Pergamon

Tetrahedron, Vol. 52, No. 11, pp. 3873-3878, 1996

0040-4020/96 $15.00 + 0.00

S0040-4020(96)00136-6

ISOLATION AND CHARACTERIZATION OF A β-1-O-ACYL-β-1,2-DIGLUCOSYL GLYCOSIDE FROM THE MEMBRANES OF A GRAM POSITIVE BACTERIUM *Sarcina ventriculi.*

Jeongrim Lee and Rawle I. Hollingsworth*

Department of Chemistry, Michigan State University, East Lansing, Michigan 48824, U.S.A.

Abstract: A new β-1-O-acyl-β-1,2-diglucosyl glycoside has been isolated from the membranes of the gram positive bacterium *Sarcina ventriculi*. Its levels are regulated by environmental conditions such as pH and temperature. The structure was determined by 2-D-NMR spectroscopy and by mass spectrometry.

INTRODUCTION

Synthetic O-glycosides and O-glycosides from natural sources are extremely important molecules for many applications. The simplest commercially important O-glycosides are the 1-alkyl glycosides used as emulsifiers in paint formulations and for the large scale purification or stabilization of proteins. Octyl glucoside is the most commonly used glycoside for the latter application. Another potential use of simple O-glycosides is as non-ionic surfactants for laundry applications. Long chain alkyl glycosides have been extensively studied from the standpoint of their crystal structure and conformational properties and their ability to form liquid crystalline phases[1-3]. One glycoside that has risen to some prominence recently is sulfoquinovosyl diacyl glycerol. This is a very important candidate as an anti-aids drug[4].

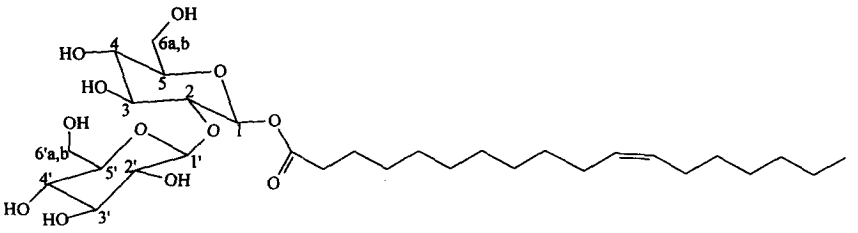
Amphiphilic molecules tend to form organized structures and are thus always interesting prospects for the preparation of liquid crystalline materials. One obvious way of controlling the properties of glycosides is to alter the hydrophilicity of the polar headgroup. Hence disaccharide-containing alkyl glycosides should have different physical properties, such as critical micelle concentration, for instance, when compared to the corresponding monosaccharide derivatives. Acyl glycosides are relatively unknown compared to long chain alkyl glycosides. From a synthetic standpoint, they represent much more of challenge. Simple alkyl glycosides can be prepared by a Fischer acid-catalysed reaction between an alcohol and a monosaccharide carbohydrate to give, in the case of glucosides, the α-anomer. This will not work with acylations since the other hydroxyl groups will also react under these conditions. A relatively elaborate scheme of protecting all of the hydroxyl groups on the carbohydrate molecule, with benzyl groups for example, activating the reducing end with a bromo functionality and doing an sn2 type displacement with a carboxylate followed by catalytic hydrogenolysis would have to be followed. Acyl glycosides with disaccharide head groups, especially those with unusual linkages, are more problematic and naturally occurring ones are immediately interesting.

We have been studying the changes in membrane chemistry that accompany the adaptation of certain bacteria to extreme changes in environment[5-8]. Such changes include pH, temperature and the presence of organic

solvents or antibiotics. Among the many fascinating modifications that occur is the chemical linking of hydrocarbon chains from opposite ends of the membrane bilayer to form transmembrane bifunctional fatty acid species that span the bilayer. These changes are important for maintaining the optimum dynamic range of molecular motion and energy transfer[9,10]. There are also several modifications to membrane lipid head group structure. We report here on the structure of an unusual 1-O-acyl-disaccharide with an unusual β-1,2-linkage (**1**) that is formed in the membranes of the gram positive organism *Sarcina ventriculi* under low pH conditions. This represents the first report of this class of molecules in bacteria.

RESULTS AND DISCUSSION

Growth of *Sarcina ventriculi* under low pH conditions led to the formation of a new slow-moving component on thin layer chromatography. Gas chromatography and GC-MS indicated that the compound contained fatty acids, predominantly hexadecanoic acid, octadecanoic acid and octadecenoic acid, and glucose. The ^{1}H-NMR spectrum contained signals between 0.5 ppm and 2.4 ppm characteristic of fatty acid groups as well as some between 3.0 ppm and 3.6 ppm characteristic of carbohydrate groups (Figure 1). A triplet at 5.14 ppm that was partially obscured by the water line but readily visible in the water-suppressed ^{1}H-NMR spectrum was assigned to the vinyl protons of unsaturated fatty acids. A multiplet at 1.82 ppm was assigned to the methylene groups adjacent to these unsaturations. Signals for the methylene groups adjacent to the carbonyl functions of fatty acid groups appeared as a multiplet at 2.14 ppm. The signals for the terminal methyl groups of fatty acid chains appeared at 0.67 ppm. Two signals of equal intensity at 5.40 and 4.36 ppm were attributed to two anomeric proton resonances. They appeared as doublets and displayed large coupling constants of 8 Hz. The large chemical shift of the one at 5.40 ppm indicated that the position was substituted by an electron withdrawing function such as an acyl group. Further proton signal assignments were accomplished through the spin connectivities in the DQF-COSY spectrum. The anomeric proton resonance at 5.40 ppm showed a cross peak with the signal at 3.36 ppm. This signal was, therefore, assigned to H-2. The H-2 signal for the other glycosyl residue was assigned in a similar fashion. It was possible to make most of the proton assignments from the COSY cross peaks and a consideration of the multiplicities and coupling constants of the signals. The complete proton and carbon assignments are given in Table 1. The ^{1}H-^{13}C-HMQC NMR spectrum (Figure 2) confirmed the assignments in the ^{1}H-spectrum. The proton signal at 5.40 ppm showed a cross peak with a ^{13}C signal at 92.5 ppm. As noted earlier, the large proton chemical shift of H-1 indicated that the anomeric position at C-1 was probably acylated by a fatty acid group. This was confirmed by the ^{13}C spectrum since the corresponding chemical shift appeared at a much more upfield position quite close to that of a free anomeric carbon. The other anomeric proton signal correlated with a ^{13}C signal at 104.4 ppm as is expected for β-glycosides. The next most downfield ^{13}C signal appeared at 81.9 ppm and



1

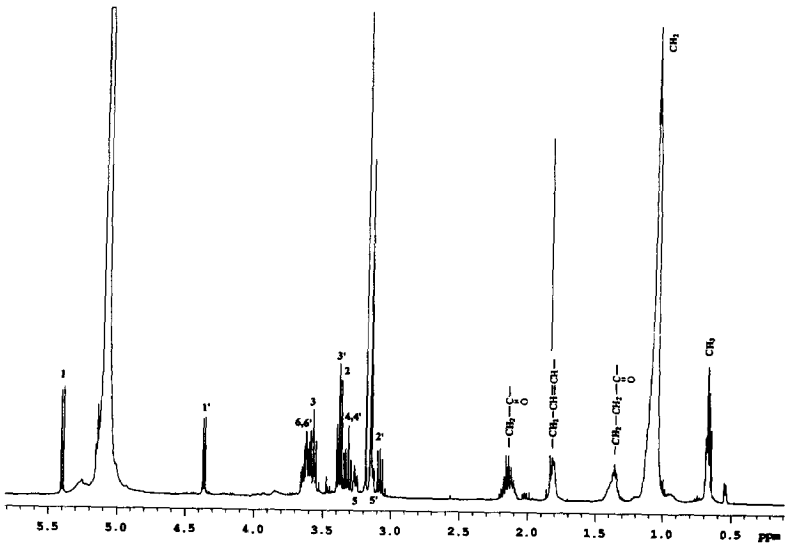


Figure 1. ^{1}H NMR spectrum of β–1–O–acyl-β–1,2–diglucosyl glycoside of *S. ventriculi*

Table 1. ^{1}H and ^{13}C chemical shifts of the disaccharide residue of β–1–O–acyl-β–1,2–diglucosyl glycoside

^{1}H(ppm)	H-1	H-2	H-3	H-4	H-5	H-6
Glu-1	5.40	3.36	3.57	3.34	3.26	3.75, 3.64
Glu-1'	4.36	3.08	3.38	3.31	3.13	3.59, 3.62
^{13}C(ppm)	C-1	C-2	C-3	C-4	C-5	C-6
Glu-1	92.5	81.9	75.5	69.0	76.7	61.1
Glu-1'	104.4	74.3	75.7	69.4	76.1	60.8

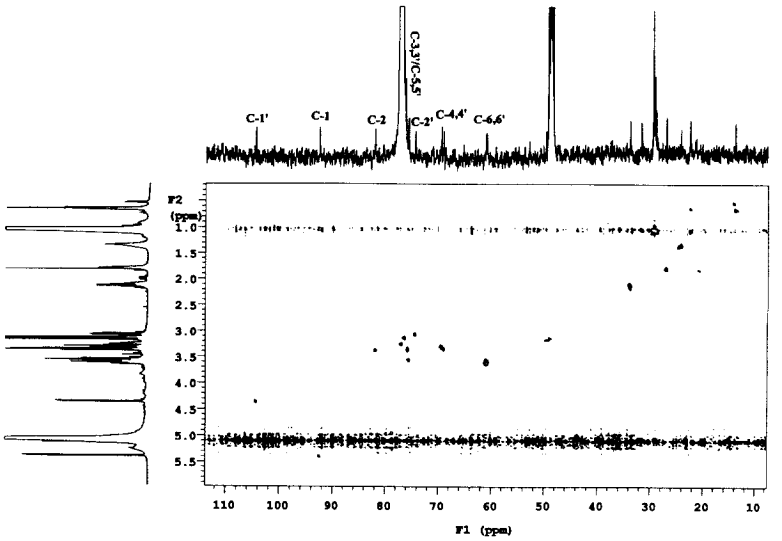


Figure 2. 2-D proton-carbon heteronuclear multiquntum coherence (HMQC) NMR spectrum

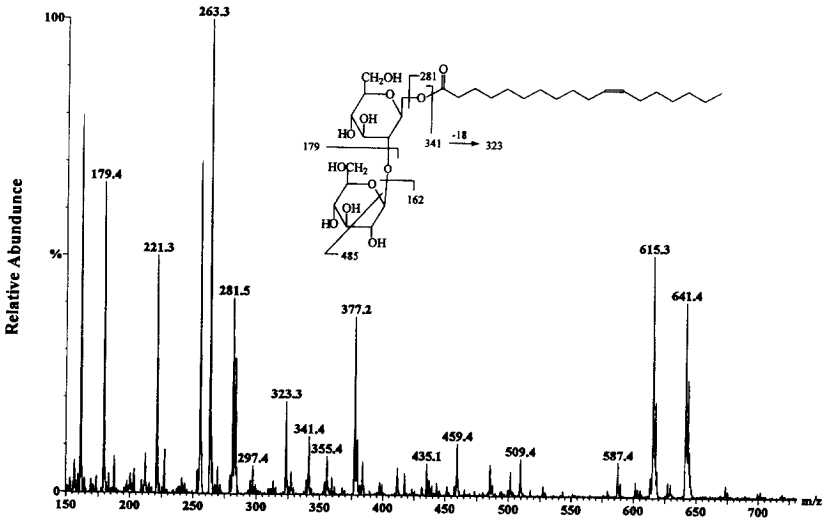


Figure 3. Negative ion electrospray ionization mass spectrum

correlated with the proton signal at 3.36 ppm which was earlier assigned to H-2 of the glucose residue bearing the acyl group. This carbon position is, therefore, involved in the linkage of two glucosyl residues as indicated by its large ^{13}C chemical shift. The signal assignments from DQF-COSY and HMQC results therefore revealed that the two glucosyl residues were linked at the 2-position and the reducing end was acylated. The other signals in the ^{13}C-NMR spectrum were also in agreement with the proposed structure (Table 1). The reported chemical shift of the non-reducing anomeric carbon of β-sophorose(a β-1,2-linked disaccharide of D-glucopyranose) is 103.9 Hz[11], quite consistent with our value.

The negative ion ESI mass spectrum contained signals for molecular ions species as chloride adducts $(M+Cl)^-$ at m/z 587, 615, 641, and 643 (Figure 3). The assignments were in agreement with ions at m/z 575, 603, 629, and 631 as $(M+Na)^+$ ions in the positive ion mode. These masses were fully consistent with the presence of four species with the general assigned structure but differing in their fatty acid chains. In addition to these peaks, the fragments showed diagnostic ions at m/z 341, 323, 281, 263, 221, 179, and 162. These ions were associated with fragment ions from the disaccharide residue[12]. The abundant ions corresponding to carboxylate anions appeared at m/z 255($C_{16:0}$), 281($C_{18:1}$) and 283($C_{18:0}$). GC and GC/MS analyses revealed that the ratio of hexadecanoic acid, octadecanoic acid and octadecenoic acid was 2.4:2.7:1.0, respectively. Also, the position and configuration of double bond of octadecenoic acid were confirmed as *cis*-11-octadecenoic acid in a previous study[7]. A signal in the mass spectrum indicating a minor amount of tetradecanoate was visible at m/z 277. Based on the NMR and MS analyses, the molecule under investigation was clearly a family of β-1-O-acyl-β-1,2-diglucosyl glycoside containing predominantly hexadecanoic acid, octadecanoic acid and octadecenoic acid.

Because of the problematic nature of their synthesis, 1-O-acyl glycosides, especially of disaccharides, have not been available for study in the many areas in which alkyl glycosides have been used. *Sarcina ventriculi* and related organisms may prove to be a very valuable source of them in the future. This unusual family of glycosides represents the major proportion of the chloroform-methanol extractable lipids formed by this organism at low pH. The order, phase behaviour and other properties of these glycosides should prove very interesting since they are synthesized in the ordered environment of a biological membrane. Their biological activity and ability to preserve the conformational integrity of membrane proteins and other macromolecules should also be interesting.

EXPERIMENTAL

Bacterial cells were grown at pH 3, harvested and extracted as described earlier[5]. The sample was purified by TLC on silica gel plates eluted with a mobile phase system consisting of chloroform/methanol/ammonia/water (3.3:1.0:0.1:0.05 by volume). NMR spectra of the purified sample were obtained in a solvent system consisting of chloroform / methanol / pyridine / 37 % HCl in the ratio 10/2/1/1 as described earlier[13]. Proton spectra were

measured at 500 MHz and ^{13}C spectra were measured at 125 MHz. Double quantum filtered J-correlated spectroscopy[14] (phase sensitive mode) and hetero-nuclear multiquantum coherence spectroscopy (HMQC)[15] experiments were performed using a total of 256 real data sets. The electrospray ionization mass spectrometry (ESI/MS) was performed on a Fisons Platform mass spectrometer. Gas Chromatography / mass spectrometry was performed by first hydrolysing the samples in 2M trifluoroacetic acid and partitioning between chloroform - water. The chloroform extract was subjected to methanolysis using 2% HCl in methanol thus converting the fatty acids to methyl ester derivatives[5]. The aqueous fraction was concentrated to dryness and redissolved in water and reduced with sodium borohydride. The alditols so formed were acetylated with acetic anhydride and pyridine and subjected to GC-MS analysis.

Acknowledgements: This work was supported by grants # FG-02-89ER14029 from the U.S. Department of Energy and IBN 9507189 from the National Science Foundation to R.I.H.

REFERENCES

1) Jeffrey, G. A.; Yeon, Y. *Carbohydr. Res.*, **1987**, 169, 1-11

2) Chung, Y. J.; Jeffrey, G. A. *Biochim Biophys. Acta*, **1989**, 985, 300-306

3) Van Doren, H. A.; Wingert, L. M. *Mol. Cryst. Liq. Cryst.*, **1991**, 198, 381-391

4) Gustafson, K. R.; Cardellina, J. H.; Fuller, R. W.; Weislow, O. S.; Kiser, K.M.; Snader, K. M.; Patterson, G. M. L.; Boyd, M. R. *J. Natl. Cancer Inst.*, **1989**, 81, 1254-1258

5) Jung, S.; Lowe, S. E.; Hollingsworth, R. I.; Zeikus, J. G. *J. Biol. Chem.* **1993**, 268, 2828-2835

6) Jung, S.; Hollingsworth, R. I. *J. Theoret. Biol.* **1995**, 172, 121-126

7) Jung, S.; Hollingsworth, R. I. *J. Lipid Res.* **1994**, 35, 1932-1945

8) Jung, S.; Zeikus, J. G.; Hollingsworth, R. I. *J. Lipid Res.* **1994**, 35, 1057-1065

9) Berube, L. R.; Hollingsworth, R. I. *Biochemistry*, **1995**, 34, 12005-12011

10) Hollingsworth, R. I. *J. Phys. Chem.*, **1995**, 99, 3406-3410

11) Bock, K.; Thogersen, H. *Ann. Rep. NMR Spectroscopy*, **1982**, 13, 1-57

12) Garozzo, D.; Impallomeni, G.; Spina, E.; Green, B. N.; Hutton, T. *Carbohydr. Res.*, **1991**, 221, 253-257

13) Wang, Y.; Hollingsworth, R. I. *Anal. Biochem.*, **1995**, 225, 242-251

14) Earnst, R. R.; Bodenhansen, G.; Wokaun, A. *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*, Oxford University Press, Oxford, **1987**, 431-440

15) Bax, A.; Subramanian, S. *J. Magn. Reson.*, **1986**, 67, 565-569

(*Received in USA* 29 *November* 1995; *accepted* 26 *January* 1996)