Surface Activities, Biodegradability and Antimicrobial Properties of n-Alkyl Glucosides, Mannosides and Galactosides

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n-Alkyl α- and β-glucopyranosides, α-D- mannopyranosides and β-D-galactopyranosides with alkyl chains having from 8 to 12 carbon atoms were synthesized and their surface properties--such as static surface tension (γ), critical micelle concentration (CMC), occupation area of molecule, dynamic surface tension and foaming properties, biodegradability and antimicrobial activities —were evaluated. Alkyl glycosides containing C8 to C12 carbon chains showed surface activities and critical micelle concentrations. D-Glucoside, D-mannoside and D-galactoside having the same alkyl chain showed similar surface tension lowering at CMC (γ_{cmc}) and occupation area of the molecule at the surface. Among the alkyl glucosides, α-anomers were less hydrophilic than β-anomers. All alkyl glycosides tested in this study were readily biodegraded by activated sludge of a municipal sewage plant compared to those of ethoxylated nonionic alcohols. The difference of the hydrophilic glycopyranoside group in biodegradability was not seen clearly. n-Alkyl glycosides containing C8 to C12 alkyl chains showed a broad spectrum of increasing antimicrobial activity. n-Dodecyl α-D-mannopyranoside was the most effective, the order of antimicrobial activity being mannopyranoside > glucopyranoside > galactopyranoside group. Members of this class of compounds exhibit the physicochemical and biological properties needed both for a wide range of applications and for environmental acceptance.

KEY WORDS: Alkyl galactoside, alkyl glucoside, alkyl glycoside, alkyl mannoside, antimicrobial activity, biodegradation, surface activity.

Alkyl glycosides are of particular interest because in addition to their potential biological and pharmaceutical applications (1) they are made from naturally occurring renewable resources of fatty alcohols and sugars. Furthermore, from an ecological point of view as well as energy considerations, alkyl glycoside will be one of the most promising candidates of new surfactants in the next generation. The preparation and physical properties of alkyl glycosides have been reported (2-4), but the discussion has been focused mainly on the alkyl glucosides, while other alkyl glycosides go largely unreported (1, 5-7). Recently, n-octyl β-D-glucopyranoside and other similar compounds have been used as detergents to solubilize membrane proteins and to study the related biological properties (8-11). However, the biodegradability and antimicrobial properties of alkyl glycosides as an interest in the industrial field have never been reported in detail.

This paper describes the synthesis of a homologous series of alkyl glycopyranosides containing D-glucose, D-mannose and D-galactose with n-alkyl chains of 8, 10 and 12 carbon atoms, and the measurement of their physicochemical properties as well as their biological properties, such as biodegradability and antimicrobial activity of the compounds.

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EXPERIMENTAL PROCEDURES

Materials. All materials were of the highest available purity and used as purchased unless stated otherwise. The water used in the measurement of surface activities was purified by passing it through an ion exchange resin column followed by distillation in an all-quartz apparatus, Auto Still, (Yamato Scientific Co., Ltd., Tokyo, Japan) (specific conductivity 1.1 $\times$ 10^{-6} ohm cm^{-1} at 25°C).

Determination of static surface tension. Static surface tension (γ) was determined by an automatic digital tensiometer, Kyowa Precise Surface Tensiometer, CBVP Method (Kyowa Kagaku Co., Ltd., Tokyo, Japan) at 25 ± 0.1°C. Measurements were done using a Wilhelmy vertical plate technique with a sandblasted glass plate. The test solutions were aged at 25 ± 0.1°C for at least 1 hr before each measurement. The measurements were repeated three times and the respective mean value was taken.

Occupation area of molecule at surface (A). The surface excess concentration (Γ_{max}) in mole/cm^2, and the corresponding occupation area of the molecule at surface (A) in nm^2, at the liquid/air interface were calculated (12) from Equations 1 and 2:

$$\Gamma_{max} = (1/2.303RT)\,(\partial\,\gamma/\partial\,\log C)_T \qquad [1]$$

and

$$A = 10^{14}/N\Gamma \qquad [2]$$

where $(\partial\,\gamma/\partial\,\log C)_T$ is the slope of the surface tension vs concentration curves below CMC at constant temperature.

Dynamic surface tension. Dynamic surface tension was determined with commercial equipment, JSTL Type (Kyowa Kagaku Co., Ltd.) using a vibrating jet procedure (13-17) at 40°C. In calculating the dynamic surface tension of the aqueous surfactant solutions, Equation 3 was used:

$$\gamma = K\rho\,(V/\lambda)^2 \qquad [3]$$

where γ is the surface tension (mN/m), ρ is the density of the solution (g cm^{-3}), V is the flow rate of the jet (mL/min), and λ is the wave length (cm). The constant, K, was found by applying the equation to the jet formed by the same orifice with pure water.

Determination of foaming power. The foaming power of the surfactants was determined by the Ross and Miles

methods at 40°C (18). The initial foam height in mm expressed the foam production, F_o, and foam height after 5 min expressed the foam stability, F_5.

Biodegradation. The five-day biochemical oxygen demand (BOD_5) was determined by the oxygen consumption method according to the Japanese Industrial Standard (JIS K 0102) using activated sludge obtained from a municipal sewage plant in Yokohama City.

Antimicrobial activity. The antimicrobial activities of alkyl glycosides were evaluated by the agar dilution method (19). Three kinds of gram-positive bacterial strains, *Staphylococcus aureus* FDA-209P, *Bacillus subtilis* PCI-219 and *Sarcina lutea* ATCC-1001, and three kinds of gram-negative bacterial strains, *Escherichia coli* 0-80, *Salmonella typhi* H-901W and *Pseudomonas aeruginosa* IF0-3080, and six kinds of fungal strains, *Candida albicans* ATCC-7491, *Saccharomyces cerevisiae* KF-25, *Trichophyton interdigitale* KF-62, *Microsporium gypseum* KF-64, *Penicillium chrysogenum* KF-97 and *Aspergillus niger* ATCC-6275, were used for the tests. Nutrient agar and Sabouraud dextrose agar were used for bacteria and fungi, respectively. These bacteria were cultured at 37°C for 48 hr, and the fungal strains were cultured at 25°C for 5 days. Antimicrobial activities are represented in terms of minimum inhibitory concentration (MIC).

Alkyl glycosides. Two separate methods were used to synthesize a series of alkyl glycosides. Alkyl α-glucosides were prepared in a one-step reaction (Method 2), and all other alkyl glycosides were prepared by the reaction of brominated sugar pentaacetate with alcohol (Method 1).

Method 1. Alkyl glucosides, alkyl galactosides and alkyl mannosides were prepared by the reaction of a fatty alcohol with brominated sugar pentaacetate reported by Rosevear *et al.* (10) and Koeltzow and Urfer (4) with several modifications.

β-D-Glucopyranose pentaacetate was prepared from D-glucose and acetic anhydride in the presence of sodium acetate according to the method of Bates (20) in 93% yield. m.p. 130.5-131.5°C. Lit. (21) 131.4-131.8°C. β-D-Galactopyranose pentaacetate was prepared in a similar procedure and purified by recrystallization from ethanol to give pure β-anomer in 57% yield. m.p. 141-142°C. Lit. (22) 142°C. D-Mannopyranose pentaacetate was also prepared in 90% yield and used as an α- and β-anomeric mixture. ($\alpha : \beta$ = 2:1 molar ratio) to give the subsequent reaction.

The preparation of n-dodecyl β-D-galactopyranoside is described as a typical procedure. β-D-Galactopyranose pentaacetate (15.0 g, 0.0384 mol) was dissolved in 38 mL of glacial acetic acid, followed by the addition of 38 mL of HBr (30% in acetic acid), and was stirred at room temperature for 45 min. Dichloromethane (150 mL) was added and the resulting solution was immediately poured over crushed ice (250 mL). The dichloromethane layer containing the brominated sugar pentaacetate was washed once with 150 mL of distilled water, 3 times with 150 mL of cold saturated aqueous sodium bicarbonate solution, and 3 times with 150 mL of distilled water. The organic layer was dried over anhydrous magnesium sulfate (35 g) and then filtered through a pad of celite. The dichloromethane layer was transferred to a foil-covered flask and diluted to a total volume of 210 mL with fresh dichloromethane. To this was added 1-octanol (5.0 g, 0.0384 mol), silver carbonate (4.5 g), iodine (0.3 g) and 4A molecular sieves (15.0 g), in that order. The mixture was stirred overnight at room temperature, filtered through a pad of celite, and washed with dichloromethane. The filtrate was evaporated to give the ortho ester as a syrup. The ortho ester was treated with 0.01 N sulfuric acid in 90% aqueous acetone (250 mL) with stirring for 30 min at room temperature. The mixture was neutralized with pyridine and concentrated to a syrup under reduced pressure.

Deacetylation was carried out by treatment of the syrup with 300 mL of methanol:triethylamine:water (2:1:1) at room temperature for 48 hr with stirring. After the reaction, methanol and triethylamine were evaporated, and the resulting aqueous solution was extracted with ether and washed with water; then the organic layer was dried over anhydrous sodium sulfate. The organic solvent and unreacted 1-octanol were distilled out under reduced pressure to give a crude product. The crude product was passed through a Dowex 1 × 2 (OH form, Dow Chemical Co., Midland, MI) chromatographic column using methanol as an eluent to give 6.84 g of n-octyl β-D-galactopyranoside in 61% yield from the pentaacetate. The isolated products were analyzed by high-performance liquid chromatography (HPLC), elemental analysis, infrared (IR), ^1H nuclear magnetic resonance (NMR) and ^{13}C NMR spectroscopy. A series of n-alkyl α-D-mannopyranoside were prepared by the same procedure; their yields and typical analytical data are shown in Table 1. In the case of the reaction with D-glucose, a small amount of α-anomer was formed as a by-product. Their yields and specific rotation are also listed in Table 1.

Method 2. n-Octyl, decyl and dodecyl α-D-glucopyranosides were synthesized in a one-step reaction using the method reported by Brown *et al.* (1) with the exception that a cation exchange resin was used in place of hydrogen chloride. The preparation of n-octyl α-D-glucopyranoside is described as a typical example. A mixture of D-glucose (10.0 g, 0.0555 mol), 1-octanol (70 mL) and a cation exchange resin (2.0 g, Amberlyst-15) was stirred at 100°C for 3 hr. After the reaction, the resin was filtered off and excess 1-octanol was distilled under reduced pressure. Saturated aqueous sodium chloride solution was added, and the remaining 1-octanol was extracted with ether. The alkyl glucoside was then extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate. After treatment with charcoal, the solvent was removed and the residue was then passed through an anion exchange resin chromatographic column (Dowex 1 × 2, OH form, Dow Chemical Co.) using methanol as an eluent to give n-octyl α-D-glucopyranoside in 26.8% yield (4.44 g) and n-octyl β-D-glucopyranoside in 13.5% yield (2.24 g). In a similar way, from 1-decanol, n-decyl α-D-glucopyranoside in 22.7% yield and n-decyl β-D-glucopyranoside in 10.4% yield, and from 1-dodecanol, n-dodecyl α-D-glucopyranoside in 15.6% yield and n-dodecyl β-D-glucopyranoside in 8.9% yield were obtained. These were analyzed by HPLC, elemental analyses, IR, ^1H NMR and ^{13}C NMR spectroscopy. Yields and analytical data are shown in Table 2.

RESULTS AND DISCUSSION

Syntheses of n-alkyl glycosides. Yields and analytical data of n-alkyl glycopyranosides obtained by two methods are shown in Table 1 and Table 2. Using Method 1, β-glucosides were predominantly obtained, but a small amount of α-anomers was simultaneously formed in the preparation of the alkyl glucosides. They could be readily separated by an ion exchange resin column. Rosevear *et al.* (10) reported that

TABLE 1

Yields and Analytical Data of Alkyl β-D-Glucopyranosides, α-D-Mannopyranosides and β-D-Galactopyranosides Obtained by the Method of Koenigs-Knorr Reaction (Method 1)

Compound	Alcohol	Sugar	Yield (%)	$[\alpha]_D^{25}$, [C = 1, MeOH]	Elemental analysis (%) C Found	C Calcd.	H Found	H Calcd.
C8βGlc	1-Octanol	D-Glucose	45.6	−27.2, (−30.3)[a]	57.65	57.41	9.44	9.65
[C8αGlc			4.5	+111.2, (+117.9)[b][c]				
C10βGlc	1-Decanol	D-Glucose	43.7	−25.4, (−27.8)[a]	60.24	59.98	9.94	10.07
[C10αGlc			4.3	+95.2, (+89.2)[b]]				
C12βGlc	1-Dodecanol	D-Glucose	35.7	−22.6, (−27.7)[a]	62.10	62.04	10.23	10.41
[C12αGlc			4.1	+97.8, (+74.9)[b]]				
C8αMan	1-Octanol	D-Mannose	36.0	+60.4,	57.64	57.51	9.20	9.65
C10αMan	1-Decanol	D-Mannose	31.5	+48.0, (+58.0)[b]	60.34	59.98	9.81	10.07
C12αMan	1-Dodecanol	D-Mannose	27.6	+43.4, (+55.9)[b]	61.99	62.04	10.16	10.41
C8βGal	1-Octanol	D-Galactose	61.0	−10.4,	57.46	57.51	9.52,	9.65
C10βGal	1-Decanol	D-Galactose	62.0	−12.4	60.38	59.98	9.77,	10.07
C12βGal	1-Dodecanol	D-Galactose	60.3	−4.0	61.92	62.04	10.14,	10.41

[a]Reference 23.
[b]Reference 1.
[c]By-products.

TABLE 2

Yields and Analytical Data of Alkyl α-D-Glucopyranosides Obtained by One-Step Reaction (Method 2)

Compound	Alcohol	Sugar	Yield (%)	$[\alpha]_D^{25}$ [C = 1, MeOH]	Elemental analysis (%) C Found	C Calcd.	H Found	H Calcd.
C8αGlc	1-Octanol	D-Glucose	26.8	+111.2	57.62	57.51	9.36	9.65
[C8βGlc			13.5	−27.2]*				
C10αGlc	1-Decanol	D-Glucose	22.7	+95.2	59.89	59.98	9.84	10.07
[C10βGlc			10.4	−25.4]				
C12αGlc	1-Dodecanol	D-Glucose	15.6	+97.8	61.88	62.04	10.20	10.41
[C12βGlc			8.9	−22.6]				

*By-products.

by Method 1 no α-anomer was detected in the reaction products. Using Method 1, α-anomers were obtained exclusively from D-mannose, but in the case of D-galactose β-anomers were obtained exclusively. n-Alkyl α-D-glucopyranosides were obtained in a one-step reaction of 1-alkanol and D-glucose in the presence of an ion exchange resin (Method 2). In this case, the molar ratio of α-anomer to β-anomer of the resultant alkyl glucosides was about 2:1.

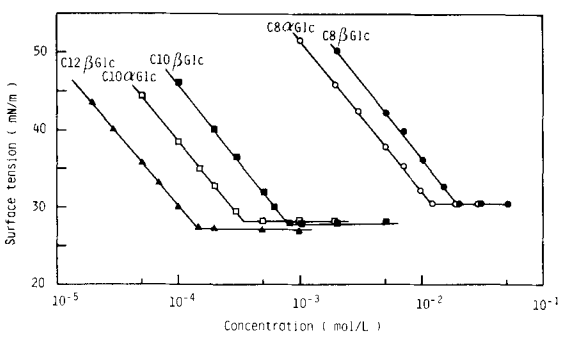

FIG. 1. Surface tension—concentration plots of alkyl glucopyranosides at 25°C.

Interfacial properties. Surface tension vs concentration plots for n-alkyl D-glucopyranosides in distilled water is shown in Figure 1 as a typical example. In general, alkyl glycosides containing a C8 to C12 carbon chain showed surface activities and critical micelle concentrations (CMC). The CMC determined from the inflection of the surface tension vs concentration curve, the surface tension at CMC (γ_{CMC}) and occupation area of a molecule at surface (A) of these compounds are summarized in Table 3. With respect to CMC, the shorter the alkyl chain, the higher the CMC values. This tendency can be seen usually in the case of polyoxyethylene type nonionic surfactants. Among the alkyl glucosides, α-anomers showed a lower CMC compared to those of the β-anomers, indicating less hydrophilicity of the α-anomers. The CMC and (γ_{CMC}) of n-dodecyl α-D-glucopyranoside could not be measured due to their lower solubilities in water. From these results, it seems that the CMC was influenced by both the length of the alkyl chain and the anomeric position of the glycoside. The alkyl glycosides of D-glucose, D-mannose and D-galactose having the same alkyl chain showed a similar γ_{CMC} and A, probably suggesting the independence of the hydrophilic character of the glycopyranosyl group from the adsorption structure at the surface. With regard to the occupation area of the molecule at surface (A), the alkyl glycosides all showed similar values of

0.47-0.54 nm². The γ_{CMC} values at the surface were determined by the alkyl group and were not affected by the structure of the hydrophilic glycoside group.

Dynamic surface tension. Dynamic surface tension curves of 5 mM n-decyl D-glycopyranosides are shown in Figure 2. It was found that the adsorption rate onto the surface of these n-decyl glycosides was similar and after 10-12 msec reached equilibrium value. In this case the difference in the dynamic surface tension between the α- and β-anomers was significant and surface tension lowering by the α-anomer of the glucosides was significantly faster than that of the corresponding β-anomer. Among them, n-decyl β-D-galactopyranoside showed the most rapid surface tension lowering.

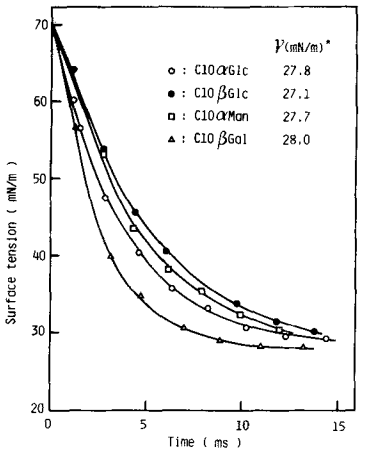

FIG. 2. Dynamic surface tension of 5 mmol/L n-decyl glycopyranosides at 40°C
***Static surface tension.**

TABLE 3

Interfacial Properties of Alkyl Glycosides at 25°C

Compound	CMC (mmol/L)	γ_{CMC} (mN/m)	A x 10^2 (nm^2)
C8αGlc	12	30.5	47
C8βGlc	20	30.5	48
C8αMan	6.0	30.5	48
C8βGal	16	30.5	48
C10αGlc	0.35	28.2	49
C10βGlc	0.80	27.8	49
C10αMan	0.25	28.5	53
C10βGal	0.70	28.0	53
C12αGlc		insoluble	
C12βGlc	0.15	27.3	51
C12αMan	0.05	28.4	54
C12βGal	(0.20	31.5)*	51

*Turbid.

Foaming properties. The difference of glycopyranosyl groups to surface properties was clearly seen in their foaming properties. Figures 3-5 show the foam production, F_o, and foam stability, F_c, vs concentration around the CMC measured by the Ross and Miles method (18). In general, foam production and foam stability of alkyl glycosides showed similar tendencies. It is known that polyoxyethylene-alkyl-ether-type nonionics show a low foaming property, but n-octyl and n-decyl glycosides show higher foaming properties. Foam production of n-octyl and n-decyl glycosides increased rapidly around the CMC, but n-dodecyl glycoside showed no prominent foaming around the CMC. This extremely low foaming compared to octyl and decyl glycosides can probably be ascribed to the low solubility (low hydrophilicity) of the compounds in water. As for their foaming properties, the n-decyl group was the best among the alkyl glycosides. Among the alkyl glycosides, β-galactoside tended to show a better foaming property. Also a remarkable difference in foaming properties between α- and β-anomers was seen in decyl glucoside shown in Figure 4.

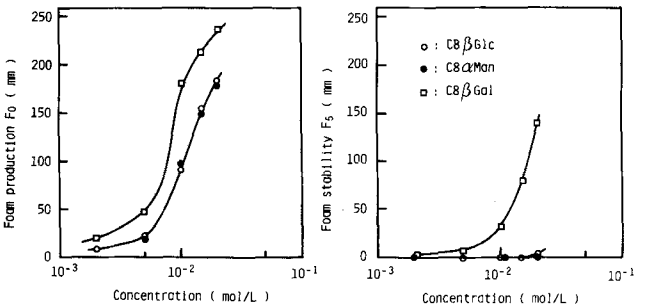

FIG. 3. Foam production and stability of n-octyl glycopyranosides by Ross-Miles test at 40°C.

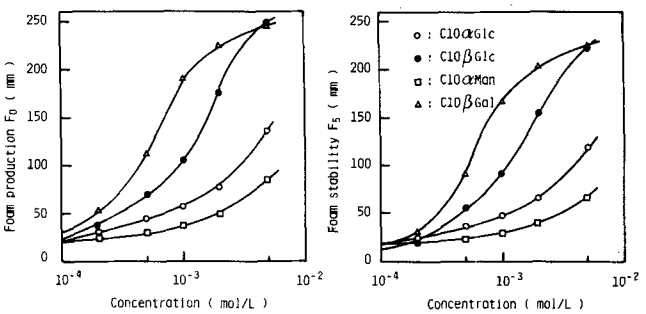

FIG. 4. Foam production and stability of n-decyl glycopyranosides by Ross-Miles foam test at 40°C.

Biodegradability. Table 4 shows the five-day biochemical oxygen demand (BOD_5) and biodegradability (BOD_5/theoretical oxygen demand [TOD]) of alkyl glycosides as well as for conventional ethoxylated nonionic alcohols. It was found that all alkyl glycosides tested in this study were biodegraded over 50% in five days, suggesting that these compounds are readily biodegradable in the environment. The influence of glycosyl residues on biodegradation was not clearly seen. This will be one of the most prominent characteristics of alkyl glycosides. On the other hand, a conventional ethoxylated alcohol shows a lower biodegradability in five days. Of course, this BOD_5 method is only a screening tool and the conventional nonionics which give a low biodegradation during testing may be more rapidly and extensively biodegraded in a system such as soil or activated sludge where

TABLE 4

Biodegradability of Alkyl Glycosides

Compound	TOD (mg O/g)	BOD_5 mg O/g)	BOD_5/TOD (%)
C8αGlc	1970	1060	53.8
C8βGlc	1970	1300	66.0
C8αMan	1970	1110	56.3
C8βGal	1970	1220	61.9
C10αGlc	2100	1220	58.1
C10βGlc	2100	1470	70.0
C10αMan	2100	1220	58.1
C10βGal	2100	1170	55.7
C12αGlc	2210	1100	55.7
C12βGlc	2210	1250	56.6
C12αMan	2210	1290	58.4
C12βGal	2210	1370	62.0
C12EO10[a]	2200	736	33.5
C18EO10[b]	2340	669	28.6

[a]C12EO10 : Dodecyl poly(oxyethylene) ether n = 10.
[b]C18EO10 : Octadecyl poly(oxyethylene) ether n = 10.

more organisms exist and more time can be provided for acclimation.

Antimicrobial activities of alkyl glycosides. Eleven n-alkyl glycosides were screened for antimicrobial activity against gram-positive and gram-negative bacterial strains and fungal strains. The MIC for the compounds tested are given in Table 5. It was generally recognized that n-alkyl glycosides containing a C8 and C12 alkyl chain showed a broad spectrum of antimicrobial activity. n-Dodecyl α-D-mannopyranoside was the most effective compound among the n-alkyl glycosides tested in this study. Among the alkyl glycosides, those with n-dodecyl groups were particularly effective against gram-positive bacterial strains as well as fungal strains. The shorter alkyl chain length glycosides although less active than the n-dodecyl derivatives are still considered to be active. It was found that the antimicrobial activities between anomeric isomers of n-dodecyl glucosides were different. The α-anomers of n-dodecyl glucosides showed better antimicrobial activities against bacterial strains than the β-isomers, whereas, the α-anomer was much less effective against fungal strains than the β-isomer. It was also found

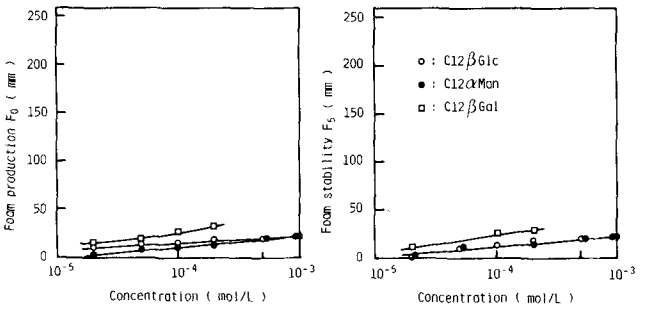

FIG. 5. Foam production and stability of n-dodecyl glycopyranosides by Ross-Miles foam test at 40°C.

TABLE 6

Antimicrobial Activity of the Conventional Ethoxylated Alcohol (24)

Compounds[a]/ Organism	MIC (μg/mL)			
	C12EO6	C12EO4	C12EO3	C14EO3
S. aureus	40	40	20	20
B. subtilis	160	80	40	160
E. coli	>500	>500	>500	>500
S. typhi	>500	>500	>500	>500

[a]C12 and C14 indicated dodecyloxy and tetradecyloxy respectively. EO 6, 4, 3 indicated the number of ethylene oxide molecule added to an alcohol.

that the antimicrobial properties were influenced by the structure of the glycopyranosyl residue of the n-alkyl glycoside. Mannopyranosyl was the most effective, the order of increasing antimicrobial activity being mannopyranosyl > glucopyranosyl > galactopyranosyl group in addition to a dependence on the alkyl chain length. Table 6 shows the antimicrobial activity of the conventional ethoxylated nonionics for a comparison. Ethoxylated dodecanol and tetradecanol showed antimicrobial activity against gram-positive bacteria, *S. aureus* and *B. subtilis,* but no antimicrobial activity toward gram-negative bacteria, *E. coli* and *S. typhi.* However, their antimicrobial activity was generally less effective against 2 gram-positive and 2 gram-negative bacteria than those of n-dodecyl glucoside and mannoside.

TABLE 5

Antimicrobial Activity of Alkyl Glycoside[a]

Compounds/ Organism	MIC (μg/mL)[b]										
	C12αGlc	C12βGlc	C12αMan	C12βGal	C10αGlic	C10βGlc	C10αMan	C10βGal	C8βGlc	C8αMan	C8βGal
S. aureus	10	25	25	200	100	100	50	100	400	100	200
B. subtilis	25	50	25	200	100	100	50	100	400	>400[c]	400
S. lutea	10	50	5	200	50	50	25	50	100	50	400
E. coli	400	>400	>400	>400	200	200	200	400	400	400	400
S. typhi	400	>400	>400	>400	400	400	400	400	400	>400	400
P. aeruginosa	200	>400	>400	>400	200	200	200	400	400	>400	400
C. albicans	400	50	200	>400	>400	200	200	200	>400	400	>400
S. cerevisiae	400	25	10	200	100	100	50	100	>400	400	>400
T. interdigitale	200	25	10	200	100	200	50	100	400	400	400
M. gypseum	200	25	25	50	200	200	50	200	400	400	400
P. chrysogenum	200	200	200	>400	>400	400	400	200	400	400	400
A. niger	200	200	400	>400	>400	400	>400	400	>400	>400	>400

[a]Control always produced growth of the microorganism.
[b]Minimum inhibitory concentration.
[c]No inhibition, maximum concentration tested listed.

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