNH_2) + k_c(D^{2-})(RNH_2) \quad (3)$$

$$k_{ap} = \frac{1}{(H^+) + K_1(H^+) + K_1K_2} \left[k_a(H^+) + k_bK_1(H^+) + k_cK_1K_2 \right] \frac{K_3}{(H^+) + K_3} \quad (4)$$

acid-base form of DIMBOA with the ε-amino group of *N*-α-acetyl-lysine were determined. The values for these rate constants were: $2.03 \pm 0.3 \times 10^2$ min^{-1} M^{-1} (DH_2); $9.6 \pm 0.7 \times 10^{-1}$ min^{-1} M^{-1} (DH^-); $1.6 \pm 0.2 \times 10^{-2}$ min^{-1} M^{-1} (D^{2-}). As expected, the neutral electrophile reacts substantially faster than the negatively charged species.

Reaction of DIMBOA analogues

The reaction of DIMBOA, 2,7-dimethoxy-4-hydroxy-1,4-benzoxazin-3-one (**6**) and 7-methoxy-2-hydroxy-1,4-benzoxazin-3-one (**7**) with butylamine were studied in ethanol. While DIMBOA and its lactam **7** reacted with second order rate constants equal to 0.49 min^{-1} M^{-1} and 0.23 min^{-1} M^{-1}, respectively, compound **6** showed no reaction. The relative rates of DIMBOA and **7** may be related to the electron-withdrawing effect of the hydroxamic hydroxyl group.

DISCUSSION

DIMBOA (**1**) equilibrates in solution with its open chain tautomer, *N*-(2-hydroxy-4-methoxy-phenyl)-glyoxylohydroxamic acid (**2**) [12], which has been implicated in the decomposition of **1** to 6-methoxy-benzoxazolin-2-one (**3**) [14, 15]. We have now shown that this intermediate is also important in the reaction of DIMBOA with amines. Thus, kinetic measurements support the scheme which includes intermediate **2** (see above) and studies with DIMBOA analogues show that the 2-hydroxy group is essential for their reactivity towards amines.

The reaction of DIMBOA with *N*-α-acetyl lysine suggests a possible way by which DIMBOA could inactivate enzymes possessing reactive lysines, such as Lys-97 of glutamate dehydrogenase [16], or lysine residues located at the active site, as in fructose-1,6-diphosphatase [17]. The inhibition of such enzymes, involved in primary metabolism, would provide further rationalization of the wide range of toxicity of DIMBOA.

Recently, the anti-inflammatory activity of 1,4-benzoxazin-3-ones was measured by the release of histamine (a primary amine) from peritoneal rat mast cells stimulated with conavalin A [18]. It was shown that such activity occurred only when a 2-hydroxy group was present in the benzoxazine ring. This finding can be rationalized through the results presented in this paper, since only when the 2-hydroxyl group is present, can the aldol form occur and react through its carbonyl group with histamine. Thus, histamine accumulation would be hindered, and the inflammatory activity suppressed.

EXPERIMENTAL

Compounds. DIMBOA was isolated as described [7] from Et_2O extracts of 6-day-old seedlings of *Zea mays* L. cv T129s, grown in a greenhouse at 25 + 3°. 2,7-Dimethoxy-4-hydroxy-1,4-benzoxazin-3-one (**6**) and 7-methoxy-2-hydroxy-1,4-benzoxazin-3-one (**7**) were synthesized essentially as described [19]. *N*-α-Acetyl-lysine was purchased from Sigma. Butylamine (Aldrich) was distilled before use.

Kinetic measurements. To 10 ml of 0.05 M buffer soln of the desired pH, amines were added to obtain solns in the range 4 to 140 mM (reference soln). Five ml of this soln were pipetted into a tube containing enough solid DIMBOA to obtain a 0.08 mM soln (sample soln). The reactions were monitored in the 240–400 nm region using quartz cells thermostatted at 31 ± 0.2°, pH was measured before and after each run.

Product analysis. DIMBOA (100 mg) was dissolved in EtOH and allowed to react with an excess of butylamine (1:100) at 30°. The reaction was monitored spectrophotometrically at 340 nm taking aliquots from the reaction mixture after different time intervals. When spectral changes had finished, the solvent was evapd. TLC (C_6H_6–Et_2O–$CHCl_3$ 1:1:1) of the residue gave compounds **3** (R_f 0.3) and **5** (R_f 0.8). Compound **3** was characterized by comparing its R_f, mp, UV and IR spectra with a pure reference sample. Compound **5** was characterized as follows: UV λ_{max}^{EtOH} nm: 340 (log ε = 4.04); IR ν_{max}^{KBr} cm^{-1}: 3300, 2950, 1570, 1470, no carbonyl absorption; ^{1}H NMR (60 MHz, CCl_4, TMS): δ 1.0 (*m*, 6H), 1.7 (*m*, 12H), 5.2 (*s*, 3H), 5.9–6.9 (*m*, 3H); EIMS (probe), 70 eV, *m/z* (rel. int.): 321 (18) $[M]^+$, 320 (20) $[M-H]^+$, 278 (35) $[M-C_3H_7]^+$. Analysis (Found: C, 63.97; H, 9.55 $C_{17}H_{27}O_3N_3$ requires: C, 63.5; H, 9.03 %).

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