JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Modes of Antifungal Action of (2*E*)-Alkenals against *Saccharomyces cerevisiae*

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A series of aliphatic (2*E*)-alkenals from C_5 to C_{14} were tested for their antifungal activity against *Saccharomyces cerevisiae* ATCC 7754. (2*E*)-Undecenal (C_{11}) was found to be the most effective with the minimum fungicidal concentration (MFC) of 6.25 μg/mL, followed by (2*E*)-decenal (C_{10}) with an MFC of 12.5 μg/mL. The time−kill curve study showed that (2*E*)-undecenal was fungicidal against *S. cerevisiae* at any growth stage, and this activity was not influenced by pH values. The (2*E*)-alkenals inhibited glucose-induced acidification by inhibiting the plasma membrane H^+-ATPase. The primary antifungal action of medium-chain (C_9−C_{12}) (2*E*)-alkenals against *S. cerevisiae* comes from their ability to function as nonionic surface-active agents (surfactants), disrupting the native membrane-associated function nonspecifically. Hence, the antifungal activity of (2*E*)-alkenals is mediated by biophysical processes, and the maximum activity can be obtained when the balance between the hydrophilic and hydrophobic portions becomes the most appropriate.

KEYWORDS: Antifungal activity; *Saccharomyces cerevisiae*; (*2E*)-alkenals; surfactant activity; H^+-ATPase; multifunction

INTRODUCTION

Recently a homologous series of acyclic α,β-unsaturated aldehydes [hereafter referred to as (2*E*)-alkenals for simplicity] was characterized as antimicrobial agents from two plants (*1*−*3*). In our previous reports on structure−antimicrobial activity relationship (SAR) studies with the same series of acyclic alcohols (alkanols), we have reported that their maximum antimicrobial activity depends on the hydrophobic alkyl (tail) chain length from the hydrophilic hydroxyl group (head). It was proposed that the hydrophilic head part binds with an intermolecular hydrogen bond like a "hook" attaching itself to the hydrophilic portion of the membrane, and then the hydrophobic tail portion of the molecule is able to enter into the membrane lipid bilayers (*4*). The result likely creates disorder in the fluid bilayer of the membrane. This concept can also be further extended to acyclic (2*E*)-alkenals. With the increase in drug resistance and prevalence of opportunistic infections, there is a great need for effective antifungal agents with new modes of action (*5*). The current study was focused on modes of the antifungal activity of (2*E*)-alkenals against *Saccharomyces cerevisiae* as a model. Accumulation of this knowledge may provide a more rational and scientific approach to the design of safe and effective antifungal agents.

We have previously reported characterization of the antifungal bicyclic sesquiterpene dialdehyde, polygodial (**1**), from various plants (*6*). This sesquiterpene dialdehyde exhibited potent antifungal activity, particularly against yeasts such as *Candida albicans* and *S. cerevisiae*, although it possessed little or no activity against bacteria (*7*, *8*). The primary discussion in this paper is centered against *S. cerevisiae*, but the same surfactant concept can be applicable to the design of antifungal agents against *C. albicans*. In fact, polygodial is now available as a commercial anti-*Candida* agent. On the basis of the potent antifungal activity, polygodial may be used as a leading compound for new antifungal drugs. This involves the study of their SAR. However, the study of SAR required the synthesis of a series of analogues differing in the hydrophobic bicyclic portion, but this may not be practical. In contrast, a series of aliphatic (2*E*)-alkenals with different chain lengths is readily available and, hence, a superior model for this study, although the antifungal action of polygodial may differ from those of the aliphatic aldehydes to some extent.

MATERIALS AND METHODS

Chemicals. A series of (2*E*)-alkenals and alkanals (*1*−*3*) and polygodial, warburganal, mukaadial, and aframodial (*9*) were available from our previous work. Cycloheximide, sorbic acid, and lactic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Anethole was obtained from Aldrich Chemical Co. (Milwaukee, WI). *N*,*N*-Dimethylformamide (DMF) was purchased from EM Science (Gibbstown, NJ).

Test Strain. The test strain *S. cerevisiae* ATCC 7754 used for this study was purchased from the American Type Culture Collection (Manassas, VA).

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10.1021/jf0211884 CCC: $25.00
Published on Web 06/07/2003

Medium. *S. cerevisiae* was maintained at −80 °C in yeast nitrogen broth (YNB; Difco Laboratories, Detroit, MI) containing 25% glycerol and subcultured at 30 °C in Sabouraud's dextrose agar (SDA) medium (Bactopeptone 1%, dextrose 4%, Bacto-agar 1.8%). A fresh culture was preincubated with shaking for 16 h at 30 °C in 2.5% malt extract (ME) medium (BBL).

Acidification Measurement. The glucose-induced medium acidification of *S. cerevisiae* was measured with a modified procedure (*10*). The test strain was cultured with shaking in YPD (glucose 2%, Bactopeptone 2%, yeast extract 1%) broth overnight at 30 °C and washed twice with cold distilled water. The cells were diluted to 5×10^7 colony forming units (CFU)/mL with cold distilled water and kept on ice. The reaction mixture contained 2.7 mL of cells and 30 μL of the inhibitor in DMF and was preincubated at 30 °C for 5 min. A 20% glucose solution of 0.3 mL was added (final = 2%) to induce acidification. After 10 min of incubation, the pH of external medium was checked (Orion 8175 Ross semimicro electrode).

Antifungal Assay. The test compounds were first dissolved in DMF, and the concentration of DMF in each medium was always 1%. The highest concentration tested was 1600 μg/mL, unless otherwise specified. The maximum extent and rate of activity are known to vary with the seed culture mediums, the physiological age of the culture, and the type of culture medium. For example, the minimum inhibitory concentration (MIC) of anethole significantly varied with the inoculum size. All antifungal susceptibility tests in this study were performed under a standard condition using fresh inoculum from a 16 h shaking culture in ME medium, final inoculum size of 10^5 CFU/mL, and 48 h stationary incubation in ME medium, unless otherwise specified. In the preculture the log-phase inoculum was used.

Broth macrodilution MICs were determined as previously described (*9*). Briefly, serial 2-fold dilutions of the test compounds were made in DMF, and 30 μL of the sample solution was added to 3 mL of ME medium. These were inoculated with 30 μL of seed culture to give the final inoculum of 10^5 CFU/mL. The assay tubes were incubated without shaking at 30 °C for 48 h. The MIC is the lowest concentration of test compound that demonstrated no visible growth. The minimum fungicidal concentrations (MFCs) were examined as follows. After the MIC had been determined, a 30 μL aliquot was taken from each clear tube and added into 3 mL of drug-free fresh medium. After 48 h of incubation, the MFC was determined as the lowest concentration of the test compounds in which no recovery of microorganism was observed. The assays were performed in triplicate on separate occasions.

Time−kill studies were performed to examine the effects of combinations of compounds in more detail. The culture tubes were prepared as described above and incubated at 30 °C for 16 h. A 30 μL aliquot of the culture was inoculated into 3 mL of ME broth containing appropriate concentrations of the test compounds. The initial population size for *S. cerevisiae* was 5.8×10^5 CFU/mL. Samples were taken at selected times during 48 h of exposure, and serial dilutions were made in sterile saline before the samples were plated onto YPD agar plates. The plates were incubated at 30 °C for 48 h before the number of CFU was determined.

Adsorption Test. The test strain was cultured with shaking in YPD broth overnight at 30 °C and washed twice with 50 mM MOPS buffer (pH 6.0). After each (2*E*)-alkenal (50, 25, 12.5, 6.3, or 3.1 μg/mL, respectively) was mixed with or without *S. cerevisiae* cells (10^8 cells/mL) in the above buffer at 30 °C, the suspension was vortexed for 2 s. Absorbance of the supernatants obtained by centrifugation at 4000*g* for 5 min was measured at 255 nm.

RESULTS

The antimicrobial activity of a homologous series of (2*E*)-alkenals (**Figure 1**) characterized from two plants has previously been reported (*1*−*3*) and is generally similar to that described for the corresponding alkanols (*4*). Their MIC and MFC values against *S. cerevisiae* are listed in **Table 1**. In general, the differences between the MIC and MFC values are not more than 2-fold, suggesting no residual fungistatic activity. As the carbon chain length increases, the activity is increased, and the activity disappears after the chain length reaches that having the maximum activity. This so-called "cutoff" is a known phenomenon. For example, (2*E*)-dodecenal (C_{12}) was found to be very effective against *S. cerevisiae* with an MIC of 12.5 μg/mL, whereas (2*E*)-tridecenal (C_{13}) no longer showed any activity up to 800 μg/mL. Interestingly, (2*E*)-dodecenal still exhibited the activity with an MIC of 12.5 μg/mL but the MFC was 100 μg/mL, indicating that residual fungistatic activity is involved. The most potent fungicide in the (2*E*)-alkenal series was found to be (2*E*)-undecenal (C_{11}) with an MFC of 6.25 μg/mL, followed by (2*E*)-decenal (C_{10}) with an MFC of 12.5 μg/mL.

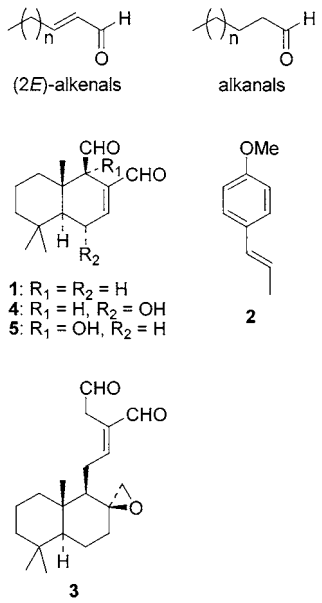

Figure 1. α,β-Unsaturated aldehydes and related compounds.

Table 1. Antifungal Activity (Micrograms per Milliliter) of Aldehydes against *S. cerevisiae*[a]

	(2*E*)-alkenal		alkanal	
aldehyde tested	MIC	MFC	MIC	MFC
C5	100	200	–	–
C6	100	200	1600	1600
C7	100	200	400	400
C8	100	100	200	200
C9	25	25	100	100
C10	12.5	12.5	25	50
C11	6.25	6.25	25	50
C12	12.5*	100	200	>800
C13	>800	>800	>800	>800
C14	>400	–	–	–

[a] The cells of *S. cerevisiae* were grown in ME broth at 30 °C without shaking. *, values are variable. –, not tested.

The fungicidal activity of (2*E*)-undecenal against *S. cerevisiae* was confirmed by the time−kill curve experiment (**Figure 2**). Cultures of (2*E*)-undecenal, with a cell density of 5.8×10^5 CFU/mL, were exposed to two different concentrations of (2*E*)-undecenal. The number of viable cells was determined following different periods of incubation with (2*E*)-undecenal. The result verifies that the MIC and 2 × MFC of (2*E*)-undecenal showed the same fungicidal effect. Notably, lethality occurred remarkably quickly, within the first 1 h after the addition of (2*E*)-undecenal. This rapid lethality very likely indicates that the antifungal activity of (2*E*)-undecenal against *S. cerevisiae* is associated in part with the disruption of the membrane (*11*).

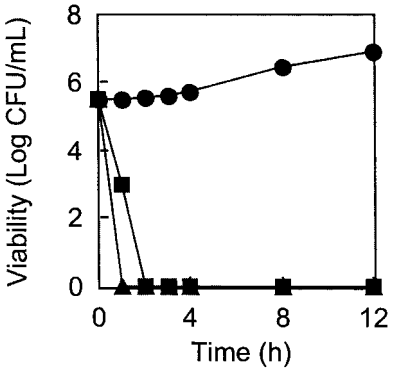

Figure 2. Time–kill curve of (2*E*)-undecenal against *S. cerevisiae*. A 16 h culture was inoculated into ME broth containing 0 μg/mL (●), 6.25 μg/mL (■), and 12.5 μg/mL (▲) of (2*E*)-undecenal.

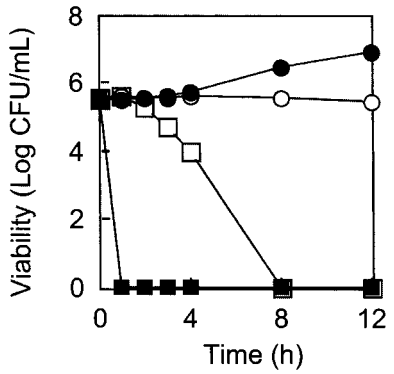

Figure 3. Fungicidal effect of (2*E*)-undecenal in cycloheximide-treated cells. After 5.8×10^5 cells had been inoculated in ME broth, compounds were added as follows: 50 μg/mL cycloheximide (○, □), 12.5 μg/mL (2*E*)-undecenal (■), no compound (●). After a further 2 h of incubation, 12.5 μg/mL (2*E*)-undecenal was added in cycloheximide-treated cells (□). Viability was estimated by the number of colonies formed on a YPD plate after incubation at 30 °C for 48 h.

Further support for the membrane action was also obtained in experiments that showed the rapid decline in the number of viable cells after the addition of (2*E*)-undecenal both at the stationary growth phase and in the presence of cell growth inhibitors, as shown in **Figure 3**. That is, (2*E*)-undecenal rapidly killed *S. cerevisiae* cells in which cell division was inhibited by cycloheximide. This antibiotic is known to inhibit protein synthesis in eukaryotes, thereby restricting cell division. The fungicidal effect of (2*E*)-undecenal appears to be independent of the necessary functions accompanying the reproduction of yeast cells, which are macromolecule biosyntheses of DNA, RNA, protein, and cell wall components. Hence, the antifungal mechanism of (2*E*)-undecenal is associated in part with membrane functions or derangement of the membrane.

In our preliminary test, octanal also showed a similar antifungal activity against *S. cerevisiae*, so that the above-mentioned antifungal activity should not be specific to (2*E*)-alkenals because the conjugated double bond is unlikely essential to elicit the activity. This prompted us to test the antifungal activity of the same series of alkanals against *S. cerevisiae* for comparison. The results are listed in **Table 1**. The activities of alkanals are slightly less than those of the corresponding (2*E*)-alkenals. Similar to (2*E*)-alkenal series, dodecanal (C_{12}) was effective with an MIC of 200 μg/mL but did not exhibit any fungicidal activity up to 800 μg/mL. Thus, *S. cerevisiae* cells appeared to adapt to dodecanal stress, eventually recovering and growing normally. In connection with this, undecanal (C_{11}) and

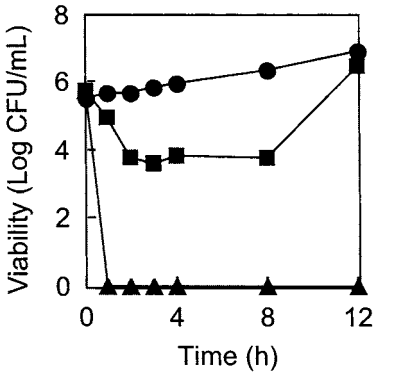

Figure 4. Time–kill curve of undecanal against *S. cerevisiae*. A 16 h culture was inoculated into ME broth containing 0 μg/mL (●), 25 μg/mL (■), and 50 μg/mL (▲) of undecanal. Viability was estimated by the number of colonies formed on a YPD plate after incubation at 30 °C for 48 h.

decanal (C_{10}) were found to be the most potent, with MFCs of 50 μg/mL. Although the current study emphasized (2*E*)-alkenals because of their greater structural similarity to polygodial, the data obtained with alkanals are basically the same as those obtained with (2*E*)-alkenals. In the case of short-chain ($<C_9$) (2*E*)-alkenals, the activity did not increase with each additional CH_2 group in the alkyl chain, indicating their mode of antifungal action may somewhat differ from that of alkanals.

The fungicidal activity of undecanal against *S. cerevisiae* was confirmed by the time−kill curve experiment as shown in **Figure 4**. Cultures of *S. cerevisiae*, with a cell density of 5.8×10^5 CFU/mL, were exposed to two different concentrations of undecanal. The number of viable cells was determined following different periods of incubation with undecanal. **Figure 4** verifies that MIC slowed growth but that the final cell count was not significantly different from the control. Notably, lethality at the MFC occurred remarkably quickly, within the first 1 h after the addition of undecanal, indicating that undecanal possesses a membrane disruptive effect, in a manner similar to that described for (2*E*)-undecenal.

It is known that *S. cerevisiae* produces the acidification of the external medium during growth on glucose. This external acidification is closely associated with the metabolism of the sugar, and its magnitude depends on the buffering capacity of the growth medium (*12*). The H^+-ATPase (P-type) is important not only in the regulation of internal pH but also in the energy-dependent uptake of various metabolites (*13*). (2*E*)-Alkenals inhibit the external acidification by inhibiting the H^+-ATPase as shown in **Figure 5**. Their antifungal activity is also partly due to the inhibition of this H^+-ATPase. Interestingly, the potency of H^+-ATPase inhibition in each (2*E*)-alkenal differs, and the cutoff phenomenon does not occur. It is an interesting question how these (2*E*)-alkenals inhibit H^+-ATPase. (2*E*)-Alkenals with chain lengths of less than C_8 and longer than C_{12} exhibited weaker inhibition activity. This inhibition pattern is not specific not only to (2*E*)-alkenals but also to alkanals. It seems that medium-chain (C_9−C_{11}) (2*E*)-alkenals have a better balance between the hydrophilic and hydrophobic portions of the molecules to act as surfactants. It should be remembered here that (2*E*)-dodecenal exhibited fungistatic activity with an MIC of 12.5 μg/mL against *S. cerevisiae* but did not show any fungicidal activity up to 100 μg/mL.

In the aforementioned acidification inhibitory activity, the effect of the fungicidal (2*E*)-undecenal was gradually enhanced, whereas cells treated with fungistatic (2*E*)-dodecenal gradually recovered with time, as shown in **Figure 6**. Yeast cells appeared

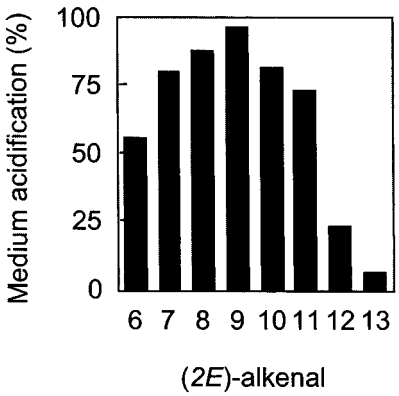

Figure 5. Inhibition of medium acidification by (2*E*)-alkenals (400 μg/mL) for short-time incubation. The acidification was assayed for 10 min. The inhibition ratio (percent) was calculated as follows: $(1 - [H^+]_{inhibitor}/[H^+]_{inhibitor\ free}) \times 100$.

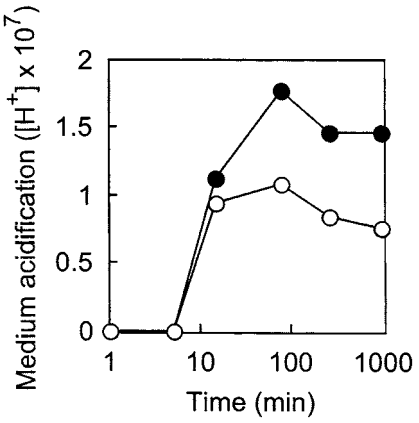

Figure 6. Effects of incubation time on the inhibition of (2*E*)-undecenal (○) and (2*E*)-dodecenal (●) to the medium acidification by the plasma membrane H^+-ATPase of *S. cerevisiae*. Alkenals were tested at the concentration of 5 mM.

to adapt to (2*E*)-dodecenal stress, eventually recovering and growing normally, similar to the behavior under weak-acid stress (*14*). Among the alkanals tested, dodecanal was still effective against *S. cerevisiae* with an MIC of 200 μg/mL, but it was not fungicidal. This can be explained in the same manner described for (2*E*)-dodecenal.

The data obtained so far indicate that the medium-chain (2*E*)-alkenals act as nonionic surfactants at the lipid−protein interface, in a manner similar to that reported for alkanols (*4*). For example, the absence of a functioning state of the H^+-ATPase could be due to its relative sensitivity to functional disruption by (2*E*)-alkenals. It is suggested that the intrinsic proteins of the membranes are held in position by hydrogen bonding, as well as by hydrophobic and electrostatic forces, and that hydrogen bonding also mediates the penetration of membranes by proteins. The binding of nonionic surfactants such as the aliphatic aldehydes can involve only relatively weak headgroup interactions, such as hydrogen bonding, so that the predominant interactions will be hydrophobic, involving the alkyl tails. As proposed above, hydrogen bonds are formed or broken by the aliphatic aldehydes and redirected. Thereby the conformation of membrane protein may be changed. For example, the H^+-ATPase in particular could lose its proper conformation, which would lead to cell death. The H^+-ATPase is the most abundant plasma membrane protein, constituting >20% of the total membrane protein in *S. cerevisiae*, but the above-mentioned fungicidal mechanism of the aliphatic aldehydes seems to be nonspecific. This can be explained as the amphipathic medium-chain aldehydes are nonionic surfactants and disrupt the hydrogen bonding in the lipid−protein interface in *S. cerevisiae*. As surfactants, the binding site of the aliphatic aldehydes should not be specific and their broad antimicrobial spectrum supports this postulate.

Further supporting generalized surfactant action at the plasma membrane is the observation that the (2*E*)-alkenals do not appear to inhibit the major energy production pathway. *S. cerevisiae* is a facultative anaerobic organism that is able to survive without a functional respiratory chain by relying on the fermentation of sugars to supply its energy demand, which is the state yeast prefer when sugars are present in significant amounts. (2*E*)-Alkenals are inhibitory to the yeast while in this fermentative state. (2*E*)-Alkenals also inhibit the growth of *S. cerevisiae* growing on nonfermentable carbon sources such as ethanol-, lactate-, acetate-, and glycerol-containing media. Because no suppression of fungicidal activity was seen as would be expected by removal of the potential target, it is unlikely that (2*E*)-alkenals' lethal action in yeast is caused by inhibiting components of the respiration or fermentation pathway.

In addition, further support for the surfactant concept was obtained in an additional experiment that indicates antifungal (2*E*)-undecenal rapidly adsorbed onto the surface of *S. cerevisiae* cells but (2*E*)-hexenal did so only slightly, as shown in **Figure 7**. It appears that *S. cerevisiae* showed different affinities to (2*E*)-alkenal having different alkyl chain lengths. The hydrophilic enal moiety was adsorbed by an intermolecular hydrogen bond by attaching itself to the hydrophilic portion of the membrane surface. The adsorbing sites may not be specific but need to be clarified.

The current SAR study of (2*E*)-alkenals was initiated largely as a model to understand the modes of the potent antifungal action of polygodial. The data described so far demonstrate the similarity between polygodial and (2*E*)-alkenals in many aspects, but there are also significant differences. For example, polygodial loses its potent antifungal activity in YPD medium but aliphatic aldehydes do not, as shown in **Table 2**. This is consistent with the previous report that a primary amino group reacts with the dialdehyde moiety of polygodial and inactivates it (*15*), but not (2*E*)-alkenals. YPD contains very high levels of components with amine groups. The result indicates that polygodial forms a pyrrole derivative with the compounds possessing a primary amino group. Therefore, the binding site of polygodial may be, at least in part, a primary amino group in living systems. On the other hand, neither (2*E*)-alkenals nor alkanals can form a pyrrole derivative. Notably, isolated mitochondrial ATPase (F-type) is strongly inhibited by polygodial (*16*), whereas it is only weakly inhibited by the (2*E*)-alkenals. In connection with this, (2*E*)-alkenals and alkanals were found to inhibit the succinate-supported respiration of intact mitochondria isolated from rat liver, similar to those found for alkanols (*17*). However, results already discussed above show that these slight mitochondrial inhibitory activities are not primarily responsible for cellular inhibition. The antifungal mechanism of polygodial seems to be associated in part with its specific dialdehyde structural features and differs from that of aliphatic (2*E*)-alkenals.

The volume of the hydrophobic portions also seems to be related to the activity because the antifungal activity of aliphatic α,β-unsaturated aldehydes is weaker than that of the bicyclic sesquiterpene, polygodial. For example, the best fungicidal activity of the (2*E*)-alkenal series against *S. cerevisiae* is that of (2*E*)-undecenal with an MFC of 6.25 μg/mL, which is 2-fold

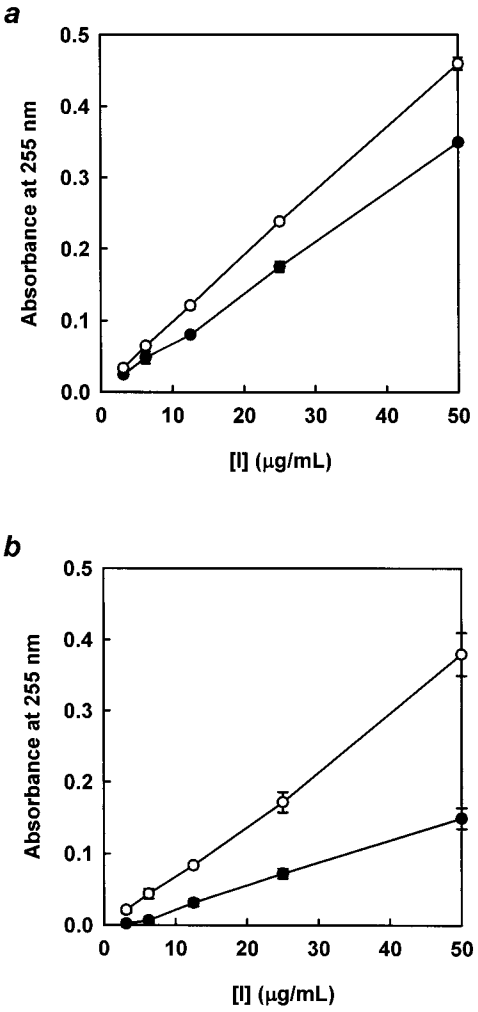

Figure 7. Binding of (a) (2*E*)-hexenal and (b) (2*E*)-undecenal to *S. cerevisiae* cells. After each (2*E*)-alkenal was mixed with (●) or without (○) yeast cells (10^8 cells/mL), the suspension was vortexed for 2 s. Absorbance of the supernatant obtained by centrifugation at 4000*g* for 5 min was measured. Each plot is the mean of triplicate determinations.

Table 2. Antifungal (MIC) Activity (Micrograms per Millilter) of Aldehydes against *S. cerevisiae* in Different Media

aldehyde tested	ME	RPMI1640	YPD
polygodial	1.56	1.56	>100
(2*E*)-undecenal	6.25	6.25	12.5
undecanal	25	25	50

less potent than that of polygodial. In the case of alkanals, the most potent undecanal is 16-fold less effective. There are two ways to increase the activity. First, the activity can be enhanced by combining with synergists. For example, the MFC of (2*E*)-undecenal against *S. cerevisiae* was enhanced 16-fold when it was combined with $^1/_2$MFC of anethole (**2**) (*1*). This combination strategy may be superior to enhance and broaden the total biological activity and, more importantly, it may hinder the development of resistant mechanisms in microorganisms. It should be noted that fungistatic compounds did not provide the stable enhancing activity in combination with other antifungal compounds. In fact, the combination data of the above-mentioned fungistatic (2*E*)-dodecenal varied. Second, the activity may be enhanced by increasing the volume of the hydrophobic portion through synthetic modification. For example, the volume of polygodial is unlikely the maximum because a bulkier

Table 3. Antifungal Activity (Micrograms per Milliliter) of Polygodial and Its Related Compounds against *S. cerevisiae*[a]

aldehyde tested	MIC	MFC
polygodial	1.56	3.13
mukaadial	>200	–
warburganal	3.13	6.25
aframodial	0.78	1.56

[a] The cells of *S. cerevisiae* were grown in ME broth at 30 °C without shaking. –, not tested.

Table 4. pH Effect of Fungicidal (MFC) Activity (Micrograms per Milliliter) of (2*E*)-Undecenal, Sorbic Acid, and Undecanal against *S. cerevisiae*

pH	(2*E*)-undecenal	sorbic acid	undecanal
3	3.13	400	25
5	6.25	1600	50
7	6.25	>1600	50
9	6.25	>1600	50

labdane diterpene dialdehyde, aframodial (**3**), exhibited even more potent activity as listed in **Table 3**. It seems that the activity increased with increasing volume of the hydrophobic portions. On the other hand, mukaadial (**4**) did not exhibit any activity up to 200 μg/mL, but warburganal (**5**) still exhibited the activity but to a lesser extent than polygodial (*9*). It is therefore apparent that the activity decreased for each additional hydroxyl group beyond polygodial. However, the rationale for these remains unclear.

Safety is a primary consideration for antifungal agents, especially concerning their use in food products. The aldehydes characterized as antifungal agents from edible plants should be superior compared to non-natural antifungal agents. In addition, aldehydes have another superior property as antifungal agents compared to sorbic acid, a common commercial antifungal agent. As a weak acid antifungal agent, the activity of sorbic acid is pH dependent and increases as the pH of the substrate decreases (*18*), as shown in **Table 4**. At higher pH values (>5), sorbic acid did not show any antifungal activity up to 1600 μg/mL due to a higher degree of dissociated molecules. In contrast, the aldehydes are not affected by pH. This would appear to be of greater overall value than other pH-sensitive antimicrobials, because many foods have near-neutral pH values.

DISCUSSION

The fluidity of the lipid bilayer is partly regulated by hydrogen bonding. For example, the hydroxyl group of ergosterol resides near the membrane−water interface in the lipid bilayer and is likely to be bonded with the carbonyl group of phospholipids (*19*, *20*). As nonionic surfactants, the aliphatic aldehydes first approach the binding site with the electron negativity of the aldehyde oxygen atom and may function by disrupting and disorganizing the hydrogen bonds such as the above-mentioned. Ergosterol is a major component of the plasma membrane of *S. cerevisiae* and owes its modulation of membrane fluidity to its rigid longitudinal orientation in the membrane. Because ergosterol has profound influences on membrane structure and function, if the hydrogen bond is broken, cell function will be impaired. If the aldehydes target the extracytoplasmic region, it is highly desirable because they do not need to enter the cell, thus avoiding most cellular pump-based resistance mechanisms. A similar hydrogen bond-breaking concept was proposed to explain the anesthesia cutoff phenomenon (*21*).

Previous papers have described the antifungal activity of the same series of alkanols against *S. cerevisiae* (*4*). Similar to (2*E*)-alkenals, the short-chain alkanols enter the cell by passive diffusion across the plasma membrane and/or through porin channels (*22*), and the long-chain alkanols enter in part into the lipid bilayers (*23*). The amount of alkanols entering into the cytosol or lipid bilayer is dependent on the length of the alkyl chain. Nonetheless, alkanols are chemically stable compounds and may not react with any biologically important substances in the cytosol or lipid bilayer. Hence, the primary antifungal action of alkanols comes largely from their ability to function as nonionic surfactants (physical disruption of the membrane). In the case of (2*E*)-alkenals, their α,*β*-unsaturated aldehyde group should not be overlooked because this group is chemically highly reactive and readily reacts with biologically important nucleophilic groups, such as sulfhydryl, amino, or hydroxyl (*24*). For example, the yeast plasma-membrane H^+-ATPase was reported to contain nine cysteines. (2*E*)-Alkenals may bind directly to the plasma membrane H^+-ATPase, probably with sulfhydryl groups of the three cysteines in the presumed transmembrane segments (C148, C312, and C867). However, Petrov and Slayman (*25*) reported that no single cysteine is required for activity on the basis of their site-directed mutagenesis study. This previous result does not exclude the possibility to assume that (2*E*)-alkenals first break the hydrogen bond as nonionic surfactants and then react with the freed sulfhydryl group of the H^+-ATPase as well as other plasma membrane proteins. This can be supported by the previous report that covalent modification of the conserved C148 in the transmembrane segment 2 may be important for inhibition of H^+-ATPase activity and cell growth (*26*). However, the observation that alkanals and (2*E*)-alkenals exhibit similar antifungal activity against *S. cerevisiae* as shown in **Table 2** and also inhibit glucose-induced acidification may not support the above assumption because the conjugated double bond is not essential to elicit the activity. The possibility of this concerted function of (2*E*)-alkenals is unlikely but cannot be ruled out.

The leakage of carboxyfluorescein (CF) in liposomes of phosphatidylcholine (PC) following exposure to (2*E*)-alkenals was previously reported (*27*), similar to those described for alkyl gallates (*11*). Interestingly, (2*E*)-alkenals tested caused rapid CF leakage from PC liposomes and the effectiveness order correlated well with the alkyl chain length. Thus, (2*E*)-nonenal was more effective in inducing CF leakage from PC liposomes than was (2*E*)-hexenal (*27*). This also supports the surfactant concept.

In summary, the antifungal activity of alkanals comes mainly from their ability to act as surfactants in which the balance between the head and tail is important to a large extent. Similarly, (2*E*)-alkenals also act as surfactants, but their α,*β*-unsaturated aldehyde moiety needs to be taken into account. (2*E*)-Alkenals may not act by a single defined process but have multiple functions. The surfactant concept, disrupting and disorganizing the lipid bilayer−protein interface nonspecifically, can be extended to answer many other problems related to membrane-bound enzymes and receptors and the fluidity of the membrane lipids. For example, the anesthesia cutoff phenomenon among alkanols is a well-known and long-standing problem. Anesthesia involves many membrane-bound proteins such as synaptosomal ATPases and acetylcholine receptor (*28*, *29*). The same surfactant concept, disrupting and disorganizing the lipid bilayer−protein interface, seems to be applicable to explain the anesthesia cutoff phenomenon of alkanols.

ACKNOWLEDGMENT

We are grateful to Dr. S. H. Lee for performing the antimicrobial assay at an earlier stage of the work.

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Received for review December 5, 2002. Revised manuscript received April 5, 2003. Accepted April 22, 2003. K.F. thanks Osaka City University for financial support during his study at the University of California, Berkeley.

JF0211884