Surface Activities, Biodegradability and Antimicrobial Properties of Glucosamine Derivatives Containing Alkyl Chains

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Three series of D-glucosamine derivatives containing an alkyl chain with 8 to 14 atoms, methyl 2-acylamino-2-deoxy-D-glucopyranosides, *n*-alkyl 2-acetylamino-2-deoxy-D-glucopyranosides and *n*-alkyl 2-amino-2-deoxy-D-glucopyranoside hydrochlorides, were synthesized, and their surface properties (such as surface tension, critical micelle concentration (CMC), dynamic surface tension and foaming properties), biodegradability and antimicrobial activities were evaluated. *n*-Alkyl 2-amino-2-deoxy-D-glucopyranoside hydrochlorides containing C8 to C12 carbon chains showed surface activities, a CMC and excellent foaming properties. The α-anomers showed a slightly lower CMC than the β-anomers, indicating less hydrophilicity of the α-anomers. On the other hand, glucosamine derivatives containing amide groups showed poor surface activities in water due to their lower solubilities in water. All glucosamine derivatives containing alkyl chains were biodegraded as well as conventional ethoxylated nonionics by activated sludge from the municipal sewage treatment plant. Methyl 2-acylamino-2-deoxy-D-glucopyranosides and *n*-alkyl 2-amino-2-deoxy-D-glucopyranoside hydrochlorides showed a broader spectrum of antimicrobial activity than the corresponding *n*-alkyl glucopyranosides. Among them the C12 derivatives showed the best results.

KEY WORDS: Alkyl glycoside, antimicrobial activity, biodegradation, glucosamine derivative, surface-active agent, surface activity, *N*-acyl glucosamine derivative.

Chitin is one of the naturally abundant polysaccharides composed of N-acetyl-D-glucosamine (2-acetamido-2-deoxy-D-glucose) and is an inexpensive, renewable material; however, most of it has been unused. Recently, much effort has been expended to develop effective utilization of chitin in both industrial and medical fields. N-Acetyl-D-glucosamine, which is readily obtained by acidic hydrolysis of chitin and has both hydroxyl and amino groups, will be one of the most important starting materials for the molecular design and production of multifunctional and physiologically active materials in the next generation. Introduction of an alkyl group into N-acetyl-D-glucosamine and its derivatives is feasible and produces novel, environmentally acceptable amphiphiles. Amphiphiles derived from amino sugars, so far, have not been extensively studied. Only a few compounds (1–3), such as 2-(N-alkyl-N,N-dimethylammonio)-2-deoxy-D-glucose (4), 2-(acylamino)-2,6-dideoxy-D-glucopyranose-6-sulfonic acid (5) and 2-acylamino-2-deoxy-glucitol (6), have been reported.

For this report, three series of D-glucosamine derivatives containing alkyl chains, such as methyl 2-acylamino-2-deoxy-D-glucopyranosides, *n*-alkyl 2-acetylamino-2-deoxy-D-glucopyranosides and *n*-alkyl 2-amino-2-deoxy-D-glucopyranoside hydrochlorides, were synthesized and their physicochemical properties as well as their biological properties, such as biodegradability and antimicrobial activity, were measured.

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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 passage through an ion exchange resin column followed by distillation in an all-quartz apparatus, Auto Still (Yamato Scientific Co., Ltd., Tokyo, Japan) (specific conductivity 1.1×10^{-6} Ohm·cm^{-1} at 25°C).

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

Dynamic surface tension. Dynamic surface tension (γ) was determined with a commercial instrument, JSTL Type (Kyowa Kagaku Co., Ltd.) by a vibrating jet procedure (7–13) at 30°C. The dynamic surface tension of the aqueous surfactant solutions was calculated from:

$$\gamma = K\varrho(V/\lambda)^2 \quad [1]$$

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 wavelength (cm). The constant K was found by applying Equation 1 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 method at 30°C (14). The initial foam height (in mm) expressed the foam production, F_0, while 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 based on OECD Guidelines for Testing of Chemicals (301D, Closed Bottle Test) (15) with activated sludge from a municipal sewage treatment plant located in Yokohama City. The concentration used in the BOD test was 0.5 to 5.0 ppm. Theoretical oxygen demand (TOD) was calculated according to the literature (15).

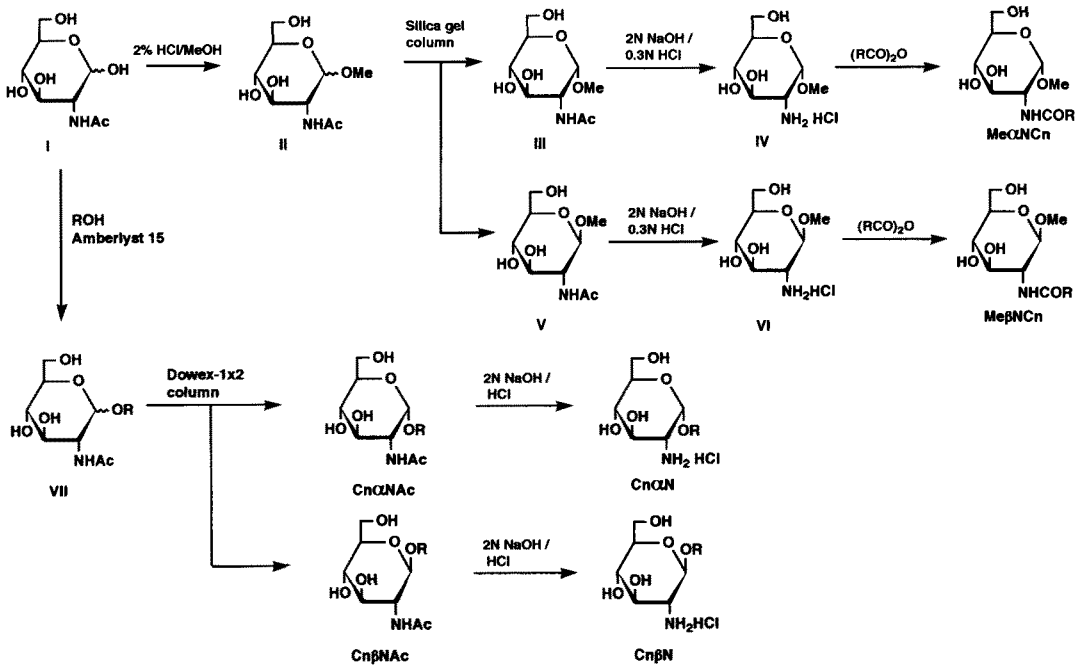
Antimicrobial activity. The antimicrobial activities of the surfactants were evaluated by the agar dilution method (16). Three kinds of gram-positive bacterial strains, *Staphylococcus aureus* FDA-209P, *Bacillus subtilis* PCI-219 and *Sarcina lutea* ATCC-1001, three kinds of gram-negative bacterial strains, *Escherichia coli* 0-80, *Salmonella typhi* H-901W and *Pseudomonas aeruginosa* IFO-3080 and six kinds of fungal strains, *Candida albicans* ATCC-7491, *Saccharomyces cerevisiae* KF-25, *Tri-*

chophyton 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. In the screening tests for antimicrobial activity, 0.4% stock solutions were prepared by dissolving 40 mg of test compound in 10 mL of distilled water or ethanol. The stock solutions were serially diluted by successive pipetting of solution in water containing nutrient agar or Sabouraud dextrose agar to obtain 400, 200, 100, 50, 25, 10, 5, 2.5 and 1 ppm concentrations of compound. After sterilization of the agar, the solutions were poured into sterile petri dishes, allowed to harden, and were then individually inoculated with one drop of cell suspension, each containing a separate test microorganism. The inoculated dishes were then incubated at 37°C for 2 d with bacterial strains and at 25°C for 5 d with fungal strains, and examined for the presence or absence of growth. Antimicrobial activities are represented in terms of minimum inhibitory concentration (MIC).

Glucosamine derivatives containing alkyl chains. Three series of glucosamine derivatives were synthesized as shown in Scheme 1 where n = number of carbon atoms in alkyl or acyl groups.

Preparation of methyl 2-acetylamino-2-deoxy-D-glucopyranoside (II) and methyl 2-amino-2-deoxy-D-glucopyranoside hydrochloride (IV, VI). Methyl 2-acetylamino-2-deoxy-D-glucopyranoside (II) was prepared from N-acetyl glucosamine (I) and methanol in the presence of 2% hydrogen chloride according to the method of Horton (17) in 49% yield. The anomeric mixture of II was separated by passage through a silica gel chromatographic column with chloroform/methanol (5:2) as an eluent to give methyl 2-acetylamino-2-deoxy-α-D-glucopyranoside (III) in 42% yield and methyl 2-acetylamino-2-deoxy-β-D-glucopyranoside (V) in 36% yield. α-Anomer (III): m.p. 191–193°C. Lit. (17) 190–193°C. $[\alpha]_D^{30}$ +124.2 (C = 1.0, water). Lit. (17) +128.2 (C = 1.0, water). β-Anomer (V): m.p. 197–201°C. Lit. (17) 196–199°C. $[\alpha]_D^{30}$ −17.8 (C = 1.0, methanol). Lit. (18) −13.5 (C = 0.7, methanol). Deacetylation of methyl 2-acetyl-amino-2-deoxy-α-D-glucopyranoside (III) was carried out by treatment with 2N sodium hydroxide according to the method of Hirano and Ishigami (19). The crude product was purified in a cation exchange column (Amberlyte CG 120 Type II, H^+ form, Rohm and Haas Co., Philadelphia, PA) with 0.3N hydrochloric acid as an eluent to give methyl 2-amino-2-deoxy-α-D-glucopyranoside hydrochloride (IV) in 95% yield. m.p. 153–156°C. $[\alpha]_D^{30}$ +124.4 (C = 1.0, water). Lit. (20) $[\alpha]_D$ +127 (C = 1.0, water). In a similar way, from methyl 2-acetylamino-2-deoxy-β-D-glucopyranoside (V), methyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (VI) was obtained in 90% yield. m.p. 180–182°C. Lit. (21) 190°C. $[\alpha]_D^{30}$ −22.7 (C = 1.0, water). Lit. (20) $[\alpha]_D$ −23.4 (C = 1.0, water).

Preparation of methyl 2-acylamino-2-deoxy-D-glucopyranoside (MeαNCn and MeβNCn). MeαNCn and MeβNCn were prepared by the reaction of methyl 2-amino-2-deoxy-D-glucopyranoside and fatty acid anhydride. The preparation of methyl 2-decanoylamino-2-deoxy-β-D-glucopyranoside (MeβNC10) is described as a typical procedure. Sodium (55 mg, 2.39 mmol) was added to methanol (10 mL) to form sodium methoxide methanolic solution. To this, methyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (500 mg, 2.18 mmol) was added at room temperature and stirred for 30 min. Precipitated sodium chloride was filtered and washed with a small amount of methanol to obtain methyl 2-amino-2-deoxy-β-D-glucopyranoside methanolic solution. Decanoic anhydride (710 mg, 2.18 mmol) was added to this methanolic solution and stirred for 3 h at 50°C. After the reaction, the



SCHEME 1

TABLE 1

Yields and Analytical Data of Methyl 2-Acylamino-2-Deoxy-D-Glucopyranosides (MeαNCn,MeβNCn), Alkyl 2-Acetylamino-2-Deoxy-D-Glucopyranosides (CnαNAc,CnβNAc) and Alkyl 2-Amino-2-Deoxy-D-Glucopyranosides Hydrochlorides (CnαN,CnβN)

Compounds	Yield (%)	m.p. (°C)	$[\alpha]_D^{30}$ [C=1, MeOH]	Elemental analysis (%) C Found	C Calcd.	H Found	H Calcd.	N Found	N Calcd.
MeαNC8	10.1	154–155	+111.2	56.05	56.41	8.82	9.15	3.98	4.39
MeαNC10	50.3	154–156	+95.5	58.45	58.77	9.13	9.57	3.88	4.03
MeαNC12	25.8	159–160	+86.9	60.74	60.77	9.61	9.93	3.67	3.73
MeαNC14	24.1	144–145	+74.9	63.01	62.50	9.95	10.24	3.10	3.47
MeβNC10	14.7	192	−26.5	58.58	58.77	9.26	9.57	4.01	4.03
MeβNC12	13.5	190	−24.2	60.66	60.77	9.65	9.93	3.81	3.73
MeβNC14	11.7	190–192	−22.9	62.20	62.50	9.95	10.24	3.44	3.47
C8αNAc	13.8	161–162	+133.8	57.53	57.64	8.99	9.37	4.13	4.20
C10αNAc	13.0	163–164	+127.4	59.63	59.81	9.49	9.76	3.93	3.87
C12αNAc	11.2	162–163	+117.6	61.46	61.67	9.78	10.09	3.63	3.60
C8βNAc	6.5	175–177	−11.0	57.26	57.64	8.97	9.37	4.13	4.20
C10βNAc	5.0	187–188	−13.8	59.58	59.81	9.47	9.76	3.85	3.87
C12βNAc	4.4	179–182	−15.5	61.68	61.67	9.64	10.09	3.65	3.60
C8αN	99.7	174–175	+115.6	51.08	51.29	9.02	9.22	4.24	4.27
C10αN	86.7	175–177	+100.9	54.24	54.00	9.30	9.63	3.95	3.94
C12αN	51.6	172–173	+95.4	55.99	56.31	9.76	9.98	3.59	3.65
C8βN	88.1	149–150	−23.5	51.50	51.29	8.99	9.22	4.30	4.27
C10βN	92.3	151–152	−21.7	53.68	54.00	9.54	9.63	4.01	3.94
C12βN	68.3	150	−20.1	56.20	56.31	9.70	9.98	3.60	3.65

solution was cooled at −20°C and the resulting crystals were collected by suction, washed with a small amount of cold methanol, then washed with a large amount of ether to obtain the acylated crude product. The crude product (320 mg) was dissolved in methanol (1 mL), then dropwise added to water (50 mL). The resultant precipitate was collected with suction and recrystallized from ethyl acetate to give MeβNC10 (111 mg) in 14.7% yield. The isolated product was analyzed by high-performance liquid chromatography (HPLC), elemental analysis, infrared (IR), ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectroscopy. Yields and analytical data are shown in Table 1.

Preparation of n*-alkyl 2-acetylamino-2-deoxy-D-glucopyranoside (CnαNAc and CnβNAc).* CnαNAc and CnβNAc were synthesized in the one-step reaction (22,23) of N-acetyl glucosamine (I) and fatty alcohol in the presence of a cation exchange resin. The preparation of *n*-octyl 2-acetylamino-2-deoxy-D-glucopyranoside is described as a typical procedure. A mixture of N-acetyl-D-glucosamine (I) (13.5 g, 60.9 mol), 1-octanol (225 mL) and a cation exchange resin (5.1 g, Amberlyst 15) was stirred at 100°C for 4 h. After the reaction, the resin was filtered off and excess 1-octanol was distilled under reduced pressure to give the crude product (5.0 g). Purification and separation of the α and β anomers were carried out in an anion exchange resin chromatographic column (Dowex 1 × 2, OH form, Dow Chemical Co.) with methanol as an eluent to give *n*-octyl 2-acetyl-amino-2-deoxy-α-D-glucopyranoside (C8αNAc) (2.80 g, 8.40 mmol) and *n*-octyl 2-acetylamino-2-deoxy-β-D-glucopyranoside (C8βNAc) (1.31 g, 3.93 mmol) as white crystals. These were analyzed by HPLC, elemental analyses, IR, ^{1}H NMR and ^{13}C NMR spectroscopy. A series of CnαNAc and CnβNAc were prepared by the same procedure; their yields and typical analytical data are shown in Table 1.

Preparation of n*-alkyl 2-amino-2-deoxy-D-glucopyranoside hydrochloride (CnαN and CnβN).* CnαN and CnβN were prepared by the deacetylation of CnαNAc and CnβNAc, respectively, with 2N sodium hydroxide. The preparation of *n*-octyl 2-amino-2-deoxy-α-D-glucopyranoside hydrochloride (C8αN) is described as a typical procedure. A mixture of C8αNAc (2.0 g, 6.00 mmol) and 2N sodium hydroxide (100 mL) was stirred at 100°C for 5 h. After the reaction, the pH of the solution was adjusted to 4 by means of 3N hydrochloric acid, then filtered through a pad of celite. The filtrate was then evaporated at reduced pressure. The obtained residue was redissolved in anhydrous ethanol, insoluble sodium chloride was filtered off, and the filtrate was evaporated at reduced pressure. This desalting procedure was repeated three times to give C8αN (1.96 g, 5.95 mmol) in 99.7% yield. The isolated product was analyzed by HPLC, elemental analysis, IR, ^{1}H NMR and ^{13}C NMR spectroscopy. Yields and analytical data of CnαN and CnβN, prepared in this way, are shown in Table 1.

RESULTS AND DISCUSSION

Syntheses of glucosamine derivatives containing an alkyl chain. Yields and analytical data of glucosamine derivatives are shown in Table 1. *n*-Alkyl 2-acetylamino-2-deoxy-D-glucopyranosides (CnαNAc, CnβNAc) were obtained in a one-step reaction of fatty alcohol and N-acetyl glucosamine (I) in the presence of a cation exchange resin, but the yield was relatively low. By this method, both α- and β-anomers were obtained simultaneously, and the molar ratio of α-anomer to β-anomer of the resultant glycoside was about 2:1 for the *n*-octyl and about 5:2 for the *n*-decyl derivatives. Long-chain alkyl glycoside can be prepared by the Koenigs-Knorr reaction (24,25), but then the α-anomer is predominantly synthesized.

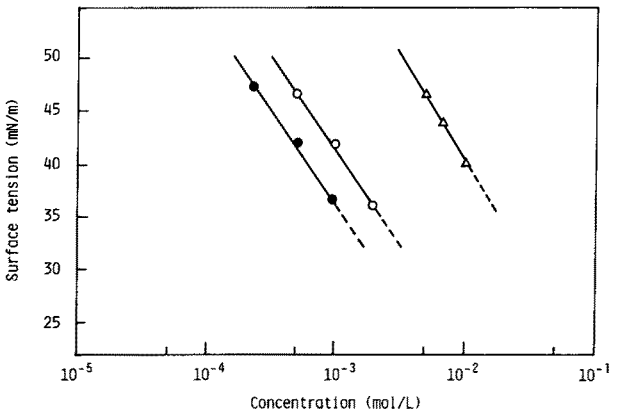


FIG. 1. Surface tension-concentration curves of N-acetyl-D-glucosamine derivatives at 25°C: ○: C8αNAc, ●: C8βNAc, △: MeαNC8.

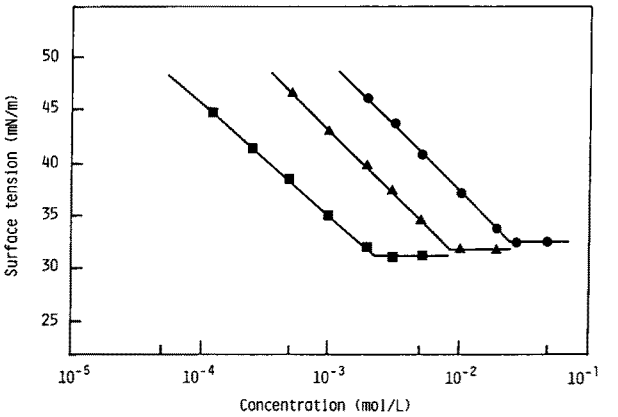


FIG. 3. Surface tension-concentration curves of alkyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochlorides at 25°C: ●: C8βN, ▲: C10βN, ■: C12βN.

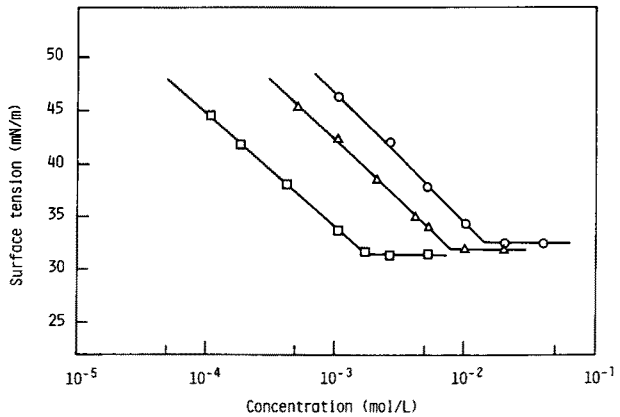


FIG. 2. Surface tension-concentration curves of alkyl 2-amino-2-deoxy-α-D-glucopyranoside hydrochlorides at 25°C: ○: C8αN, △: C10αN, □: C12αN.

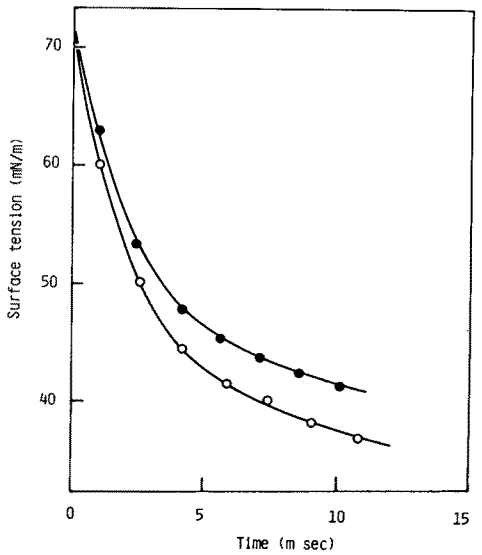


FIG. 4. Dynamic surface tension of 4 mmol/L dodecyl 2-amino-2-deoxy-D-glucopyranoside hydrochlorides at 30°C. Static surface tension for C12αN: 31.7 mN/m, C12βN: 30.8 mN/m at 30°C: ○: C12αN, ●: C12βN.

Interfacial properties. Surface tension *vs.* concentration plots for glucosamine derivatives containing an alkyl chain in distilled water are shown in Figures 1-3. MeαNCn and MeβNCn, and CnαNAc and CnβNAc and CnβNAc containing longer than C10 alkyl groups, could not be measured due to their lower solubilities in water. This is probably ascribed to the strong hydrogen bonding by the C-2 amide group. To improve the hydrophilicity of the N-acetyl derivatives (CnαNAc, CnβNAc), the C-2 amido group was deacylated by aqueous sodium hydroxide and acidified by hydrochloric acid to form *n*-alkyl 2-amino-2-deoxy-D-glucopyranoside hydrochloride (CnαN, CnβN). These hydrochlorides containing a C8 to C12 carbon chain showed surface activities and critical micelle concentrations (CMC). With respect to CMC, the shorter the alkyl chain, the higher the CMC values. This tendency can usually be seen in polyoxyethylene-type nonionic surfactants. Among them, α-anomers showed a slightly lower CMC than the β-anomers, indicating less hydrophilicity of the α-anomers. The CMC was influenced by both the length of the alkyl chain and the anomeric position of the glucosamine derivatives. Similar tendencies were observed for *n*-alkyl glycopyranosides (22). Dynamic surface tension curves of 4 mM C12αN and C12βN are shown in Figure 4. The adsorption rate onto the surface of C12αN was faster than that of the corresponding β-anomer (C12βN). C12αN reached an equilibrium value after 20 msec and C12βN after 25 msec. It seems that this difference in dynamic surface tension lowering between the α- and β-anomers was ascribed to the greater hydrophobicity of the α-anomer.

Foaming properties. Foaming properties of glucosamine derivatives were measured by the Ross and Miles method (14). Alkyl glucosaminide hydrochlorides (CnαN, CnβN) showed good foam production as well as better foam stability above the CMC. On the other hand, N-acetyl or acyl glucosamine derivatives (MeαNCn, MeβNCn, CnαNAc, CnβNAc) showed poor foaming properties. Extremely low foaming can probably be ascribed to the low solubility (low hydrophilicity) of the compounds in water. Figure 5 shows foam production, F_0 and foam stability,

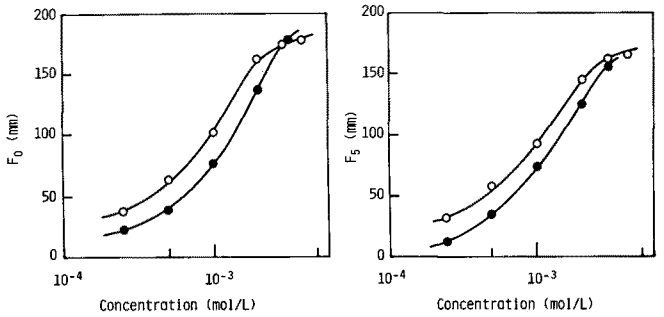


FIG. 5. Foam production (F_0) and stability (F_5) of dodecyl 2-amino-2-deoxy-D-glucopyranoside hydrochlorides by the Ross-Miles test at 30°C: ○: C12αN, ●: C12βN.

F_5, *vs.* concentration around the CMC for C12αN and C12βN as measured by the Ross and Miles method (10). Foam production of C12αN and C12βN increased around the CMC, and the α-anomer tended to show better foaming than the β-anomer.

Biodegradability. Table 2 shows the five-day biochemical oxygen demand (BOD_5) and biodegradability [BOD_5/TOD] of glucosamine derivatives as well as that for con-

TABLE 2

Biodegradability of Glucosamine Derivatives[a]

Compounds	TOD (mg O/g)	BOD_5 (mg O/g)	BOD_5/TOD (%)
MeαNC8	1930	590	30.6
MeαNC10	2052	400	19.5
MeαNC12	2150	240	11.2
MeβNC10	2052	700	30.7
C8αNAc	1991	430	21.6
C10αNAc	2103	410	19.5
C8βNAc	1991	574	28.8
C10βNAc	2103	400	19.0
C8αN	2050	350	17.1
C10αN	2158	555	25.7
C12αN	2250	645	28.7
C8βN	2050	672	32.8
C10βN	2158	1010	46.8
C12βN	2250	916	40.7
C10αGlc	2100	1220	58.1
C12E010	2200	736	33.5
C18E010	2340	669	28.6

[a]TOD, theoretical oxygen demand; BOD_5, five-day biochemical oxygen demand; C10αGlc: Decyl α-D-glucopyranoside, C12EO10: Dodecyl poly(oxyethylene) ether n=10, C18EO10: Octadecyl poly (oxyethylene) ether n=10.

TABLE 3

Antimicrobial Activity of Glucosamine Derivatives Containing Alkyl Chain and Alkyl Glucopyranosides (I)[a]

Organisms	Compounds [MIC(μg/mL)[b]]									
	MeαNC8	MeαNC10	MeαNC12	MeαNC14	MeβNC10	MeβNC12	MeβNC14	C8βGlc[c]	C10βGlc[c]	C12βGlc[c]
S. aureus	>400[d]	>400	100	200	400	50	100	400	100	10
B. subtilis	>400	>400	100	400	400	50	>400	400	100	25
S. lutea	10	10	10	10	10	10	10	100	50	10
E. coli	10	10	10	10	10	10	10	400	200	400
S. typhi	400	>400	400	200	400	50	>400	400	400	400
P. aeruginosa	10	5	5	5	5	25	10	400	200	200
C. albicans	>400	>400	200	100	100	100	>400	>400	200	400
S. cerevisiae	>400	>400	50	200	200	50	>400	>400	100	400
T. interdigitale	50	100	50	50	50	50	25	400	200	200
M. gypseum	50	100	50	50	50	50	25	400	200	200
P. chrysogenum	50	100	50	50	50	50	25	400	400	200
A. niger	>400	>400	>400	200	200	400	>400	>400	400	200

[a]Control always produced growth of the microorganism.
[b]MIC, minimum inhibitory concentration.
[c]C8βBlc:*n*-octyl β-D-glucoside, C10βGlc:*n*-decyl β-D-glucoside, C12βGlc:*n*-dodecyl β-D-glucoside (22).
[d]No inhibition, maximum concentration tested listed.

TABLE 4

Antimicrobial Activity of Glucosamine Derivatives (II)[a]

Organisms	Compounds [MIC(μg/mL)[b]]											
	C8αNAc	C10αNAc	C12αNAc	C8βNAc	C10βNAc	C12βNAc	C8αN	C10αN	C12αN	C8βN	C10βN	C12βN
S. aureus	400	200	>400	> 400[c]	>400	>400	400	100	10	400	50	10
B. subtilis	>400	200	>400	>400	>400	>400	>400	100	10	400	100	10
S. lutea	>400	200	>400	>400	>400	>400	400	50	10	400	100	10
E. coli	>400	>400	>400	>400	>400	>400	400	50	10	400	50	25
S. typhi	>400	>400	>400	>400	>400	>400	400	100	10	>400	200	25
P. aeruginosa	400	>400	>400	>400	400	400	400	200	50	>400	200	50
C. albicans	>400	>400	>400	>400	>400	>400	>400	400	200	>400	400	400
S. cerevisiae	>400	>400	>400	>400	>400	>400	>400	100	10	400	100	25
T. interdigitale	400	400	>400	400	>400	400	400	50	25	400	100	25
M. gypseum	400	400	>400	400	>400	400	400	100	25	400	200	50
P. chrysogenum	>400	400	>400	400	>400	400	>400	400	200	>400	400	200
A. niger	>400	400	>400	400	>400	>400	>400	400	400	>400	>400	400

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

ventional ethoxylated nonionics (22). All surfactants tested in this study were biodegraded by the environmental microbes in 5 d. The BOD_5 method is only a screening tool, and the compounds that give a low biodegradation during testing may be more rapidly and extensively biodegraded in soil or activated sludge, where more organisms exist and more time can be provided for acclimation.

Antimicrobial activities. Nineteen glucosamine derivatives and the corresponding *n*-alkyl glucopyranosides containing C8, C10 and C12 alkyl chains (22) were screened for antimicrobial activity against gram-positive and gram-negative bacterial strains and fungal strains. The minimum inhibitory concentrations for the compounds tested are given in Tables 3 and 4. Methyl N-acyl glucosaminides (MeαNCn, MeβNCn) and *n*-alkyl glucosaminide hydrochlorides (CnαN, CnβN) showed a broad spectrum of antimicrobial activity and much stronger antimicrobial activity than the corresponding *n*-alkyl glucopyranosides. Among them, the glucosamine derivatives containing a C12 alkyl chain were the best. On the other hand, *n*-alkyl N-acetyl glucosaminides (CnαNAc, CnβNAc) showed no significant antimicrobial activities.

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