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Structural Functions of Antimicrobial Long-chain Alcohols and Phenols†

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Abstract—Antimicrobial activity of a series of long-chain alcohols and common naturally occurring alcohols were tested against 15 selected microorganisms in order to gain new insights into their structural functions. The maximum activity seems to depend on the hydrophobic chain length from the hydrophilic hydroxyl group, and also the microorganisms being tested. The results obtained with the alcohols exhibit a generally applicable rule to many other compounds.

Introduction

Alcohols are among the most versatile of all organic compounds. Free and esterified alcohols are known to occur widely in nature, e.g. in fruit.[1] In our continuing search for antimicrobial agents from edible plants, food spices and beverages, a large number of secondary metabolites have been characterized as active principles.[2–4] Among them, many are alcohols. For example, linalool (**1**), geraniol (**2**), nerolidol (**3**), α-terpineol (**4**) and 1-octanol (**5**), which are the five most abundant alcohols in green tea flavor,[5] were found to exhibit antimicrobial activity.[3] In addition, crinitol (**6**) isolated as an antibacterial agent from a marine brown alga, *Sargassum tortile*,[6] is an acyclic diterpene alcohol. The antimicrobial effects of alcohols on microorganisms have been reported. However, studies have been limited to short-chain (< C_6) alcohols, almost exclusively ethanol.[7] Antimicrobial activity of long-chain (> C_6) alcohols, on the other hand, has been demonstrated only with restricted microorganisms because of their limited solubility in water.

Although the alcohols have higher activity compared to the corresponding acids and aldehydes,[8] the difficulty associated with the use of the alcohols as antimicrobial agents is the lack of their potency. Therefore, studies to enhance the activity are needed. Combining alcohols with other substances in order to enhance the total biological activity seems to be a most promising strategic approach to this problem,[4,6,9–13] although the rationale for selecting the 'other substances' is still in a preliminary stage. In order to proceed with this, understanding the effects of alcohols on microbial systems and the biochemical changes involved is essential. This led us to investigate antimicrobial activities of alcohols, using a series of simple aliphatic alcohols as a model in order to gain new insights into their actions on a molecular basis. The current study was focused towards understanding the role of the hydrophobic portion, the alkyl group, since all the molecules possess the common hydrophilic portion, the hydroxyl group. The work has been briefly communicated,[14] and is now described in detail.

Results and Discussion

The study has been conducted primarily with a series of C_6 to C_{20} straight chain alcohols of which the structures consist simply of a head and tail. Moreover, the emphasis has been placed on primary alcohols not only due to their structural simplicity, but also largely because of their availability. The initial aim was to analyze the susceptibility patterns of the 15 selected microorganisms to the 14 long-chain primary alcohols. The microorganisms used for the assay were selected in light of the application of antimicrobial agents from edible plants, food spices, and beverages to food and cosmetic products. The results are listed in Table 1. The alcohols were effective primarily against Gram-positive bacteria, but there was also some activity against yeasts and molds. However, they possessed little or no activity against Gram-negative bacteria. In fact, none of the alcohols tested were active against *Pseudomonas aeruginosa* up to 800 μg mL^{-1}. Gram-negative bacteria seem to be more affected by short-chain (< C_6) rather than long-chain (> C_6) alcohols.[7]

The alcohols of the C_7 to C_{16} chain lengths exhibited activity against all or at least one of the Gram-positive bacteria tested, among which *Propionibacterium acnes* was the most sensitive and *Staphylococcus aureus* was the least. In the case against the latter bacterium, the minimum inhibitory concentrations (MICs) ranged between 12.5 and > 800 μg mL^{-1}, and those of the former were between 0.78 and > 800 μg mL^{-1}. The

†In honor of Professor Koji Nakanishi's seventieth birthday.

Table 1. Antimicrobial activity of the primary alcohols

	MIC, μg mL^{-1}													
	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{20}
B. subtilis	>800	>800	400	200	50	25	12.5	6.25	>800	-	>800	-	>800	>800
B. ammoniagenes	>800	>800	400	200	50	25	12.5	6.25	6.25	6.25	>800	-	>800	>800
S. aureus	>800	>800	800	200	50	25	12.5	>800	>800	-	>800	-	>800	>800
S. mutans	>800	800	400	100	50	25	12.5	6.25	>800	-	>800	-	>800	>800
P. acnes	>800	400	200	100	25	12.5	3.13	1.56	1.56	0.78	0.78	>800	>800	>800
P. aeruginosa	>800	>800	>800	>800	>800	>800	>800	>800	>800	-	>800	-	>800	>800
E. aerogenes	>800	800	>800	>800	>800	>800	>800	>800	>800	-	>800	-	>800	>800
E. coli	>800	800	400	>800	>800	>800	>800	>800	>800	-	>800	-	>800	>800
S. cerevisiae	>800	800	400	100	50	50	>800	>800	>800	-	>800	-	>800	>800
C. utilis	>800	400	200	100	50	25	>800	>800	>800	-	>800	-	>800	>800
P. ovale	800	200	100	100	50	100	100	100	800	>800	>800	-	>800	>800
P. chrysogenum	800	400	200	50	25	12.5	12.5	800	>800	-	>800	-	>800	>800
T. mentagrophytes	>800	400	200	50	12.5	12.5	3.13	800	>800	-	>800	-	>800	>800
A. niger	>800	800	400	200	50	25	>800	>800	>800	-	>800	-	>800	>800
M. mucedo	400	200	100	50	100	200	>800	>800	>800	-	>800	-	>800	>800

- Not tested.

maximum activity against *P. acnes* occurred at 1-pentadecanol (C_{15}) and 1-hexadecanol (C_{16}) with a MIC of both being 0.78 μg mL^{-1}. The most notable observation was that the activity dropped off suddenly above C_{16}. Thus, 1-heptadecanol (C_{17}) no longer showed any activity up to 800 μg mL^{-1}. In the case against *S. aureus*, the optimum activity was found in 1-dodecanol (C_{12}) with a MIC of 12.5 μg mL^{-1}. Again, the activity dropped off suddenly above C_{12} against this bacterium. Thus, 1-tridecanol (C_{13}) no longer exhibited any activity up to 800 μg mL^{-1}.[14] Similarly, the maximum activity against *Bacillus subtilis* and *Streptococcus mutans* occurred at 1-tridecanol (C_{13}) with a MIC of 6.25 μg mL^{-1} for each bacterium, while 1-tetradecanol (C_{14}) no longer showed any activity against both bacteria up to 800 μg mL^{-1}. The optimum activity against *Brevibacterium ammoniagenes* occurred at 1-tridecanol (C_{13}), 1-tetradecanol (C_{14}) and 1-pentadecanol (C_{15}) with each MIC of 6.25 μg mL^{-1}. However, 1-hexadecanol (C_{16}) did not exhibit any activity up to 800 μg mL^{-1}. The maximum activity against the five Gram-positive bacteria tested was found between the C_{12} and C_{16} chain lengths. Noticeably, *P. acnes* was most inhibited by alcohols with chains slightly longer than those inhibiting the other Gram-positive bacteria. This may allow us to better differentiate toxic effects against the specific target bacterium, that is *P. acnes*. Thus, both 1-pentadecanol and 1-hexadecanol showed potent activity against *P. acnes* but not the other Gram-positive bacteria tested. In contrast, 1-dodecanol exhibited activity against all the Gram-positive bacteria tested.

In the case against yeasts, the maximum activity against *Saccharomyces cerevisiae* was found in 1-decanol (C_{10}) and 1-undecanol (C_{11}) with a MIC of both being 50 μg mL^{-1}, and against *Candida utilis* was found in 1-undecanol (C_{11}) with a MIC of 25 μg mL^{-1}. 1-Tridecanol (C_{13}) no longer showed any activity against both yeasts up to 800 μg mL^{-1}. The remaining yeast, *Pityrosporum ovale* was susceptible to a series of alcohols, from the C_6 to C_{14} chain lengths, among which 1-decanol (C_{10}) showed maximum activity with a MIC of 50 μg mL^{-1}. Again, 1-pentadecanol (C_{15}) no longer exhibited any activity against this dermatomycotic yeast up to 800 μg mL^{-1}.

The four molds tested, *Penicillium chrysogenum*, *Aspergillus niger*, *Mucor mucedo* and *Trichophyton mentagrophytes*, were found to be susceptible to the alcohols of the C_6 to C_{13} chain lengths. Among these molds, *T. mentagrophytes* was the most sensitive, while *M. mucedo* was the least. In the case against the food born mold, *A. niger*, the optimum activity occurred at 1-undecanol (C_{11}) with a MIC being 25 μg mL^{-1}. However, 1-dodecanol (C_{12}) did not show any activity up to 800 μg mL^{-1}. The maximum activity against *M. mucedo* was found at 1-nonanol (C_9) with a MIC of 50 μg mL^{-1}. 1-Decanol (C_{10}) and 1-undecanol (C_{11}) still showed activity against this mold at 100 and 200 μg mL^{-1}, respectively, but 1-dodecanol (C_{12}) no longer exhibited any activity up to 800 μg mL^{-1}. *Penicillium chrysogenum* and a dermatomycotic mold, *T. mentagrophytes*, were more sensitive to these alcohols. The optimum activity against *P. chrysogenum* occurred at 1-undecanol (C_{11}) and 1-dodecanol (C_{12}) with a MIC of both being 12.5 μg mL^{-1}, and that against *T. mentagrophytes* was found at 1-dodecanol (C_{12}) with a MIC of 3.13 μg mL^{-1}. The maximum activity against the four molds tested was found between the C_9 and C_{12} chain lengths.

These data indicated that the maximum activity of the carbon chain lengths differed between the microorganisms tested, reflecting differences in their cell-envelope structures.[15] Lien *et al.* reported that the lipophilic character as expressed by octanol–water partition coefficients (log P) is the most important factor in determining the antibacterial activity and the optimum lipophilic character (log P_0) for Gram-positive bacteria was about 6.[15] The log P_0 values of the long-chain alcohols should now be obtained more

specifically to each bacterium. For example, log P_0 for *S. aureus* was found to be 5.1 (reported as log P for 1-dodecanol[16,17]), while that for *P. acnes* was 6.6–7.1 (calculated values for 1-pentadecanol and 1-hexadecanol according to the formulae described by adding 0.5 for each CH_2[17]).

The notable observation was that both yeasts and molds were also inhibited by long-chain alcohols but with somewhat shorter chains than those inhibiting Gram-positive bacteria. Hence, the log P_0 for yeasts and molds should be smaller than 6. The differences in the chemical properties of these alcohols are undoubtedly responsible. They can be classified as nonionic surface-active compounds.

The MIC, which was obtained by measuring the turbidity after 2 days (by naked eye observation after 5 days for molds) of incubation, does not fully characterize the activity. There is no doubt that it would be superior if the activity is lethal rather than growth inhibitory. Therefore, the minimum bactericidal concentrations (MBCs) of several alcohols (C_7–C_{16}) were further investigated. The results are listed in Table 2. With the exception against the spore forming bacterium *B. subtilis*, the alcohols showed bactericidal activity against all the Gram-positive bacteria tested and MBC to MIC ratios were no greater than four in all alcohols tested. In the case against *B. subtilis*, all the alcohols at the concentration up to 800 μg mL^{-1} were not bactericidal. Alcohols are known to have little effect on spores.[18]

In contrast to fatty acids, alcohols possess many more diverse structures. Thus, alcohols are subdivided into three classes: primary, secondary, and tertiary alcohols. All varieties are known to occur in nature. For example, geraniol (**2**), 1-octanol (**5**) and farnesol (**7**) are primary; crinitol (**6**), ipsdienol (**8**) and farnesylacetol (**9**) are secondary; and linalool (**1**), nerolidol (**3**) and α-terpineol (**4**) are tertiary alcohols. Since many naturally occurring alcohols belong to the secondary and tertiary alcohols, some aliphatic secondary alcohols were also tested for comparison, although only limited analogues were available. The results are listed in Table 3. Similar results observed with the primary alcohols were also obtained.

There is an apparent correlation between the activity of the long-chain alcohols and the alkyl chain lengths (tail) from the hydrophilic hydroxyl group (head). This rule seems to be generally applicable to naturally occurring isoprene long-chain alcohols. Table 4 shows antimicrobial activity of common natural alcohols. Among them, farnesol (**7**) exhibited the most potent activity against several Gram-positive bacteria, followed by nerolidol (**3**). This can now be understood since these sesquiterpene alcohols have chain lengths comprised of 12 and 10 carbon atoms from the hydrophilic hydroxyl group, respectively. Moreover, geraniol (**2**) showed more potent activity than linalool (**1**), and the latter exhibited stronger activity than ipsdienol (**8**). This is also evident because the chain lengths from the hydroxyl group decrease in this order ($C_8 \rightarrow C_6 \rightarrow C_5$) as illustrated in Figure 1. Similarly,

Table 2. Bactericidal activity of the primary alcohols against five Gram-positive bacteria

	MBC, μg mL^{-1}									
	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}
B. subtilis	>800	>800	>800	>800	>800	>800	>800	-	-	-
B. ammoniagenes	-	400	200	50	25	25	6.25	6.25	6.25	-
S. aureus	-	800	800	100	25	25	-	-	-	-
S. mutans	>800	800	400	100	100	12.5	6.25	-	-	-
P. acnes	800	400	200	50	25	6.25	3.13	1.56	1.56	1.56

- Not tested since the MIC was > 800 μg mL^{-1}.

Table 3. Antimicrobial activity of the secondary alcohols

	MIC, μg mL^{-1}							
	C_6	C_7	C_8	C_9	C_{10}	C_{12}	C_{14}	C_{16}
B. subtilis	>800	>800	800	400	200	25	>800	>800
B. ammoniagenes	>800	>800	800	400	200	25	>800	>800
S. aureus	>800	>800	>800	800	200	25	>800	>800
S. mutans	800	>800	800	800	100	12.5	>800	>800
P. acnes	800	800	800	400	100	12.5	3.13	0.78
P. aeruginosa	>800	>800	>800	>800	>800	>800	>800	>800
E. aerogenes	>800	>800	800	>800	>800	>800	>800	>800
E. coli	>800	>800	800	>800	>800	>800	>800	>800
S. cerevisiae	>800	>800	800	400	200	>800	>800	>800
C. utilis	>800	>800	400	200	400	>800	>800	>800
P. ovale	>800	400	200	100	100	25	100	>800
P. chrysogenum	>800	800	200	200	50	25	200	>800
T. mentagrophytes	>800	800	400	100	50	6.25	100	>800

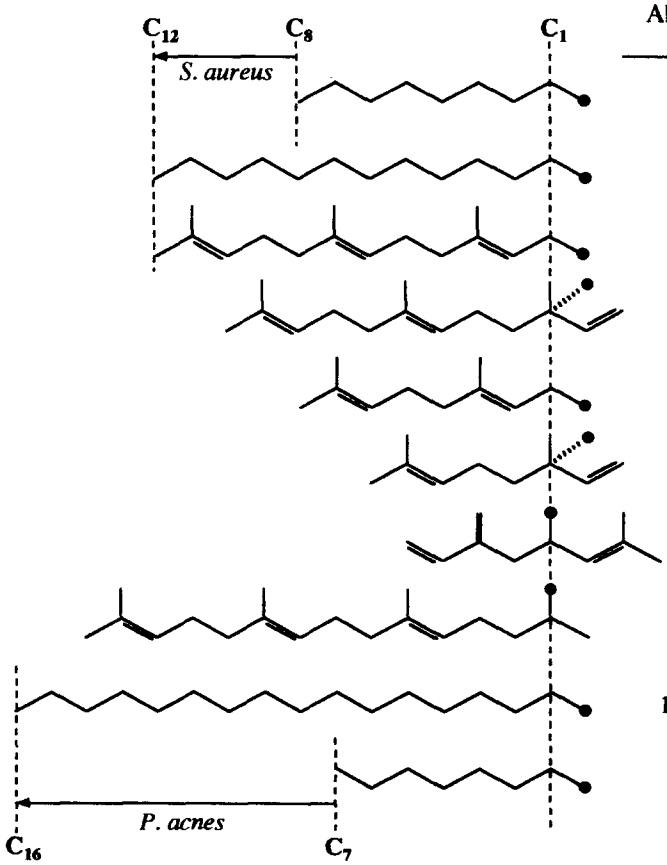
geranylgeraniol (**10**) did not show any activity up to 800 μg mL^{-1}, while crinitol (**6**) showed activity against the Gram-positive bacteria.[6] In the case of these two diterpene alcohols, the chain lengths from the hydrophilic hydroxyl group differed from C_{16} to C_8.

Since the alcohols are known to have a higher activity compared to the corresponding acids and aldehydes,[8] farnesylacetone (**11**) was reduced by $LiAlH_4$ to farnesylacetol (**9**). As a result, while farnesylacetone did not show any activity up to 800 μg mL^{-1}, farnesylacetol exhibited potent activity against *P. acnes* with a MIC of 1.56 μg mL^{-1}. It can be predicted that this MIC value is comparable to that of 2-pentadecanol, the corresponding straight chain alcohol (Table 3). As expected, besides this bacterium, farnesylacetol did not

Table 4. Antimicrobial activity of common naturally occurring alcohols

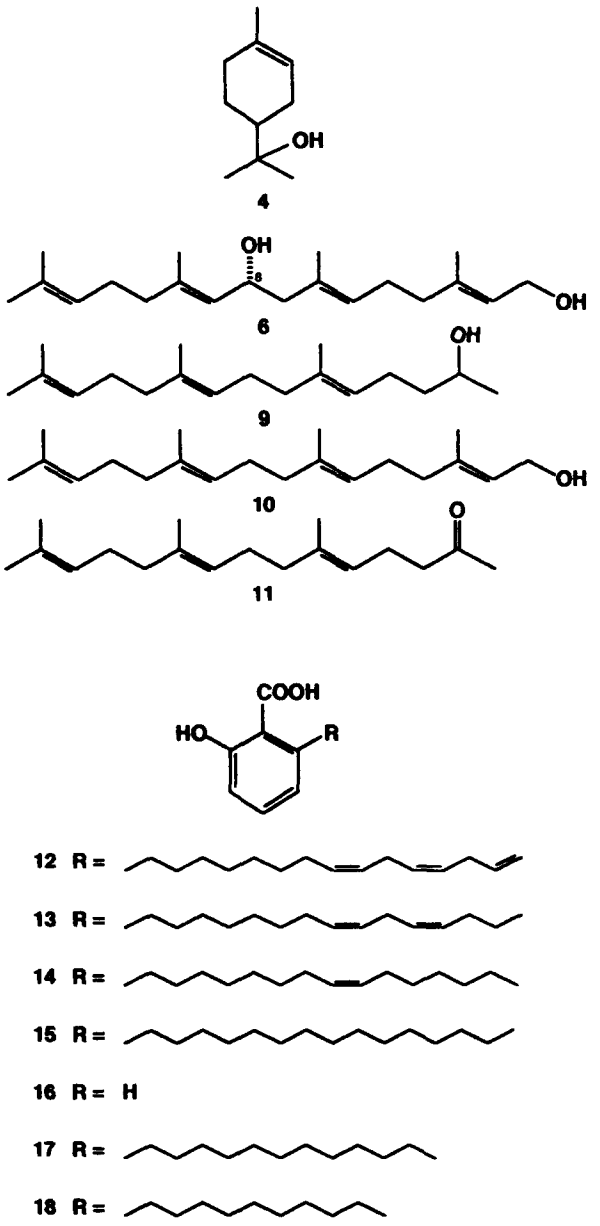
	MIC, μg mL^{-1}									
	7	**3**	**2**	**1**	**8**	**6**	**9**	**22**	**23***	**24**
B. subtilis	12.5	25	400	800	>800	50	>800	1.56	12.5	200
B. ammoniagenes	12.5	25	400	800	>800	100	>800	0.78	12.5	200
S. aureus	25	50	800	>800	>800	400	>800	1.56	12.5	200
S. mutans	12.5	25	400	1600	>800	50	>800	0.78	12.5	200
P. acnes	6.25	25	400	200	200	25	>800	0.39	6.25	200
P. aeruginosa	>800	>800	>800	>800	>800	>800	>800	>800	>100	>800
E. aerogenes	>800	>800	>800	>800	>800	>800	>800	>800	>100	400
E. coli	>800	>800	800	>800	>800	>800	>800	>800	>100	200
S. cerevisiae	>800	>800	400	800	>800	>800	>800	>800	>100	200
C. utilis	>800	>800	400	400	>800	>800	>800	>800	>100	200
P. ovale	>800	800	200	400	400	>800	>800	>800	>100	25
P. chrysogenum	>800	800	200	800	800	>800	>800	>800	>100	200
T. mentagrophytes	12.5	12.5	200	200	400	25	>800	-	6.25	-

- Not tested.
* The highest concentration tested was 100 μg mL^{-1}.



Alcohols tested	MIC (μg/mL) *S. aureus*	MIC (μg/mL) *P. acnes*
1-Octanol	800	200
1-Dodecanol	12.5	3.13
7	25	6.25
3	50	25
2	800	400
1	>800	200
8	>800	200
9	>800	1.56
1-Hexadecanol	>800	0.78
1-Heptanol	>800	400

Figure 1. Antibacterial activity of long-chain alcohols against *S. aureus* and *P. acnes* (●) represents the hydrophilic hydroxyl group, and (←) indicates more potent activity.

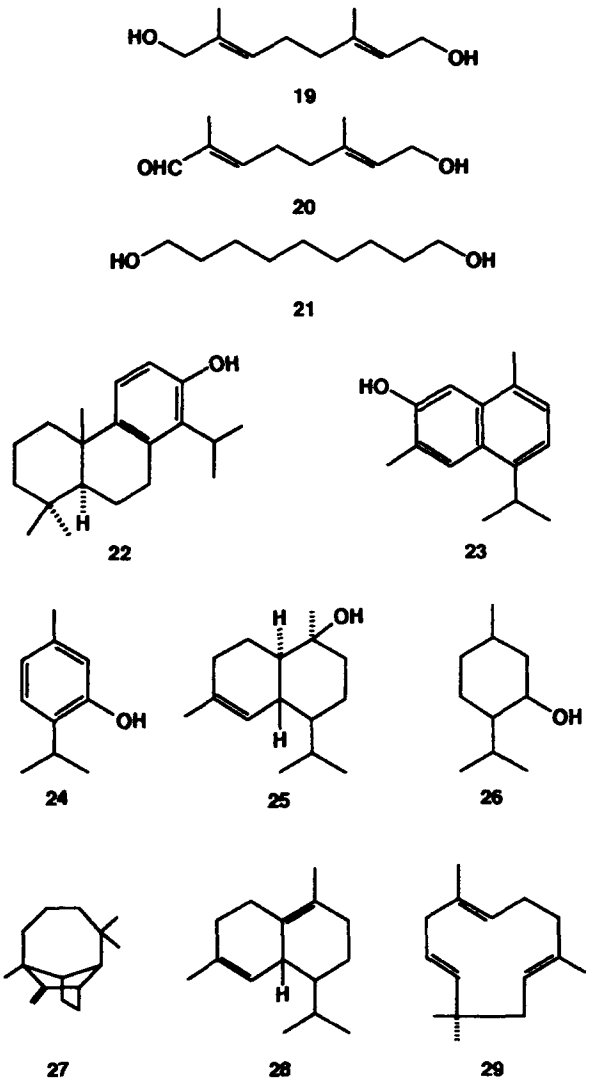


show any activity against the other Gram-positive bacteria tested because its chain length (C_{14}) from the hydroxyl group may be too long as illustrated in Figure 1. Or in other words, its lipophilic character, log *P*, is greater than the maximum value, log P_0.

The head and tail structure and antimicrobial activity relationships observed with long-chain alcohols can be extended to include many other types of compounds. We would like to add some discussion particularly regarding phenolic compounds in this report. Thus, we have recently described similar results based on the comparative study using phenolic compounds, more specifically a series of anacardic acids.[19] Needless to say, phenolic compounds are well known to have antimicrobial activity and some of them have been used in ways such as preservatives in food and cosmetic products, and as disinfectants.[20] However, the comparative study was described based on rather limited molecules. By comparison of the anacardic acids **12–15** isolated from various parts of the cashew *Anacardium occidentale* (Anacardiaceae) fruit, to their parent compound, salicylic acid (**16**), an addition of a C_{15} non-isoprenoid alkyl side chain to **16** resulted in a dramatic change in the antimicrobial activity.[21] Thus, salicylic acid, which has no alkyl side chain, exhibited weak but broad antimicrobial activity against almost all of the microorganisms tested. In contrast, the anacardic acids **12–15** exhibited a narrow spectrum of activity mainly against Gram-positive bacteria, but this activity is much more potent than that of salicylic acid.[21] For example, the activity against *S. mutans* of the anacardic acid, 6-[8(*Z*),11(*Z*),14-pentadecatrienyl]salicylic acid (**12**) was 2048-times more effective than salicylic acid (**16**). This suggested that an alkyl side chain plays an important role in increasing the antibacterial activity. To understand this role, in addition to natural anacardic acids **12–15**, a series of their analogs with different side-chain lengths were synthesized and assayed for comparison. Similar results as described with long chain alcohols were also observed. For example, in the case against *S. mutans* and *P. acnes*, the anacardic acid **17** having the C_{12} alkyl side chain was found to be most active with MICs of 1.56 and 0.39 μg mL^{-1}, while against *S. aureus*, the anacardic acid **18** possessing the C_{10} alkyl side chain was found to be most effective with a MIC of 3.13 μg mL^{-1}.[19] This indicates that the lipophilic character, log P_0, for each bacterium seems to be somewhat different.

In addition, two alcohols **19** and **20** obtained as intermediates during synthetic study of crinitol (**6**) as well as 1,9-nonanediol (**21**) were also tested against the same 15 microorganisms for comparison. None of them showed any activity up to 800 μg mL^{-1}. Hence, it appears that a head and tail structure is essential to have activity. A similar observation with totarol (**22**), characterized as an antibacterial agent from the root bark of *Podocarpus nagi* (Podacarpaceae), and its derivatives has been previously reported. Totarol exhibited potent antibacterial activity against the five Gram-positive bacteria but this activity was completely lost when the 4β-methyl group was oxidized.[13]

These results obtained so far were based largely on a series of the simple saturated long-chain alcohols. The position, number and stereochemistry of double bonds also seems to affect the activity in some way. Although unsaturated long-chain alcohols are rare in nature, the above mentioned anacardic acids **12–15** isolated from the cashew fruit provide us with an excellent example on this subject. These anacardic acids possess a C_{15} alkyl side chain with zero to three double bonds. Importantly, these double bonds are all *cis* configuration except the exomethylene group in **12**. Gellerman *et al.* previously reported that a decrease in the number of double bonds in the side chain of anacardic acids decreases the antibacterial activity against Gram-positive bacteria.[22] Our data also indicated a similar result with the exception against *P. acnes*.[19] The C_{15} saturated hydrocarbon side chain in **15** can be assumed to be in the extended form since it



requires the least amount of energy. The unsaturated hydrocarbon side chain in **14**, on the other hand, has a bend of about 30°, imposed on the molecule by the *cis* configuration of the double bond. Two double bonds in **12** and **13** in *cis* configuration create more bends and significantly shortens the length of the molecule. These conformational features of unsaturated side chains with their bends and shorter lengths, creates more disorder in the fluid bilayer membrane, resulting in more potent activity. For example, the MICs against *S. mutans* differed greatly between $C_{15:1}$ anacardic acid **14** and $C_{15:0}$ anacardic acid **15**. More precisely, **15** did not exhibit any activity against this cariogenic bacterium up to 800 μg mL^{-1}, while the MIC of **14** was as low as 3.13 μg mL^{-1}.[19] In addition, a similar result has recently been reported with long chain unsaturated fatty acids and their antibacterial activity against two strains of *S. aureus* including a methicillin-resistant *S. aureus* (MRSA).[23] Thus, stearic acid ($C_{18:0}$) did not exhibit any activity up to 400 μg mL^{-1} while linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$) showed activity. Needless to say, the double bonds in these unsaturated fatty acids are known as *cis* configuration. The location of double bonds to obtain the maximum activity remains to be investigated.

In addition, the volume of the hydrophobic portions also seems to be related to the activity. For example, a bicyclic 7-hydroxycadalene (**23**) isolated from the dried flowers of a Mexican medicinal plant, *Heterotheca inuloides*,[24] exhibited stronger activity than the monocyclic thymol (**24**), while a tricyclic totarol (**22**) showed even more potent activity as shown in Table 4. Thus, the monocyclic thymol exhibited weak but broad antimicrobial activity against almost all of the microorganisms tested. In contrast, the tricyclic totarol exhibited a narrow spectrum of activity only against Gram-positive bacteria,[13] but this activity was dramatically increased compared to that of thymol. In addition, a cyclic alcohol, α-cadinol (**25**) (a bicyclic sesquiterpene) exhibited more potent activity against Gram-positive bacteria than menthol (**26**) (a monocyclic monoterpene). For example, the MIC of α-cadinol for *S. aureus* was 12.5 μg mL^{-1} while that of menthol was 800 μg mL^{-1}. However, β-sitosterol and cholesterol did not show any activity up to 800 μg mL^{-1}. Notably, when a molecule possesses a certain volume, it shows some activity without the hydrophilic portion in the molecule. For example, sesquiterpene hydrocarbons, longiforene (**27**), δ-cadinene (**28**) and α-humulene (**29**) exhibited weak activity against *S. mutans*[25] and potent activity against *P. acnes*.[26] The role of the molecule's volume in the activity remains to be investigated.

The mechanisms of antibacterial activity of long-chain alcohols are due to a balance between the polar hydrophilic (head) and nonpolar hydrophobic (tail) portions of the molecule. The hydrophobic portion of the molecule seems to be the important constituent since all the compounds tested possess the common hydrophilic hydroxyl group with various hydrophobic alkyl groups. Their activities differed significantly. The fluidity of the cell membrane can be disturbed maximally by hydrophobic compounds of particular hydrophilic hydroxyl groups. They could enter the molecular structure of the membrane with the polar hydroxyl group oriented into the aqueous phase by hydrogen bonding and nonpolar carbon chain aligned into the lipid phase by dispersion forces. If so, interestingly, why does the addition of CH_2 make a big difference in their activity? For example, in the case of *S. aureus*, the optimum activity against this bacterium was found in 1-dodecanol (C_{12}) with the MIC of 12.5 μg mL^{-1} but no activity was observed in 1-tridecanol (C_{13}) up to 800 μg mL^{-1}. The same rapid drop off was observed with almost all the other microorganisms tested. It is not illogical to suppose that a hydrophobic chain length greater than that for the maximum activity disperses into the lipid layer, resulting in the breaking of the hydrogen bond. Thus, when the balance between hydrophilic and hydrophobic portions of the alcohol was destroyed by the dispersion forces, or in other words when the hydrophobic character, log *P*, exceeded the maximum value, log P_0, the activity disappeared. This may be the answer to the above question. Needless to say, more work is needed to prove this hypothesis. Their modes of action, especially involving the membrane

remains much to be explored. Nevertheless, accumulation of this kind of knowledge will provide a more rational and scientific approach to design safe but as yet efficient antimicrobial agents.

The antimicrobial activity of long-chain alcohols is usually slightly weaker compared to that of phenols. This may be explained by other parameters such as electron density, especially on the oxygen atom. The role of the hydrophilic part to the activity will be described separately.

Among the alcohols tested, some of them may be potent enough for further investigation involving, for example, the modes of action and combinations with other substances. The following is an example. The combination of the above mentioned two compounds, farnesol (**7**) and anacardic acid **12**, was found to show synergistic activity by the broth checkerboard method.[26] Thus, the latter was found to increase the antibacterial activity of the former against *P. acnes*. More specifically, in combination with 0.39 μg mL^{-1} of anacardic acid, the MIC of farnesol was lowered from 6.25 to 0.78 μg mL^{-1}. This synergism was found to be *vice versa*; the MIC of anacardic acid was reduced from 0.78 to 0.2 μg mL^{-1} when it was combined with 3.13 μg mL^{-1} of farnesol.[26] The mode of action of this combination, however, remains unclear.

In conclusion, the long-chain alcohols are effective against all the Gram-positive bacteria, yeasts and molds tested, but not as much against Gram-negative bacteria. Some of these alcohols can be considered for practical use. For example, since some alcohols have already been used as flavor components in common foods such as chewing gum, candy and baked goods,[27] their use, especially 1-dodecanol which showed the maximum activity against *S. mutans*, for oral care products as anticavity agents should be admissible. They can now be considered, in addition to their use as flavor and fragrance, to serve an antimicrobial function.

Experimental

Chemicals

All the C_6 to C_{20} straight chain alcohols and 1,9-nonanediol used for the study were purchased from Aldrich Chemical Co. (Milwaukee, WI). These alcohols were used for the assay without purification. Linalool, geraniol, nerolidol, geranylacetone, crinitol, geranylgeraniol, totarol and 7-hydroxycadalene were from our previous studies.[3,6,13,24] Farnesol, ipsdienol, thymol and menthol were obtained from Sigma Chemical Co. (St Louis, MO), Bedoukian Research Inc., (Danbury, CT) and Aldrich Chemical Co., respectively. The two alcohols **19** and **20** were synthetic intermediates from the total synthesis of crinitol (to be published). *N,N*-Dimethylformamide (DMF) was purchased from EM Science (Gibbstown, NJ).

Microorganisms and media

All microorganisms used for the assay were purchased from American Type Culture Collection (Rockville, MD). They are *B. subtilis* ATCC 9372, *B. ammoniagenes* ATCC 6872, *S. aureus* ATCC 12598, *S. mutans* ATCC 25175, *P. acnes* ATCC 11827, *Escherichia coli* ATCC 9637, *P. aeruginosa* ATCC 10145, *Enterobacter aerogenes* ATCC 13048, *S. cerevisiae* ATCC 7754, *C. utilis* ATCC 9226, *P. ovale* ATCC 14521, *P. chrysogenum* ATCC 10106, *T. mentagrophytes* ATCC 18748, *A. niger* ATCC 16404, and *M. mucedo* ATCC 20094.

The culture medium for the bacteria consisted of 0.8% nutrient broth (BBL), 0.5% yeast extract (Difco) and 0.1% glucose, with the exception of *S. mutans*. For the culture of *S. mutans*, 3.7% brain heart infusion broth (Difco) was used. The culture medium for the yeasts and molds was 2.5% malt extract broth (BBL), with the exception of *P. ovale* and *T. mentagrophytes*. For the culture of *P. ovale*, 1% bactopeptone (Difco), 0.5% yeast extract, 1% glucose and 0.1% corn oil were used, and for *T. mentagrophytes*, 1% bactopeptone and 4% glucose were utilized.

Antimicrobial assay

The highest concentration tested was 800 μg mL^{-1}, unless otherwise specified, because of their limited solubility in the water based media. The solubility limitation in water also limits selection of assay methods for their evaluation. For example, the paper disk method is not relevant since water insoluble substances do not diffuse into the media. This has made the past study limited. Throughout this experiment the broth dilution method was employed. The test compound was first dissolved in DMF and serial two-fold dilutions were performed in DMF, and then 30 μL of the sample solution was added to sterile media resulting in 1% DMF concentration which did not affect the growth of any of the microorganisms employed. The growth test tube was inoculated with 1% of a 2-day-old culture of the test organisms (5-day-old for molds) and then incubated at 30 or 37 °C. All microorganisms were cultured stationary except molds, which were cultured with shaking. After 2 days of cultivation (3 days for *P. ovale* and 5 days for molds), the growth of the microorganisms, except *P. ovale* and molds, was examined by turbidity (OD at 660 nm). That of *P. ovale* and molds was examined with the naked eye. The MIC was the lowest concentration of the test compound that completely prevented growth.

The bactericidal effects of some alcohols were examined against Gram-positive bacteria. After determining the MIC, a 30 μL aliquot was taken from each clear tube and added into 3 mL of the alcohol-free fresh medium. After 2 days of incubation, the MBC was determined as the lowest concentration of the alcohol in which no recovery of bacteria was observed.

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