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Naturally Occurring Phenolic Antibacterial Compounds Show Effectiveness against Oral Bacteria by a Quantitative Structure−Activity Relationship Study

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Natural and synthetic phenolic compounds were evaluated against oral bacteria. A quantitative structure−active relationship approach was applied to the germ-kill activity for a range of phenolic compounds. The lipophilicity and steric effects were found to be two key factors in determining germ-kill activity. The optimum lipophilicity, measured by the logarithm of the octanol/water partition coefficient, or log *P*, was found to be 5.5 for *Fusobacterium nucleatum*, a Gram-negative type of oral bacteria that causes bad breath. The optimum log *P* was found to be 7.9 for *Streptococcus mutans*, a Gram-positive type of oral bacteria that causes tooth decay. The steric effect of substituents ortho to the phenolic group was found to be critical in reducing antibacterial activity despite having increased lipid solubility approaching the optimum lipophilicity value. The antibacterial activity of phenolic compounds is likely exerted by multiple functions, primarily comes from its ability to act as a nonionic surface-active agent therefore disrupting the lipid−protein interface.

KEYWORDS: Antibacterial; oral malodor; magnolia bark extract; octanol−water partition coefficient; quantitative structure−activity relationship

INTRODUCTION

Oral malodor, also referred to as bad breath or halitosis, is a major social and psychological problem that affects the majority of the general population (*1*). Volatile sulfur compounds such as hydrogen sulfide, methyl mercaptan, and dimethyl sulfide, are the principle materials which impart oral malodor. The malodorous volatile sulfur compounds are generated through the metabolic activities of oral microorganisms on substrates available in the oral cavity. Gram-negative bacteria, predominantly at the dorsum of the tongue, are considered to be the most important group of microorganisms in the production of oral malodor (*2*−*6*).

In the past few years, many antimicrobial agents have been developed for the inhibition of halitosis bacteria and thus for the treatment of oral malodor (*7*−*10*). Antibacterial compounds such as chlorhexidine, cetylpyridinium chloride, triclosan, and chlorine dioxide have been tested either alone or in different combinations. However, most compounds have been known to induce side effects. For example, chlorhexidine stains the teeth. Triclosan was recently observed to react with free chlorine in drinking water to form toxic substances in a recent study (*11*).

Previously, we reported that magnolia bark extract (MBE), a traditional Chinese medicine isolated from the stem bark of *Magnolia officinalis*, demonstrated strong germ-kill activity against oral bacteria (*12*). MBE consists primarily of magnolol and honokiol, the two phenolic isomers. During our ongoing studies, we observed that a number of phenolic compounds in natural botanic extracts and flavors demonstrated antimicrobial activity. Among them, eugenol, magnolol, honokiol, thymol, and xanthorrhizol showed strong activity against oral bacteria. This led us to perform a systematic evaluation of various phenolic compounds to develop a quantitative structure−active relationship (QSAR).

The QSAR techniques involve correlating the logarithm of the reciprocal molar concentration of a bioactive compounds required for a specific biological response such as ED_{50} or LD_{50} values with linear free energy constants such as the Hammett constant σ, a measure of aromatic substituent electronic effects, Taft polar constant σ^*, a measure of substituent polar effects, the logarithm of the 1-octanol/water partition coefficient (log *P*), a measure of hydrophobic−hydrophilic effects, and the Taft steric constant E_s, a measure of substituent steric effects, etc. With the use of the QSAR techniques, a variety of correlations have been developed in the past. For example, Greenberg correlated the odor intensity and the hydrophobic properties of molecules and discovered good linear and parabolic correlations of the odor threshold and log *P* values (*13*). Selassie et al. (*14*) studied the cytotoxicity of various of phenolic compounds by QSAR, which led to a linear correlation equation:

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$$\log 1/C = -0.19\text{BDE} + 0.21 \log P + 3.11 \quad (1a)$$

where C is the molar concentration resulting in 50% inhibition of cell growth; BDE is the dissociation energy of the OH bond. Hansch (*15*) studied the correlation of a group of antibacterial agents and found the following correlation:

$$\log 1/C = -k(\log P)^2 + k' \log P + k'' \quad (2a)$$

Again, C is the molar concentration; k, k', and k'' are correlation parameters. The parabolic relation led to an optimum value of P_0: if P_0 is near zero, the compound will be so water-soluble it will not be able to across a lipophilic membrane, and the compound will be localized in the first aqueous phase. As P_0 approaches to infinity, the compound becomes so tightly bound to lipophilic phases that it cannot cross aqueous barriers. The authors discovered the optimum value of $P_0 = 4$ for Gram-negative bacteria and $P_0 = 5$ for Gram-positive bacteria.

The objective for this study is to report the applicability of QSAR techniques in establishing the primary physical−chemical property important for determining germ-kill activity and to rationalize how this contributes to the antibacterial property. A secondary objective is to determine the mode of action based on the QSAR and kill-time assay techniques.

MATERIALS AND METHODS

Chemicals. Magnolol (5,5′-di-2-propenyl-(1,1′-biphenyl)-2,2′-diol) and honokiol (3′,5-di-2-propenyl-(1,1′-biphenyl)-2,4′-diol) were purchased from Waco Chemicals (Japan). Magnolia bark extract was received from Masson Pharma (China) without further purification. The MBE contains 94% magnolol and 1.5% honokiol. Xanthorrhizol (99%) was obtained from LG Healthcare Company (South Korea). All other materials were purchased from Sigma-Aldrich Chemicals (Milwaukee, WI) and from TCI Chemicals (Portland, OR).

Cultures and Microorganisms. The microorganisms for this study were purchased from American Type Culture Collection (ATCC, Maryland). The bacterial stains used in this study were *Streptococcus mutans* (ATCC no. 25175) and *Fusobacterium nucleatum* (ATCC no. 10953). All microorganisms were taken from the frozen cultures, passed twice in Schaedler broth containing 1 mg/L menadione and 10 mg/L hemin, and maintained by serial passages. For each test, the inoculum consisted of cultures in late log or early stationary phase that had been subcultured by inoculating 0.5 mL of culture into 9.5 mL of Schaedler broth. The culture was used within 12 h after inoculation. All Gram-positive microorganisms were inoculated under aerobic condition for 16−25 h at 37 °C. All Gram-negative microorganisms were inoculated under anaerobic condition in AnaeroPack jar. The *F. nucleatum* were inoculated for 48 h.

Minimum Inhibitive Concentration and Minimum Bactericidal Concentration Tests. The minimum inhibitive concentration (MIC) was determined by the broth dilution method as described previously. Briefly, 5 mL of Schaedler broth supplemented with hemin (10 μg/mL) and vitamin K (1 μg/mL) incorporating serial 2-fold dilution of the test compounds was inoculated with 100 μL of a 2 day old culture of bacteria strains. They were anaerobically incubated (*F. nucleatum*) or aerobically incubated (*S. mutans*) at 37 °C for 2 days. The MIC was recorded as the lowest concentration of test compound to inhibit growth. The initial viable bacteria colony confirmation unit (CFU)/mL was counted. For *S. mutans*, it was 1 to ∼2×10^6 CFU/mL. The minimum bactericidal concentration (MBC) was determined as follows. After the determination of the MIC, 100-fold dilutions with fresh Shaedler broth containing hemin and vitamin K from each tube showing no turbidity were incubated at 37 °C for 2 days. The MBC was the lowest concentration of the test compound that showed no visible growth in the culture.

Kill-Time Assay Test. The kill-time curve assay method was used to study the bactericidal effects of MBE and its two main components. A total of 200 μL of a 2 day old culture of *S. mutans* was incubated into 10 mL of Schaedler broth containing 10 μg/mL of hemin and 1 μg/mL of menadione. The initial bacterial population was 1×10^6 microorganisms/mL. The testing compound was added, and all test cultures were incubated stationary at 37 °C for a 24 h period. At selected time intervals, samples from the test culture were taken, serially diluted in sterile water, and plated in Shaedler agar containing hemin and menadione. All plates were incubated at 37 °C for 2 days before the CFU was counted.

Table 1. Molecular Weight, log P, E_s, and MIC Values of Phenolic Compounds

compd	mol wt	log P	E_s	MIC (μg/mL) *S. mutans*	MIC (μg/mL) *F. nucleatum*
phenol	94	1.46	1.24	10 000	5000
p-cresol	108	1.94	1.24	2000	1250
2,6-dimethylphenol	122	2.36	0.0	2000	1250
2,6-diisopropylphenol	178	4.36	−0.47	500	250
2-allylphenol	134	2.72	1.24	500	200
2,2′-biphenol	186	2.73	1.24	500	500
eugenol	164	2.77	1.24	1000	
4-ethylphenol	122	2.5	1.24	1000	5000
4-propylphenol	136	3.03	1.24	500	1000
4-butylphenol	150	3.56	1.24	250	125
4-hexylphenol	178	4.62	1.24	25	10
4-heptylphenol	192	5.15	1.24	10	5
4-nonylphenol	220	6.21	1.24	5	40
2-phenylphenol	170	3.16	1.24	250	250
thymol	150	3.3	1.24	250	125
carvacrol	150	3.3	1.24	250	125
tert-butylhydroxyanisole	180	3.56	1.24	500	50
tert-butylhydroxytoluene	220	6.27	−1.54	>5000	>1%
octyl gallate	282	4.15	1.24	50	12.5
magnolol	268	5.25	1.24	31	12.5
honokiol	268	5.25	1.24	16	12.5
magnolia bark extract				31	16
xanthorrhizol	234	5.57	1.24	7.8	12.5

Partition Coefficients and Steric Parameter. The log[1-octanol/water partition coefficients] (log P) for compounds selected for this study were obtain from those reported by Hansch et al. (*16*) or were calculated from fragmental constants as reported by Nys and Rekker (*17*). The Taft steric parameter (E_s) was originally derived from the rate of ester hydrolysis hindered by different steric groups (*18*). It was extended by other researchers (*19, 20*). For small substituents, the agreement of modified E_s and the Taft's value was very close. In this study, we used E_s values reported by Taft. It was found to be 0 for methyl group and 1.24 for unsubstantiated hydrogen, respectively.

Regression Study. For each compound, the reciprocal millimolar concentration required to produce the MIC value [log(1/C)] was correlated to the corresponding log P and Taft steric constant values. Regression analysis was conducted using a stepwise multiple regression program from Microsoft Excel software. Correlation coefficient, optimum log P (log P_0), and 95% confidence intervals for coefficients were determined.

RESULTS

Table 1 lists the molecular weight, log P, and E_s values of a list of phenolic compounds as well as their MIC values against *S. mutans* and *F. nucleatum*. It is seen that common phenolic compounds including phenol, cresol, 4-ethyl phenol, and *t*-butylhydroxytoluene (BHT) showed a very weak germ-kill effect with MIC above 1000 μg/mL. Butylhydroxyanisole (BHA), octyl gallate, 4-heptylphenol, 4-nonlyphenol, magnolol, honokiol, and xanthorrhizol showed strong germ-kill activity with MIC < 50 μg/mL. **Figure 1** shows the chemical structures for some phenolic compounds.

Table 2 presents the QSAR regression analysis results of log(1/C) values for *F. nucleatum*, a Gram-negative oral bacteria. Log P and (log P)^2 account for 64% of the variance (R^2) in the model (eq 2). Addition of the steric parameter for the smallest

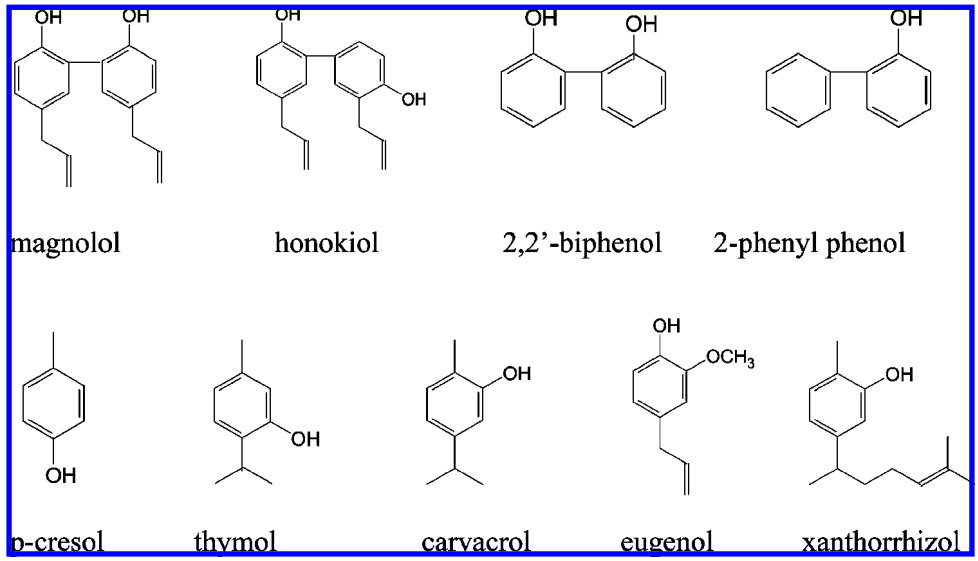

Figure 1. Chemical structures of phenolic compounds.

Table 2. Results of QSAR Regression Analysis on *F. nucleatum*, where *C* Is the Molar Concentration (mmol/L) and *P* Is the 1-Octanol/Water Partition Coefficient

eq 1: $$\log(1/C) = 0.49(\pm 0.28)\log P - 1.81 \quad N = 21, \quad R = 0.64 \quad (1)$$

eq 2: $$\log(1/C) = -0.28(\pm 0.16)[\log P]^2 + 2.69(\pm 1.38)\log P - 5.69 \quad N = 21, \quad R = 0.80 \quad (2)$$

eq 3: $$\log(1/C) = -0.20(\pm 0.12)[\log P]^2 + 2.20(\pm 0.96)\log P + 0.71(\pm 0.32)E_s - 5.73 \quad N = 21, \quad R = 0.92, \quad \log P_0 = 5.5 \quad (3)$$

eq 4: $$\log(1/C) = 0.62(\pm 0.21)\log P + 0.86(\pm 0.39)E_s - 3.14 \quad N = 21, \quad R = 0.85 \quad (4)$$

eq 5: $$\log(1/C) = 0.53(\pm 0.30)\log P + 0.13(\pm 0.24)\sum E_s - 2.09 \quad N = 21, \quad R = 0.67 \quad (5)$$

Table 3. Results of QSAR Regression Analysis on *S. mutans*, where *C* Is the Molar Concentration (mmol/L) and *P* Is the 1-Octanol/Water Partition Coefficient

eq 6: $$\log(1/C) = 0.54(\pm 0.23)\log P - 2.17 \quad N = 22, \quad R = 0.73 \quad (6)$$

eq 7: $$\log(1/C) = -0.18(\pm 0.16)[\log P]^2 + 1.98(\pm 1.31)\log P - 4.72 \quad N = 22, \quad R = 0.80 \quad (7)$$

eq 8: $$\log(1/C) = -0.09(\pm 0.08)[\log P]^2 + 1.42(\pm 0.66)\log P + 0.82(\pm 0.22)E_s - 4.77 \quad N = 22, \quad R = 0.96, \quad \log P_0 = 7.9 \quad (8)$$

eq 9: $$\log(1/C) = 0.68(\pm 0.12)\log P + 0.89(\pm 0.24)E_s - 3.56 \quad N = 22, \quad R = 0.94 \quad (9)$$

eq 10: $$\log(1/C) = 0.62(\pm 0.22)\log P + 0.19(\pm 0.18)\sum E_s - 2.61 \quad N = 22, \quad R = 0.79 \quad (10)$$

functional group ortho to the phenolic group, E_s, increases the amount of variation accounted by the model to 85%.

E_s alone poorly correlates to the activity. Summing the E_s values for each of the groups ortho to the phenolic group produces eq 5 which has an inferior correlation to eq 4 which only accounts for the steric hindrance of the smallest group ortho to the phenolic group. This indicates that one large bulky group does not hurt germ-kill activity as long as there is a small second group ortho to the phenolic group. On the basis of the statistical significance of the coefficients at high R in eq 3, both steric and lipophilic properties determine germ-kill activity of phenolic compounds.

Table 3 presents the QSAR regression analysis results of $\log(1/C)$ values for *S. mutans*, a Gram-positive oral bacteria. Again, $\log P$ and $(\log P)^2$ accounts for 64% of the variance in the model (eq 7). Addition of the steric parameter for the smallest functional group ortho to the phenolic group, E_s, increases the amount of variation accounted by the model to 92%. However, addition to E_s to eq 6 produced eq 9, which accounted for 88% of the variation. The correlation coefficients for eqs 8 and 9 are not significantly different. Most likely eq 8 would have produced significance if we studied phenolic compounds with $\log P > 7$. These compounds would have proved to be difficult to test due to poor water solubility.

We observed again that E_s alone poorly correlates to the activity. Summing the E_s values for each of the group ortho to the phenolic group produces eq 10. This equation has an inferior correlation to eq 9 which only accounts for the steric hindrance of the smallest group ortho to the phenolic group. This indicates that one large bulky group does not hurt germ-kill activity as long as there is a small second group ortho to the phenolic group. On the basis of the coefficients in eqs 8−10, both steric and lipophilic properties determine germ-kill activity of phenolic compounds.

Table 4 shows the correlation coefficient matrixes for both *F. nucleatum* and *S. mutans*. The matrix table summarizes the correlations between $\log(1/C)$, $\log P$, and E_s. The table indicates that E_s and $\log P$ are not colinear. In addition, the values show poor correlations with $\log(1/C)$ and E_s alone.

Table 4. Squared Correlation Coefficient Matrices on (a) *F. nucleatum* (b) *S. mutans*

(**a**) Squared Correlation Coefficient Matrices on *F. nucleatum*

	log(1/*C*)	E_s	log *P*
log(1/*C*)	1.00	0.13	0.41
E_s	0.13	1.00	0.08
log *P*	0.41	0.08	1.00

(**b**) Squared Correlation Coefficient Matrices on *S. mutans*

	log(1/*C*)	E_s	log *P*
log(1/*C*)	1.00	0.12	0.53
E_s	0.12	1.00	0.09
log *P*	0.413	0.089	1.00

Table 5. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Magnolia Bark Extract

microorganism	MIC (μg/mL)	MBC (μg/mL)
S. mutans (ATCC 25175)	31	31
F. nucleatum (ATCC 10953)	16	16

Equations 1, 2, 6, and 7 are statistically significant at the 95% level of confidence. Thus the lipophilicity plays a key role, the addition of steric effects as seen in eqs 3, 4, 8, and 9 shows that steric effects can be critical in reducing antibacterial activity despite having increased lipid solubility approaching the optimum lipophilicity value.

To explore the antibacterial mechanism, we conducted a more elaborative germ-kill study on two phenolic compounds, magnolol and honokiol, which are present in the extract of magnolia bark at 94% and 1.5%, respectively. **Table 5** presents the MIC and MBC values of MBE against *S. mutans* and *F. nucleatum*. We observed that both MIC and MBC for Gram-positive bacteria and Gram-negative bacteria are the same, indicating the bactericidal effect of the extract and no residual bacteriostatic activity involved. We observed the same MIC and MBC values for *P. gingivalis*, *V. parvula*, *S. sobrinus*, and *S. salivarius* species (data not presented).

The bactericidal activity of magnolol, honokiol, and MBE against *S. mutans* was further investigated by the time-kill curve experiment as shown in **Figure 2**. Cultures of *S. mutans* with a cell density of 1×10^6 CFU/mL were exposed to 0.5, 1, and 2 times MIC levels of magnolol, honokiol, and MBE. We observed that the lethality for magnolol and MBE occurred quickly at the MIC concentration, within 5 min after addition of magnolol or MBE. However, for honokiol, the bactericidal activity was shown to be both time- and concentration-dependent. The bactericidal activities were observed at less than 30 min of incubation for double or 5× MIC values, respectively. No more living bacteria were observed after 30 min of incubation at these concentrations, and regrowth was not observed. At the MIC, the bacterial growth was inhibited for 8 h and eventually killed after 24 h.

The effects of MBE against *S. mutans* were tested during holding viable cell number in the presence of chloramphenicol, a material that restricts cell division by inhibiting protein synthesis (*21*). The MIC of chloramphenicol was found to be 6.25 μg/mL against *S. mutans*. **Figure 3** shows that the effect of chloramphenicol against *S. mutans* is bacteriostatic for the first 6 h of incubation after addition of the drug. The viable

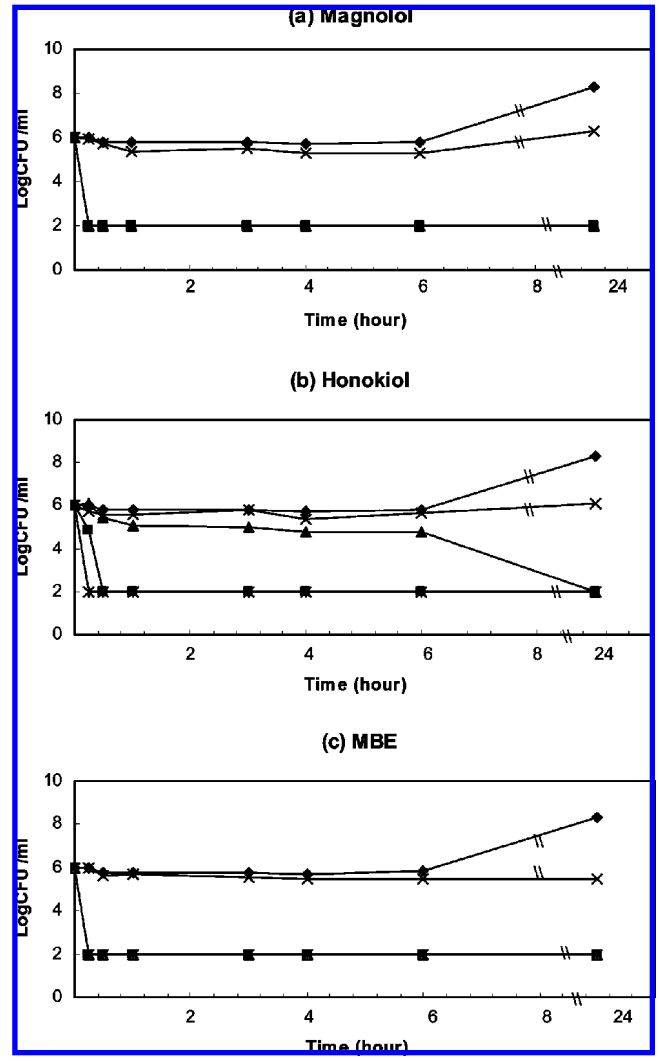

Figure 2. Kill-time assay of magnolol, honokiol, and MBE on *S. mutans*: (**a**) magnolol; (**b**) honokiol; (**c**) MBE. ◆, control; ■, 2 times MIC; ▲, 1 times MIC; ×, 0.5 times MIC.

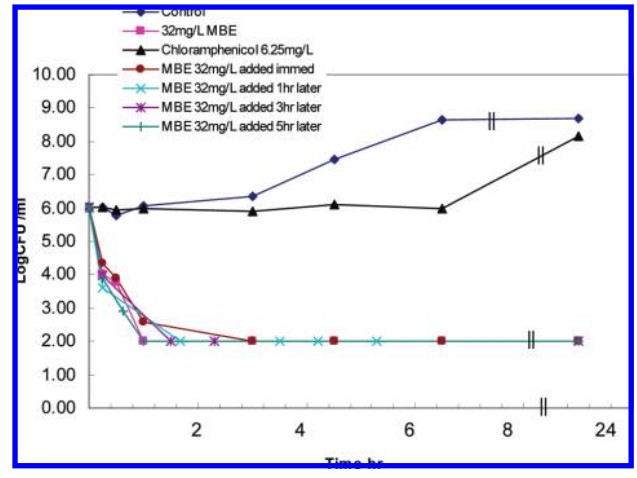

Figure 3. Kill-time assay of MBE on *S. mutans* after introducing 6.25 mg/L chloramphenicol.

cell count did not change appreciably after adding chloramphenicol up to 6 h. However, the viable bacterial counts decreased rapidly after addition of MBE to the *S. mutans* in the presence of 6.25 μg/mL chloramphenicol for various periods of time. In the presence of chloramphenicol, MBE decreased viable cell numbers a little faster than in its absence. However,

the inhibition of cell division by chloramphenicol did not influence the bactericidal effect of MBE.

DISCUSSION

The movement of an antimicrobial agent through tissue to its action site has long been regarded as important in explaining antimicrobial action (*22*−*26*). Several different attitudes have been assumed toward this problem by researchers in the field. Some have attempted to ignore the effect of small changes in structure on this process. Others have assumed a linear relationship between movement and partition coefficient, and still others have considered the problem too complicated to yield a rational solution by present knowledge.

By means of substituent constants and regression analysis, we have found the lipophilic character of the molecule, or substituent as expressed by log *P*, the partition coefficient, is the most important factor in determining the activities of the compounds examined. Steric effect of substituents ortho to the phenolic group can be critical in reducing antibacterial activity, particularly when lipid solubility approaches to the optimum value. In our case, we observed strong germ-kill by BHA with one bulky *t*-butyl group ortho to the phenolic group; however, we observed very low germ-kill effect by BHT with two bulky *t*-butyl groups. Numerous other examples in **Table 1** demonstrate that one large bulky group still allows for germ-kill activity. Equations 3 and 8 are the parabolic fits of log(1/*C*) with respect to log *P* on *F. nucleatum* and *S. mutans*, respectively. The correlation coefficients were found to be 0.85 for *F. nucleatum* and 0.92 for *S. mutans*. If the partial derivative of eq 2 log(1/*C*)/log *P* is taken and set equal to zero, one can solve for the constant log P_0. This gives the apex of the parabola. The log P_0 represents the ideal lipophilic character for a set of antimicrobial compounds. The P_0 was found to be 5.5 for *F. nucleatum*. and 7.9 for *S. mutans*. The value for *F. nucleatum* was in good agreement with Lien's discovery on Gram-negative bacteria. For *S. mutans*, the P_0 was above 7. Compounds with log $P > 7$ would have been difficult to study due to extremely poor water solubility. We also evaluated a series of similar phenolic compounds against other Gram-positive and Gram-negative oral bacteria including *S. sobrinus*, *S. salivarius*, and *P. gingivalis* (data not presented) and observed very similar results. Lien et all attributed the lower log P_0 for Gram-negative bacteria to the higher lipid content of the cell wall (up to 25% dry weight) compared to that of the Gram-positive species (0−3%). Lipophilic substances bind the outer cell wall more tightly for Gram-negative bacteria compared to Gram-positive bacteria. Therefore, they cannot penetrate the cell membrane easily, thus rendering them relatively less efficient for killing Gram-negative bacteria.

Antibacterial compounds may have different action mechanisms. Some of them will inhibit cell wall synthesis and activate enzymes that destroy the cell wall. Some increase cell membrane permeability. Still some may interfere with protein synthesis and nucleic acid metabolism (*21*). For example, heavy metals including zinc, copper, or silver and their salts were reported to interact with hydrosulfide group or −S−S− bond in bacterial enzymes and deactivate bacteria. Surface-active agents including sodium lauryl sulfate, cetylpyridium chloride, and sodium sorbate kill bacteria by disrupting the cell membrane and causing a rapid leakage of cell substances.

The surface-active types of antibacterial compounds share several common characters: they are usually nonselective with a broad spectrum of antibacterial effects on both Gram-negative and Gram-positive bacteria; they show the same or very close MIC and MBC concentrations as their acting mode is on the bactericidal effect on the cell membrane; they show rapid and irreversible bactericidal effect (*22*−*24*). In addition, the surfactant type of antibacterial agents show strong correlation of the hydrophilicity and/or the hydrophobicity of the molecule characterized by the water−octanol partition coefficient *P*. The time-kill kinetics of surfactant-type antimicrobials will not be influenced by the treatment of bacteriostatic agents such as chloramphenicol, etc.

Kubo and Fujita studied the mode of action of polygodiol, a natural occurring flavor substance, on salmonella bacteria (*22*). The authors discovered that polygodiol showed the same MIC and MBC concentrations. Polygodiol also showed rapid kill against salmonella bacteria. In the presence of chloramphenicol, polygodiol did not change the time-kill assay at different bacteria growth stages.

In a separate paper, Kubo et al. studied the mode of action of a serial of fatty alcohols against *S. mutans* (*23*). The authors discovered the germ-kill activity correlated well with the chain length of fatty alcohol, being lowest on carbon number below 6 and/or above 14 and optimum carbon number at 12 or 13. The authors found the same MIC and MBC concentrations for 1-tridecanol and concluded that the primary mode of action of dodecanol and tridecanol was a surfactant-like mechanism.

Shapiro and Guggenheim studied the antibacterial mechanism of thymol, a FEMA GRAS, phenolic plant-derived compound, on *P. gingivalis*, *S. artemidis*, and *S. sobrinus* (*24*). The authors concluded that the action of antibacterial activity was mainly caused by the rapid disruption of cell membrane, or surfactant type of action.

The present study shows that magnolol or MBE was bactericidal against *S. mutans* and killed the cells quickly. Magnolol and/or MBE showed the same concentration of MIC and MBC on a range of bacteria. MBE showed also a broad germ-kill spectrum including various oral bacteria, *S. aureus*, and various fungi (data not included). It has recently found against *H. pylori* (*25*), *P. acnes* (*26*), and *P. gingivalis* (*27*). All studies showed that MBE kills the bacteria and fungi within a very short period of time, typically in a few minutes. MBE very likely targets the extracytoplasmic region as a nonionic surfactant and thus does not need to enter the cell, thereby avoiding most cellular pump-based resistance mechanisms.

In addition, we observed that the inhibition of cell division by chloramphenicol did not influence the bactericidal effect of MBE. Chloramphenicol is a known bacteristat agent. It restricts cell division by inhibiting protein synthesis (*19*). The presence of MIC level of chloramphenicol did not change the time-kill assay profile of MBE. It is, thus, not likely that the reduced bacterial viability is due to interaction with the synthesis of macromolecules such as DNA, RNA, and proteins. Our finding agreed with Kubo and co-worker's discovery of polygodiol's mode of action against salmonella and fungi (*22, 28*).

LITERATURE CITED

(1) Tonzetich, J. Production and Origin of Oral Malodor: A Review of Mechanisms and Methods of Analysis. *J. Periodontol.* **1977**, *48*, 13–19.

(2) Quirynen, M. Management of Oral Malodour. *J. Clin. Periodontol.* **2003**, *30* (Suppl. 5), 17–18.

(3) Pitts, P.; Pianotti, R.; Feary, T. W.; McGuiness, J.; Masurat, T. The in Vivo Effects of an Antiseptic Mouthwash on Odor-Producing Microorganisms. *J. Dent. Res.* **1981**, *60*, 1891–1895.

(4) Yaegaki, K.; Sanada, K. Volatile Sulfur Compounds in Mouth Air from Clinically Healthy Subjects and Patients with Periodontal Disease. *J. Periodontal Res.* **1992**, *27*, 233–238.

(5) Loesche, W.; Kazor, C. Microbiology and Treatment of Halitosis. *Periodontology* **2002**, *28*, 256–279.
(6) McNamara, T. F.; Alexander, J. F.; Lee, M. The Role of Microorganisms in the Production of Oral Malodor. *Oral Surg.* **1972**, *34*, 41–48.
(7) Hayashi, Y.; Ohara, N.; Ganno, T.; Yamaguchi, K.; Ishizaki, T.; Nakamura, T.; Sato, M. Chewing Chitosan-Containing Gum Effectively Inhibits the Growth of Cariogenic Bacteria. *Arch. Oral Biol.* **2007**, *52*, 290–294.
(8) Loesche, W. Clinical and Microbiological Aspects of Chemotherapeutic Agents Used According to Specific Plaque Hypothesis. *J. Dent. Rev.* **1979**, *58*, 2404–2412.
(9) Giertsen, E. Effects of Mouthrinses with Triclosan, Zinc Ions, Copolymer, and Sodium Lauryl Sulphate Combined with Fluoride on Acid Formation by Dental Plaque in Vivo. *Caries Res.* **2004**, *38*, 430–435.
(10) Greenstein, R. B.-N.; Goldberg, S.; Marku-Cohen, S.; Sterer, N.; Rosenberg, M. Reduction of Oral Malodor by Oxidizing Lozenges. *J. Periodontol.* **1997**, *68*, 1176–1181.
(11) Rule, K. L.; Ebbett, V. R.; Vikes, P. J. Formation of Chloroform and Chlorinated Organics by Free-Chlorine-Mediated Oxidation of Triclosan. *Environ. Sci. Technol.* **2005**, *39*, 3176–3185.
(12) Greenberg, M.; Urnezis, P.; Tian, M. Compressed Mints and Chewing Gum Containing Magnolia Bark Are Effective against Bacteria That Are Response for Oral Malodor. *J. Agric. Food Chem.* **2007**, *55*, 9465–9469.
(13) Greenberg, M. Dependence of Odor Intensity on the Hydrophobic Properties of Molecules. A Quantitative Structure Odor Intensity Relationship. *J. Agric. Food Chem.* **1979**, *27*, 347–352.
(14) Selassie, C.; Verma, R. P.; Kapur, S.; Shusterman, A. J.; Hansch, C. QSAR for the Cytotoxicity of 2-Alkyl or 2,6-Dialkyl, 4-X-phenols: The Nature of the Radical Reaction. *J. Chem. Soc., Perkin Trans. 2* **2002**, 1112–1117.
(15) Hansch, C. A Quantitative Approach to Biochemical Structure−Activity Relationships. *J. Med. Chem.* **1969**, *2*, 232–239.
(16) Hansch, C.; Leo, A.; Elkins, D. Partition Coefficents and Their Uses. *Chem. Rev.* **1971**, *71*, 525.
(17) Nys, G. G.; Rekker, R. F. *Eur. J. Med. Chem.* **1974**, *9*, 375.
(18) Taft, R. W. In *Steric Effects in Organic Chemistry*; Newman, M. S., Ed.; Wiley: New York, 1956; p 556.
(19) Hansch, C.; Leo, A. *Steric Effects on Organic Reactions in Exploring QSAR*; American Chemical Society: Washington, DC, 1995; Chapter 3, pp 69−96.
(20) Fujita, T.; Iwamura, H. In *Topics in Current Chemistry*; Charton, M.; Motoc, I., Eds.; Springer-Verlag: Berlin, Germany, 1983; Vol. 114, p 119.
(21) Beers, M. H., MD.; Porter, R. S., MD.; Jones, T. V., MD., MPH.; Kaplan, J. L., MD.; Berkwits, M., MD., MSCE. Bacteria and Antibacterial Drugs. *Merck Manuals* [Online], 18th ed. http://www.merck.com/mmpe/sec14/ch170/ch170a.html.
(22) Kubo, I.; Fujita, K.-I. Naturally Occurring Anti-Salmonella Agent. *J. Agric. Food Chem.* **2001**, *49*, 5750–5754.
(23) Kubo, I.; Muroi, H.; Kubo, A. Antibacterial Activity of Long-Chain Alcohols against *Streptococcus mutans*. *J. Agric. Food Chem.* **1993**, *41*, 2447–2450.
(24) Shapiro, S.; Guggenheim, G. The Action of Thymol on Oral Bacteria. *Oral Microbiol. Immunol.* **1995**, *10*, 241–246.
(25) Bae, E. A.; Han, M. J.; Kim, N. J.; Kim, D. H. Anti-*Helicobacter pylori* Activity of the Herbal Medicines. *Biol. Pharm. Bull.* **1989**, *21*, 990–992.
(26) Park, J.; Lee, J.; Jung, E.; Park, Y.; Kim, K.; Park, B.; Jung, K.; Park, E.; Kim, J.; Park, D. In Vitro Antibacterial and Anti-Inflammatory Effects of Honokiol and Magnolol against *Propionibacterium* sp. *Eur. J. Pharmacol.* **2004**, *496*, 189–195.
(27) Ho, K. Y.; Tsai, C. C.; Chen, C. P.; Huang, J. S.; Lin, C. C. Antimicrobial Activity of Honokiol and Magnolol Isolated from *Magnolia officinalis*. *Phytother. Res.* **2001**, *15*, 139–141.
(28) Kubo, I.; Xiao, P.; Nihei, K.-I.; Fujita, K.-I.; Yamagiwa, Y.; Kamikawa, T. Molecular Design of Antifungal Agents. *J. Agric. Food Chem.* **2002**, *50*, 3992–3998.

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