THE GLYCOLIPIDS FROM A ROUGH STRAIN OF PNEUMOCOCCUS TYPE I

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Received November 30, 1964

An investigation into the chemical constitution of the lipids from a rough strain of Pneumococcus Type I has revealed the presence of considerable quantities of glycolipids that have been isolated and purified. Deacylation yielded two glycosides characterised as O-α-D-galactopyranosyl-(1→2)-O-α-D-glucopyranosyl-(1→1)-glycerol and 1-O-α-D-glucopyranosylglycerol.

The lipid fraction was obtained from whole dry cells by extraction with chloroform-methanol mixtures and non-lipid contaminants removed by the method of Wells and Dittmer (1963). Acid hydrolysis (2N HCl at 100° for 3 hr.) followed by paper chromatography showed the presence of substantial quantities of glucose and galactose together with glycerol and its phosphates. Chromatography on silicic acid removed the neutral lipids but only partially separated the glycolipid and phospholipid components. Further purification was achieved by fractionation on DEAE cellulose (Rouser, Bauman, Kritchersky, Heller, and O'Brien, 1961) yielding two distinct glycolipid components in an approximate weight ratio of 100:1. The total yield of glycolipids was 190 mg. from 10 g. of dry cells.

The major component was sparingly soluble in chloroform and methanol, readily soluble in chloroform-methanol mixtures and hot acetone, but insoluble in cold acetone. Analysis gave a fatty acid:hexose ratio of 0.83:1. Deacylation (Carter, McCluer, and Slifer, 1956) of the glyco-

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lipid yielded a gum which crystallised on trituration with ether. The non-reducing glycoside so obtained was recrystallised from moist ethanol: it had m.p. 165-167°, with softening, and $[\alpha]_D$ 176 ± 5° (c, 4.4 in water). Examination of an acid hydrolysate (2N HCl at 100° for 3 hr.) on paper revealed only glucose, galactose, and glycerol and analysis gave the ratio D-glucose:D-galactose:glycerol:total hexose as 1.00:1.04:1.27:1.93. Elementary analysis gave C, 43.1; H, 6.9. $C_{15}H_{28}O_{13}$ requires C, 43.2; H, 6.8%. Mild acid hydrolysis (0.015N HCl for 5 hr. at 100°) yielded galactose and a glucosylglycerol which was chromatographically identical to a synthetic sample of 1-O-α-D-glucopyranosylglycerol but was different from 2-O-α-D-glucopyranosylglycerol. Furthermore, both the glucoside and the galactosylglucoside rapidly gave a purple colour with the periodate-Schiff's reagents (Baddiley, Buchanan, Handschumacher, and Prescott, 1956) characteristic of formaldehyde which would be produced from a 1-substituted glycerol (Roberts, Buchanan, and Baddiley, 1963). The glucosylglycerol was unaffected by treatment with β-glucosidase, thus establishing the α-configuration. Treatment of the original glycoside with almond emulsin which has a high α-galactosidase content but no α-glucosidase, also yielded galactose and glucosylglycerol. This suggests that both glycosidic linkages have the α-configuration, a conclusion which is in accord with the high positive rotation of the compound.

The glycoside consumed 1.98 moles of sodium periodate for each hexose unit, and produced 0.47 moles of formaldehyde and 0.42 moles of formic acid. These results are only compatible with a galactosyl-glucosyl-(1→1)-glycerol containing either a 1→2 or 1→4 galactose-glucose linkage. When the glycoside was treated with an excess of sodium periodate, the product reduced with sodium borohydride and then hydrolysed with acid, glycerol and ethylene glycol were the only products detected: this indicates the presence of a 1→2 linkage between galactose and glucose, as erythritol, which would be the expected product of a 1→4 linkage, was not detected in the hydrolysate.

The glycoside is thus characterised as O-α-D-galactopyranosyl-(1→2)-O-α-D-glucopyranosyl-(1→1)-glycerol. The fatty acids were isolated from the lipid by saponification and extraction with light petroleum. After conversion to their methyl esters, analysis by gas chromatography showed the following compositions: 12:0, 6.9%; 14:0, 13.6%; 14:1(?), 6.5%; 16:0, 33.8%; 16:1, 25.9%; 18:0, 2.9%; 18:1, 10.4%. The location of the two fatty acids on the glycolipid has not been established although comparison with similar compounds, e.g. the galactosyl diglycerides isolated by Carter, Hendry, and Stanacev (1961) from plant sources, suggests esterification on the 2- and 3-positions of glycerol.

The minor glycolipid component, after deacylation and acid hydrolysis, yielded only glucose and glycerol in the ratio 1.00:1.15. It was unchanged following treatment with β-glucosidase and was chromatographically indistinguishable from authentic 1-O-α-D-glucopyranosylglycerol.

Whilst this work was in progress, Distler and Roseman (1964) reported the incorporation of [^{14}C] glucose and galactose into a butanol extract of a cell-free system derived from Pneumococcus Type XIV. Their adjoining communication reports the isolation, characterisation and biosynthesis of a galactosylglucosyldiglyceride and a glucosyldiglyceride from lipid extracts of this organism. The structures proposed are identical to those described here for the glycolipids of Pneumococcus Type I.

Lennarz (1964) has partially characterised a dimannosyldiglyceride from Micrococcus lysodeikticus and Reeves, Latour, and Lonsteau (1964) have isolated 1-O-β-D-galactofuranosylglycerol from the lipids of Bacteriodes symbiosus. Further investigations in progress reveal the widespread occurrence of similar glycolipids in strains of Pneumococci and other bacteria suggesting an important metabolic function which as yet remains unknown.

We acknowledge the technical assistance of Mr. A.W. Lewis in the analysis of the fatty acids and also grants from the U.S. Public Health

Service (AI-05237-02-BIO) and the Department of Scientific and Industrial Research.

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