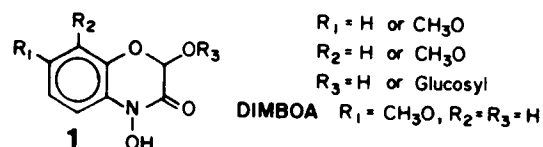


HYDROXAMIC ACIDS FROM GRAMINEAE: THEIR ROLE IN APHID RESISTANCE AND THEIR MODE OF ACTION

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Hydroxamic acids (Hx, 1) isolated from extracts of cereals such as maize, wheat and rye (Willard & Penner, 1976) have been suggested as resistance factors against several organisms. In this paper we summarize evidence showing that Hx are involved in cereal resistance to aphids and describe biochemical and chemical bases for this biological activity.



Role of hydroxamic acids in host plant resistance. Different wheat, maize and rye cultivars showed varying Hx levels up to 1.2 g/kg fr. wt. Inverse relationships were obtained between these levels and growth rates of populations of the aphids *Rhopalosiphum maidis* (Long et al., 1977), *Metopolophium dirhodum* (Argandona et al., 1980), *Schizaphis graminum* (Corcuera et al., 1982) and *Sitobion avenae* (Bohidar et al., 1986). Comparisons made were intraspecific or interspecific with respect to the plant.

Hx content varied with plant age, showing a steep increase a few days after germination and a subsequent slow decrease. Inverse correlations were found between Hx in wheat and rye plants of different ages and growth rate of populations of *M. dirhodum* (Argandona et al., 1980).

Hx content was higher in younger than in older tissues. Young wheat leaves were more resistant than old leaves towards *S. graminum* (Argandona et al., 1981).

DIMBOA, the main hydroxamic acid in wheat and maize extracts, decreased survival and reproduction rate of aphids fed with holidic diets, at concentrations comparable to those found in the plant (Corcuera et al., 1982).

These facts support the idea that hydroxamic acids constitute a chemical defense of cereals against aphids.

Additionally, hydroxamic acids have been claimed as cereal resistance factors towards fungal diseases such as *Puccinia graminis* (ElNaghy & Shaw, 1966) and *Diplodia zeae* (BeMiller & Pappelis, 1965) and towards the insect *Ostrinia nubilalis* (Robinson et al., 1978).

Biochemical bases of DIMBOA toxicity. Hydroxamic acids are toxic towards an ample range of organisms that include bacteria (Corcuera et al., 1978; Lacy

et al., 1979) fungi and insects. A biochemical basis for such widespread toxicity among aerobic organisms was sought for by examining the effects of DIMBOA on energy-linked reactions in submitochondrial particles from bovine heart (Niemeyer et al., 1986).

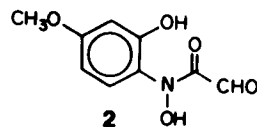
Electron transport from NADH, succinate and ascorbate + N,N,N,N-tetramethylphenylenediamine (TMPD) both in coupled and in uncoupled particles was reversibly inhibited by DIMBOA ($I_{50} = 11$ mM). This effect may be located at complex IV of the respiratory chain.

DIMBOA also inhibited reversibly the ATPase complex, as reflected by inhibitions of ATP synthesis, P_i -ATP exchange reaction and ATPase activity ($I_{50} = 4, 2$ and 6 mM, respectively).

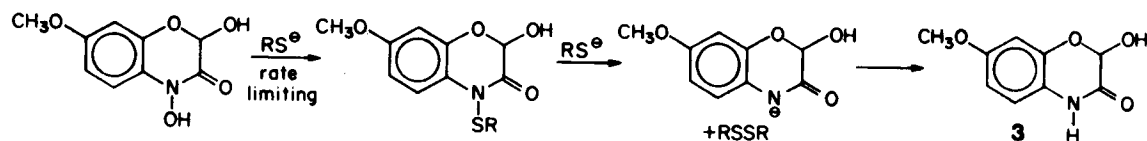
More importantly, incubation of submitochondrial particles with DIMBOA resulted in an irreversible inhibition of electron transport when either NADH or succinate were used as substrates (loss of 50% activity was obtained with 4 mM DIMBOA in 40 min). Furthermore, in DIMBOA-treated particles cytochromes **b**, **c** and **a** were not reduced by succinate whereas cytochromes c and a were reduced by ascorbate + TMPD. Hence, this irreversible inhibition by DIMBOA may be located at complex III of the respiratory chain.

This latter effect would produce an inactivation of ATP synthesis leading to increased ineffectiveness of the energy metabolism of aerobic organisms and could be responsible for the toxicity of DIMBOA.

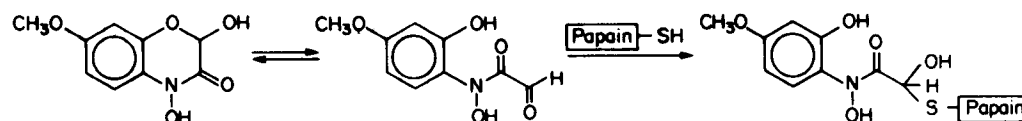
Chemical bases for enzymatic inhibitions by DIMBOA. DIMBOA and the open-chain analogue **2** in equilibrium with it (Copaja et al., 1986) possess several centers potentially reactive towards nucleophiles. These nucleophiles may be residues from aminoacids such as cysteine (SH groups) or lysine (NH_2 groups) related to the activity of enzymes. The reaction of DIMBOA with thiols and amines was studied in further detail.



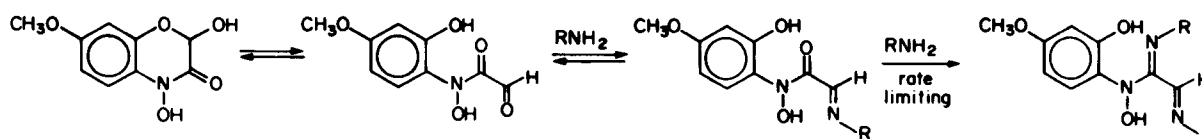
Reaction of DIMBOA with thiols. The main product from the reaction of DIMBOA with thiols was the reduction product, lactam **3**. Other products were hemithioacetals and reduced hemithioacetals (Niemeyer et al., 1982). Rate-pH profiles showed that the active species were undissociated DIMBOA and thiolate anion. Higher nucleophilicity of thiolate anion, as reflected by higher pK_a of the parent thiol, enhanced its rate of reaction with DIMBOA. The analogue of DIMBOA lacking the 7-methoxy group reacted slower than DIMBOA. Finally, CNDO/2 molecular orbital calculations indicated that nucleophilic superdelocalizability, a measure of the tendency of an atom to interact with a nucleophile, was highest at the nitrogen atom. The following mechanism was proposed for the predominant reaction (Perez & Niemeyer, 1985):



The reactivity of DIMBOA towards a sulfhydryl group in an enzyme was studied using papain. This enzyme has a single free cysteine residue which is located at the active site. The enzyme was irreversibly inhibited by DIMBOA. Disappearance of titratable SH groups and of enzyme activity were synchronous. Inactivation by DIMBOA was protected by substrate and could be reverted by dithiothreitol. The rate of inactivation-pH profile showed 2 inflections corresponding to the 2 dissociation constants associated with the cysteine residue at the active site. HPLC analysis of the organic products of an equimolar mixture of DIMBOA and papain failed to show evidence for lactam or free DIMBOA, suggesting that DIMBOA remained attached to the inactivated enzyme. The DIMBOA analogue lacking the 2-hydroxy group, and hence unable to form an open-chain compound, showed no effect on enzyme activity. The following mechanism accounts for the experimental observations (Perez & Niemeyer, to be published):



Reaction of DIMBOA with amines. The main products of the reaction of DIMBOA with *n*-butylamine were the Schiff bases arising from nucleophilic addition to the aldehydic and hydroxamic carbonyl groups in the open-chain analogue of DIMBOA. Only the ϵ -amino group in lysine was reactive between pH 5 and 12. This was shown through the use of α - and ϵ -*N*-acetylated lysine as model compounds, and is in agreement with the higher nucleophilicity of the ϵ -amino group, as reflected by the higher pK_a of its conjugate acid.



Distribution of hydroxamic acids in wheat. A screening for Hx levels of *Triticum* species and their immediate *Aegilops* ancestors was carried out. Hx were found in all the accessions analyzed, concentrations varying within a wide range. Extreme values were observed in the diploid *Aegilops* and *Triticum* accessions. Interestingly, tetraploid wheats arising from two diploids with high Hx levels have not been described. This possibility might be worthwhile considering for the production of high Hx-level wheats (Niemeyer, to be published).

Acknowledgements

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