AFRL-ML-TY-TP-2006-4530

SILANOLS, A NEW CLASS OF ANTIMICROBIAL AGENT

Yun-mi Kim, Samuel Farrah and Ronald H. Baney

Department of Materials Science and Engineering
University of Florida
Gainesville, FL 32611

Interim Paper, April 2006

Air Force Research Laboratory
Materials and Manufacturing Directorate
Airbase Technologies Division
139 Barnes Drive, Suite 2
Tyndall AFB, FL 32403-5323

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
07-04-2006	Technical Paper	30-09-2004 – 01-03-2006

4. TITLE AND SUBTITLE
Silanols, a New Class of Antimicrobial Agent

5a. CONTRACT NUMBER
F08637-02-C-7021

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
106013

6. AUTHOR(S)
Yun-mi Kim, Samuel Farrah and Ronald H. Baney

5d. PROJECT NUMBER
ARMT

5e. TASK NUMBER
00

5f. WORK UNIT NUMBER
ARMT0047

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Department of Materials Science and Engineering
University of Florida,
Gainesville, FL 32611

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Air Force Research Laboratory
Materials and Manufacturing Directorate
139 Barnes Drive, Suite 2
Tyndall AFB, FL 32403-5323

10. SPONSOR/MONITOR'S ACRONYM(S)
AFRL/MLQL

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
AFRL-ML-TY-TP-2006-4530

12. DISTRIBUTION/AVAILABILITY STATEMENT
Distribution Statement A: Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES
MLQ Public Affairs Case # 06-031.

14. ABSTRACT

Alkyldimethylsilanols, $R(CH_3)_2SiOH$, were recently reported to exhibit unexpectedly strong antimicrobial effects. The antimicrobial activities of alkyldimethylsilanols were significantly higher than their analogous alcohols. A study of structural dependence of their antimicrobial activity was conducted with four bacteria, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis.* Silanols, alcohols with structures analogous to the silanols, $R(CH_3)_2COH$, and substituted phenols were evaluated as a single class of materials. The minimum lethal concentrations (*MLC*) defined as the concentration required for a 7-log reduction in viable bacteria after a 1-hour exposure period were used to measure the antimicrobial activity. Octanol/water partition coefficients (log *P*) and H-bond acidities (Continued on p. ii)

15. SUBJECT TERMS
Antimicrobial, Bacteria, Gram-negative, Gram-positive, QSAR, silanol

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Joseph D. Wander
U	U	U	UU	17	19b. TELEPHONE NUMBER *(Include area code)* 850-283-6240

Reset

Standard Form 298 (Rev. 8/98)
Prescribed by ANSI Std. Z39.18

13. (CONTINUED)

(Δν) measured as the shift in frequency of O-H stretching bands between free OH and hydrogen bonded OH to diethyl ether oxygen by infrared spectroscopy were utilized as a dispersive and polar structural parameter. The correlation established by multiple regression analysis between antimicrobial activities and structural properties of silanols and carbinols against the four bacteria was $\log(1/MLC) = 0.670 \log P + 0.0035\ \Delta\nu - 1.836$, $n = 282$, $r = 0.96$, $s = 0.22$. This equation and a significantly high correlation coefficient r supported the hypothesis that the lipophilic properties and the H-bond acidities are primary factors for antimicrobial action of silanols and carbinols.

1. Introduction

Understanding the mechanisms of antimicrobial action is critical for the design of improved antimicrobial agents. The quantitative relationship between chemical structure and biological activity has received considerable attention in recent years because it allows one to predict chemical toxicity or bioactivity without an inordinate amount of time and effort. A method for quantitative biological activity and chemical structure relationship was introduced by Hansch and Fujita in 1964. This method is based on a linear free energy-related approach, called Hansch analysis [1, 2]. All parameters used in Hansch analysis are linear free-energy-related values derived from rate or equilibrium constants.

Structural dependence studies with antimicrobial activity have been mainly focused on the effects of the hydrophobicity through testing a homologous series of samples, for example, aliphatic alcohols, alkylated phenol derivatives, and quaternary ammonium compounds [3–6]. Tanner and Wilson examined the antimicrobial activity of aliphatic alcohols containing from 1 to 11 carbon atoms by employing nine different strains of bacteria [6]. They showed that the bioactivity increased as the alkyl chain length increases from methyl to pentyl, then decreases through the primary-normal hexyl, heptyl and octyl alcohols as a result of a decrease in solubility. The relationship between the chemical structure and antimicrobial activity of substituted phenol compounds has been reported [5, 7]. The bactericidal actions of alkyl substituted phenol [5] and the normal alkyl derivatives of *p*-chlorophenols [7] were examined against Gram-negative and Gram-positive bacteria. They reported that an increase in the alkyl chain length led to an increase of antimicrobial activity, but there existed a cut-off point where the activity began to fall off as the alkyl chain continued to increase. It was also reported that the cut-off points varied with tested microorganisms.

Hansch and Lien summarized the structure–activity relationship of antimicrobial agents by means of equations [8] based on a method proposed by Hansch and Fujita in 1964 [1]. This multiple regression analysis method was used for establishing the correlation between structural properties and activities [9]. They observed that the correlations varied with bacteria and type of antimicrobial agents. These authors suggested that the lipophilic property of the molecule was the most important factor for the antimicrobial activities of the compounds with a relatively minor contribution from electronic properties [8]. Daoud demonstrated a parabolic relationship between log P and log $(1/MLC)$ of a homologous series of alkyldimethylbenzylammonium chlorides, quaternary ammonium salt compounds [3]. The parabolic relationship obtained implies that the antimicrobial activity increases as the alkyl chain length increases, then, as the activity passes an optimum point, the antimicrobial activity begins to decrease with increasing alkyl chain.

Silanols, *i.e.*, silicon alcohols, R_3SiOH, are a new class of antimicrobial agents [10]. Their antimicrobial activity appears to be stronger than their analogous alcohols show. Silanols are environmentally friendly materials because they are rather quickly degraded into environmentally benign silica, carbon dioxide and water in the environment [11]. Silanols have a hydrophilic portion, the hydroxyl group, and a hydrophobic region, the organic substituents, similar to alcohols and phenols widely used as antimicrobial agents.

It is reasonable to predict that the mode of antimicrobial actions of silanols would resemble those of the alcohols and phenols because of the similarity of the chemical structures of the materials. The antimicrobial actions of alcohols and phenols are generally explained as protein denaturing and membrane damage although there are further actions regarding inhibition of enzymatic action or protein synthesis [12–15]. The degree of membrane disruption of cells is reported to be related to the hydrophobic property of the agents [16, 17].

The relationship between the physicochemical properties and the bioactivities reported earlier was mainly established using a homologous series of agents such as alcohols and phenol derivatives separately. Our study examines the relationship between antimicrobial activity and physicochemical properties for alcohols, phenols, and previously unreported silanols as a single class of agents. The parameters utilized in our study were the partition coefficient and the H-bond acidity that represented, respectively, the dispersive or lipophilic property and the polar property of the hydroxyl-containing antimicrobial agents.

2. Materials and methods

2.1. Preparation of alkyldimethylsilanols and their purities

Alkyldimethylsilanols, $R(CH_3)_2SiOH$, shown in fig. 1.a., were prepared by the hydrolysis of organosilicon halides [18, 19]. Organosilicon halides and water were mixed for 15–30 minutes in diethyl ether solution with ammonium hydroxide. The analogous alcohols, $R(CH_3)_2COH$ and substituted phenols shown in fig. 1.b. and 1.c. were obtained from Acros Organics. The R substituents were methyl, ethyl, *n*-propyl, *n*-butyl, phenyl, vinyl, benzyl, and phenethyl. The substituents for the phenols were 4-methyl, 4-ethyl, 4-propyl, 4-butyl, 4-pentyl, 4-hexyl, 3-chloro, and 2-phenyl. The purities of the silanols measured by a ^{29}Si and ^{1}H NMR (nuclear magnetic resonance spectroscopy) method [20] were 95 ± 3 %, with the impurity consisting of disiloxanes arising from condensation of silanols. The antimicrobial effects of disiloxanes were also tested. The alcohols and phenols were used as received and their purities were greater than 97 %.

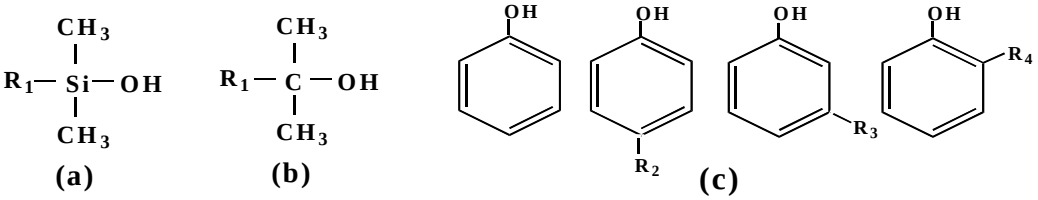

Fig. 1. Chemical structures (a) Alkyldimethylsilanols (b) Alkyldimethylcarbinols, (c) Substituted phenols

2.2. Preparation of the bacteria and procedures of the antimicrobial tests

The bacterial strains employed were *Escherichia coli* (*E. coli*) C3000 (ATCC 15597), a laboratory strain of *Staphylococcus aureus* (*S. aureus*) (Department of Microbiology, University of Florida), *Pseudomonas aeruginosa* (*P. aeruginosa*) type strain (ATCC 10145), and *Enterococcus faecalis* (*E. faecalis*) type strain (ATCC 19433). Suspensions of the bacteria were prepared according to the procedure by Rincon [21]. The bacteria were inoculated under aerobic conditions in a nutrient, Columbia broth, overnight at 37 °C with constant agitation. The bacterial cells were collected by centrifugation at 500 *rcf* (relative centrifugal force) for ten minutes at 4°C and washed three times with sterilized distilled water. A bacterial pellet was resuspended in the sterilized water after final washing. Concentrations of the prepared bacteria suspension were 2–6 $\times 10^8$ cfu/mL (colony forming units).

The antimicrobial activity tests of the materials were carried out by adding a given concentration of antimicrobial agent into 9 g of deionized water and 1 g of bacterial suspension containing a concentration of 2–6 $\times 10^8$ cfu/mL. The solution was stirred for an hour. Minimum lethal concentration (*MLC*) in our studies is defined as the maximum dilution of the product that kills the test organisms by more than a 7-log reduction after a one-hour exposure. The *MLC*s were utilized as the measure of the biocidal activity of silanols, alcohols, and phenols. Samples collected after the 1-hour treatment were diluted by a phosphate-buffered saline and then plated on a plate-count agar (Difco)[22]. After incubation of the plates for 24 hours at 37 °C, the colonies that grew on the medium were counted to estimate the number of viable bacteria. The standard deviation values in table 1 were determined by taking a mean of three tests.

2.3. Measurement of Hydrogen-bond acidity (H-bond acidity)

Relative H-bond acidities of a series of silanols, analogous alcohols, and phenols were determined by measuring $\Delta\nu$, defined as the shift in frequency of the O–H stretching band between free OH and OH hydrogen-bonded to diethyl ether oxygen [23]. The shift, $\Delta\nu$, is proportional to the strength of the hydrogen bond. The strength of the hydrogen bond is based on the ability of the proton of the hydroxyl compound to associate with a proton acceptor site in the hydrogen bond base, diethyl ether. The shift is a measure of the relative proton-donating nature of the OH-containing compounds, because the proton-accepting ability remains constant since the same base diethyl ether was employed.

Solutions of the silanols, the alcohols, and the phenols were prepared at 0.04*M* in carbon tetrachloride. The Lewis base, anhydrous diethyl ether, was prepared at 0.5*M* in carbon tetrachloride. One-to-one mixtures of the base and the silanols or alcohols were prepared for the infrared spectroscopy study. The possibility of the self-association bands was not a concern at the low concentration of silanols and alcohols [23].

A transmission sampling technique with a NaCl window material was utilized for the infrared spectroscopy measurements. Two OH bands in the 3800–3200 cm^{-1} region were observed. A broad OH band was detected at lower frequency range, 3500–3200 cm^{-1}, due to OH hydrogen bonded to the ether oxygen, whereas a sharp free-OH band was observed

at higher frequency, 3800–3550 cm^{-1}. The difference in frequency between two bands, $\Delta\nu$, measured the relative H-bonding acidity of the silanols and alcohols [23].

2.4. Calculation of the octanol–water partition coefficient.

The lipophilic nature was determined by partitioning a compound between an aqueous and a non-aqueous phase. The octanol–water partition coefficient, log $P_{o/w}$, is defined as the ratio of the concentration of a solute in a non-polar solvent (1-octanol) and the concentration of the same species in a polar solvent (water) under equilibrium conditions [24]. Log *P* of the silanols, alcohols, and phenols was calculated by using the demo program LogKow, provided by Syracuse Research Corporation. The program was used to estimate log *P* by using a new fragment-constant approach which is the atom/fragment contribution (AFC) method [25]. In the fragment-constant approach, proposed by Hansch and Leo [26, 27], a chemical structure is divided into fragments such as atoms or larger functional groups. The values of the fragments are summed together with structural correction factors to estimate the value of log *P*.

The AFC method was developed through multiple linear regressions of experimental log *P* values. The first regression analysis was correlated to the atom/fragment values without correction factors. Correction factors were derived from the difference between the value of log *P* estimated from the first regression and the measured log *P* values. The log *P* of a compound was then estimated by simply summing all atom/fragment values and correction factors contained in a structure.

3. Results

3.1. Characterization of the partition coefficient, the H-bond acidity for the silanols, the alcohols, and the phenols and determination of the purity of the silanols

The purities of the silanols, measured by ^{29}Si and ^{1}H NMR, were 95 ± 3 %. The impurities of the silanols were identified as the silanol condensation products, dialkyltetramethyldisiloxanes. Antimicrobial activities of disiloxanes such as the hexamethyldisiloxane impurity in trimethylsilanol and diphenyltetramethyldisiloxane in phenyldimethylsilanol were evaluated to determine their contribution to the antimicrobial activities of the silanols. The experiments performed using 10% of the disiloxanes tested against the four bacteria of the study showed less than a 1-log reduction, demonstrating that the silanols and not their condensation products, the disiloxanes, were responsible for the observed antimicrobial activity.

The physicochemical properties, the H-bond acidity and the octanol–water partition coefficient of the tested materials are shown in table 1. The H-bond acidities of the silanols are almost two times higher than their analogous alcohols due to the electron back donation through π-bonding from a *p* orbital of oxygen to a vacant *d* orbital of silicon [23]. This greater acidity occurs even though the silicon atom is more electropositive than a carbon atom, which would lead one to predict that silanols should be less acidic than their analogous carbinols. We propose that the higher acidities of the

silanols play an important role to their enhanced biocidal activity. The H-bond acidities of phenol derivatives were generally higher than those of most silanols and alcohols.

The partition coefficients estimated by the atom/fragment-contribution method [25] showed a gradual increase as the alkyl chain increased for silanols, alcohols and phenols presented in table 1. The partition coefficients of the silanols were higher than the analogous alcohols as shown table 1. It is well known that silicon compounds including the silanols exhibit higher hydrophobic properties than analogous organic compounds due to flexible molecular chains and lower group rotation energy barriers than the carbon bond [28]. 4-Pentylphenol and 4-hexylphenol, which contain longer alkyl chains, exhibited the highest partition coefficients.

3.2. Antimicrobial activities of silanols, alcohols, and phenols against two Gram-negative and two Gram-positive bacteria.

The minimum lethal concentrations (*MLC*) of the silanols, alcohols, and phenols were determined against two Gram-negative bacteria, *E. coli* and *P. aeruginosa* and two Gram-positive bacteria, *S. aureus* and *E. faecalis*, as summarized in table 1. Fig. 2. is an example illustrating that the minimum lethal concentration decreases as the alkyl chain length of silanols increases. The experimental results with alcohols and phenols were similar to that of silanols except their *MLC* values were different from the values for silanols. A lower minimum lethal concentration implies a higher antimicrobial activity. Consequently, a change of substituents from a short alkyl chain to a longer alkyl chain of silanols, alcohols, and phenols resulted in increased biocidal activity.

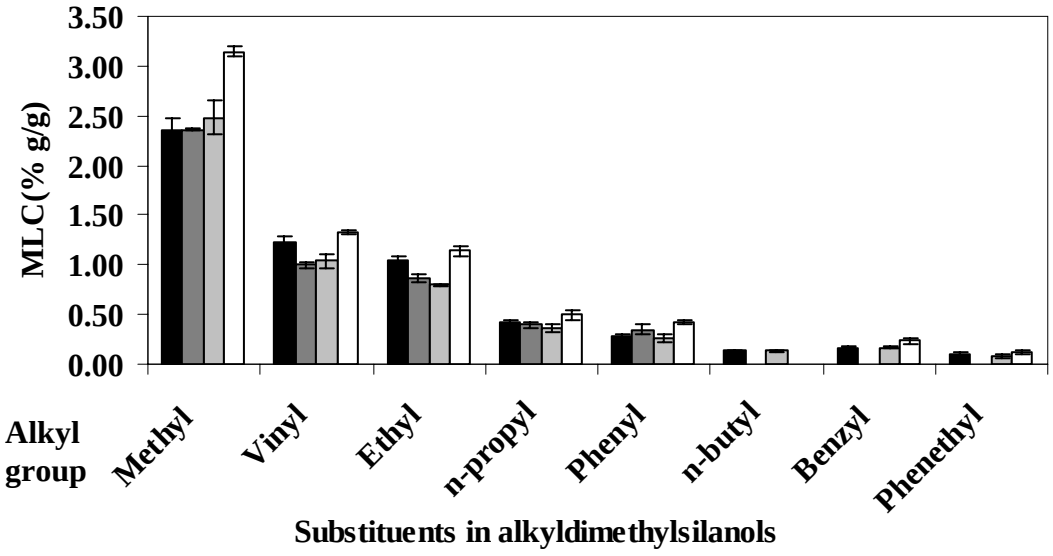

Fig. 2. Minimum lethal concentration of alkyldimethylsilanols against Gram-negative bacteria, ▢ *Escherichia coli* and ▢ *Pseudomonas aeruginosa* and Gram-positive bacteria, ■ *Staphylococcus aureus* and ▩ *Enterococcus faecalis*.

Table 1. Minimum lethal concentrations (% g/g), H-bond acidity in ether (Δν) and octanol–water partition coefficient (log *P*) of silanols, alcohols, and phenols against *Escherichia coli*, *Staphylococcus aureus*, *Psudomonaos aeruginosa*, and *Enterococcus faecalis.* Each minimum lethal concentration is the averaged value of three data points.

Materials	R	Minimum lethal concentration (% g/g)				log *P*	Δν
		E. coli	*S. aureus*	*P. aeruginosa*	*E. faecalis*		
Silanols $R(CH_3)_2SiOH$	Methyl	2.36±0.12	2.48±0.17	2.36±0.01	3.15±0.05	1.14	239
	Vinyl	1.23±0.06	1.04±0.07	1.0±0.03	1.32±0.03	1.5	260
	Ethyl	1.04±0.04	0.80±0.02	0.87±0.04	1.14±0.05	1.63	237
	n-Propyl	0.43±0.03	0.36±0.04	0.40±0.03	0.50±0.05	2.12	237
	Phenyl	0.27±0.03	0.26±0.03	0.35±0.05	0.42±0.03	2.36	267
	Benzyl	0.17±0.01	0.16±0.01	×	0.23±0.03	2.85	257
	n-Butyl	0.14±0.01	0.14±0.01	×	×	2.62	236
	Phenethyl	0.10±0.02	0.08±0.02	×	0.12±0.02	3.34	252
Alcohols $R(CH_3)_2COH$	Methyl	13.54±1.27	10.61±0.19	9.79±0.19	13.33±0.42	0.75	126
	Vinyl	5.23±0.06	4.37±0.05	3.67±0.08	5.63±0.18	1.08	130
	Ethyl	5.09±0.04	4.17±0.12	3.96±0.04	5.67±0.15	1.22	125
	n-Propyl	1.68±0.04	1.69±0.04	1.03±0.03	1.76±0.04	1.71	125
	Phenyl	0.96±0.06	0.78±0.03	0.71±0.03	0.93±0.03	1.95	137
	n-Butyl	0.67±0.03	0.65±0.02	0.53±0.02	0.76±0.06	2.2	126
	Benzyl	0.7±0.05	0.59±0.02	0.63±0.03	0.75±0.05	2.44	129
	Phenethyl	0.26±0.01	0.26±0.01	1.33±0.28	0.32±0.02	2.93	129
Phenols RC_6H_5OH	[1]Hydrido	0.70±0.01	0.61±0.02	0.62±0.08	0.98±0.03	1.51	278
	4-Methyl	0.410±0.01	0.35±0.02	0.35±0.05	0.45±0.05	2.06	270
	3-Chloro	0.13±0.02	0.11±0.01	0.11±0.01	0.14±0.01	2.16	318
	4-Ethyl	0.13±0.02	0.14±0.02	0.11±0.01	0.17±0.03	2.55	269
	4-Propyl	0.053±0.006	0.045±0.005	0.052±0.008	0.055±0.005	3.04	270
	2-Phenyl	0.12±0.03	0.085±0.015	0.13±0.03	0.13±0.03	3.28	252
	4-Butyl	0.013±0.002	0.015±0.005	0.060±0.01	0.017±0.003	3.53	270
	4-Pentyl	0.010±0.002	0.008±0.002	×	0.012±0.002	4.02	269
	4-Hexyl	0.055±0.005	0.004±0.001	×	0.006±0.001	4.52	271

[1]Hydrido : unsubstituted phenol.

× indicates no minimum lethal concentration was obtained.

The susceptibility of bacteria to antimicrobial agents can be compared based on the relative minimum lethal concentration of the antimicrobials. The resistances of the four bacteria against silanols, alcohols, and phenols slightly varied. *E. faecalis* was overall the least susceptible bacterium followed by *E. coli*, *P. aeruginosa*, and *S. aureus*. It is significant to note that the alkyldimethylsilanols showed at least two times more effective antimicrobial activities than the analogous alcohols against all four bacteria. For the *E. coli* test, trimethylsilanol and phenethyldimethylsilanol showed *MLC* of 2.4 % and 0.1%, respectively, whereas the corresponding alcohols, *t*-butyl alcohol and phenethyldimethyl-carbinol, exhibited *MLC*s of 14.51% and 0.25%, respectively. 4-Hexylphenol, which contains the most carbon atoms among the materials tested in this study, showed the lowest *MLC*, 0.004% against *S. aureus* and 0.006% against *E. faecalis*.

A fall-off of antimicrobial activity was observed as the number of carbon atoms of the substituent of silanols, alcohols, and phenols increased. The antimicrobial activity against *E. coli* obtained for 4-hexylphenol, *MLC* = 0.055%, was lower than that of 4-entylphenol, *MLC* = 0.011%. Benzyldimethylcarbinol, having *MLC* = 0.58% against *P. aeruginosa*, showed lower activity than the *MLC* obtained for butyldimethylcarbinol, 0.52%. Phenethyldimethylcarbinol also exhibited a continuous decrease of the activity after benzyldimethylcarbinol against *P. aeruginosa*, as shown in table 1.

The minimum lethal concentrations for 4-hexyl and 4-pentylphenol and *n*-butyl, benzyl, and phenethyldimethylsilanol were not determined against *P. aeruginosa* because of a significant reduction of antimicrobial activity of the materials as the alkyl chain length increases beyond this cut-off point.

3.3. Correlation between antimicrobial activity and structural parameters.

We propose that the physicochemical parameters of the silanols showing a higher hydrophobicity and a higher H-bond acidic property compared to the alcohols, contribute to the enhanced antimicrobial activity of the silanols. Since phenol derivatives are also composed of the hydrophilic hydroxyl group and the hydrophobic alkyl chains similar to that of silanols and alcohols as shown in fig. 1 the three types of chemical agents were treated as a single class of antimicrobial agents. In this study, we demonstrated a quantitative structure–activity relationship using silanols, alcohols, and phenols. The correlation equations between their antimicrobial activities and structural properties, log *P* and H-bond acidity, were created by a multiple regression analysis and are summarized in table 2.

The minimum lethal concentration (*MLC*) values were converted into the logarithms of (1/*MLC*), which can demonstrate a linear free-energy relationship with the structural parameters [2, 29]. Hansch and associates proposed that a linear free-energy relationship exists between the lipophilicity and biological activity [2, 29]. The plot of log (1/*MLC*) as a function of the partition coefficient produced a linear relationship as presented in table 2, equations 1, 3, 5 and 7, with statistically significant values of the correlation coefficient *r*, standard deviation *s*, and *F* value reported in table 2.

The significance and validity of the regression models or equations can be evaluated by assessing the correlation coefficient *r*, the standard deviation *s*, and the *F* values [9, 30].

Table 2 Correlation equations for antimicrobial activities and the physicochemical properties of silanols, alcohols, and phenols against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis.*

Materials	Alkyldimethylsilanols, Alkyldimethylcarbinols, Phenol Derivatives	
Gram-negative bacteria	*Escherichia coli*	log (1/*MLC*) = 0.721 log *P* -1.242, *n* = 75, *r* = 0.91, *s* = 0.32, *F* = 352 (1) log (1/*MLC*) = 0.614 log *P* + 0.0036 Δν -1.777, *n* = 75, *r* = 0.95, *s* = 0.24, *F* = 333, partial *F*-test = 55 (2)
	Pseudomonas aeruginosa	log (1/*MLC*) = 0.645 log *P* - 1.072, *n* = 60, *r* = 0.83, *s* = 0.34, *F* = 128 (3) log (1/*MLC*) = 0.525 log *P* + 0.0039 Δν -1.645, *n* = 60, *r* = 0.93, *s* = 0.23, *F* = 183, partial *F*-test = 74 (4)
Gram-positive bacteria	*Staphylococcus aureus*	log (1/*MLC*) = 0.825 log *P* -1.384, *n*=75, *r*=0.95, *s*=0.26, *F*=676 (5) log (1/*MLC*) = 0.722 log *P* + 0.0034 Δν -1.8934, *n* = 75, *r* = 0.99, *s* = 0.17, *F* = 873, partial *F*-test = 105 (6)
	Enterococcus faecalis	log (1/*MLC*) = 0.809 log *P* -1.477, *n* = 72, *r* = 0.95, *s* = 0.26, *F* = 648 (7) log (1/*MLC*) = 0.718 log *P* + 0.0031 Δν -1.935, *n*=72, *r* = 0.98, *s* = 0.17, *F* = 837, partial *F*-test = 101 (8)
Overall equation		log (1/*MLC*) = 0.764 log *P* -1.318, *n* = 282, *r* = 0.92, *s* = 0.30, *F* = 1543 log (1/*MLC*) = 0.670 log *P* + 0.0035 Δν -1.836, *n* = 282, *r* = 0.96, *s* = 0.22, *F* = 1639, partial *F*-test = 268 (9)

Log *P* is the partition coefficient, Δν is the H-bond acidity, *r* is the correlation coefficient, *s* is standard deviation, and *n* is the number of data points.

The correlation coefficient *r* is a measure of how well the predicted values from a model fit with the actual data. If the correlation coefficient *r* is 0.9 or larger for *in vitro* data, they are significant. The standard deviation *s* is an absolute measure of the quality of fit. If the standard deviation *s* is not much larger than the standard deviation of the biological data—normally around 0.3—the model is acceptable. The *F* value is a measure of statistical significance of the regression model. If *F* values are larger than the 95% significance limit the overall significance of a regression equation is proven [30]. The values of *r*, *s*, and *F* from the statistical analysis of data shown in table 2 confirmed the significance of the regression equations achieved in this study. It was thus revealed that the hydrophobicity is a major contributor to the antimicrobial effect against Gram-positive bacteria and Gram-negative bacteria.

In our study, the structural parameters involved with the antimicrobial activity were not only the partition coefficient but also the H-bond acidity. It was mentioned that the partition coefficient is related to the lipophilicity of the materials and the H-bond acidity is dependent on the polar properties of materials similar to pK_a or the Hammett constant σ. The significance of establishing the correlation with both of the H-bond acidity and log *P* was clearly demonstrated by an increase of the correction coefficient to close to 0.99 from 0.95, as well as of the *F* value for *S. aureus*, as shown in table 2. The other bacterial tests also showed an improvement in the statistical values when two parameters were employed for the correlation.

Partial *F* value was estimated to assess the significance of introducing a new variable. Addition of the H-bond acidity as a structural parameter can be justified if the partial *F* value is larger than 95% significance levels. The partial *F* values estimated exceeded the 95% confidence levels, suggesting that the H-bond acidity and the partition coefficient are primary contributors to the antimicrobial activity. The overall equation for the four bacteria against the antimicrobial agents was also significant: $\log(1/MLC) = 0.670 \log P + 0.0035 \Delta\nu - 1.836$, $n = 282$, $r = 0.96$, $s = 0.22$, $F = 1639$, partial F-test = 268.

A linear free-energy relationship between antimicrobial activity, and the partition coefficient and the H-bond acidity has been demonstrated. The correlations were achieved over a wide range of structural variations and microbes. Twenty-five chemical agents were tested against four bacteria. A wide range of the partition coefficient from 0.73 to 4.52 was covered. Their biological responses, minimum lethal concentrations, varied from 14.51% to 0.01% against *E. coli* and from 10.67% to 0.004% against *S. aureus*.

3.4. Variation of the cut-off points

It has been previously reported that antimicrobial activity of aliphatic alcohols falls off as the alkyl chain increases to more than six carbon atoms [31]. This is the so called “cut-off point,” at which biological activity falls off rapidly or disappears as chain length increases in a homologous series. The cut-off points varied with bacteria. In the case of the Gram-negative bacterium *E. coli*, a clear cut-off point of antimicrobial activity can be seen in fig. 3.a. The activity began to decrease as the partition coefficient increased beyond a point. In contrast, no cutoff point was detected for *S. aureus*, as shown in fig. 3.b. A disappearance of antimicrobial activity was also detected in *P. aeruginosa* and *E. faecalis* tests.

In the case of *P. aeruginosa* the minimum lethal concentration values of *n*-butyl-, benzyl-, phenethyldimethylsilanol and 4-pentyl- and 4-hexyl- phenol were not detected under the condition mentioned earlier. *n*-Butyldimethylsilanol did not achieve 7-log reduction against *E. faecalis*. It was found that *n*-butyldimethylsilanol required more than an hour to show a 7-log reduction against *E. faecalis.* It should be pointed out that all the cut-off points were observed under the limited experimental conditions, *i.e.*, 1-hour exposure time at a given concentration of bacteria in deionized water.

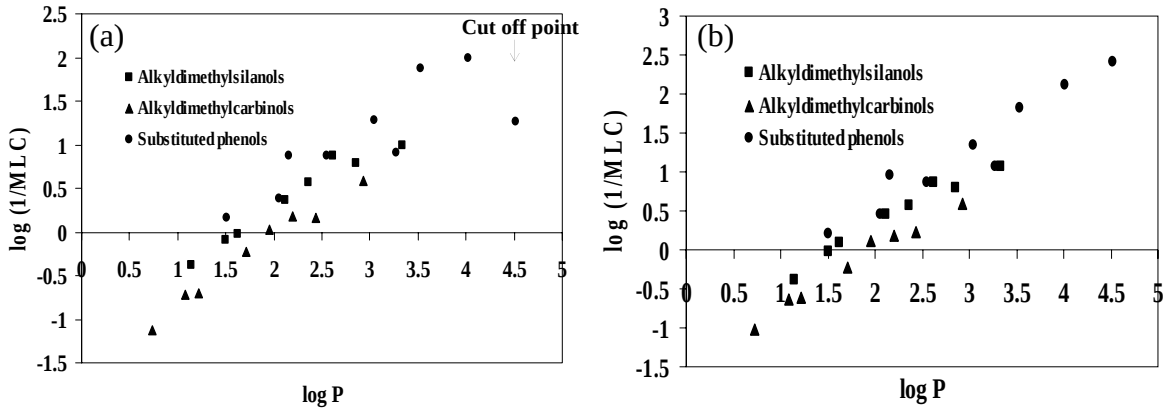

Fig. 3. Correlation between antimicrobial activities and the partition coefficient, (a) *Escherichia coli*, (b) *Staphylococcus aureus*; 25 data points are used, from table 1.

4. Discussion

4.1. Structure and antimicrobial activity relationship

In this study, the hydrogen-bonding acidity as the polar contribution and the partition coefficient as the dispersive component are the key parameters for consideration. The data reported above reveal that the two parameters are primary contributors to the antimicrobial activity. The hydrophobicity of materials is an important parameter with respect to such bioactivity as toxicity or alteration of membrane integrity, because it is directly related to membrane permeation [16]. Hunt also proposed that the potency of aliphatic alcohols is directly related to their lipid solubility through the hydrophobic interaction between alkyl chains from alcohols and lipid regions in the membrane[17]. We suggested that a similar hydrophobic interaction might occur between the alkyl chain of silanols or phenols accumulated in the lipid like nature of the bacteria membranes. As a consequence of their hydrophobic interaction, bacteria lose their membrane permeability, ultimately causing death of the bacteria [16, 17].

Kubo pointed out that antimicrobial effects may be due to a balance between the hydrophilic and hydrophobic portions of the molecule [32–34]. This concept is reasonable because antimicrobial agents having not only a hydrophobic portion but a hydrophilic region or relatively long alkyl chains in their chemical structure showed either reduced or no bioactivities [6]. Condensation by-products, disiloxanes, did not show antimicrobial activity either.

The hydroxyl function of the alcohols is to orient and preferentially localize the materials near the membrane by virtue of hydrogen bonding with ester linkages of fatty-acyl residues and with water molecules [12]. The hydrogen-bonding forces are directly related to the H-bond acidity [23]. It is reasonable to suggest that the higher H-bond acidity of the silanols compared to the analogous alcohols has contributed to a better balance through strong hydrogen bonding.

Even though it is minor, a contribution of the H-bond acidity was clear when we compared agents with lower log *P* and higher H-bond acidity. Alcohols such as

ethyldimethylcarbinol and vinyldimethylcarbinol, having a higher partition coefficient compared to that of trimethylsilanol, demonstrated lower antimicrobial activity as shown in table 1. *n*-Butyldimethylsilanol, with log *P* = 2.85, also showed lower activity than 4-ethylphenol, log *P* = 2.55. The higher activity of trimethylsilanol and 4-ethylphenol appears to be attributed to their higher H-bond acidity, implying that the contribution of the H-bond acidity is significant. It is reasonable to suggest that the silanols may disrupt the cell membrane more efficiently than the analogous alcohols due not only to their higher hydrophobicity but also the higher H-bond acidity.

Hansch reported a number of correlation equations involving antimicrobial activity with alcohols and phenols [8]. For benzyl alcohols against *E. coli*, log (1/*C*) = 0.539 log *P* + 0.531 σ + 4.001, *n* = 14, *r* = 0.939 was observed. For *S. aureus* and *E. faecalis*, log (1/*C*) = 0.599 log *P* + 0.421 σ + 4.069, *n* = 18, *r* = 0.906 was obtained. Substituted phenols tested against *P. aeruginosa* displayed log (1/*C*) = 0.684 log *P* - 0.921 σ + 0.265, *n* = 21, *r* = 0.847 [8]. *C* is the molar concentration of the drug necessary to cause a standard biological response. For the electronic property Hansch used the Hammett σ constant, whereas the H-bond acidity was employed in our study. The value of the Hammett σ constant is dependent on the electronic properties of the substituent *X* relative to the substituted H [8].

The H-bond acidity employed depends not only on the electronic property of the substituents but also the parent molecule. As a result the correlations obtained from the previous studies were limited to a homologous series of chemicals such as substituted phenols or benzyl alcohols individually, and did not allow for combining different series of chemicals. In our study, silanols, alcohols, and phenols were considered as one group of chemicals that contain alkyl chains and a hydroxyl group in their chemical structure. The correlation coefficients acquired from our study as shown table 2 were significant enough to reveal that the antimicrobial activities of silanols, alcohols, and phenols were dependent on their lipophilic nature and their H-bond acidity.

4.2. The cut-off point

There are several concepts to explain the occurrence of the cut-off point. One suggested by Hansch is that the maximum activity of the substituted phenols varies with bacteria [1, 8]. Hansch suggested that it was related to the rate of diffusion of the molecule into the cell. The rate depends upon how strongly molecules are bound by the protein or lipid they interact with in the membrane section. Hansch, *et al.*, concluded that the fall-off in activity with increase in hydrophobicity was due to a slow diffusion rate of molecules strongly bound in the membrane. Consequently, it was not possible to accumulate a sufficient concentration at the reaction site to produce the particular biological response within a test period [1, 8]. Additionally Ferguson suggested that the limiting solubility of materials in the hydrophilic phase led to the fall-off of the activity [1, 35, 36]. Drug molecules will not be able to cross the aqueous-phase barrier of the membrane when the partition coefficient of the drug reaches a point at which a micelle begins to form or drug molecules are localized in the first lipophilic phase of membrane.

Klarmann reported that for Gram-negative bacteria the maximum activity was reached at the amyl derivative of chlorophenol against *Eberthella typhi* and the hexyl derivative

against *Eberthella paradysenteriae*. However, in the case of the Gram-positive bacterium *S. aureus*, maximum activity was observed with the *n*-octyl derivative [7]. Hansch also reported that Gram-positive bacteria showed a higher cut-off point—optimum partition coefficient value = 6—than the value of 4 for Gram-negative bacteria [8]. This author claimed that the lower cut-off point with Gram-negative bacteria was due to their higher lipid content contributing to a higher resistance of the bacteria. Our study showed a similar result that *E. coli* and *P. aeruginosa*, Gram-negative bacteria, showed lower cut-off points than those of the Gram-positive bacteria *S. aureus* and *E. faecalis*. For 4-hexylphenol, the Gram-positive bacteria did not exhibit the fall-off whereas the Gram-negative bacteria, with the same substituents, displayed either reduction or disappearance of antimicrobial activity. Fig. 3. is an example showing the cut-off point against *E. coli* in comparison with *S. aureus*.

5. Conclusion

Antimicrobial activities of alkyldimethylsilanols were measured and compared with their analogous alcohols, alkyldimethylalcohols. Both the higher lipophilic nature of the silanols and the higher H-bond acidity induced enhanced activities of the silanols over their analogous alcohols. A structure–activity dependence study with four bacteria was carried out to determine the relationship between physicochemical properties of silanols, alcohols, and phenols and their antimicrobial activities. In this study silanols, alcohols, and phenols were treated as belonging to the same group of chemicals, which have hydrophobic regions and a hydrophilic portion containing an hydroxyl group. This study revealed that a linear free-energy relationship exists between the partition coefficient and the H-bond acidity and their antimicrobial activities. The cut-off points found appear to vary with tested bacteria.

Acknowledgments

The authors acknowledge the financial support of Air Force Research Laboratory, Tyndall Air Force Base, Florida, the Particle Engineering Research Center (PERC), and Dr. Koopman's microbiology laboratory for characterization and biological activity tests.

References

[1] Hansch C, Fujita T, Rho–Sigma–Pi Analysis . Method for Correlation of Biological Activity + Chemical Structure. *J Am Chem Soc* 1964**; 86:** 1616–1626.

[2] Hansch C, Hoekman D, Leo A, Zhang LT, Li P, "The Expanding Role of Quantitative Structure–Activity Relationships (QSAR) in Toxicology." *Toxicol Lett* 1995; **79:** 45–53.

[3] Daoud NN, Dickinson NA, Gilbert P, "Anti-Microbial Activity and Physicochemical Properties of Some Alkyldimethylbenzylammonium Chlorides." *Microbios* 1983**; 37:** 73–85.

[4] **Klarmann EG, Shternov VA, Gates LW**, "The alkyl derivatives of halogen phenols and their bactericidal action. 1. Chlorophenols. *J Am Chem Soc* 1933**; 55:** 2576–2589.

[5] Suter CM, ”Relationships between the structure and bactericidal properties of phenols.” *Chem Rev* 1941; **28:** 269–299.

[6] Tanner FW, Wilson FL, "Germicidal action of aliphatic alcohols." *Proc soc exp biol med* 1943**; 52:** 138–140.

[7] Klarmann EG, Shternov VA, Gates LW, "Halogen derivatives of monohydroxyl-diphenylmethane and their antibacterial action." *J Am Chem Soc* 1932; **54:** 3315–3328.

[8] Lien EJ, Hansch C, Anderson SM, "Structure–Activity Correlations for Antibacterial Agents on Gram-Positive and Gram-Negative Cells." *J Med Chem* 1968; **11:** 430–441.

[9] Kubinyi H, *QSAR: Hansch Analysis and Related Approaches.* New York: VCH Publishers, 1993.

[10] Kim Y, Farrah S, Baney R, Silanol—"A novel Class of Antimicrobial Agent.**"** *Electron J Biotech* 2006; accepted Sep, 2005.

[11] Graiver D, Farminer KW, Narayan R, "A review of the fate and effects of silicones in the environment." *J Poly Environ* 2003; **11:** 129–136.

[12] Dombek KM, Ingram LO, "Effects of Ethanol on the *Escherichia coli* Plasma-Membrane." *J Bacteriol* 1984; **157:** 233–239.

[13] Lucchini JJ, Corre J, Cremieux A, "Antibacterial Activity of Phenolic-Compounds and Aromatic Alcohols." *Res Microbiol* 1990; **141:** 499–510.

[14] Lucchini JJ, Bonnaveiro N, Cremieux A, Legoffic F, "Mechanism of Bactericidal Action of Phenethyl Alcohol in *Escherichia Coli*." ***Curr Microbiol*** 1993; **27:** 295–300.

[15] Denyer SP, "Mechanisms of Action of Biocides." *International Biodeterioration* 1990; **26:** 89–100.

[16] McKarns SC, Hansch C, Caldwell WS, Morgan WT, Moore SK, Doolittle DJ, "Correlation between hydrophobicity of short-chain aliphatic alcohols and their ability to alter plasma membrane integrity." *Fundam Appl Toxicol* 1997; **36:** 62–70.

[17] Hunt WA, "The effects of aliphatic alcohols on the biophysical and biochemical correlates of membrane function." *Adv Exp Med Biol* 1975; **56:** 195–210.

[18] Kantor SW, "The Hydrolysis of Methoxysilanes—Dimethylsilanediol." *J Am Chem Soc* 1953; **75:** 2712–2714.

[19] Sauer RO, "Derivatives of the Methylchlorosilanes. Trimethylsilanol and its simple ethers." *J Am Chem Soc* 1944; **66:** 1707–1710.

[20] Brook MA, *Silicon in Organic, Organometallic, and Polymer Chemistry:* John Wiley & Sons, Inc., 2000.

[21] Rincon AG, Pulgarin C, "Photocatalytical inactivation of *E. coli*: effect of (continuous–intermittent) light intensity and of (suspended–fixed) TiO_2 concentration."

*App Catal B-Environ 2003***; 44:** 263–284.

[22] Collines CH, Lyne PM, Grange JM, *Microbiological Methods, 7th edition.* Butterworth–Heinemann Ltd., 1995; 151–154.

[23] West R, Baney RH, "Hydrogen Bonding Studies .2. The Acidity and Basicity of Silanols Compared to Alcohols." *J Am Chem Soc* 1959; **81:** 6145–6148.

[24] Kubinyi H, *QSAR: Hansch Analysis and Related Approaches, Vol. 1.* Weinheim, New York, Basel, Cambridge, Tokyo: VCH, 1993.

[25] Meylan WM, Howard PH, "Atom Fragment Contribution Method for Estimating Octanol–Water Partition-Coefficients." *J Pharm Sci* 1995; **84:** 83–92.

[26] Hansch C, Leo A, Unger SH, Kim KH, Nikaitan.D, Lien EJ, "Aromatic Substituent Constants for Structure–Activity Correlations." *J Med Chem* 1973; **16:** 1207–1216.

[27] Hansch C, Rockwell SD, Jow PYC, Leo A, Steller EE, "Substituent Constants for Correlation Analysis." *J Med Chem* 1977; **20:** 304–306.

[28] Owen MJ, "Siloxane Surface-Activity." *Adv Chem Ser* **1990:** 705–739.

[29] Silverman RB, *The Organic Chemistry of Drug Design and Drug Action. 2nd Edition* Elsevier Academic Press, 2004; 51–55.
[30] Livingstone D, *Data Analysis for Chemists- Applications to QSAR and Chemical Product Design.* Oxford, New York, Tokyo: Oxford University Press, 1995.

[31] Rotter ML, "Hygienic Hand Disinfection." *Infect Control Hosp Epidemiol* 1984; **5:** 18–22.

[32] Kubo I, Muroi H, Kubo A, "Antibacterial Activity of Long-Chain Alcohols against *Streptococcus mutans*." *J Agric Food Chem* 1993; **41:** 2447–2450.

[33] Kubo I, Muroi H, Himejima M, Kubo A, "Antibacterial Activity of Long-Chain Alcohols—the Role of Hydrophobic Alkyl-Groups." *Bioorg Med Chem Lett* 1993**; 3:** 1305–1308.

[34] Kubo I, Muroi H, Kubo A, "Structural Functions of Antimicrobial Long-Chain Alcohols and Phenols." *Bioorg Med Chem* 1995; **3:** 873–880.

[35] Burger A, *Medicinal Chemistry Second Edition***.** Interscience Publishers, Inc., New York, 1960; 48–52.

[36] Ferguson J, "The use of chemical potentials as indices of toxicity." *Proc Roy Soc Lond B* 1939; **127:** 387–403.