A Novel Monoacyldiglycosyl−Monoacylglycerol from *Flavobacterium marinotypicum*

Hisaaki Yagi* and Akihiko Maruyama

National Institute of Bioscience and Human Technology, 1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan

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A monoacyldiglycosyl−monoacylglycerol was isolated from the Gram-negative bacterium *Flavobacterium marinotypicum* (ATCC 19260). The structure was determined, mainly by spectral data, to be [α-glucopyranosyl-(1→3)-*O*-(6-*O*-acyl-α-mannopyranosyl)]-*O*-acylglycerol.

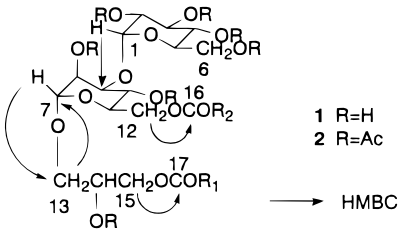
Monoacyldimannopyranosyl−monoacylglycerol acylated at the *sn*-1 (or *sn*-3) and the C-6 positions of internal mannopyranose was recently found in the *Saccharopolyspora* genus,[1] the Coryneform bacterium *Arthrobacter atrocyaneus*,[2] and the sponge-associated bacterium *Micrococcus luteus*.[3] It is unusual with respect to the location of fatty acids at *sn*-1 (or *sn*-3) of glycerol and the C-6 position of the internal hexopyranose moiety, placing this glycolipid in the class of lyso-glycerols.

We have isolated a monoacyldiglycosyl−monoacylglycerol from the Gram-negative bacterium *Flavobacterium marinotypicum* (ATCC 19260). It contains both glucopyranose and mannopyranose in the disaccharide unit and is acylated at the C-6 position of mannopyranose and the methylene position of glycerol. In this paper, we present the isolation and elucidation of the structure of this glycolipid. To our knowledge, this is the first report of its isolation from a Gram-negative bacterium.

The positive FABMS spectrum of glycolipid fraction **1** (Figure 1) showed three pseudomolecular ion peaks at *m*/*z* 887, 901, and 915 $[M + Na]^+$. The presence of spin systems corresponding to two sugar units (H1−H6, H7−H12), glycerol (H13−H15), and fatty acids were suggested from $^1H-^1H$ COSY and HOHAHA spectra. The HMQC spectrum gave the assignment of carbons directly bonded to protons. After conversion to methylesters of fatty acids by methanolysis, fatty acid moieties were determined to be C15, C16, and C17 by GC−MS analysis, when mass fragmentation gave molecular ions of *m*/*z* 256, 270, and 284.

The NMR spectra revealed a number of oxymethines and methylenes (δ_H 4.09−4.95, δ_C 62.6−83.2), together with two anomeric methines, δ_H 5.34 and 5.67, attached to carbon resonating at 102.4 and 102.9 ppm, suggesting the presence of two sugar units (Table 1). Starting from the two anomeric protons (H1, H7), interpretation of COSY and HOHAHA spectra revealed spin systems corresponding to two sugar units. The relative configuration of the sugars was determined using coupling constants and NOE cross peaks. The high values of the coupling constants J_{2-3} 10.11 Hz, J_{3-4} 9.80 Hz, and J_{4-5} 9.84 Hz in the acetyl derivative indicated an axial orientation of H2, H3, H4, and H5. Then, the sugar H1−H6 was assigned as glucopyranose. An anomeric proton H1 was in the α-configuration, as judged from the coupling constant of J_{1-2} 3.86 Hz. The high values of the coupling constants J_{9-10} 9.41 Hz and J_{10-11} 9.83 Hz indicated an axial orientation of H9, H10, and H11. In contrast, J_{8-9} 2.98 Hz showed an equatorial orientation of H8, indicating that the sugar H7−H12 had a mannopyranose configuration. The absence of NOE cross peaks H7/H9 and H7/H11 suggested that H7 was equatorial.

Two oxymethylenes (δ_H 3.87, 4.18, and 4.53) and oxymethines (δ_H 4.44) were assigned to the glycerol moiety on the basis of spin systems from $^1H-^1H$ COSY and their directly bonded carbons. The downfield shift of the H-14



COR_1, COR_2	*m/z* $[M+Na]^+$(for **1**)
C15, C15	887
C15, C16	901
C15, C17	915
C16, C16	915

Figure 1. The structure of the glycolipid fraction **1**:R=H and **2**:R=Ac. The positional distribution of fatty acids was not determined.

Table 1. NMR Data on Monoacyldiglycosyl−Monoacylglycerol (Glycolipid Fraction **1**) and Its Acetyl Derivative (**2**) in Pyridine-d_5[a]

	1			2	
no.	^{13}C	^1H	HMBC	^{13}C	^1H
1	102.9	5.67 (d, 3.86)	3, 9	97.2	5.62 (d, 3.71)
2	74.2	4.11 (dd, 3.86, 9.12)		71.4	5.24 (dd, 3.71, 10.11)
3	75.4	4.59 (dd, 9.12, 9.19)	2, 4	69.9	5.89 (dd, 10.11, 9.80)
4	72.0	4.09 m	3, 6	69.1	5.47 (dd, 9.80, 9.84)
5	74.6	4.74 m		69.0	4.67 m
6	62.6	4.24 (dd, 5.89, 11.5)	4, 5	62.5	4.46 m
		4.44 m	4		4.60 m
7	102.4	5.34 s	8, 9, 11, 13	98.4	5.24 s
8	70.9	4.78 m	7, 9, 10	71.2	5.71 m
9	83.2	4.47 (dd, 2.98, 9.41)	1	73.3	4.67 m
10	67.2	4.69 (dd, 9.41, 9.83)	9, 11, 12	68.7	5.84 (dd, 9.91, 9.93)
11	72.3	4.43 m	9	69.5	4.31 m
12	64.4	4.72 m		62.5	4.52 (dd, 2.31, 12.2)
		4.95 (d, 10.34)	10, 16		4.60 m
13	69.8	3.87 (dd, 10.16, 6.30)	7, 14, 15	66.7	3.93 (dd, 6.34, 10.98)
		4.18 (dd, 10.16, 5.08)	14, 15		4.14 (dd, 4.44, 10.98)
14	68.5	4.44 m	13, 15	70.7	5.61 m
15	66.4	4.53 m	14, 17	62.5	4.46 m
					4.65 m

[a] Chemical shifts are referenced to solvent peaks; δ_H 7.2 and δ_C 123.5 ppm for pyridine-d_5.

* To whom correspondence should be addressed. Tel.: +81-298-54-6162. Fax: +81-298-54-6172. E-mail: hyagi@ccmail.nibh.go.jp.

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proton in the fully acetylated compound suggested that the hydroxyl at C-14 of **1** was not acylated.

HMBC experiments furnished correlations between H1/C9 and H9/C1, which established unambiguously the 1−3 linkage of the two sugar moieties. Correlations between H13/C7 and H7/C13 showed that the glycerol moiety was attached to anomeric carbon C-7. Long-range correlations of H12/C16 and H15/C17 demonstrated that the two acyl groups were located on C-12 and C-15 oxygen (Figure 1). The glycolipid fraction was thus determined to be [α-glucopyranosyl-(1→3)-*O*-(6-*O*-acyl-α-mannopyranosyl)]-*O*-acyl glycerol.

The positive FABMS spectrum of the acetyl derivative showed three pseudomolecular ion peaks *m*/*z* 1181, 1195, and 1209 $[M + Na]^+$, which suggested that the acetyl derivative was a heptaacetyl derivative. The HMBC correlation from H6 to carbonyl carbon of an acetyl group was observed, hence OH-6 was acetylated. No prominent downfield shift of H6 was observed, however.

In recent studies, *Hyphomonas jannaschiana* was found to be devoid of phospholipids and contained almost entirely glycolipids as polar lipids.[4,5] Although phospholipids are thought to be a common component of the cell membrane, this may not be obligatory when there are substitute components such as glycolipids or *N*-acylornithine. Further instances of cell membranes lacking phospholipids may exist. Although the *F. marinotypicum* described in this paper also contained quite a large amount of glycolipid, it probably also contains phospholipid, suggested by a positive spot with the Dittmer reagent.

Experimental Section

General Experimental Procedures. ^1H and ^{13}C NMR spectra were recorded on a Bruker DMX-500 spectrometer. All chemical shifts are given in parts per million (ppm) relative to pyridine-d_5 (δ_H 7.2 and δ_C 123.5) and couplings in Hertz. The FABMS were obtained on a JEOL JMS−HX110 mass spectrometer using *m*-nitrobenzyl alcohol as a matrix.

To analyze fatty acid components, the compound was methanolyzed at 90 °C for 2 h in 0.7 M HCl in MeOH and the reaction solution extracted with hexane. The hexane layer was examined by GC−MS (HP 5971A and HP 5890).

Bacterial Material. *Flavobacterium marinotypicum* (ATCC 19260) was purchased from American Type Culture Collection.

Extraction and Isolation of Monoacylglycosyl−Monoacylglycerol. The bacterium was grown at 25 °C on a medium containing 1% glucose, 0.3% peptone, and 0.1% yeast extract in artificial seawater. Cells were collected in the stationary phase and, after centrifugation, washed with distilled H_2O, then lyophilized. Lyophilized cells were extracted with a $CHCl_3$−MeOH solution (1:2 v:v) and the extract evaporated under reduced pressure. The extract was fractionated by flash chromatography on Si gel with $CHCl_3$, $CHCl_3$−MeOH (95:5), $CHCl_3$−MeOH (9:1), $CHCl_3$−MeOH (8:2), $CHCl_3$−MeOH (1:1), and $CHCl_3$−MeOH−H_2O (7:3:0.5). The fraction eluted with $CHCl_3$−MeOH (8:2) was evaporated, then dissolved in MeOH. The monoacylglycosyl−monoacylglycerol fraction was obtained as MeOH-insoluble material.

To obtain the acetyl derivative of monoacylglycosyl−monoacylglycerol, the glycolipid fraction was dissolved in pyridine (0.5 mL) and Ac_2O (0.25 mL) and stirred overnight. The solution was evaporated in a N_2 stream and the residue examined as the acetyl derivative of glycolipid fraction.

References and Notes

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