Calorimetric Study of the Antimicrobial Action of Various Polyols Used for Cosmetics and Toiletries

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(Received Nov. 24, 1998, Accepted Jan. 9, 1999)

The inhibitory effect of some polyols which are added in various cosmetics and toiletries as humectants, emulsion stabilizers and for some other purposes against the growth activity of *Escherichia coli* in liquid media was studied by a calorimetric technique. The polyols studied were 1,2-ethanediol (ethylene glycol), 1,2-propanediol (propylene glycol), 1,2-butanediol (1,2-butylene glycol), 1,3-butanediol (1,3-butylene glycol), 1,2,3-propanetriol (glycerol), 1,2-pentanediol (1,2-hydroxypentane), 3-(3-hydoxypropanoxy)propan-1-ol (di(propylene glycol)) and polyethylene glycol. The growth thermograms observed in the absence and the presence of increasing amounts of the polyols were analyzed to determine the inhibitory parameters, the polyol concentration which inhibits the growth of the microbes by 50 % (K_μ or K_θ) and the concentration which completely prevents the growth (MIC_μ or MIC_θ). All of the polyols studied were found to exert the inhibitory effect with the 50 % inhibitory concentrations between 1 ~ 17 % (W/V) for *E. coli*. It was also shown that the apparent affinity of the polyols to the microbial cell calculated on the basis of the Gibbs equation linearly increases with the length of their alkyl chains, indicating that the inhibitory effect of the polyols is related to their hydrophobicity.

1. Introduction

Two different problems arise when preservatives and disinfectants are used in cosmetics and toiletries. First is that microorganisms easily contaminate the cosmetics and toiletries when the amounts of antimicrobial agents are kept low for safety and economy, and second is that serious problems of skin reactions produced by antimicrobial agents are caused when their amounts are increased for preventing microbial contamination. In any case, only the minimum effective quantities of antimicrobial agents must be employed. If some of the

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This work was conducted as a part of the cooperative research programs (Projects #98025 and #98034) at the Center for Research and Development of Bioresources, Research Institute for Advanced Science and Technology, Osaka Prefecture University.

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ingredients used in cosmetics and toiletries for other purposes had some antimicrobial activity or increased the activity of antimicrobial agents, it would be possible to reduce the required amounts of antimicrobial agents.

In our preceding paper[1] we reported that the antimicrobial actions of 1,3-butanediol and 1,2-pentanediol exert a significant inhibitory effect against the growth activity of various microbial strains. Other than these two alcohols, polyols are widely added in various cosmetics and toiletries as humectants, which is their fundamental function, solvents, emulsion stabilizers, and so on. It was reported that some polyols, for example propylene glycol or 1,3-butanediol, when used in high concentrations in patch tests under closed conditions produced certain levels of skin reaction[2]; however these levels are low in comparison with other chemical compounds. Polyols are considered safe for use in cosmetics and toiletries mainly under open conditions.

Our previous findings on the siginificant antimicrobial effect of 1,3-butanediol and 1,2-pentanediol[1] which are used for their properties mainly as solvents in cosmetics and toiletries suggest that there might be a possibility that other polyols widely used may also have an antimicrobial activity.[3,4] In this context, the antimicrobial actions of various polyols, mainly diols, whichi are commonly used in cosmetics and toiletries have been investigated by a microbial calorimetry technique and the results obtained are reported below.

2. Materials and Methods

All microorganisms employed in this study were stock cultures of Mandom Central Research Laboratory, Osaka, Japan. *Escherichia coli* IFO 3972, *Saccharomyces cerevisiae* IFO 0234 and *Aspergillus niger* ATCC 6275 were employed as representative strains of bacteria, yeasts and fungi, respectively.

1,2-pentanediol was the product of Dragoco Japan. Ethylene glycol, propylene glycol, 1,2-butanediol, 1,3-butanediol, di(propylene glycol) and glycerol were obtained from Wako Pure Chemical Industries, Ltd. Polyethylene glycol 200 was the product of Sanyo Chemical Industries, Ltd. All of these chemicals were of certified reagent grade.

Brain heart infusion broth (purchased from Wako Pure Chemical Industries, Ltd., Japan) was used as a liquid growth medium for bacteria (containing 7.5 $g\,l^{-1}$ beef brain extract, 8.0 $g\,l^{-1}$ beef heart extract, 10.0 $g\,l^{-1}$ peptone, 2.0 g l-1 glucose, 5.0 $g\,l^{-1}$ NaCl, 2.5 $g\,l^{-1}$ KH_2PO_4, pH 7.2). Glucose-peptone broth (obtained from Nissui Pharmaceutical Co., Ltd., Japan; abbreviated GPB below when necessary) was employed as a liquid growth medium for yeast and fungus (containing 20 $g\,l^{-1}$ glucose, 2 $g\,l^{-1}$ yeast extract, 1.5 $g\,l^{-1}$ $MgSO_4$, 5 $g\,l^{-1}$ polypeptone, 1 $g\,l^{-1}$ KH_2PO_4, pH 5.6).

For each strain the experimental procedure was applied as follows. In case of bacteria, cells taken from the stock culture were preincubated at 37 ℃ for 20 hours. After that, the culture was diluted with 0.85 % sterile physiological saline in order to obtain initial cell number of the order of 10^6 cells ml^{-1} which was checked by CFU counting. In the same way, yeast cells taken from the stock culture were preincubated at 30 ℃ for 20 hours. In this case, the preincubated culture was not diluted and the very thing was employed in order to obtain initial cell number of the order of 10^4 cells ml^{-1}. In case of fungi, sporules were taken 5 times with the tip of the inoculating needle and put into 9 ml 0.85 % sterile physiological saline. The cell suspensions obtained as described above were employed as inocula.

Glass vials of 30 ml volume were sterilized by dry heat sterilization and then a total of 5 ml of culture broth were aseptically added in each vial. These 5 ml were composed of: 1 ml of growth medium (prepared five times more concentrated than the value intended in the actual culture), plus various amounts of other solutions of chemicals, sterilized by passing through a 0.2 μm filter. Usually, two vials were prepared with the same concentration of each chemical, in order to assess the reproducibility of the growth process easily.[5] Then, each vial was inoculated with 50 μl of the microbial solution. Sets of 24 vials thus prepared were introduced in the calorimetric units and incubated at 30 ℃ until the calorimetric signal of all samples monitored by the calorimeter returned to baseline, *i.e.* until growth ceased in all vials.

Calorimeter used for the observation of growth was a multiplex isothermal batch calorimeter having 24 calorimetric units.[5] The growth activity of the microorganisms was monitored by detecting the metabolic heat evolved during their growth in liquid medium. The calorimeter had the sensitivity $A_s = 14.2\ \mu W\ \mu V^{-1}$

at steady heat effect; the output signal obtained is expressed in voltage units and can be transformed into units of heat by multiplication with the parameter β = 5.9 mJ μV^{-1}. One growth experiment can provide enough data for a good estimation of inhibitory parameters, such as the 50 % inhibitory concentration and minimum inhibition concentration. However, during this work two or more experiments were performed for each set of conditions. Details of the apparatus were described elsewhere [5] and the experimental procedures employed were substantially the same as those described in the preceding paper.[1]

3. Results and Discussion

Figure 1 shows three typical example of growth thermograms, hereafter also named $g(t)$ curves, provided by the calorimeter during* growth of cultures at 30 ℃ in the presence of various concentrations of polyols. As can be understood from **Fig.1**, the pattern of the thermograms depends on the polyol concentration. As a general characteristic, with increasing the amount of polyols in the medium, the growth thermograms significantly broadened, *i.e.* their initial shape decreased and the peak height shifted towards longer incubation times.

However, these thermograms represent only the apparent calorimetric output, because the calorimeter is of the conduction type and heat exchange takes place between the calorimetric cell and the surroundings through the wall of the calorimetric unit. Accordingly, for each $g(t)$ curve an actual heat evolution curve, namely $f(t)$, is determined after correction for the heat exchange, using the previously reported equation[5-8];

$$f(t) = g(t) + K \int g(t)\mathrm{d}t \qquad (1)$$

where K is the heat conduction constant of the apparatus. The values of K for each calorimetric unit were determined by the previously reported method [5,8].

In previous works it was shown that the amount of heat generated by microorganisms $f(t)$ during the exponential growth phase can be expressed as an exponential function given by the equation [8-11] ;

$$f(t) = A_0N_0 \exp(\mu t) + B_0N_0 \qquad (2)$$

where μ is the growth rate constant, N_0 is the number of viable cells at the start of the measurement (the inoculum size) and A_0 and B_0 are constants.

It has been well established that a good correlation

* One of the referees of this paper suggested that the unit of calorimetric signal in **Fig.1** should be given in mW rather than μV. The Interunion Commission on Biothermodynamics certainly made the decision (*Pure & Applied Chemistry* **54**, 671-679 (1982).) that the power unit must be used to show the amplitude of calorimetric signals when the growth behavior of microbes is observed with a calorimeter. We disagree with this decision for the reason given below. If a heat evolution process is observed in a calorimeter with a very short time constant, the calorimeter output signal should be very close to the thermogenesis expressed in watts. However, what is actually observed with any calorimeter is the temperature difference between the calorimeter and its surroundings (or the reference vessel), and not the "power, $\mathrm{d}Q/\mathrm{d}t$". The signal is proportional to the power only in the particular case where the instrumental time constant of the calorimeter used is practically zero (the heat conduction constant is infinite). This relation will be clear if the use of an adiabatic calorimeter is considered. Because the heat conduction constant of the ideal adiabatic calorimeter is practically zero, the heat evolution results in a rise in the temepratrue of the calorimetric vessel, the extent of which is directly proportional to the amount of heat evolved. The calorimetric signal (deg or K) gives the amount of heat evolved (J) when multiplied by the heat capacity (J deg^{-1} or J K^{-1}) of the system. Strictly speaking, with actual calorimeters, the signal (K) is converted to the power unit by the following procedure: the signal (K) is converted to units of power by correction for heat leakage and conversion to Joules, followed by differentiation with respect to time (s) to give the "power (J s^{-1} = watts)". In other words, simple graphic integration of the so-called "power-time-curve" does not give a true thermokinectic curve, and we believe that the use of the power unit to express the amplitude of calorimetric signal may lead to erroneous conclusions. For this reason, in all of our works including this paper, we simply use "μV", the unit of thermopile terminal voltage.

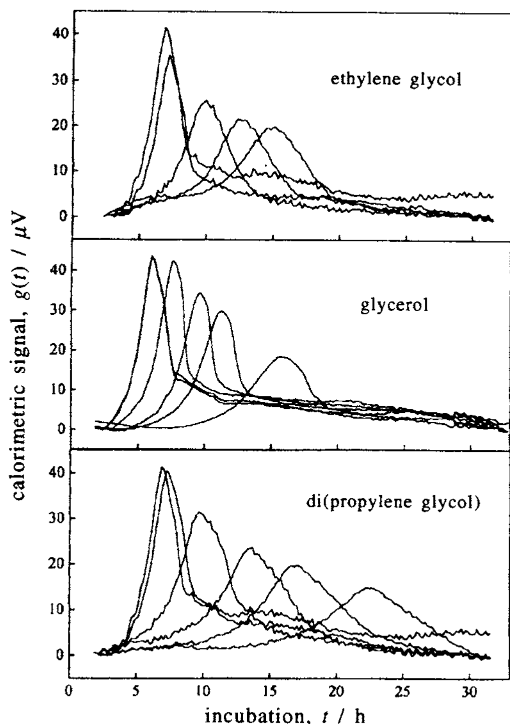

Fig.1 Representative examples of the growth thermograms recorded as the calorimetric output during the incubation of *E. coli* cultures with addition of 1,2-ehtanediol (ethylene glycol, 0 ~9.0 %(w/v), 1,2,3-propanetriol (glycerol, 0 ~ 16.0 %(w/v) and di(propylene glycol) (3-(3-hydroxy-propanoxy)propan-1-ol (0 ~ 8.0 %(w/v)

exists between this $f(t)$ function and the growth curve. The $f(t)$ curve was found to be in very good agreement with the number of cells in the culture and turbidity of the cultures.[8,10] Accordingly, the conclusion was that the $f(t)$ curves (not shown in this paper), as derived from the growth thermograms using equation (1), are suitable for the determination of the specific growth rate constant.

Hereafter the notation μ_m will be employed to denominate the maximum value of the growth rate constant observed in the absence of inhibitor (polyols in this case). The growth rate constant affected by the polyol concentration i, will be designated by μ_i. Thus, the parameter μ_i/μ_m represents the specific growth activity of the microbial cells in the presence of inhibitor at concentration i and is given by the following equation [8,10-13];

$$\mu_i/\mu_m = 1/\{1+(1/K_\mu)^{m_\mu}\} \quad (3)$$

Similar parameters can be derived if, instead of μ, one takes into consideration the duration of time required for a certain culture to reach a given level of calorimetric output. For a culture in the presence of an inhibitor at concentration i the incubation time necessary to reach the level α is designated as $t_\alpha(i)$. The shortest duration is $t_\alpha(0)$ (observed in the absence of an inhibitor), and consequently the parameter $t_\alpha(0)/t_\alpha(i)$ can be regarded as the specific retardation of growth for the microbial cells in the presence of an inhibitor at concentration i and is given by the following equation[8,10-13];.

$$t_\alpha(0)/t_\alpha(i) = 1/\{1+(1/K_\theta)^{m_\theta}\} \quad (4)$$

In this case, K_θ signifies the concentration of polyols which double the time required for the culture to reach a certain level and m_θ is the cooperativity parameter of the polyol action.

The data obtained in each experiment (values of i, μ_i, μ_m, $t_\alpha(0)$, $t_\alpha(i)$, μ_i/μ_m and $t_\alpha(0)/t_\alpha(i)$ for each of the calorimetric units) are then analyzed as presented below. Regression analysis on the basis of equations (3) and (4), applied on the data set of i, μ_i/μ_m and those of i, $t_\alpha(0)/t_\alpha(i)$, respectively, allows computation of the so-called drug potency curves.[8-13] Examples are represented in **Fig.2** (solid lines). The regression also leads to the values of K_μ, K_θ, m_μ and m_θ.[8-13] The values of m_μ and m_θ are measures of the cooperativity in the action of drugs (polyols in this case). Larger values of m_μ and m_θ corresponds to greater cooperativity, expressed in the form of steeper drug potency curves.

The minimum inhibition concentration of drugs (MIC) can also be determined from the same data sets using the previously reported mathematical treatment.[8-13] It was shown that μ_i/μ_m is an expression of the specific growth activity of the microorganisms in the presence of an inhibitor at concentration i. Under the hypothesis that the loss of growth activity caused by drugs ($1-\mu_i/\mu_m$) is proportional to the m_1-th power of drug concentration i, then we have the equation

$$1-\mu_i/\mu_m = k_1\, i^{m_1} \quad (5)$$

where k_1 and m_1 are constants. Thus the concentration

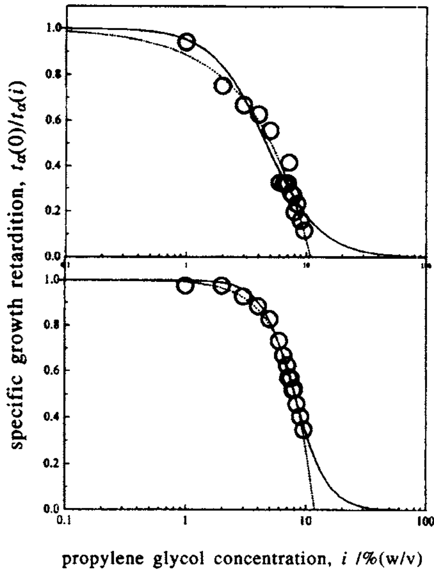

Fig.2 Representative plots of (μ_i/μ_m and $t_\alpha(0)/t_\alpha(i)$ versus 1,2-propanediol (propylene glycol) concentration i added in the culture medium. The solid lines, also called "drug potency curve" were drawn as the best fitted curves on the basis of equations (3) and (4). If a horizontal line is drawn at μ_i/μ_m = 0.5 or $t_\alpha(0)/t_\alpha(i)$ = 0.5, it intersects the drug potency curves in a point which corresponds, on the horizontal axis, to the 50 % inhibitory concentration K_μ or K_θ, respectively. The dotted lines are "MIC curves" fitted to the experimental data on the basis of equations (5) and (7). Their point of intersection with the horizontal axis provides the concentration which totally inhibits growth (MIC_μ and MIC_θ, respectively).

i at which the growth activity is completely inhibited (hereafter designated as MIC_μ is given by

$$MIC_\mu = (1/k_1)^{m_1} \quad (6)$$

Using the same analogy as before, if the loss of growth activity is expressed as $(1 - t_\alpha(0)/t_\alpha(i))$, then an equation similar to (5) can be written in terms of $t_\alpha(0)$ and $t_\alpha(i)$:

$$1 - t_\alpha(0)/t_\alpha(i) = k_2\ i^{m_2} \quad (7)$$

with the corresponding value:

$$MIC_\theta = (1/k_2)^{m_2} \quad (8)$$

By definition, the parameters MIC_μ and MIC_θ are described as values of concentration at which the growth activity of the microorganisms is completely inhibited, and therefore they have the meaning of MIC.

Examples of MIC curves drawn on the basis of equations (5) and (7) are also presented in **Fig.2** (dotted lines). All of the values for K_μ, K_θ, m_μ, m_θ, MIC_μ and MIC_θ of the polyols determined by the method described above are summarized in **Table 1**. In our previous study,[1] the antimicrobial properties of 1,2-pentanediol and 1,3-butanediol against 6 microorganisms were studied and it was found that the antimicrobial activity of 1,2-pentanediol against *E. coli* is stronger than that of 1,3-butanediol by a factor of 4 to 5. As is known from **Table 1**, all of the polyols studied in this work were found to exert the antimicrobial activity against *E. coli*, although their extents were weaker compared to that of 1,2-pentanediol and comparable with that of 1,3-butanediol by the order of magnitude which were obtained in the

Table 1 Inhibitory parameters determined calorimetrycally for the action of polyols at 30℃.

polyol	microbe	determined from μ_i/μ_m			determined from $t_\alpha(0)/t_\alpha(i)$		
		m_μ	K_μ /%(w/v)	MIC_μ /%(w/v)	m_θ	K_θ /%(w/v)/	MIC_θ /%(w/v)
1,2-ethanediol	*E.coli*	1.45 ±0.23	6.79 ±0.42	17.26 ±2.12	4.04 ±0.32	9.63 ±0.12	13.06 ±0.23
1,2-propanediol	*E.coli*	1.95 ±0.21	4.79 ±0.24	11.29 ±0.45	3.20 ±0.13	8.33 ±0.06	12.22 ±0.19
1,2-butanediol	*E.coli*	2.68 ±0.29	2.30 ±0.08	4.49 ±0.10	3.90 ±0.33	3.81 ±0.07	4.95 ±0.08
1,3-butanediol	*E.coli*	2.45 ±0.34	5.44 ±0.25	10.95 ±0.53	2.82 ±0.23	7.44 ±0.14	11.66 ±0.20
1,2,3-propanetriol	*E.coli*	3.15 ±0.40	17.54 ±0.47	25.74 ±1.38	1.74 ±0.17	17.42 ±0.52	32.69 ±1.64
1,2-pentanediol	*E.coli*	2.67 ±0.43	1.63 ±0.07	2.50 ±0.17	2.78 ±0.31	1.89 ±0.06	2.65 ±0.13
	S.cereviasiae	1.85 ±0.06	2.06 ±0.02	3.81 ±0.20	2.52 ±0.27	2.04 ±0.06	3.40 ±0.05
	Asp.niger	2.05 ±0.24	1.44 ±0.06	2.38 ±0.23	2.12 ±0.10	1.63 ±0.03	2.45 ±0.10
di(propylene glycol)	*E.coli*	2.18 ±0.33	4.35 ±0.25	9.78 ±0.45	2.31 ±0.17	5.94 ±0.12	10.63 ±0.14
polyethylene glycol	*E.coli*	3.07 ±0.74	7.83 ±0.41	12.96 ±1.01	3.02 ±0.32	9.44 ±0.19	14.09 ±0.25

Values are the average ±SD

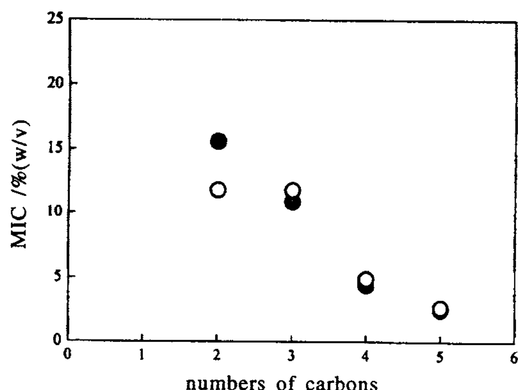

Fig.3 Values of MIC_μ (●) and MIC_θ (○) for *E. coli* plotted against the number of carbon atoms of polyols. Number of carbon atoms corresponds to the polyol: 2, 1,2-ethanediol (ethylene glycol); 3, 1,2-propanediol (propylene glycol); 4, 1,2-butanediol (1,2-butylene glycol); 5, 1,2-pentanediol (1,2-hydroxypentane).

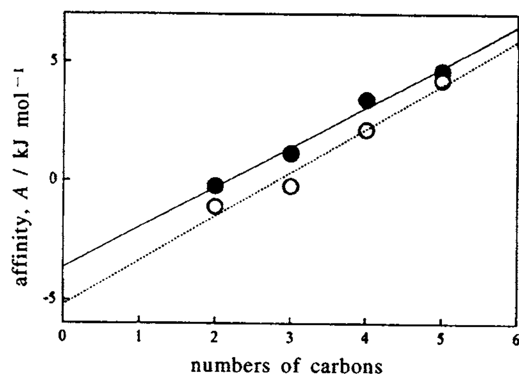

Fig.4 Values of affinity A plotted against the number of carbon atoms of polyols. Number of carbon atoms corresponds to the polyol: 2, 1,2-ethanediol (ethylene glycol); 3, 1,2-propanediol (propylene glycol); 4, 1,2-butanediol (1,2-butylene glycol); 5, 1,2-pentanediol (1,2-hydroxypentane).

previous study.[1]

If the antimicrobial effect of 1,2-pentanediol is regarded as 1, those of the other polyols were calculated to be between 0.11 and 0.55, indicating that 1,2-pentanediol has obviously the strongest antimicrobial effect.

The action mechanism of the antimicrobial activity of polyols is assumed to be based on chemical structure.[4] In order to characterize them more quantitatively the MIC values were plotted against the number of carbon atoms of polyols having two hydroxyl groups at the positions 1 and 2 and are shown in **Fig.3**. It is obvious that the larger the number of carbon atoms of polyols, the stronger are their antimicrobial effects.

K_i (K_μ or K_θ : the 50 % inhibitory concentration) was determined, as mentioned above, when calculating the drug potency curves. It seems reasonable to assume that there are multiple binding modes in the actual process of the drug binding to microbial cells. Here the term "binding" was used to express not a simple physical binding process of a drug on the cell surface but a process associated with the biological effect. If we assume that all of such the binding process are identical, the value of K_i (either K_μ or K_θ) defined above corresponds to the average dissociation constant per binding site. This K_i is related to the equilibrium constant K_b for binding process per binding site by the following equation:

$$K_i = 1/K_b \tag{9}$$

and the Gibbs energy change upon binding is given by the Gibbs equation;

$$\Delta G = RT \ln K_i \tag{10}$$

If A is the affinity defined by the relation $A = -\Delta G$, then the equation (10) can be transformed to:

$$A = -RT \ln K_i \tag{11}$$

From equation (11), the affinity A is estimated, representing the average affinity of drugs against the cell of microorganisms.[14] The values of the affinity A calculated on the basis of equation (11) are plotted against the number of the carbon atoms of polyols. The plot is given in **Fig.4**. From the plot it can also be known that the larger is the number of carbon atoms of polyols, the larger becomes the affinity. Accordingly, with increasing the number of carbon atoms of polyols, they showed stronger antimicrobial effects. The slope of the straight line in the plot of **Fig.4**, calculated by regression analysis, shows the force of affinity corresponding to an alkyl group. In the plot only the data set found for polyols having two hydroxyl groups at the positions 1 and 2 is given. From the plot it was found that the binding

activity of polyols for bacteria increased by 1.60 kJ mol^{-1} for every alkyl group.

On the other hand, comparison of the MICs of 1,2-butanediol and 1,3-butanediol together with those of other polyols given in **Table 1** suggested that their antimicrobial effects were also different due to the different position of the hydroxyl group. Thus it is very interesting to know that the antimicrobial effects of polyols are not only due to the alkyl chain bound, but also to the relative position of hydroxyl groups.

Furthermore, from the present finding that all of the polyols studied here showed antimicrobial activity to some extent, it may be reasonably concluded that the required amounts of antimicrobial agents will be reduced when they are used as the ingredients for the cosmetics and toiletries.

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要　旨

保湿剤，乳化安定剤，その他の目的で各種化粧品に添加されるポリオール類について，それらの抗微生物活性を微生物熱測定法を用いて検討した。微生物としては大腸菌を選び，ポリオールとしてはエチレングリコール，1,2-プロパンジオール，1,2-ブタンジオール，1,3-ブタンジオール，グリセロール，1,2-ペンタンジオール，ジプロピレングリコール，ポリエチレングリコールを対象とした。種々の濃度の各ポリオールの存在下で観測した増殖サーモグラムを解析し，それぞれの50 %生育阻止濃度（K_μ or K_θ）および最小生育阻止濃度（MIC_μ or MIC_θ）を決定した。全てのポリオールについて抗微生物作用が認められ，その大きさは50 %生育阻止濃度にして1～17 % (w/v)の範囲であった。ギブズの式に基づいて微生物細胞に対するポリオールの親和力を求めたところ，アルキル炭素鎖の長さに比例して親和性が増し，これらの抗微生物活性に疎水性が寄与していることが明らかとなった。