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Structure−Function Analysis of the Vanillin Molecule and Its Antifungal Properties

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The aim of the present study was to evaluate which structural elements of the vanillin molecule are responsible for its observed antifungal activity. MICs of vanillin, its six direct structural analogues, and several other related compounds were determined in yeast extract peptone dextrose broth against a total of 18 different food spoilage molds and yeasts. Using total mean MICs after 4 days of incubation at 25 °C, the antifungal activity order was 3-anisaldehyde (1.97 mM) > benzaldehyde (3.30 mM) > vanillin (5.71 mM) > anisole (6.59 mM) > 4-hydroxybenzaldehyde (9.09 mM) > phenol (10.59 mM) > guaiacol (11.66 mM). No correlation was observed between the relative antifungal activity of the test compounds and log $P_{o/w}$. Furthermore, phenol (10.6 mM) was found to exhibit a greater activity than cyclohexanol (25.3 mM), whereas cyclohexanecarboxaldehyde (2.13 mM) was more active than benzaldehyde (3.30 mM). Finally, the antifungal order of isomers of hydroxybenzaldehyde and anisaldehyde was found to be 2- > 3- > 4- and 3- > 2- > 4-, respectively. In conclusion, the aldehyde moeity of vanillin plays a key role in its antifungal activity, but side-group position on the benzene ring also influences this activity. Understanding how the structure of natural compounds relates to their antimicrobial function is fundamentally important and may help facilitate their application as novel food preservatives.

KEYWORDS: Aldehyde; molds; natural antimicrobials; structure−function; vanillin; yeasts

INTRODUCTION

In the food industry there is a growing interest in naturally occurring compounds that exhibit antimicrobial activity and therefore provide a potential source of novel preservatives (*1*, *2*). This interest has been driven by consumer desire for high-quality foods that are deemed to be more natural, minimally processed, and preservative-free together with the tighter legislation governing the use of current preservatives (*1*−*3*). Naturally occurring antimicrobial compounds are produced by animals, for example, lysozyme from eggs and milk, microbes, for example, the bacteriocin nisin from *Lactococcus lactis*, and plant-derived components (*2*). Indeed, plants probably represent the most diverse source of potential antimicrobials, and examples of these compounds include cinnamaldehyde (cinnamon), carvacrol (oregano), eugenol (cloves), thymol (thyme), and vanillin (vanilla). However, their application to food products as preservatives remains to be fully exploited.

Vanillin (4-hydroxy-3-methoxybenzaldehyde), a major constituent of vanilla pods, has generally regarded as safe (GRAS) status and is one of the world's principal flavoring compounds. Currently it is added to foods such as ice cream, liquors, soft drinks, and confectionary in concentrations ranging from 1 to 26 mM depending on the nature of the product (*4*). Vanillin is produced naturally via a multistep curing process of the green vanilla pods of the orchid plant *Vanilla planifolia*, *Vanilla pompona*, or *Vanilla tahitiensis* (*5*). However, the majority (90%) of vanillin currently in use is synthetically produced (nature identical) from eugenol, lignin, or guaiacol (*4*, *6*).

Vanillin has been shown to exhibit antioxidant properties (*7*), antimutagenic properties (*8*), and, moreover, antifungal activity (*9*−*11*). Vanillin inhibited the growth of *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Debaryomyces hansenii*, and *Zygosaccharomyces rouxii* in culture medium and apple puree for 40 days when used at a concentration of ∼13 mM. However, it was less effective in banana puree, in which ∼20 mM was insufficient to inhibit the growth of *Z. bailii*, the authors concluding that the higher lipid/protein levels in bananas interfered with vanillin's antifungal activity (*10*). The incorporation of vanillin (∼3−7 mM) into fruit-based agars (apple, banana, mango, papaya, and pineapple) was sufficient to inhibit the growth of *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus*

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Table 1. Microorganisms Used in This Study

microorganism	source
mold strains	
Aspergillus oryzae (CBS 108.24)	Centraalbureau voor Schimmel cultures, Utrecht, The Netherlands
Aspergillus sojae (ATCC 20245)	American Type Culture Collection, Manassas, VA
Penicillium corylophilum (Cz 1)	Unilever R&D, Sharnbrook, U.K.
Penicillium expansum (Pol 4)	Unilever R&D, Sharnbrook, U.K.
Penicillium expansum (Pol 11)	Unilever R&D, Sharnbrook, U.K.
Penicillium funiculosum (IMI 134756)	International Mycological Institute, Egham, U.K.
Penicillium spinulosum (Vru 32)	Unilever R&D, Sharnbrook, U.K.
Penicillium sp. (Vru 35)	Unilever R&D, Sharnbrook, U.K.
Rhizomucor racemosus (It 2)	Unilever R&D, Sharnbrook, U.K.
yeast strains	
Candida lipolytica (205)	Unilever R&D, Sharnbrook, U.K.
Candida rugosa (154)	Unilever R&D, Sharnbrook, U.K.
Candida sake (165)	Unilever R&D, Sharnbrook, U.K.
Kloeckera apiculata (127)	Unilever R&D, Sharnbrook, U.K.
Pichia pastoris (219)	Unilever R&D, Sharnbrook, U.K.
Saccharomyces cerevisiae (NCYC 956)	National Collection of Yeast Cultures, Norwich, U.K.
Saccharomyces cerevisiae (176)	Unilever R&D, Sharnbrook, U.K.
Zygosaccharomyces bailii (NCYC 1427)	National Collection of Yeast Cultures, Norwich, U.K.
Zygosaccharomyces bailii (80)	Unilever R&D, Sharnbrook, U.K.

ochraceus, and *Aspergillus parasiticus* for 2 months (*9*). We were recently able to show that vanillin at concentrations of 10−20 mM was sufficient to achieve the complete inhibition of growth of *Candida parapsilosis* and *S. cerevisiae* in two different soft drinks over an 8 week storage period at 25 °C (*11*). These inhibitory concentrations could be reduced to between 1 and 5 mM when the storage temperature was lowered to 8 °C. A recent study on the mode of action of vanillin against *Escherichia coli*, *Lactobacillus plantarum*, and *Listeria innocua* suggested that it is primarily a membrane active compound, but it may also have other target sites within the bacterial cells (*12*). To date, relatively little is known about the function the different structural elements of the vanillin molecule play with respect to its antifungal activity.

The objective of this study therefore was to evaluate which structural elements of the vanillin molecule are important in its observed antifungal property. The relative antifungal activity of vanillin and its six direct structural analogues was determined against a range of food spoilage molds and yeasts. The structural analogues consist of each of the three vanillin side groups, hydroxy (OH), methoxy (OCH_3), and aldehyde (CHO), bound to the basic benzene ring structure either singularly or in combinations. Additionally, the antifungal activities of several other related compounds were also determined.

MATERIALS AND METHODS

Microorganisms and Culture Conditions. The food spoilage associated mold and yeast strains used in this study are listed in **Table 1**. The microorganisms were maintained at 2 °C on yeast extract peptone dextrose (YEPD) agar containing 20 g of glucose, 20 g of bacteriological peptone, 10 g of yeast extract, 15 g of agar, and water to 1 L. Glucose was purchased from BDH (Poole, U.K.), and all other components of YEPD were obtained from Becton Dickinson (Cowley, U.K.).

Chemical Preparation. The following compounds were purchased from Sigma (Poole, U.K.) and were of the highest grade available (96−100% pure) (common names are stated in parentheses): 4-hydroxy-3-methoxybenzaldehyde (vanillin); benzaldehyde; phenol; 2-methoxyphenol (guaiacol); methoxybenzene (anisole); 2-methoxybenzaldehyde (2-anisaldehyde); 3-methoxybenzaldehyde (3-anisaldehyde); 4-methoxybenzaldehyde (4-anisaldehyde); 2-hydroxybenzaldehyde (salicylaldehyde); 3-hydroxybenzaldehyde; 4-hydroxybenzaldehyde; cyclohexanol; and cyclohexanecarboxaldehyde (**Figure 1**). Stock solutions (10% v/v or w/v) of all the test compounds were prepared in 99.7−100% ethanol to final volumes of 10 mL with the following exceptions: anisole (30% v/v), guaiacol (20% v/v), and salicylaldehyde (1% v/v). These stocks were stored overnight at 2 °C in the dark prior to use.

Antifungal Assays. Each of the 13 test compounds was added to 10 mL volumes of YEPD broth (pH 4.0) in 30 mL screw-capped bottles to the following final concentration ranges: vanillin (3.0−18.5 mM); benzaldehyde (1.0−14.6 mM); phenol (5.5−25.6 mM); guaiacol (0.0−32.7 mM); anisole (5.0−10.6 mM); 2-anisaldehyde (0.2−8.1 mM); 3-anisaldehyde (0.6−10.8 mM); 4-anisaldehyde (1.0−14.6 mM); salicylaldehyde (0.2−0.85 mM); 3-hydroxybenzaldehyde (1.5−17.5 mM); 4-hydroxybenzaldehyde (2.0−21.3 mM); cyclohexanol (10.0−33.1 mM); and cyclohexanecarboxaldehyde (1.0−14.6 mM). A total of 12 concentrations covered each range. Cultures grown in the presence of maximum ethanol levels (3.8% v/v) only acted as controls. For the preparation of inocula, mold agar slopes were washed with 9 mL of maximum recovery diluent (8.5 g of NaCl, 1 g of peptone, and water to 1 L; Oxoid, Basingstoke, U.K.) containing 0.1% (v/v) Tween 80 to collect the spores. The yeast cells were grown overnight in YEPD (pH 4.0) at 25 °C. Spore/cell counts were performed by enumeration using a counting chamber. The microorganisms were then inoculated to a final concentration of 1.0×10^3 cells or spores/mL. The cultures were incubated at 25 °C without agitation for a period of 12 days. The MIC was recorded visually after days 4 and 12.

Chemical Properties. The octanol/water partition coefficient (log $P_{o/w}$) of the test compounds referred to in this paper was provided by the ChemDraw Ultra 7.0 software package (ChemOffice 2001, CambridgeSoft, Cambridge, MA).

RESULTS

The MIC data presented in **Tables 2** and **3** indicate that vanillin and all of its analogues exhibited antifungal activity, whereas results from the controls indicated that the presence of ethanol (maximum = 3.8% v/v) had no appreciable effect on the growth of any of the microorganisms investigated. The activity of all the compounds was found to vary among different genera of both molds and yeasts, whereas variation in activity was also found among different strains of the same species. Furthermore, the relative sensitivity or resistance exhibited by any mold or yeast strain against one particular compound was not always reflected against another. However, in general, using total mean MIC values after day 4, salicylaldehyde exhibited the greatest activity with an MIC of 0.32 mM. The antifungal activity order of the remaining compounds was 3-anisaldehyde (1.97 mM) > benzaldehyde (3.30 mM) > vanillin (5.71 mM) > anisole (6.59 mM) > 4-hydroxybenzaldehyde (9.09 mM) > phenol (10.59 mM) > guaiacol (11.66 mM). Although MIC

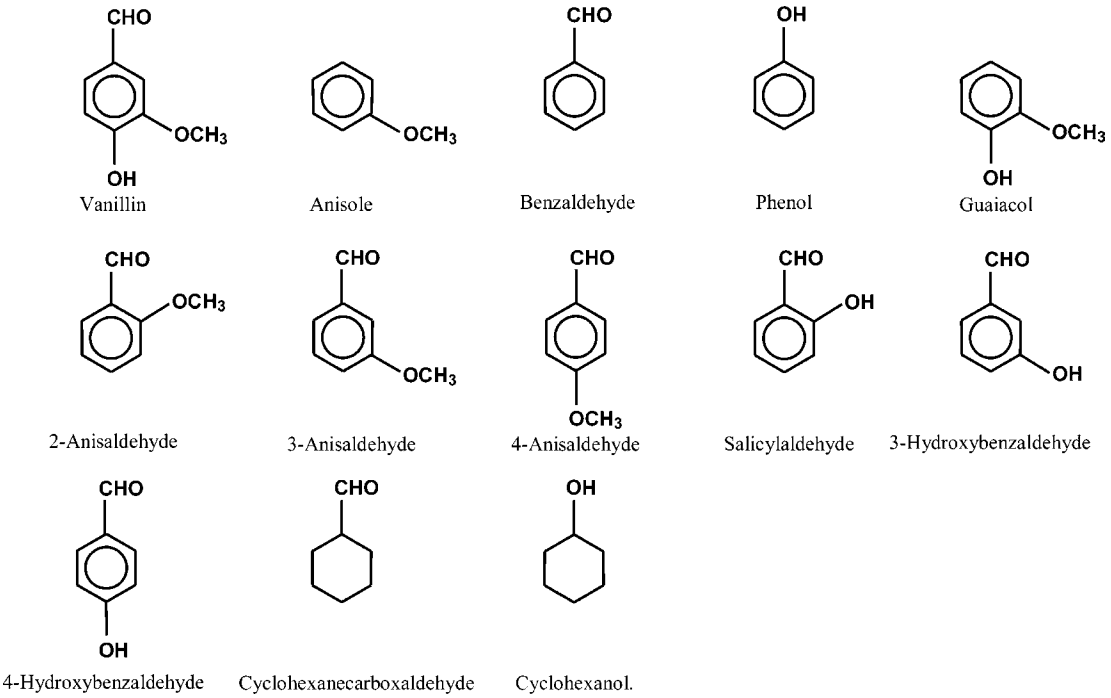

Figure 1. Chemical structures of vanillin and other compounds tested for their antifungal activities.

values varied, the average yeast/mold MIC ratios remained constant, in the range from 1.29 for 4-hydroxybenzaldehyde to 1.71 for benzaldehyde, indicating a higher sensitivity of the molds to all of the test compounds compared to the yeasts. For all of the compounds examined, the total mean MIC values increased between the day 4 and day 12 sampling points, but the antifungal order of activity of the compounds remained unchanged. Yeast/mold ratios of all the compounds also increased with the exception of guaiacol, for which a slight decrease was observed.

The relationship between log $P_{o/w}$ and the relative activity of each test compound, taken as the total mean MIC after day 12, are presented in **Figure 2**. The observed distribution of the data points and the subsequent R^2 value of the linear trend line (0.0056) indicated that there was no correlation between these parameters.

Further studies were carried out to determine whether the benzene ring structure plays a role in the antifungal activity of vanillin. Structural analogues of hydroxybenzaldehyde and anisaldehyde were also investigated to determine whether the position of side groups could influence this activity. Saturating the cyclic ring structure decreased the overall antifungal activity when a single hydroxyl group is attached. The observed total mean MIC of cyclohexanol (25.3 mM, **Table 3**) was ~2.5 times greater than the total mean MIC of phenol (10.6 mM, **Table 2**). In contrast, the replacement of a monosubstituted hydroxyl group with a single aldehyde group in cyclohexanol and phenol resulted in an increase in antifungal activity, with total mean MICs of 2.13 and 3.30 mM recorded for cyclohexanecarboxaldehyde and benzaldehyde, respectively (**Tables 2** and **3**). Using total mean MICs, the antifungal activity order of the anisaldehyde and hydroxybenzaldehyde isomers was 3- > 2- > 4- and 2- > 3- > 4-, respectively. The saturated cyclic compounds, the anisaldehyde and hydroxybenzaldehyde isomers, were again found to be more inhibitory toward the molds than toward the yeasts (yeast/mold ratios ranged between 1.35 and 1.77).

DISCUSSION

Vanillin and its direct structural analogues exhibited varying degrees of antifungal activity against the 18 different food spoilage molds and yeasts investigated. The use of the total mean MICs for the structure−function analysis gave a good indication of the overall antimicrobial effectiveness of each test compound and circumvented any potential anomalies as a result of specific sensitivity or resistance exhibited by an individual organism to certain compounds. For example, the *Rhizomucor racemosus* (It 2) strain appeared to exhibit a particular resistance to most of the compounds with an aldehyde moeity (**Tables 2** and **3**). Generally, the mold strains investigated were found to be more sensitive to all of the test compounds than the yeast strains. This may indicate that yeast physiology may be better equipped to counteract the antifungal properties of these compounds and with time can overcome them to a greater degree than the molds.

The antifungal activity order of the compounds was shown to be 3-anisaldehyde > benzaldehyde > vanillin > anisole > 4-hydroxybenzaldehyde > phenol > guaiacol. These results suggest that the aldehyde moiety plays a key role in the antifungal activity of vanillin. A total of four of the seven compounds feature an aldehyde group in their structure, and with the exception of anisole, these represented the most active antifungal compounds. Other researchers using prokaryotic bacteria reported that 4-hydroxybenzaldehyde demonstrated a greater antimicrobial activity than either vanillin (*13*) or benzaldehyde (*14*), respectively. Recently, Friedman et al. (*15*) reported that the antimicrobial activity order was benzaldehyde > 4-hydroxybenzaldehyde > vanillin > 3-anisaldehyde in bactericidal assays against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. Fundamental physiological differences, for example, in cell wall and/or cell membrane structures, between the prokaryotic and eukaryotic microorganisms could account for the observed differences.

A number of studies using both prokaryotic and eukaryotic microorganisms have suggested that the inhibitory action of phenolic compounds is due to the presence of the hydroxyl

Table 2. MIC Values of Vanillin and Its Six Direct Structural Analogues against the Selected Range of Mold and Yeast Strains Grown in YEPD (pH 4.0) at 25 °C

	compound													
	vanillin		anisole		benzaldehyde		phenol		3-anisaldehyde		guaiacol		4-hydroxybenzaldehyde	
microorganism	4 days	12 days	4 days	12 days	4 days	12 days	4 days	12 days	4 days	12 days	4 days	12 days	4 days	12 days
mold strains														
A. sojae	6.35	6.9	5.8	6.6	2.0	3.0	8.7	9.6	1.3	1.7	7.8	7.8	11.9	13.9
A. oryzae	5.8	7.5	5.4	6.6	2.0	3.0	8.4	11.1	1.5	1.7	8.85	12.6	9.0	12.6
Pn. corylophilum	4.2	5.8	8.7	8.7	2.0	2.4	2.7	12.7	1.5	1.7	14.3	20.3	12.6	17.2
Pn. expansum (Pol 4)	4.2	4.2	5.0	5.0	1.6	1.6	7.3	7.3	1.4	1.5	9.9	9.05	5.9	9.0
Pn. expansum (Pol 11)	3.0	4.2	5.0	5.0	1.3	2.4	6.8	7.3	0.85	1.7	6.2	7.8	2.5	5.0
Pn. funiculosum	3.0	4.2	5.0	6.6	1.7	2.0	6.3	7.3	0.85	1.5	6.2	9.9	5.9	5.9
Pn. spinulosum	3.25	4.0	5.4	5.0	2.2	2.4	5.5	5.9	1.3	1.5	9.9	9.9	7.3	7.7
Penicillium sp.	5.45	5.2	5.0	5.0	1.6	1.6	5.5	5.5	1.15	1.3	7.8	9.05	7.3	6.6
Rz. racemosus	5.8	5.35	5.8	6.6	7.5	7.5	9.8	11.9	4.35	4.9	7.8	8.0	9.0	9.0
mean mold MIC	4.56	5.26	5.68	6.12	2.43	2.88	7.89	8.73	1.58	1.94	8.75	10.5	7.93	9.66
yeast strains														
C. lipolytica	3.85	8.45	6.6	6.6	2.0	2.0	9.6	11.1	1.3	1.7	9.9	9.9	9.0	11.2
C. rugosa	3.0	3.0	5.0	5.0	1.0	1.0	7.3	7.3	0.6	0.6	6.2	7.0	5.0	5.3
C. sake	7.5	10.0	8.7	8.7	4.8	4.8	16.8	18.0	1.7	2.9	16.0	18.0	11.2	13.9
K. apiculata	7.5	8.85	8.7	8.7	3.8	3.8	14.6	16.8	1.7	2.2	20.3	18.0	11.2	23.0
P. pastoris	6.9	8.85	6.6	8.7	4.8	4.8	11.1	11.6	2.2	2.9	11.3	16.0	7.3	9.0
S. cerevisiae (NCYC 956)	7.5	10.0	8.7	11.5	6.75	9.9	16.8	22.3	4.35	6.4	20.3	25.8	6.25	11.2
S. cerevisiae (176)	6.9	11.3	10.1	11.5	7.5	9.3	18.1	20.0	4.9	5.65	20.3	22.7	11.2	13.9
Z. bailii (NCYC 1427)	8.9	11.3	6.6	7.65	3.8	6.0	12.7	13.2	2.39	3.8	14.3	16.0	13.9	17.2
Z. bailii (80)	9.6	12.3	6.6	8.7	3.0	6.0	12.7	14.6	2.2	3.35	12.6	17.0	17.2	21.3
mean yeast MIC	6.85	9.34	7.51	8.56	4.16	5.29	13.3	15.0	2.37	3.28	14.6	16.7	10.3	14.0
total mean MIC	5.71 ± 2.09	7.30 ± 2.94	6.59 ± 1.66	7.34 ± 2.09	3.30 ± 2.14	4.08 ± 2.68	10.6 ± 4.05	11.9 ± 4.93	1.97 ± 1.27	2.61 ± 1.62	11.7 ± 4.90	13.6 ± 5.76	9.09 ± 3.60	11.8 ± 5.31
yeast/mold ratio	1.50	1.78	1.32	1.40	1.71	1.84	1.69	1.72	1.50	1.69	1.67	1.59	1.29	1.45

Table 3. MIC Values of Selected Isomers Anisaldehyde and Hydroxybenzaldehyde, and of Two Cyclohexane Derivatives against a Range of Mold and Yeast Strains Grown in YEPD (pH 4.0) at 25 °C

	compound											
	2-anisaldehyde		4-anisaldehyde		salicylaldehyde		3-hydroxy-benzaldehyde		cyclohexane-carboxaldehyde		cyclohexanol	
microorganism	4 days	12 days	4 days	12 days	4 days	12 days	4 days	12 days	4 days	12 days	4 days	12 days
mold strains												
A. sojae	2.1	3.0	3.8	4.8	0.23	0.34	8.1	11.2	1.7	2.4	19.2	21.4
A. oryzae	2.1	2.1	3.8	4.8	0.23	0.39	7.2	11.2	1.7	3.0	21.4	28.1
Pn. coryophilum	1.3	2.1	3.0	4.3	0.24	0.34	7.2	7.2	1.6	2.0	29.7	33.1
Pn. expansum (Pol 4)	1.5	1.5	2.4	3.0	0.31	0.37	3.7	4.25	1.6	2.0	20.3	21.4
Pn. expansum (Pol 11)	1.3	2.1	2.0	3.0	0.2	0.23	2.3	3.7	1.0	1.7	15.5	15.5
Pn. funiculosum	1.3	1.5	3.0	4.05	0.2	0.2	1.7	7.6	1.0	2.0	17.2	18.2
Pn. spinulosum	1.5	1.5	3.0	4.05	0.22	0.26	5.7	5.7	1.15	2.0	20.3	21.4
Penicillium sp.	1.1	1.1	2.7	2.7	0.28	0.39	2.9	5.15	1.15	1.8	16.4	18.2
Rz. racemosus	3.0	4.1	7.5	9.3	0.58	0.74	4.6	5.7	5.4	6.75	21.4	22.7
mean mold MIC	1.69	2.11	3.47	4.44	0.28	0.36	4.82	6.86	1.81	2.63	20.2	22.2
yeast strains												
C. lipolytica	1.5	2.1	3.8	4.05	0.23	0.26	6.1	6.45	3.0	4.8	22.7	23.9
C. rugosa	1.35	1.5	1.8	2.0	0.2	0.23	1.5	1.5	1.3	1.6	15.5	15.5
C. sake	3.0	3.0	6.0	6.0	0.2	0.24	7.2	8.9	2.0	3.0	35.0	35.0
K. apiculata	2.1	2.55	3.0	5.4	0.54	0.57	4.6	5.7	2.2	2.7	35.0	35.0
P. pastoris	2.55	3.0	4.8	6.0	0.28	0.39	7.2	8.9	1.6	2.0	26.6	29.7
S. cerevisiae (NCYC 956)	4.1	5.8	7.5	8.4	0.52	0.67	7.2	11.2	3.0	3.8	19.8	35.0
S. cerevisiae (176)	4.1	5.8	7.5	9.3	0.46	0.67	10.1	11.2	2.2	3.0	40.0	37.0
Z. bailii (NCYC 1427)	4.1	5.8	6.0	7.5	0.52	0.67	8.9	11.2	3.8	3.8	40.0	37.0
Z. bailii (80)	4.1	4.1	6.0	7.5	0.41	0.65	8.9	11.2	3.0	3.2	40.0	37.0
mean yeast MIC	2.99	3.74	5.16	6.24	0.37	0.48	6.85	8.47	2.46	3.1	30.5	31.7
total mean MIC	2.34 ± 1.12	2.93 ± 1.57	4.31 ± 1.95	5.34 ± 2.26	0.32 ± 0.14	0.42 ± 0.19	5.84 ± 2.61	7.66 ± 3.10	2.13 ± 1.14	2.86 ± 1.30	25.3 ± 8.91	27.0 ± 7.97
yeast/mold ratio	1.77	1.77	1.49	1.40	1.35	1.34	1.42	1.24	1.36	1.18	1.51	1.43

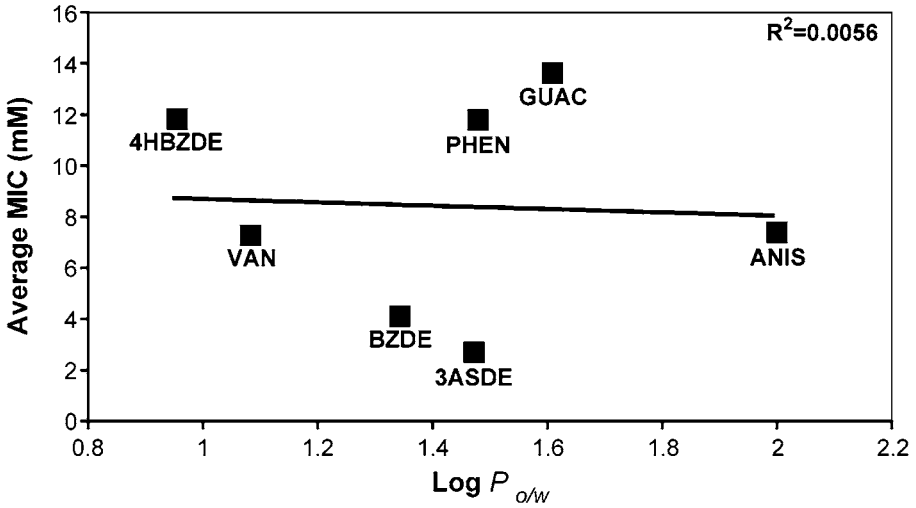

Figure 2. Analysis of the correlation between octanol/water partition coefficient (log $P_{o/w}$) and total mean MICs of vanillin (VAN), anisole (ANIS), benzaldehyde (BZDE), phenol (PHEN), guaiacol (GUAC), 4-hydroxybenzaldehyde (4HBDE), and 3-anisaldehyde (3ASDE) established after 12 days of incubation at 25 °C. The linear trend line and R^2 value are indicated.

group (*16*−*19*). The authors reasoned that the hydroxyl group either reacts with enzyme active sites through the formation of hydrogen bonds (*16*, *17*) or acts as a transmembrane carrier for monovalent cations (*18*). However, in this study, the two least effective compounds were shown to be phenol and guaiacol, both of which possess a hydroxyl moiety within their structures. Removal of the hydroxyl group from vanillin, 4-hydroxybenzaldehyde, or guaiacol resulted in a slight improvement in activity. However, its presence in salicylaldehyde as an ortho substitution resulted in a >10-fold higher activity than the meta- or para-substituted analogues 3- and 4-hydroxybenzaldehydes. This result is consistent with earlier observations (*15*, *20*) that ortho substitution in benzaldehydes increased their antimicrobial action against *Listeria monocytogenes* and other food-poisoning bacteria. Structural comparison also revealed that, in general, addition of an aldehyde group to a compound (e.g., anisole to 3-anisaldehyde or guaiacol to vanillin in **Table 1**) resulted in a significant improvement in activity. In contrast, replacement of an aldehyde group in cyclohexanecarboxaldehyde with a hydroxyl group in cyclohexanol resulted in decreased activity.

The importance of the aldehyde group in cinnamaldehyde, citral, citronellal, perillaldehyde, and salicylaldehyde was also observed (*21*) in antimicrobial assays against numerous bacterial strains. Similarly, Chang et al. (*14*) reported that cinnamaldehyde demonstrated greater antimicrobial activity against a range of bacterial strains than cinnamic acid, cinnamyl alcohol, or cinnamyl acetate. The presence of the polarized carbon−oxygen double bond of aldehyde groups probably accounts for their

greater antimicrobial activity. It is known that aldehyde groups are very reactive and can form covalent bonds with DNA and proteins, thereby potentially interfering with their normal functions (*22*).

The presence of the benzene ring appears to be important when a single hydroxyl group is attached. Phenol exhibited greater antifungal activity than cyclohexanol (**Tables 2** and **3**). This could be attributed to the delocalized electron cloud of the unsaturated benzene ring allowing the hydrogen of the hydroxyl group to act in an acidic manner. However, total mean MICs of 2.13 and 3.30 mM were established for cyclohexanecarboxaldehyde and benzaldehyde, respectively. This would indicate that the importance of the benzene ring was less significant when an aldehyde group was present in the structure. These results again appear to correlate the presence of the aldehyde moeity with increased antifungal activity.

Analysis of isomers of anisaldehyde and hydroxybenzaldehyde also suggested that side-group position can influence antifungal activity. Generally, the closer the side groups were to the aldehyde moiety, the greater was the antifungal activity exhibited, and this held particularly true for the hydroxybenzaldehyde isomers.

No correlation was observed between the relative antifungal activity of vanillin, its six direct structural analogues, and log $P_{o/w}$. The antimicrobial activity of phenolic compounds usually correlates well with their intrinsic hydrophobicity and, therefore, their respective log $P_{o/w}$ values (*13*, *19*, *23*, *24*). However, there was no evidence to support this conclusion using vanillin and its structural analogues. Investigations using prokaryotic bacteria have demonstrated that eugenol exhibits a greater antimicrobial activity compared to vanillin (*25*). However, they are structurally similar and differ only in the functional group at the C-1 position, aldehyde (CHO) in vanillin or allyl ($CH_2{=}CHCH_2$) in eugenol. The authors attributed the increased activity of eugenol over vanillin to the greater hydrophobicity of the former, whereas the more potent phenolics such as carvacrol and thymol have even higher log $P_{o/w}$ values. These data suggest that log $P_{o/w}$ is a good indicator of antifungal activity between fairly distinct compounds, but not for compounds with identical structural components, for which activity appears to correlate better with the type and location of the various side groups.

In conclusion, this study was designed to provide a better understanding of the structure−function relationships of the different substituent groups within the vanillin molecule. Clearly the presence of an aldehyde group has the greatest influence on its antifungal property. This observation is interesting because this aldehyde group is also a key component in determining its function as a flavor compound. The absence of this structural moiety in guaiacol resulted in a reduction in antifungal activity. In our previous study (*26*), oxidation of vanillin to vanillic acid or its reduction to vanillyl alcohol resulted in a dramatic decrease in its antifungal potency. The flavor intensity of the three compounds, guaiacol, vanillic acid, and vanillyl alcohol, is several orders of magnitude less than that of vanillin. The effect of the hydroxyl group was variable depending on its position within the benzene ring, but the 2-OH position was beneficial to antifungal activity. Understanding the influence of the phenolic structures is important if we are to design and develop more effective natural preservative processes.

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