Production and Structure Elucidation of Glycoglycerolipids from a Marine Sponge-Associated *Microbacterium* Species

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The bacterium *Microbacterium* sp., isolated from the sponge *Halichondria panicea*, produced four unusual cell-associated glycoglycerolipids and one diphosphatidylglycerol when grown on marine broth and on artificial seawater media. The lipids were isolated by chromatography on silica columns and their structures elucidated using a combination of multidimensional NMR and MS techniques. The main compound was 1-*O*-acyl-3-[α-glucopyranosyl-(1−3)-(6-*O*-acyl-α-mannopyranosyl)]glycerol (GGL.2) with 14-methyl-hexadecanoic acid and 12-methyl-tetradecanoic acid positioned at C-6 of the mannose unit and at the glycerol moiety. Glycolipid production was correlated with growth and reached a maximum value of 200 mg/L when grown on artificial seawater medium with 20 g/L glucose. The main compound decreased the surface tension of water down to 33 mN/m and the interfacial tension of the water/*n*-hexadecane system down to 5 mN/m. In addition to this good surface-active behavior, the main glycoglycerolipid showed antitumor activities.

It is well-known that marine sponges are a rich source of new bioactive compounds.[1−3] As sponges are mostly associated with microorganisms, there is a great deal of interest in marine bacteria, as original producers of the metabolites,[4,5] perhaps because they are the natural source organism and because of their easier cultivation compared to their hosts.[6,7] During a screening of sponge-associated bacteria for metabolites with unknown chemical structures and antimicrobial activities, a new *Microbacterium* sp. (DSM 12583) isolated from *Halichondria panicea* was found to produce several interesting compounds. This paper deals with cultivation conditions of the bacterium and the isolation, identification, and structure elucidation of these compounds, as well as their fundamental surfactant properties and biological activity.

Results and Discussion

To isolate pure cultures of *Microbacterium* sp., pieces of fresh material from *H. panicea* were squeezed under sterile conditions and plated on marine broth agar. After incubation at 27 °C for 3 days, single yellow colonies were obtained and transferred to new plates to enrich pure cultures of *Microbacterium* sp. The classification of the bacterium occurred by sequencing of the 16S ribosomal RNA.

When grown on marine broth containing peptone and yeast extract as carbon and nitrogen sources, *Microbacterium* sp. produced anthranilic acid and phenylacetic acid. Both well-known aromatic compounds[8] were released into the supernatant and reached amounts up to 14 mg/L in our experiments.

In addition, *Microbacterium* sp. produced at least four glycoglycerolipids and one diphospatidylglycerol. Centrifugation of culture broth after cultivation and separate extraction of the supernatant and the cells showed that all the lipids were associated with the cells. Initial studies on growth and lipid production were performed in 100-mL shake-flask cultures. An incubation temperature of 30 °C showed the best growth and glycolipid production, while at lower temperatures (27, 20, 10, and 4 °C) growth occurred more slowly and at higher temperatures (34 and 37 °C) growth ceased. The addition of 20 g/L glucose to the medium caused improved growth and a higher yield of glycoglycerolipid production. Exchange of the basic medium marine broth by artificial seawater with optimized amounts of yeast extract (3.5 g/L) and peptone (3.5 g/L) afforded the best results for both growth and lipid production. A comparison of various nitrogen sources indicated that the highest glycoglycerolipid yield was reached with ammonium chloride rather than with ammonium sulfate, ammonium nitrate, or sodium nitrate.

Figure 1 shows a batch cultivation in a 40-L bioreactor using the optimized artificial seawater medium. With an initial glucose concentration of 20 g/L, *Microbacterium* sp. ceased growth with 11−12 g/L dry biomass after 30 h when the carbon source was exhausted. Glycoglycerolipid formation was growth-associated and reached a maximum yield of 200 mg/L after 27 h. The physiological activity indicated by the pO_2-electrode data, as well as the oxygen consumption rate (QO_2) and carbon dioxide production rate (QCO_2), were in agreement with cell growth and showed the highest values during the exponential phase of growth.

The qualitative analysis of the crude cellular extract by TLC showed one diphosphatidylglycerol (DPG) at R_f 0.16 and four glycoglycerolipid fractions at R_f values of 0.31 (GGL.1), 0.45 (GGL.2), 0.56 (GGL.3), and 0.6 (GGL.4). The main product was compound GGL.2, all other components occurred only in trace amounts.

After separation and purification by chromatography on Si gel columns and plates, the structures of all components were elucidated by multidimensional ^1H and ^{13}C NMR spectroscopy in combination with both positive- and negative-ion ESIMS that included MS/MS analyses. ^1H (1D and 2D COSY) and ^{13}C (1D and DEPT-135) spectra allowed identification of the sugar, glycerol, and fatty-acid units in the molecule. The anomericity of the sugars in the compounds (detailed in the legend of Figure 2) was deduced

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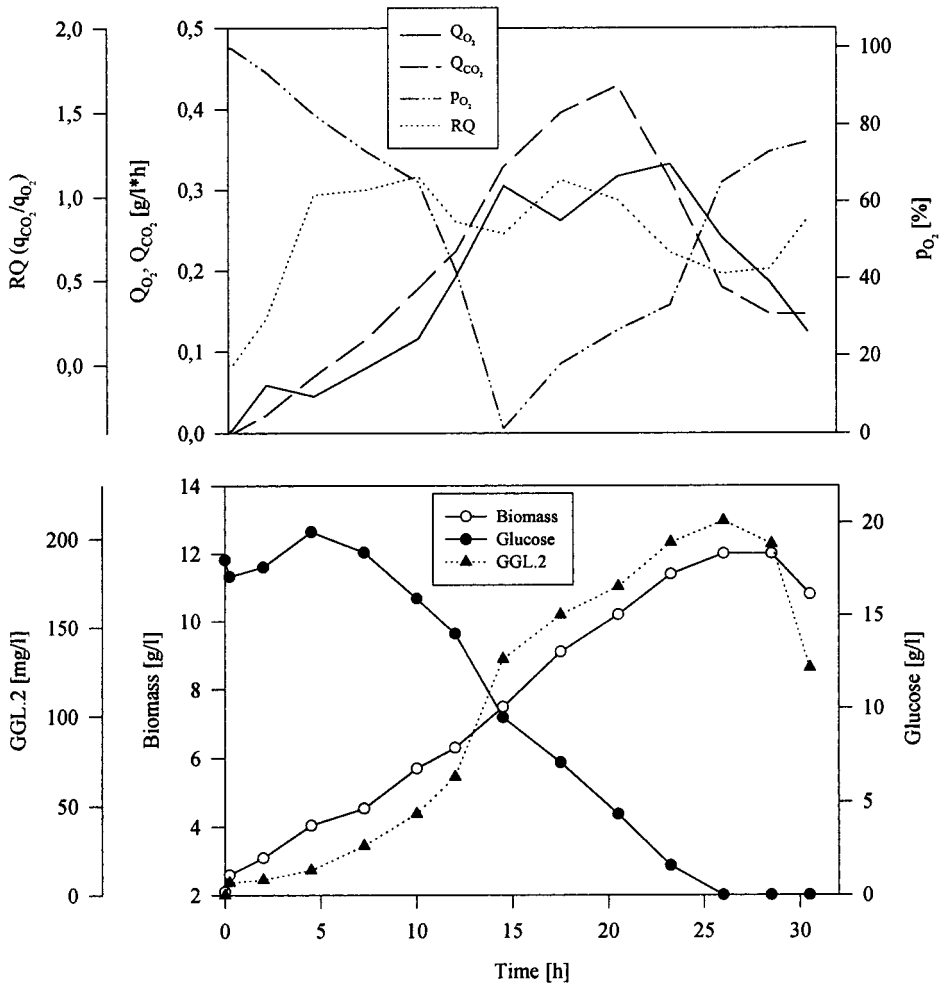


Figure 1. Growth, glycoglycerolipid production, oxygen consumption rate (QO_2), and carbon dioxide production rate (QCO_2) of *Microbacterium* sp. in a batch cultivation on glucose 20 g/L. Conditions: 40-L bioreactor, artificial seawater medium, 30 °C, 500 rpm, 0.4 v/vm, pH adjusted at 7.5.

Table 1. ^{1}H NMR Data[a] of the Sugar and Glycerol Systems of GGL.1, GGL.2, and GGL.3 in $CD_3OD/CDCl_3$ (30:70)

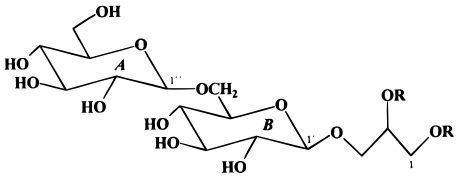
	GGL.1		GGL.2		GGL.3	
	chemical shift	coupling constant	chemical shift	coupling constant	chemical shift	coupling constant
sugar A	H-1 4.35 d	(1−2) 7.8	H-1 5.12 d	(1−2) 3.9	H-1 5.14 d	(1−2) 3.9
	H-2 3.30 dd	(2−3) 8.6	H-2 3.46 dd	(2−3) 10.5	H-2 3.45 m	(2−3) 9.5
	H-3 3.45−3.39 m		H-3 3.70 t	(3−4) 9.3	H-3 3.73 t	(3−4) 9.5
	H-4 3.45−3.39 m		H-4 3.24 t	(4−5) 9.4	H-4 3.31 t	(4−5) 9.5
	H-5 3.32 m		H-5 3.86 m		H-5 3.99 m	
	H-6A 3.87 dd	(6A-5) 2.5	H-6A 3.89 dd	(6A-5) 2.2	H-6A 4.38 dd	(6A-5) 2.1
	H-6B 3.73 m	(6B-5) 5.5	H-6B 3.63 dd	(6B-5) 7.0	H-6B 4.25 dd	(6B-5) 5.9
		(6A−6B) 12.0		(6A−6B) 11.6		(6A−6B) 11.6
sugar B	H-1 4.28 d	(1−2) 7.8	H-1 4.77 d	(1−2) 1.3	H-1 4.79 d	(1−2) 1.6
	H-2 3.26 dd	(2−3) 9.2	H-2 4.12 m	(2−3) [b]	H-2 4.07 m	(2−3)[b]
	H-3 3.47 t	(3−4) 9.2	H-3 3.85−3.82 m		H-3 3.85−3.75 m	
	H-4 3.38 t	(4−5) 9.0	H-4 3.85−3.82 m		H-4 3.76 m	
	H-5 3.46 m		H-5 3.79 m		H-5 3.82 m	
	H-6A 4.15 dd	(6A-5) 2.0	H-6A 4.42 dd	(6A-5) 1.6	H-6A 4.44 m	
	H-6B 3.82 dd	(6B-5) 4.7	H-6B 4.28 dd	(6B-5) 6.2	H-6B 4.29 dd	(6B-5) 5.2
		(6A−6B) 11.3		(6A−6B) 11.8		(6A−6B) 11.8
glycerol	H-1A 4.40 dd	(1A-2) 3.0	H-1A 4.10 dd	(1A-2) 5.7	H-1A 4.10 dd	(1A-2) 5.8
	H-1B 4.23 dd	(1B-2) 6.7	H-1B 4.14 dd	(1B-2) 4.8	H-1B 4.15 dd	(1B-2) 4.8
		(1A−1B) 12.1		(1A−1B) 11.5		(1A−1B) 11.4
	H-2 5.27 m		H-2 3.98 m		H-2 3.99 m	
	H-3A 3.97 dd	(3A-2) 5.5	H-3A 3.44 dd	(3A-2) 3.9	H-3A 3.48 dd	(3A-2) 3.7
	H-3B 3.74 m	(3B-2) 5.5	H-3B 3.76 dd	(3B-2) 3.9	H-3B 3.76 m	(3A−3B) 9.7
		(3A−3B) 11.0		(3A−3B) 10.3		

[a] Abbreviations: s, singlet; d; doublet; t, triplet; m, multiplet. [b] From the COSY spectrum this coupling is small.

from the vicinal ^{1}H−^{1}H coupling constants to H-1 (Table 1), and this was confirmed for GGL.1 and GGL.2 from the magnitudes of ^1J(CH) (Table 3). The configurations of the sugars in GGL.1 to GGL.3 were evident from the vicinal couplings of the ring protons and/or from the characteristic ^{13}C chemical shifts. ^{1}H-detected long-range ^{13}C−^{1}H (HMBC)

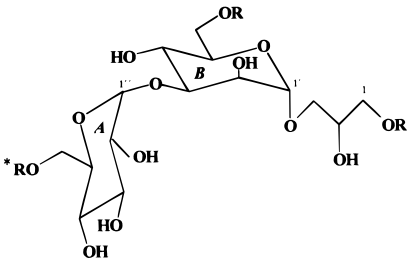
Table 2. ^{1}H NMR Data of the Sugar and Glycerol Components of GGL.4 and DPG in $CD_3OD/CDCl_3$ (30:70)

	GGL.4		DPG	
	chemical shift	coupling constant	chemical shift	coupling constant
sugar	H-1 4.93 s	(1−2) < 2		
	H-2 3.99 m			
	H-3 3.73 m			
	H-4 3.95−3.53 m			
	H-5 3.95−3.53 m			
	H-6A 3.95−3.53 m			
	H-6B 3.95−3.53m			
glycerol	H-1A 4.33 m	(1A-2) 3.4	H-1 3.62 m	
	H-1B 4.16 dd	(1B-2) 6.5	H-2 3.92 m	
		(1A−1B) 11.8	H-3AB 3.96 m	
	H-2 5.22 m		H-4 5.24 m	
	H-3A 3.78 dd	(3A-2) 5.4	H-5A 4.19 dd	(5A-4) 6.6
	H-3B 3.61 m	(3A−3B) 10.9	H-5B 4.40 m	(5A−5B) 11.7



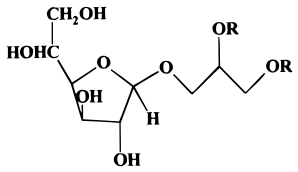
GGL.1

R = anteiso - C15:0 and - C17:0; but also iso - C16:0



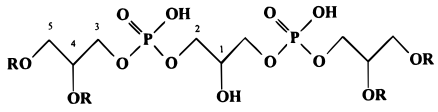
GGL.2, GGL.3

R = anteiso - C15:0 and - C17:0; but also iso - C16:0
GGL.2: *R = H, GGL.3 *R = $COCH_3$



GGL.4

R = anteiso - C15:0 and - C17:0; but also iso - C16:0



DPG

R = anteiso - C15:0 and - C17:0; but also iso - C16:0

Figure 2. Structures of the glycoglycerolipids and the cardiolipin formed by *Microbacterium* sp. GGL.1 1,2-*O*-diacyl-3-[*β*-glucopyranosyl-(1−6)-*β*-glucopyranosyl)]glycerol, GGL.2 1-*O*-acyl-3-[α-glucopyranosyl-(1−3)-(6-*O*-acyl-α-mannopyranosyl)]glycerol, GGL.3 1-*O*-acyl-3-[6-*O*-acetyl-α-glucopyranosyl-(1−3)-(6-*O*-acyl-α-mannopyranosyl)]glycerol, GGL.4 1,2-*O*-diacyl-3-[*β*-galactofuranosyl)]glycerol, and DPG tetraacyldiphosphatidylglycerol.

correlations for GGL.1 and GGL.2 afforded the unambiguous assignment of the ^{13}C signals and the points of attachment and hence sequence of the various units from the observation of ^3J(CH). These same spectra showed a complex set of correlations in the aliphatic region that were

Table 3. ^{13}C Chemical Shifts of the Sugar and Glycerol Components of the Lipids in $CD_3OD/CDCl_3$ (30:70)

	sugar A	sugar B	glycerol
GGL.1	C-1 103.8[a]	C-1 104.1[a]	C-1 63.2
	C-2 74.0	C-2 74.0	C-2 70.8
	C-3 76.9	C-3 76.9	C-3 68.5
	C-4 70.6[b]	C-4 70.2[b]	
	C-5 76.9	C-5 75.8	
	C-6 62.1	C-6 69.2	
GGL.2	C-1 100.8[a]	C-1 101.3[a]	C-1 65.5
	C-2 72.8	C-2 69.9	C-2 68.6
	C-3 74.1	C-3 80.5	C-3 69.4
	C-4 71.1	C-4 66.5	
	C-5 73.0	C-5 71.2	
	C-6 62.3	C-6 64.3	
GGL.3	C-1 101.4	C-1 101.3	C-1 65.7
	C-2 72.7	C-2 70.4	C-2 68.6
	C-3 73.8	C-3 80.8	C-3 69.4
	C-4 70.8	C-4 66.6	
	C-5 70.7	C-5 71.4	
	C-6 64.3	C-6 64.3	
GGL.4	C-1 108.8		C-1 63.9
	C-2 81.2		C-2 70.5
	C-3 77.9		C-3 66.1
	C-4 85.3		
	C-5 71.5		
	C-6 63.1		
DPG			C-1 70.5[c]
			C-2 66.7[c]
			C-3 64.0[c]
			C-4 70.9
			C-5 63.1

[a] The ^1J(CH) to these carbons were determined from the residual cross-peaks in the HMBC spectrum and had magnitudes for GGL.1 of 160 Hz for both signals and for GGL.2 of 169 Hz for both signals allowing confirmation of the anomericity of the sugar units.[22] [b] These assignments are interchangeable. [c] C-1 is a broader signal than C-4, and C-2 and C-3 are broader than C-5 through coupling to ^{31}P.

compatible only with a mixture of terminally branched (anteiso and iso) fatty acids (Table 4). Comparison of the ^{1}H and ^{13}C shifts of GGL.2 and GGL.3 indicated the position of the third acyl group at C-6 of ring A, and it was assumed that this was the new acetyl system. For GGL.4, the NMR data indicated the presence of a *β*-galactofuranose unit that was particularly evident from a comparison of the ^{13}C shifts with data for the methyl aldosides.[19] This was confirmed from the observation of an excess of galactofuranose forms in the component monosaccharide analysis. The resulting structures of all compounds are shown in Figure 2.

The exact nature and percentages of the fatty-acid components present in each system could be unambiguously deduced from an extensive MS analysis that is only

Table 4. ^{1}H and ^{13}C NMR Data for the Acyl Moieties of All Lipids in $CD_3OD/CDCl_3$ (30:70)

^{1}H NMR[a] chemical shift		coupling constant		^{13}C NMR chemical shift[b]	
H-2 2.35 m				C-1[c]	
H-3 1.62 m				C-2 34.4	
CH_2(rest) 1.35−1.23				C-3 25.2	
				C-4 29.5	
				CH_2 (rest) 29.6−30.3	
Anteiso-form	Iso-form	Anteiso-form	Iso-form	Anteiso-form	Iso-form
H-ω 0.86 t	0.86 d	[ω-(ω-1)] 6.8	6.8	C-ω 11.5	22.8
H-(ω-1)A 1.26 m 1.52 m				C-(ω-1) 29.9	28.3
H-(ω-1)B 1.12 m				C-(ω-2) 34.7	39.4
H-(ω-2) 2.35 m	1.16 m			C-(ω-3) 37.0	
H-(ω-3) 1.26 m				C-(ω-4) 25.2	
(ω-2)-CH_3 0.88 d		[CH_3-(ω-2)] 6.5		(ω-2)-CH_3 19.4	

[a] The methyl shift of the acetyl group in GGL.3 is at 2.10 ppm. [b] The shifts of Shirahashi et al.[18] require correcting. [c] The exact shift of the carbonyl group was dependent on the immediate environment, and each compound showed two signals for the long-chain acyl groups in GGL.1 at 174.6 and 174.3 ppm, in GGL.2 at 174.9 and 174.7, in GGL.3 at 175.0 and 174.8 with an additional signal of the acetyl group at 172.3 and the corresponding methyl at 20.9, in GGL.4 at 174.5 and 174.1, and in DPG at 174.4 and 174.0.

described in outline here. The relative abundance of the fatty-acid components was determined by integration of their methyl ester peaks obtained after methanolysis and GC/MS. The branching points within the alkyl chains were deduced from their characteristic fragmentation patterns. The majority of fatty acids with an even number of carbon atoms were of the iso-form, while the rest were of the anteiso-form. The intact glycolipid fractions were then subjected to ESIMS/MS analysis in the positive or negative ion mode. The abundance and number of components (Table 5), differing in the length of the alkyl chain of the fatty acid residues, was determined from the resulting pattern of molecular ions (sodium adducts in positive mode and the deprotonated molecular species in the negative mode). The molecular mass of the individual fatty-acid constituents could be easily determined from the intense fragment ions in the daughter-ion spectra formed by elimination of these residues in the positive-ion mode [M − fatty acid + Na]$^+$ or the corresponding ions of the eliminated deprotonated fatty acids in the negative-ion mode. Even isomeric forms of components having the same nominal total molecular mass in which there were different combinations of fatty acids could be differentiated in this way (Table 5). In addition the presence or absence of a substituent at the terminal glucose of fractions GGL.1−3 was readily confirmed from the characteristic fragment ion generated by the loss of this residue in the positive-mode spectra. Interestingly, the respective fragment ion had a much higher relative intensity in the case of the Glcα1−3 linkage found in GGL.2+3 than in isomeric structures with a Glcβ1−6 linkage as found in GGL.1.

Glycoglycerolipids are widely found among microorganisms as components of the cell wall.[9−11] It is well-known that bacteria of the genus *Microbacterium* contain diglycosyldiacylglycerol (mainly dimannosyldiacylglycerol), monoglycosyldiacylglycerol, and DPG.[12] The major fatty acids in many strains are 12-methyltetradecanoic (anteiso-C_{15}) and 14-methylhexadecanoic (anteiso-C_{17}) acids.[13] Similarly, glycoglycerolipids have been found in some species of *Arthrobacter*, where the same fatty-acid compositions predominate.[14] A recent analysis of the polar lipids of *A. atrocyaneus*[15] also identified a diglycosyldiacylglycerol with nearly the same structure and fatty-acid composition as GGL.2, but it possesses two mannose units instead of one mannose and one glucose unit in the sugar moiety found here.

The main compound GGL.2 decreased the surface tension of water from 72 mN/m to 33 mN/m at a concentration of approximately 200 mg/L (Figure 3). This is a good value and comparable to other microbial surfactants, which decrease the surface tension of water to within the 29−38 mN/m range.[16] Similarly, the interfacial tension of the

Table 5. Mass Spectrometric Analysis of the Lipid Mixture by Negative- and Positive-Ion ESIMS

molecular weight of component	positive-ion [M + Na]$^+$	negative-ion [M − H]$^-$	absolute % of acyl components[a]	absolute % of component
GGL.1				
864	887		ai-C 15:0	24
878	901		ai-C 15:0(50) i-C 16:0 (50)	20
892	915		ai-C 15:0 (50) ai-C 17:0 (50)	34
906	929		i-C 16:0 (50) ai-C 17:0 (50)	6
920	943		ai-C 17:0	5
GGL.2				
864	887	863	ai-C 15:0	21
878	901	877	ai-C 15:0 (50) i-C 16:0 (50)	27
892	901	891	ai-C 15:0 (50) ai-C 17:0 (50)	44
906	929	905	i-C 16:0 (50) ai-C 17:0 (50)	4
920	943	919	ai-C 17:0	4
GGL.3				
906	929	905	ai-C 15:0	6
920	943	919	ai-C 15:0 (50) i-C 16:0 (50)	32
934	957	933	ai-C 15:0 (50) ai-C 17:0 (50)	50
948	971	947	i-C 16:0 (50) ai-C 17:0 (50)	7
962	985	961	ai-C 17:0	5
GGL.4				
702	725		ai-C 15:0	26
716	739		ai-C 15:0(50) i-C 16:0 (50)	23
730	753		ai-C 15:0 (50) ai-C 17:0 (50)	46
744	767		i-C 16:0 (50) ai-C 17:0 (50)	3
758	781		ai-C 17:0	2
DPG				
1310	1333	1309	ai-C 15:0 (75) i-C 16:0 (25)	13
1324	1347	1323	ai-C 15:0 (75) ai-C 17:0 (25)	38
1338	1361	1337	ai-C 15:0 (50) i-C 16:0 (25 ai-C 17:0 (25)	31
1352	1375	1351	ai-C 15:0 (50) ai-C 17:0 (50)	11
1366	1389	1365	ai-C 15:0 (25) i-C 16:0 (25) ai-C 17:0 (50)	3

[a] Abbreviations: ai, anteiso; i, iso.

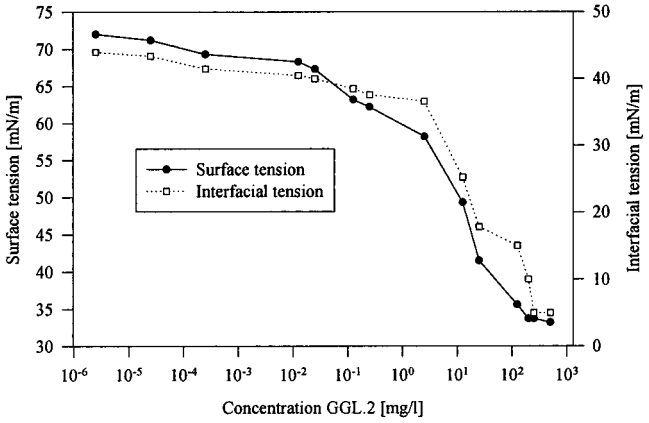


Figure 3. Influence of purified glycoglycerolipid GGL.2 from *Microbacterium* sp. on the surface tension of water and the interfacial tension of the water/*n*-hexadecane system at 25 °C.

water/*n*-hexadecane system was reduced from 44 mN/m to 5 mN/m. Consequently, this glycoglycerolipid shows considerable potential for surfactant applications.

Screening for antimicrobial activities against *Bacillus megaterium*, *Escherichia coli*, *Ustilago violacea*, and *Chlorella fusca* showed no pronounced effects, but in initial studies of antitumor activities positive results were found for GGL.2 and the related glycoglycerol.[17] This result and the fact that there are data in the literature suggesting possible antitumor activities for other related glycoglycerolipids and glycoglycerols provide incentives for further studies.[18]

Experimental Section

General Experimental Procedures. For biomass measurement, 10 mL of whole broth was centrifuged for 20 min at 13 000 rpm, dried at 105 °C, and determined gravimetrically. The glucose concentration was measured with Accutrend analytical test strips (Boehringer, Mannheim, Germany). To determine the content of anthranilic acid and phenylacetic acid, 200 mL of whole broth was centrifuged for 20 min at 6000 rpm. The supernatant was adjusted to pH 3.0 and extracted three times with the same volume of ethyl acetate. Quantitative measurement was performed by TLC/densitometer CD 60 (Desaga, Heidelberg) with RP_{18} Si gel as stationary phase and acetonitrile/water/acetic acid (30:65:5) as solvent system, with detection at 235 nm.

To determine the content of glycoglycerolipid, 100 mL of culture broth was centrifuged for 20 min at 6000 rpm. The cells were extracted by stirring with 100 mL of CH_2Cl_2/CH_3OH (2:1) for 12 h. Quantitative measurement was performed by TLC/densitometer CD 60 with Si gel 60 as stationary phase, chloroform/methanol/water (65:15:2) as solvent system, and α-naphthol/sulfuric acid as detecting reagent at 580 nm.

The crude lipids were purified using liquid chromatography with a stationary phase of Si gel 60 and a solvent system consisting of various proportions of CH_2Cl_2/CH_3OH (from 70:30 to 50:50).

Structure analysis of all compounds was performed using NMR spectroscopic and mass spectrometric techniques. 1D (^1H and ^{13}C) and 2D (^1H COSY, inverse ^1H-detected one-bond and multiple-bond $^{13}C-^1H$ correlations[20,21]) NMR spectra were recorded at 300 K on Bruker ARX 400 and DPX 600 NMR spectrometers locked to the major resonance of the mixed solvent, $CD_3OD/CDCl_3$ (30:70). Chemical shifts were referenced to the residual methanol signal (3.35 ppm), and coupling constants are given in hertz. Fatty-acid and monosaccharide components were analyzed after methanolysis (0.625 M HCl in MeOH at 70 °C for 12 h) and pertrimethylsilylation on a Finnigan gas chromatograph interfaced to a GCQ ion trap mass spectrometer (Finnigan) running in the electron impact mode (EI). Negative- and positive-ion-electrospray mass spectra and MS/MS experiments were recorded on a Finnigan MAT TSQ 700 triple quadrupole mass spectrometer.

To estimate the antimicrobial activity of all compounds, the organic extracts of the supernatant and the cells were applied to 5-mm filter-paper disks. Disks were then placed on agar plates [CP agar for *C. fusca*: 10 g/L glucose, 10 g/L yeast extract, pH adjusted to 6.2; NB agar (nutrient broth, Difco) for *B. megaterium* and *E. coli*; MPY agar for *U. violacea*: 20 g/L malt extract, 2.5 g/L peptone, 2.5 g/L yeast extract] and were spread with a fresh culture of each test organism. Plates were incubated up to 5 days to allow growth. Zones of inhibition were measured as excess diameter.

The surface tension of aqueous solutions and the interfacial tension of the *n*-hexadecane/aqueous system of the purified glycoglycerolipid were determined with a tensiomat (MGW Lauda, Königshofen, Germany) at 25 °C, using the ring method.

Isolation and Culture Conditions. The sponge *H. panicea* (Porifera, Demospongiae, Halichondriida, Halichondriidae), a gift of Prof. Dr. W. E. G. Müller, University of Mainz, Germany, was collected in August 1995, along the Adriatic coast, Rovinj, Croatia. Bacteria were collected by squeezing the sponge, and initial growth was achieved on marine broth agar (Difco).The bacterial strain isolated in this study was identified by sequencing the 16S ribosomal RNA as *Microbacterium* sp. (DSM 12583) by the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) and shown to be a new species of the genus *Microbacterium*. Slants were stored at 4 °C and transferred at 2-month intervals. Liquid cultures (500-mL Erlenmeyer flasks, 100 mL broth volume) were incubated at 30 °C in a shaking incubator rotating at 100 rpm until cells reached the stationary phase. The artificial seawater medium contained (per liter): NaCl 23.0 g, KCl 0.75 g, $CaCl_2 \cdot 2H_2O$ 1.47 g, $MgCl_2 \cdot 6H_2O$ 5.08 g, $MgSO_4 \cdot 7H_2O$ 6.16 g, NH_4Cl 5 g, yeast extract 3.5 g, peptone 3.5 g, $Na_2HPO_4 \cdot 2H_2O$ 0.89 g, and glucose 20 g. Batch cultivation on marine broth was carried out in a 10-L bioreactor (Braun, Melsungen, Germany). The conditions were as follows: stirring 800 rpm, temperature 30 °C, aeration rate 0.1 v/vm, and incubation time up to 170 h. For batch cultivation on artificial seawater medium a 40-L bioreactor was used with the following conditions: stirring 500 rpm, temperature 30 °C, aeration rate 0.4 v/vm, pH adjusted at 7.5, and incubation time up to 31 h. Both bioreactors were equipped with an intensor system, and the physiological activity was followed by the use of a pO_2-electrode, and by oxygen and carbon dioxide gas analyzers (Oxygor and Unor, Maihak, Hamburg, Germany).

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