The Occurrence of an Acylated Inositol Mannoside in the Lipids of Propionic Acid Bacteria

By N. SHAW and F. DINGLINGER

Microbiological Chemistry Research Laboratory, Department of Organic Chemistry, University of Newcastle upon Tyne, NE1 7RU

(Received 10 July 1968)

Glycolipids of the diglycosyl diglyceride type are widely distributed in Gram-positive bacteria and their structure may have taxonomic significance (Shaw & Baddiley, 1968). A study of the lipids of some propionic acid bacteria has led to the isolation of a new glycolipid that contains mannose and inositol.

The total lipid was extracted from freeze-dried cells of *Propionibacterium shermanii* PZ3 with chloroform-methanol (2:1, v/v) and purified by the method of Wells & Dittmer (1963). A glycolipid fraction was isolated from chromatography on a column of silicic acid by elution with acetone according to the method of Vorbeck & Marinetti (1965). Examination of this fraction by thin-layer chromatography in the system chloroform-methanol-water (65:25:4, by vol.) showed a single component, R_F 0.55, which gave a negative reaction to phospholipid reagents but with the periodate-Schiff reagents gave a slow-developing blue colour typical of glycolipids (Shaw, Heatherington & Baddiley, 1968). Hydrolysis with alkali yielded a non-reducing glycoside whose paper chromatographic properties (R_{Glc} 0.18 in the system butan-1-ol-pyridine-water, 6:4:3, by vol.) did not correspond to any known diglycosylglycerol. Moreover it did not give the rapid purple colour with the periodate-Schiff reagents characteristic of formaldehyde produced from a 1-substituted glycerol but gave an initial yellow colour that subsequently turned blue-grey. Total acid hydrolysis of the glycoside (2N-HCl for 3 hr. at 100°) and examination of the products by paper chromatography confirmed the absence of glycerol and showed the presence of mannose and inositol. Acetylation of the glycoside with acetic anhydride in pyridine yielded a crystalline acetate, m.p. 173–174°, $[\alpha]_D + 21^\circ$. *O*- α -D-Mannopyranosyl-(1 $\rightarrow$ 2)-myoinositol, m.p. of the crystalline acetate 176–178° and $[\alpha]_D + 20.5^\circ$, has been isolated as a degradation product of phosphatidylmyoinositol mannoside from mycobacteria (Ballou & Lee, 1964) and it is probable that the glycoside from *P. shermanii* has the same structure. The number and location of the fatty acids have yet to be determined, but the reaction of the glycolipid to the periodate-Schiff reagents suggests that they are probably esterified to the inositol. The initial

yellow colour produced by the glycoside with these reagents is indicative of the formation of a substituted malondialdehyde residue from the oxidation with periodate (Hardy & Buchanan, 1963). Both inositol and phosphatidylmyoinositol give a similar reaction. The glycolipid, however, did not give this yellow colour, suggesting that the formation of a substituted malondialdehyde residue was prevented by the presence of fatty acids on the inositol.

We have also isolated from *P. shermanii* St 33, *P. arabinosum* K7, *P. arabinosum* N.C.I.B. 5958 and *P. freudenreichii* N.C.I.B. 5959 a similar glycolipid that on hydrolysis with alkali yielded a single glycoside with chromatographic properties identical with those described above; total acid hydrolysis gave mannose and inositol. The concentrations of the glycolipids varied in the different strains: *P. freudenreichii* and *P. arabinosum* contained only small amounts whereas in *P. shermanii* it represented about 40% of the total lipid.

Brennan & Ballou (1968) have presented evidence for the presence in and biosynthesis by *P. shermanii* of a phosphatidylmyoinositol mannoside. The isolation from *P. freudenreichii* by chromatography on silicic acid of a phospholipid fraction that on acid hydrolysis gave glycerol, inositol and mannose has also led Laneelle & Asselineau (1968) to suggest the occurrence of a similar lipid in this organism. The biosynthetic route envisaged by Brennan & Ballou (1968) is similar to that previously proposed for the phosphatidylmyoinositol mannoside from mycobacteria (Hill & Ballou, 1966), namely the transfer of mannose from GDP-mannose to phosphatidylmyoinositol. The occurrence of an inositol mannoside glycolipid in propionic acid bacteria suggests an alternative route involving the transfer of the phosphatidic acid residue of CDP-diglyceride to the inositol mannoside glycolipid. This route would give an initial product containing a fatty acid ester:phosphorus ratio greater than 2:1, and it is significant that Brennan & Ballou (1968) observed a labelled product formed by *P. shermanii* with the endogenous acceptor that they thought may have been a more highly acylated phosphatidylmyoinositol mannoside. Such compounds have been found in mycobacteria and phosphatidylmyoinositol mannosides containing 4 fatty acid residues/

phosphate residue have been isolated (Pangborn & McKinney, 1966). The glycolipids of mycobacteria have been extensively studied (Lederer, 1967), but so far as we are aware no similar glycolipid containing inositol has been isolated, although, if present, it may not accumulate in large amounts.

The biosynthesis of the glycolipid probably involves the transfer of mannose from GDP-mannose to an inositol acceptor. The acylation could take place either before or after transfer of the sugar residue. If this glycolipid is involved in the biosynthesis of phosphatidylmyoinositol mannosides, the stimulation in the rate of biosynthesis of phosphatidylmyoinositol mannoside in *P. shermanii* by the addition of yeast phosphatidylmyoinositol observed by Brennan & Ballou (1968) may indicate a lack of specificity in the nature of the inositol acceptor.

F.D. thanks the Deutscher Akademischer Austauschdienst-Bad Godesburg for the award of a Kurzstipendium and

Professor J. Baddiley, F.R.S., for the provision of laboratory facilities. The strains of *P. shermanii* and *P. arabinosum* K7 used in this work were kindly provided by Professor K. Bernhauer, Technischen Hochschule Stuttgart, Germany.

Ballou, C. E. & Lee, Y. C. (1964). *Biochemistry*, **3**, 682.

Brennan, P. & Ballou, C. E. (1968). *Biochem. biophys. Res. Commun.* **30**, 69.

Hardy, F. E. & Buchanan, J. G. (1963). *J. chem. Soc.* p. 5881.

Hill, D. L. & Ballou, C. E. (1966). *J. biol. Chem.* **241**, 895.

Lancelle, M. A. & Asselineau, J. (1968). *C.R. Acad. Sci., Paris*, **266** D, 1901.

Lederer, E. (1967). *Chem. Phys. Lipids*, **1**, 294.

Pangborn, M. C. & McKinney, J. A. (1966). *J. Lipid Res.* **7**, 627.

Shaw, N. & Baddiley, J. (1968). *Nature, Lond.*, **217**, 142.

Shaw, N., Heatherington, K. & Baddiley, J. (1968). *Biochem. J.* **107**, 491.

Vorbeck, M. L. & Marinetti, G. V. (1965). *Biochemistry*, **4**, 296.

Wells, M. A. & Dittmer, J. C. (1963). *Biochemistry*, **2**, 1259.