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ANTIBACTERIAL ACTIVITY OF LONG-CHAIN ALCOHOLS: THE ROLE OF HYDROPHOBIC ALKYL GROUPS

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Abstract: The role of hydrophobic alkyl groups of long-chain alcohols to antibacterial activity against *Staphylococcus aureus* and *Propionibacterium acnes* is discussed. The maximum activity was found to depend on the chain length from the hydrophilic hydroxyl group, and also the bacteria tested.

In our continuing search for antimicrobial agents, a number of secondary metabolites have been characterized in various plants.[1-3] Based on continuing accumulation of this kind of data, we have become aware of an ambiguous rule in their structure-activity relationships. Thus, antimicrobial activity seems to be due to a balance between the hydrophilic and hydrophobic portions of the molecule. In order to gain new insights into the effect of the hydrophobic portion to the activity, a series of long-chain alcohols was studied. The hydrophilic hydroxyl group is common among all so that only the hydrophobic alkyl groups need to be investigated. This selection was based not only on their structural simplicity, but also largely on their availability. Although the 16 selected microorganisms [1-4] were tested, the two Gram-positive bacteria, *Staphylococcus aureus* ATCC 12598 and *Propionibacterium acnes* ATCC 11827, are discussed in this communication to make the story simple.

Among the Gram-positive bacteria tested, *P. acnes* was the most sensitive and *S. aureus* was the least. The maximum activity against the latter bacterium occurred at 1-dodecanol (C_{12}) with the minimum inhibitory concentration (MIC)[5] of 6.25 μg/ml, while that against the former bacterium occurred at 1-pentadecanol (C_{15}) and 1-hexadecanol (C_{16}) with the MICs of both being 0.78 μg/ml, as listed in Table 1. Notably, 1-dodecanol also exhibited activity against two strains of methicillin resistant *S. aureus* (MRSA) with the same MICs. The maximum activity of the chain lengths differed between the microorganisms being tested.[6] The C_8 to C_{12} chain lengths exhibited activity against *S. aureus*, while the C_7 to C_{16} showed activity against *P. acnes*, as illustrated in Figure 1. Thus, *P. acnes* was affected by more compounds with chain lengths longer than those affecting the other Gram-positive bacteria. A similar result was also obtained with 6-alkylsalicylic acids; *P. acnes* was the most sensitive while *S.*

aureus was the least susceptible. Thus, 6-decylsalicylic acid was the most active against the latter bacterium with the MIC of 3.13 μg/ml, while 6-pentadecylsalicylic acid was the most effective against the former bacterium with the MIC being 0.78 μg/ml.[7] This may allow us to differentiate toxic effects against the specific target microorganism, *P. acnes*.

The results seem to be generally applicable to naturally occurring isoprene long-chain alcohols, among which, for example, a sesquiterpene alcohol, farnesol (**1**), can easily be selected as a potent antibacterial agent against *S. aureus* since its structure is comprised of the C_{12} chain length which was found to exhibit the maximum activity. A similar sesquiterpene alcohol, nerolidol (**2**), exhibited less activity against this bacterium since its chain length consisted of C_{10}, while a monoterpene alcohol, linalool (**3**) of which chain length consisted of C_6 did not show any activity. Similarly, a monoterpene alcohol, geraniol (**4**) of which chain length comprised of C_8, showed even less effective than nerolidol against *S. aureus*.[8] Thus, the MICs of these terpene alcohols are comparative to the corresponding straight chain alcohols. Interestingly, an acyclic diterpene alcohol, crinitol (**5**) isolated as an antibacterial principle from a marine alga,[9] showed activity with the same MIC of 1-octanol since the chain length from the C-9 hydroxyl group of crinitol consisted of C_8.

In addition, since alcohols are known to have a higher activity compared to the corresponding acids or aldehydes,[10] farnesylacetone (**6**) was reduced by $LiAlH_4$ to farnesylacetol (**7**) and tested. As a result, farnesylacetol exhibited potent activity against *P. acnes* with the MIC of 1.56 μg/ml, while farnesylacetone did not show any activity up to 800 μg/ml. As expected, this alcohol did not show any activity against *S. aureus* because its chain length comprised of C_{14} which exceeds the maximum chain length of C_{12} for this bacterium. On the other hand, geranylacetol (**8**) which comprised of C_{10} showed activity against both bacteria.

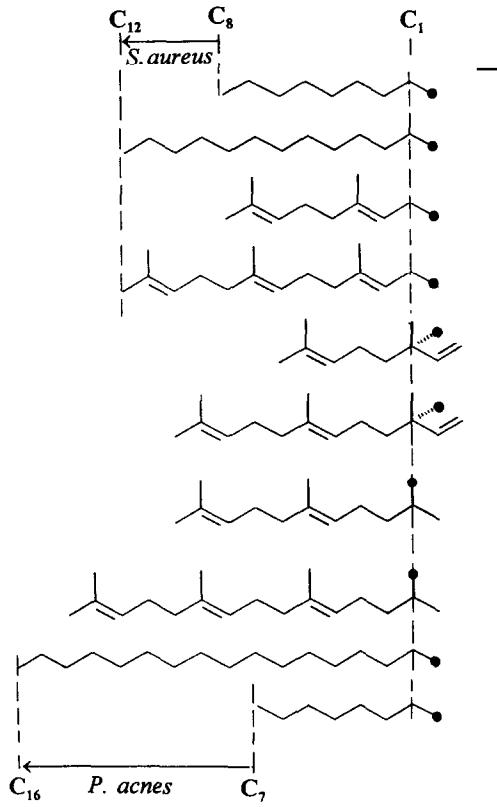
The above discussion is based on the MIC values, however, the MIC, which is determined by measuring turbidity after 48 hrs of incubation, does not fully characterize the activity. Therefore, 1-dodecanol and 1-hexadecanol were tested by the time-kill curve method against *S. aureus* and *P. acnes*, respectively. As a result, the alcohols were confirmed as bactericidal. The ratio between the MIC and minimum bactericidal concentration (MBC) did not differ more than two-fold.

Noticeably, the activity dropped off suddenly above the MIC chain lengths. For example, as mentioned above, 1-dodecanol (C_{12}) was the most effective against *S. aureus* with the MIC of 6.25 μg/ml, while 1-tridecanol (C_{13}) did not show any activity up to 800 μg/ml. Thus, the carbon chain length for the maximum activity against *S. aureus* should be less than C_{13} but as close to C_{13} as possible. The same rapid drop off was observed with almost all the other microorganisms tested. The fluidity of the cell membrane can be disturbed maximally by the lipophilic alcohols 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 the nonpolar carbon chain aligned into the

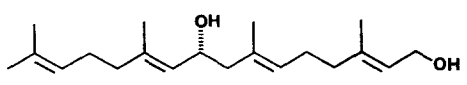
Table 1. Antibacterial activity of 14 long-chain alcohols against *S. aureus* and *P. acnes*.

Alcohols	MIC (μg/ml)		Alcohols	MIC (μg/ml)	
	S. aureus	*P. acnes*		*S. aureus*	*P. acnes*
1-Hexanol	>800	>800	1-Tridecanol	>800	1.56
1-Heptanol	>800	400	1-Tetradecanol	>800	1.56
1-Octanol	800	200	1-Pentadecanol	>800	0.78
1-Nonanol	200	100	1-Hexadecanol	>800	0.78
1-Decanol	50	25	1-Heptadecanol	>800	>800
1-Undecanol	25	12.5	1-Octadecanol	>800	>800
1-Dodecanol	6.25	3.13	1-Eicosanol	>800	>800

Figure 1. Structure-activity relationship of long-chain alcohols, (•) represents the hydrophilic hydroxyl group, and (←) indicates more potent activity.



Alcohols tested	MIC (μg/ml)	
	S. aureus	*P. acnes*
1-Octanol	800	200
1-Dodecanol	6.25	3.13
4	800	400
1	6.25	6.25
3	>800	>800
2	50	25
8	50	25
7	>800	1.56
1-Hexadecanol	>800	0.78
1-Heptanol	>800	400



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lipid phase by dispersion forces.[10] If so, it may not be illogical to suppose that a lipophilic chain length greater than the chain length of the MIC disperses more easily into the lipid layer, resulting in the breaking of the hydrogen bond. Thus, when the balance between the hydrophilic and hydrophobic portions of the compounds was destroyed by the dispersion forces, the activity disappeared.

The position, number and stereochemistry of double bonds seem to affect the activity in some way.[11,12] Obviously, more work is needed to clarify this. The results obtained so far are somehow similar to those of long-chain fatty acids.[11-13] In contrast to fatty acids, alcohols have many more diverse structures. Most significantly, cyclic alcohols occur more commonly in nature. The volume of the hydrophobic portions seems to affect the activity in some way. However, the role of the molecules' volume to the activity remains to be studied.

References and Notes

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5. The highest concentration tested was 800 μg/ml because of solubility limitation in the water based media. The MICs were determined by the broth dilution method.[1-4]
6. Although the data is not shown here, both yeasts and fungi were also affected by long-chain alcohols but with somewhat shorter chains than those affecting Gram-positive bacteria. They possess little or no activity against Gram-negative bacteria which seem to be more affected by short-chain (C_6) rather than long-chain alcohols.[14]
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