Antimicrobial Activity of Natural 2-Benzoxazolinones and Related Derivatives

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Natural 2-benzoxazolinones BOA and MBOA and related synthetic derivatives inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Both the electrophilic character of the nitrogen atom of the heterocyclic ring and the lipophilic character of the substituents of the aromatic ring are involved in the antifungal activity. These two effects are evaluated by the shift of the infrared N−H frequencies and by the correlation between the antifungal activity and the π parameter, respectively.

Keywords: *2-Benzoxazolinone; Staphylococcus aureus; Escherichia coli; Candida albicans; antimicrobial activity*

INTRODUCTION

6-Methoxy-2-benzoxazolinone (MBOA) and the demethoxylated analogue (BOA) (Figure 1) occur in several species of higher plants (Virtanen et al., 1956; Chen and Chen, 1976; Wolf et al., 1985; Warner et al., 1993). In gramineae species such as maize, wheat, and rye, it is well-known that these compounds are products of the degradation of the cyclic hydroxamic acids, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 2,4-dihydroxy-1,4-benzoaxzin-3-one (DIBOA), respectively (Figure 1) (Woodward et al., 1978; Bravo and Niemeyer, 1985; Grambow et al., 1986; Niemeyer, 1988).

The possible roles of MBOA and BOA as host plant resistance factors toward various pest and pathogens of these cereals has been investigated (Argandoña et al., 1980; Beck and Smissman, 1961; Kubo and Kamikawa, 1983; Beck and Stauffer, 1957). The range of known activities of these molecules has widened substantially: allelopathy (Barnes et al., 1987; Barnes and Putnam, 1987), auxin inhibitors (Kosemura et al., 1994; Sakoda-Hoshi et al., 1994), anti-inflammatory (Otsuka et al., 1988), mammal reproduction stimulation (Sanders et al., 1981; Berger et al., 1981), and antimicrobial activity toward bacteria and fungi pathogenic to plant and mammals (Wahltroos and Virtanen, 1958; Whitney and Mortimore, 1959, 1961; Virtanen et al., 1957) are some of their properties. Although they have attracted attention because of their interesting biological activities, the chemical mechanisms of the actions are not well understood. However, some of their activities in plants have been associated to fundamental processes of energy metabolism (Niemeyer et al., 1987). The effect of the side chain length at the C_6 position in the aromatic ring on the auxin-inhibiting activity has been established (Sakoda-Hoshi et al., 1994).

The antimicrobial properties of these secondary metabolites are particularly interesting owing to their potential roles similar to natural antipathogens in food plants of agricultural importance.

In this manuscript we report the electronic and lipophilic effects of substituent at C_6 position in 2-benzoxazolinones on the antibacterial and antifungal activities. The bacteria *Staphyloccus aureus* and *Escherichia coli* and the fungi *Candida albicans* were chosen as model systems for this study. They grow rapidly and are easily manipulated. The chemical basis for the activity was rationalized on the basis of structure−activity relationships.

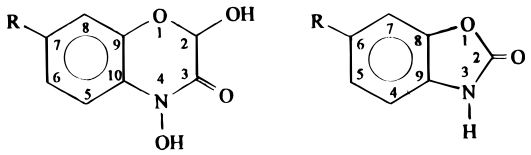

Figure 1. (Left) DIBOA, R = H; DIMBOA, R = MeO. (Right) BOA, R = H; MBOA, R = MeO.

MATERIALS AND METHODS

Synthesis. MBOA and BOA were obtained from DIMBOA and DIBOA as already reported (Niemeyer et al., 1982). The BOA obtained in this manner was identical to the commercial product (Aldrich Chemical Co., Milwaukee, WI). All other compounds were synthesized by the method of Nachman (1982). Melting points and chromatographic and spectroscopic data were consistent with those reported (Woodward et al., 1978; Nachman, 1982; Lyons et al., 1988).

Antimicrobial 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 of 50−1000 μg/mL using increments of 50 μg/mL. This increment decreases to values of 20 μg/mL in the region close to the I_{50} and the minimum inhibitory concentrations (MIC) values. Mueller−Hinton nutrient medium (DIFCO) was used for *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922). DIFCO plus 5% dextrose mixture was used for the fungi *C. albicans* (Faculty of Medicine, University of Valparaiso, Chile). Samples were incubated at 35 °C for 24 h in test tubes containing 10^4 colony-forming units (CFU). Approximate I_{50} values were obtained from the percentage inhibition which was measured spectrophotometrically. Replicated values showed errors below 10% in all cases.

RESULTS AND DISCUSSION

Minimum inhibitory concentration for *S. aureus*, *E. coli*, and *C. albicans* growth are reported in Table 1. All compounds display moderate activity against all test microorganisms. In that series, bacteria are less sensitive to structural modifications of the 2-benzoxazolinones. However, the electron-acceptor properties of the aromatic ring substituents increase their activity. *C. albicans* was more sensitive to the structural changes.

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S0021-8561(96)00858-8 CCC: $14.00

Table 1. Antimicrobial Activity of 2-Benzoxazolinones (Figure 1) against *S. aureus, E. coli, C. albicans,* and ν_{N-H} in Dioxane Solutions, Respectively

		minimum inhibitory concentration (μg/mL)			
compd	R_1	*S. aureus*	*E. coli*	*C. albicans*	ν_{N-H} (cm^{-1})[a]
1	H	>1000	>1000	650	3572
2	MeO	>1000	>1000	450	3578
3	F	700	1000	500	3586
4	CH_3	>1000	500	350	nd[b]
5	Cl	350	300	250	3580
6	NO_2	150	300	125	3587

[a] ν_{N-H}, infrared frequencies of the N−H bonds. [b] nd, not determined.

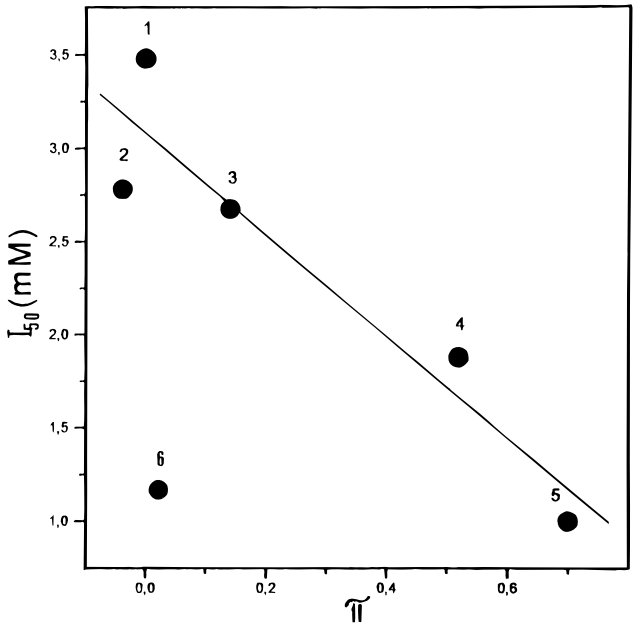


Figure 2. Structure−activity relationships using π parameter for 6-substituted 2-benzoxazolinones and I_{50} values obtained from *C. albicans* (**1** = H; **2** = MeO; **3** = F; **4** = CH_3; **5** = Cl; **6** = NO_2).

The effect on the antifungal activity was rationalized in terms of lipophilic and electronic character of aromatic ring substituents.

The lipophilic effect was evaluated through structure−activity relationships (SARs) by 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 linearly by increasing the lipophilic character of the substituents ($r = 0.94$). The nitro derivative displays an antifungal activity higher than that predicted.

The antifungal activity of the 2-benzoxazolinones can also be associated with the electrophilic character of the nitrogen atom. Structure−activity studies on analogs of six-membered heterocyclic compounds (Hashimoto et al., 1991; Weiss-Lopez and Bravo, 1994; Perez and Niemeyer, 1985; Bravo and Lazo, 1993, 1996) suggest that the influence of substituents on the electrophilicity of this nitrogen atom has a profound effect on the biological activity.

The electrophilic character of the 2-benzoxazolinones can then be increased when the free electrons of the nitrogen atom in the lactam group are delocalized toward both the carbonyl fragment and the aromatic ring. In extreme, B and A forms could be formed. Polarized forms are stabilized by electron-donor and electron-acceptor substituents, respectively.

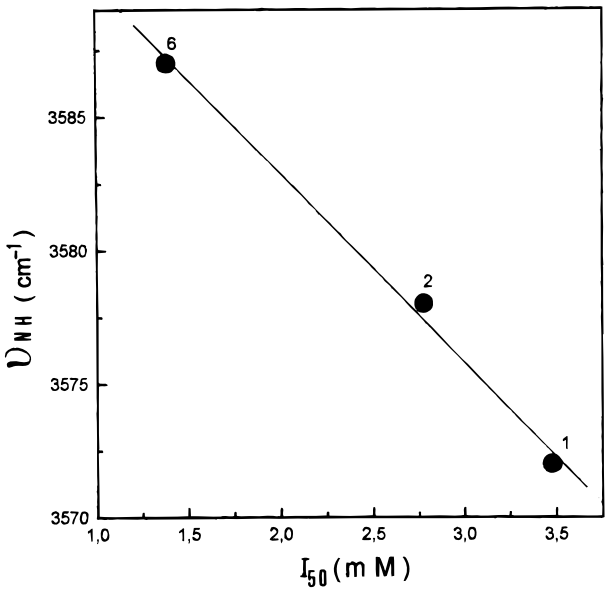
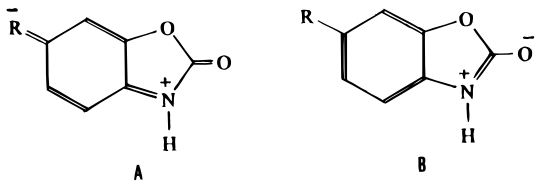


Figure 3. Structure−activity relationship using infrared ν_{N-H} (Table 1) of the 2-benzoxazolinone (**1**) and 6-NO_2 (**6**) and 6-MeO (**2**) derivatives and I_{50} values obtained from *C. albicans*.



Polarization is supported by several modifications observed in the infrared spectra. Frequencies of the N−H bond in the 2-benzoxazolinones are reported in Table 1. It is known that the polarization of the N−H bond in the analogue amides produces an increasing of the frequency (Bellamy, 1958). Similarly, the ν_{N-H} modes were more energetic in the substituted derivatives of BOA. Consistent with this hypothesis, the substituted derivatives show more activity than the unsubstituted 2-benzoxazolinone.

More specific evidences are obtained comparing the π and σ_p values of the H, MeO, and NO_2 substituents. These three groups have similar lipophilia ($\pi_H = 0.0$; $\pi_{NO_2} = 0.06$; $\pi_{MeO} = -0.04$) (Hansch and Leo 1979); however, they display different σ_p values ($\sigma_{pH} = 0.0$; $\sigma_{pNO_2} = 0.81$; $\sigma_{pMeO} = -0.27$). σ_p values are a measure of the electronic properties of the aromatic substituents (Johnson, 1973). The different antifungal activities of these three compounds should be associated only with the electronic effect of the substituents. Moreover, the MeO and NO_2 groups preferentially stabilize the B and A forms, respectively. Thus major activity of these derivatives is expected and the I_{50} values and the ν_{N-H} frequencies of these compounds should correlate as observed in Figure 3 ($r = 0.98$).

In conclusion, the results suggest that the antifungal activity of the 2-benzoxazolinones can be determined by the electronic and lipophilic properties imposed by the aromatic ring substituents.

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Received for review November 12, 1996. Revised manuscript received May 2, 1997. Accepted May 7, 1997.[⊗] We are pleased to acknowledge financial support of DTI of the Universidad de Chile, Grants Q.-3070 and Q.-3545-9313-V.

JF9608581

[⊗] Abstract published in *Advance ACS Abstracts*, July 1, 1997.