Antialgal and Antifungal Activity of Natural Hydroxamic Acids and Related Compounds

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Natural hydroxamic acids and related compounds derived from 1,4-benzoxazin-3-one structures inhibited the growth of the alga *Chlorella xanthella* and the fungus *Candida albicans.* On the basis of structure−activity relationships the lipophilic character of the substituent in the aromatic ring and the electrophilic character of the hydroxamic function are suggested to be responsible for antialgal and antifungal activity, respectively.

Keywords: *Hydroxamic acids; Chlorella xanthella; Candida albicans; antialgal/antifungal activity*

INTRODUCTION

Cyclic hydroxamic acids derived from 1,4-benzoxazin-3-one structures (Figure 1) are secondary metabolites of Gramineae and present a number of different biological activities (Niemeyer, 1988). The aglucone 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) is the main component in maize and wheat extracts, while in rye the main component is the demethoxylated analogue DIBOA. These compounds occur initially as glucosides in live plants (Viertanen and Hietala, 1960; Hofman and Hofmanova, 1969). When plant tissue is injured, aglucones are released through the action of the hydrolytic enzyme β-glucosidase (Hofman and Hofmanova, 1971). The hydroxamic acid function seems to play a fundamental role in the bioactivity of these compounds. Particularly, substitution in the aromatic ring and C2 of the heterocyclic ring produces compounds with modified antimicrobial (Bravo and Lazo, 1993) and anti-inflammatory activity (Otsuka et al., 1988), respectively.

In this paper, we report the antialgal and antifungal activity of naturally occurring hydroxamic acids and related synthetic derivatives. *Chlorella xanthella*, a unicellular green alga that grows rapidly and is easily manipulated, was chosen as a model system to study the antialgal activity, and *Candida albicans* was chosen to study antifungal activity.

MATERIALS AND METHODS

Isolation of Natural Hydroxamic Acids. DIMBOA-glucoside and the aglucone were isolated from maize (*Zea mays* L. cv. T-128), and DIBOA-glucoside and the aglucone were isolated in a similar manner from rye (*Secale cereale* L. cv. Tetra) as described (Queirolo et al., 1983; Lyons et al., 1988; Leighton et al., 1994).

Synthesis of Hydroxamic Acid Derivatives. Derivatives of aglucone DIBOA substituted at position 7 of the aromatic ring and C2 of the heterocyclic ring were synthesized according to methods previously described (Atkinson et al., 1991; Matlin et al., 1979; Quiroz and Niemeyer, 1991), and the *N*-acetoxy derivative was obtained according to the method of Hashimoto (Hashimoto et al., 1991).

Antialgal and Antifungal Test. Test compounds were dissolved in nutrient media with the aid of either ultrasound or gentle heating. *In vitro* serial dilutions were prepared in the concentration range from 30 to 1000 μg mL^{-1}, with increments of 50 μg mL^{-1}. This increment decreased to values from 10 to 20 μg mL^{-1} in the region close to the I_{50} and MIC values. *C. xanthella* was grown in nutrient broth medium (Gibco). Samples were incubed at 25 °C for 10 days in test tubes containing 4.0 × 10^4 colony forming units (CFU) with continuous cold white fluorescent light with an intensity of 200 ft-c. *C. albicans* was grown in Mueller-Hinton nutrient medium (Difco) plus 5% dextrose. Samples were incubated at 35 °C for 24 h. Approximate I_{50} values were obtained from the percentage inhibition measured spectrophotometrically. These results were obtained from average below 10% in all cases.

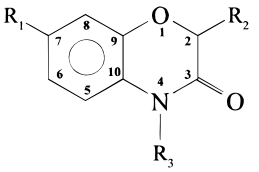
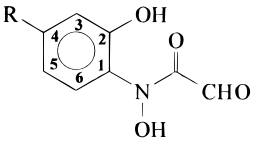


Figure 1. DIMBOA-Glc: $R_1 = OCH_3$; $R_2 = O$-glucosyl; $R_3 =$ OH. DIMBOA: $R_1 = OCH_3$; $R_2 = R_3 =$ OH. DIBOA-Glc: $R_1 =$ H; $R_2 = O$-glucosyl; $R_3 =$ OH. DIBOA: $R_1 =$ H; $R_2 = R_3 =$ OH.

RESULTS AND DISCUSSION

Antialgal Activity. Minimum inhibitory concentrations for *C. xanthella* growth are reported in Table 1. DIMBOA-glucoside (**2**) and DIBOA-glucoside (**1**) showed no activity in the concentration range studied. DIMBOA (**4**) and DIBOA (**3**) aglucones showed moderate MIC values.

Aglucones possess a hemiacetal function at C2 which in solution is in equilibrium with the open form **24**



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(Copaja et al., 1982). Part of the activity of the aglucones may arise from the aldehyde group, which reacts with nucleophiles such as thiols (Niemeyer et al., 1982) and amines (Pérez and Niemeyer, 1989).

Blocking the aperture of DIBOA (compounds **5** and **6**) decreases the antialgal activity significantly; however, other blocked derivatives (compounds **6**−**13**) also show moderate MIC values. This suggests that the opening of the hemiacetal is not an essential requirement to show antialgal activity in these compounds. Hence, a possible reason for the substantial differences between the activities of glucosides and aglucones may be related to the hydrophilic property of the glucosyl

S0021-8561(95)00345-1 CCC: $12.00

Table 1. Antialgal and Antifungal Activity of Hydroxamic Acids and Related Compounds (Figure 1) against *C. xanthella* and *C. albicans*, Respectively

				MIC (μg mL^{-1})	
compd	R_1	R_2	R_3	*C. xanthella*	*C. albicans*
1	H	glucosyl	OH	>1000	>1000
2	MeO	glucosyl	OH	>1000	>1000
3	H	OH	OH	50	666
4	MeO	OH	OH	150	500
5	H	MeO	OH	500	650
6	H	H	OH	200	350
7	CN	H	OH	650	nt[a]
8	Cl	H	OH	50	nt
9	CH_3	H	OH	60	350
10	MeO	H	OH	75	150
11	F	H	OH	150	500
12	MeO_2C	H	OH	300	nt
13	HOOC	H	OH	500	nt
14	CN	H	OH	>500	nt
15	H	OH	AcO	>500	666
16	H	OH	H	400	>1000
17	F	OH	H	>500	nt
18	CN	OH	H	>500	>1000
19	MeO	OH	H	nt	1000
20	CH_3	OH	H	nt	>1000
21	MeO_2C	OH	H	nt	1000
22	NO_2	OH	H	nt	>1000
23	H	H	H	nt	1000

[a] nt; not tested.

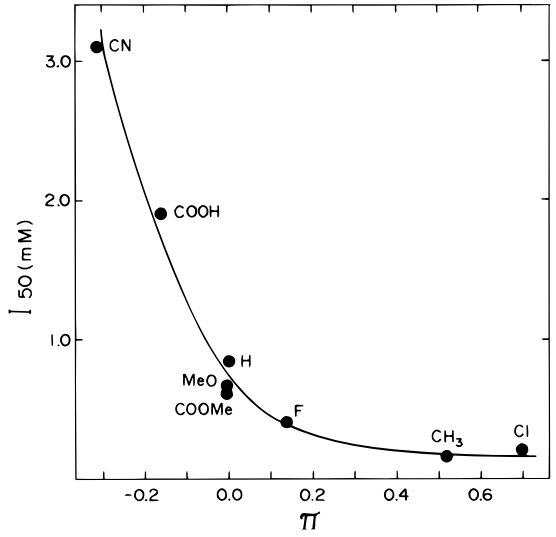

Figure 2. Structure−activity relationships using π parameter for 4-hydroxy-7-substituted-2*H*-1,4-benzoxazin-3-ones and I_{50} values obtained from *C. xanthella*.

residue, which may be involved in transport processes of the whole molecule through the plant tissues. Support for this hypothesis is obtained from structure−activity relationships (SARs) using the π parameter as a measure of the hydrophobic properties of the aromatic substituents (Hansch and Leo, 1979). As shown in Figure 2, the activities increase with the lipophilic character of the substituents, with an optimum π value of approximately 0.5.

Some of the chemical and biological properties of these molecules have been associated with the strong electrophilic character of the hydroxamic acid function. This electrophilic character is a function of the leaving capability of the substituents at the nitrogen atom (Hashimoto et al., 1991) and electronic effects of the substituents in the aromatic ring (Bravo and Lazo, 1993; Weiss and Bravo, 1994).

However, antialgal activity cannot be rationalized in terms of these effects. The I_{50} values do not correlate with the σ_p Hammett constant (Johnson, 1973). This parameter is a measurement of the substituent electronic properties.

When the hydroxyl group at the nitrogen atom in DIBOA is substituted by an acetoxy group (see **15**), which is a more efficient leaving group, or by a hydrogen atom (see **16**), which is a negative leaving group, both derivatives show a significant decrease in the activity. Hence, these results show that the antialgal activity depends on the lipophilic character of the molecules and the hydroxamic function properties.

The alga *Chlorella* has been recommended as a model system to study the herbicidal properties of chemicals since it presents various similarities to higher plants (Somida and Ueda, 1974).

Accordingly DIBOA, the more active inhibitor of *C. xanthella* growth, also has been considered to be responsible for the allelopathy of rye, and it is an active interferent in the growth of several monocots and dicots (Barnes et al., 1987; Barnes and Putman, 1987).

Antifungal Activity. Inhibitory activity on the growth of *C. albicans* is reported in Table 1. Structural modifications of the 1,4-benzoxazin-3-ones show modifications in the antifungal activity, similar to those observed in the antialgal activity. In effect, DIMBOA-glucoside and DIBOA-glucoside (**1** and **2**) show no antifungal activity. The other hydroxamic acids (**3**−**11**) show more activity than the lactams (**16**−**23**), and when the hydroxyl group at the nitrogen atom in **3** is replaced by an acetoxy group (see **15**), no differences in the MIC values are observed.

DIMBOA, DIBOA, and lactam **16** show less activity than the blocked derivatives of **24** (see **10**, **6**, and **23**); it seems then that the opening of the hemiacetal does not play a significant role in the antifungal activity. The lipophilic character of the ring substituent is not clear and, from these results, could not be evaluated.

All of this evidence suggests that the antifungal activity should be mainly controlled by the hydroxamic functionality.

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Received for review June 6, 1995. Accepted February 12, 1996.[⊗] We are pleased to acknowledge financial support of DTI of the Universidad de Chile, Grant Q-3070.

JF950345E

[⊗] Abstract published in *Advance ACS Abstracts,* April 1, 1996.