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Low-molecular Carbohydrates in Algae

IX *. Structure of the Glyceric Acid Mannoside from Red Algae

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The structure of the D-glyceric acid α-mannoside occurring in various red algae has been investigated. Methylation, reduction of the methyl ester-methyl ether with lithium aluminium hydride followed by further methylation and hydrolysis yielded a mannose tetramethyl ether and a glycerol dimethyl ether. The two last named substances were shown to be 2,3,4,6-tetra-*O*-methyl-D-mannopyranose and 1,3-di-*O*-methyl-glycerol, and the glycoside is therefore 2-D-glyceric acid α-D-manno-pyranoside.

Colin and Augier [1], in 1939, isolated a new glycoside from the red alga *Polysiphonia fastigiata*, which they demonstrated to be an α-mannoside of D-glyceric acid. They assumed the glyceric acid to be linked in 2-position, but gave no experimental evidence for this assumption. Later Augier investigated a large number of red algae and isolated the same glycoside from several of them (Ref.[2] and preceding papers). In the present paper the elucidation of its structure, using the methylation technique, is reported.

The glycoside, isolated as its sodium salt from *Ceramium rubrum*, was methylated with methyl sulphate and sodium hydroxide and the methylated acid esterified with diazomethane. The ester was then reduced with lithium aluminium hydride. A small amount of the reduced product was hydrolysed and investigated by paper chromatography, using as solvent butanol-ethanol-water, 5:1:1, and as reference substances 2,3,4,6-tetra-*O*-methyl-D-mannose, 1-*O*-methyl-glycerol and 2-*O*-methyl-glycerol. Two spots were obtained, identical in R_F-values and colour reactions with the first two reference substances. As expected 2-*O*-methyl-glycerol could not be developed with the reagents used, silver nitrate-sodium ethoxide [3] and lead tetraacetate in benzene [4]. Indications of the presence of 1-*O*-methyl-glycerol in the hydrolysate were thus obtained and this compound should be optically active. In order to obtain inactive

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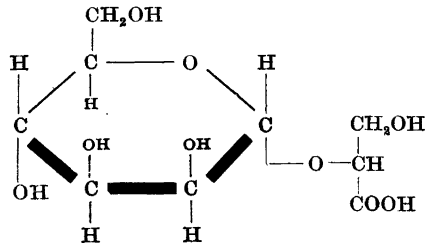
1,3-di-*O*-methyl-glycerol, which is more easily available as reference substance, the reduced material was further methylated before hydrolysis. The products of hydrolysis were separated by distillation under reduced pressure. The 1,3-di-*O*-methyl-glycerol was characterized as its 3,5-dinitrobenzoate and the 2,3,4,6-tetra-*O*-methyl-D-mannose as its anilide, both being identical with authentic specimens.

According to this study and the previous investigations of Colin and Augier [1], it can be concluded that the structure of the mannoside is 2-D-glyceric acid α-D-mannopyranoside (I).*



I

EXPERIMENTAL

The isolation of the glycoside, as its sodium salt, will be described in a coming publication. Its melting point, 255° (decomp.), and specific rotation, +106° (in water), were in good agreement with the values recorded by Colin and Augier [1].

Methylation of the mannoside. The sodium salt (2.0 g) was methylated with methyl sulphate (11 ml) and sodium hydroxide (9 g) in water (11 ml). The reaction time was 4 hours and the temperature 55–65°. After cooling, the mixture was neutralized with 2 *N* sulphuric acid, diluted with ethanol after slight acidification and the precipitated sodium sulphate was removed by filtration. The solution was neutralized and concentrated and subjected to a second methylation under the same conditions. After neutralization and removal of sodium sulphate as before, the solution was concentrated to a small volume, acidified with sulphuric acid and continuously extracted with chloroform. The chloroform solution was dried over magnesium sulphate and concentrated, yielding a light-coloured syrup (1.87 g).

Esterification and reduction of the methylated product. The methylated product (1.87 g) was dissolved in ether and treated with diazomethane in ether over-night. Excess of diazomethane and ether were evaporated, and the product in dry ether (50 ml) added to a slurry of lithium aluminium hydride (7 g) in dry ether (150 ml). The mixture was vigorously stirred for 2 hours and then water (5 ml) was carefully added. The ether was evaporated, the reaction mixture acidified with 2 *N* hydrochloric acid and extracted with chloroform (3 × 50 ml). The chloroform solution was dried and concentrated, yielding a dark-coloured syrup (1.40 g).

Methylation of the reduced product. The reduced product (1.10 g) was dissolved in dioxan (50 ml), powdered sodium hydroxide (5.5 g) was added and the mixture vigorously stirred for 4 hours at 70–75° C. During the first 45 minutes methyl suphate (6.5 ml) was added in portions. After cooling, the mixture was filtered, the filter cake washed with dioxan and benzene, and the combined filtrates were concentrated and the methylation

* *Added in proof.* Kawaguchi, K., Yamada, S. and Miyama, S. (*Bull. Japan Soc. Sci. Fisheries* **19** (1953) 481. *C. A.* **49** (1955) 4803.) have recently investigated the oxidation of the mannosido-glycerate with lead tetraacetate and arrived at the same conclusion regarding the structure of the substance.

procedure repeated, yielding the crude glycerol mannoside hexamethyl ether as a syrup (1.04 g).

Hydrolysis of the glycerol mannoside hexamethyl ether. The crude hexamethyl ether (1.04 g) was dissolved in 0.5 *N* hydrochloric acid (14 ml), kept at 100° for 22 hours, neutralized by filtering through a column of Amberlite IR 4B, concentrated to a small volume and continuously extracted with chloroform. The chloroform solution was dried over magnesium sulphate and the chloroform evaporated. The product (0.90 g) was fractionated by distillation under reduced pressure (10 mm), yielding 1,3-di-*O*-methyl-glycerol (97 mg, bath temp. 80—100°) and 2,3,4,6-tetra-*O*-methyl-D-mannose (150 mg, bath temp. 125—140°). A considerable residue from the distillation showed that the hydrolysis had not been complete.

Anilide of 2,3,4,6-tetra-O-methyl-D-mannose. (1) A mixture of authentic 2,3,4,6-tetra-*O*-methyl-D-mannose (200 mg), freshly distilled aniline (7 ml) and anhydrous ethanol (7 ml) was refluxed for 8 hours. Ethanol and excess of aniline were evaporated under reduced pressure and the residue crystallized from ethanol to constant m. p., 146—147°. Yield, 40 mg. This procedure is essentially the same as that of Haworth *et al.*[5], but our product has a somewhat higher m. p. (2) The mannose tetramethyl ether from the hydrolysis (40 mg) yielded the same anilide (18 mg), m. p. 146—147°, undepressed on admixture with the authentic material.

3,5-Dinitrobenzoate of 1,3-di-O-methyl-glycerol. (1) A mixture of authentic 1,3-di-*O*-methyl-glycerol [6] (150 mg), 3,5-dinitrobenzoylchloride (310 mg), pyridine (0.2 ml) and Drierite (150 mg) in anhydrous chloroform (2.0 ml) was kept at 50° for 48 hours. The Drierite was filtered off and a small amount of water was slowly added to the mixture followed by ether (10 ml). The solution was extracted successively with water, 1 *N* sulphuric acid, 0.5 *M* sodium hydrogen carbonate and water, and the residue concentrated to dryness. The crude product (204 mg) was crystallized from ethanol to constant m. p., 86—87°. Yield, 60 mg. (Found: N 9.05. Calc. for $C_{12}H_{14}O_8N_2$; N 8.94). (2) The glycerol dimethyl ether from the hydrolysis (97 mg) yielded the same 3,5-dinitrobenzoate (35 mg), m. p. 86—87°, undepressed on admixture with authentic material.

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