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Structure of a Glyceritol Glycoside from *Polysiphonia fastigiata* and *Corallina officinalis*

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The O-α-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→1)-glyceritol previously isolated from wheat flour lipids, has been found in the red algae *Polysiphonia fastigiata* and *Corallina officinalis*. The glyceritol residue has been shown to have the D-configuration.

Investigation of the red alga *Polysiphonia fastigiata*[1] gave a glycoside (A), m. p. 196—198°, $[\alpha]_D^{23}$ +88°, which after acid hydrolysis gave spots indistinguishable from those given by D-galactose and glyceritol on paper chromatograms. The same glycoside has now been obtained in small quantities from another red alga, *Corallina officinalis, Florideae*, order *Cryptonemiales*, which was previously found to contain floridoside [2]. The structure of glycoside A has now been investigated in greater detail.

A quantitative determination of the products of hydrolysis, which were assumed to be galactose and glyceritol, analysis for carbon and hydrogen and the results of periodate oxidation, including periodate consumption and formic acid and formaldehyde liberated, indicated that A could be assigned the general structure O-galactopyranosyl-(1 → 6)-O-galactopyranosyl-(1 → 1)-glyceritol. Provided the galactose belongs to the D-series the moderately high optical rotation of A is consistent with the presence of one α- and one β-linkage in the molecule.

Glycoside A was strikingly similar to a glycoside (B), O-α-D-galactopyranosyl-(1 → 6)-O-β-D-galactopyranosyl-(1 → 1)-glyceritol, m. p. 182—184°, $[\alpha]_D$ + 86.4°, which had recently been isolated from wheat flour lipids by Carter *et al.* [3] A second glycoside (C), O-β-D-galactopyranosyl-(1 → 1)-glyceritol, m. p. 139—140°, $[\alpha]_D$ + 3.77° was also isolated from the same source. The anomeric configurations of B and C were evident from enzymatic degradations; B, though unaffected by β-galactosidase from *E. coli*, was split by α-galactosidase in a yeast hexokinase preparation to yield D-galactose and glycoside C. The latter compound was hydrolysed by β-galactosidase to give D-galactose and glyceritol.

As glycosides A and B might have differed in the position of the α- and β-linkages, A was incubated with α- and β-galactosidase preparations from sweet almonds, and the reaction was followed qualitatively on paper chromatograms. It was found that A was attacked very slowly by a β-galactosidase with low α-activity, which rapidly hydrolysed a synthetic specimen of O-β-D-galactopyranosyl-(1 → 1)-D, L-glyceritol. An enzyme preparation with about equal α- and β-galactosidase activity liberated galactose and later also glyceritol. The identity of A with B indicated by these experiments, was later confirmed by direct comparison with a sample of glycoside B *. The corrected melting point of B determined with the same apparatus used for A was 195—197°, the mixed melting point showed no depression and the infrared spectra of the two samples were identical.

The question of the configuration of the glyceritol residue, which is optically active due to substitution in the 1-position, has been solved by synthesis of O-β-D-galactopyranosyl-(1 → 1)-D- and -L-glyceritol [4]. The D-glyceritol galactoside was found to be identical with a sample of glycoside C* and therefore the glyceritol residue in A also has D-configuration.

EXPERIMENTAL **

The isolation of glycoside A from *Polysiphonia fastigiata* was described in a previous paper [1]. It was purified by chromatography on Whatman 3 MM thick filter paper and recrystallised from aqueous ethanol, m. p. 196—198°, $[\alpha]_D^{23}$ + 88° (water, c = 1.8). (Found: C 43.5; H 6.68. Calc. for $C_{18}H_{28}O_{18}$: C 43.3; H 6.78).

A sample of *Corallina officinalis* (575 g) was extracted with ether and methanol and the methanol extract was worked up as described earlier [1] yielding a syrup (6.1 g). This was separated on a carbon — Celite column in the usual way [1]. Paper chromatograms of the eluate indicated the presence of floridoside and a number of other carbohydrates. One fraction gave a spot with about the same R_F value as glycoside A. This fraction was concentrated, the residue (100 mg) was dissolved in aqueous ethanol and the solution was seeded with a trace of glycoside A. The crystals separating (41 mg), after recrystallisation had m. p. 194—196.5° and $[\alpha]_D^{20}$ +90° (water, c = 1.8). The melting point was undepressed on admixture with glycoside A or B, and the infrared spectra of the three preparations were identical.

Molar ratio of D-*galactose and glyceritol in glycoside A.* The glycoside (*ca.* 5 mg) was hydrolysed in 0.2 N hydrochloric acid (0.15 ml) at 105° for 17 h. The hydrolysate was passed through a small column of Amberlite IR 4B and was then put on a strip of Whatman 3 MM filter paper which had been prewashed with water and then dried. The chromatogram was developed with ethyl acetate — acetic acid — water (3:1:1) and the appropriate areas were then cut out and extracted with water; adjoining empty areas of the paper were treated in the same way and these extracts were used as blanks. Suitable amounts of the extracts were oxidised with sodium metaperiodate overnight and the excess of periodate was determined essentially as described in a previous paper [5]. The glyceritol and galactose content of the hydrolysate was calculated empirically by comparison with synthetic mixtures of glyceritol and D-galactose which were separated and oxidised in the same way. The amounts of glyceritol and galactose thus obtained were 98 and 97 %, respectively, of the theoretical calculated for a glyceritol digalactoside.

Periodate oxidations. a) The consumption of periodate, determined as described earlier [5], was *ca.* 4.9 moles/mole of glycoside A after 3 h and 5.0 moles/mole after 21 h.

* A sample was kindly supplied by Dr. Carter.
** All melting points are corrected.

b) The formaldehyde was determined as its dimedone compound, essentially by the method of Reeves [6]. Aqueous sodium metaperiodate (0.2 M, 0.5 ml) was added to a solution of glycoside A (7.08 mg) in water (4 ml) and after 4.5 h the oxidant was reduced by the addition of 0.8 M aqueous disodium arsenite (0.5 ml), 0.8 N hydrochloric acid (0.5 ml) and 1 N aqueous sodium acetate (0.5 ml). After the addition of a 5 % ethanolic solution of dimedone (0.5 ml) the mixture was heated at 100° for 10 min and left in a cool place for 1 h. The sticky precipitate (10 mg) was collected on a filter stick, washed with water and after drying sublimed at *ca.* 0.01 mm pressure. Yield 3.75 mg corresponding to 0.75 mole of formaldehyde/mole of glycoside A; m. p. 186—193°. Recrystallisation from aqueous ethanol gave a product with m. p. 191—194°, alone or mixed with an authentic sample.

c) A sample of glycoside A (6.95 mg) was oxidised in 0.2 M sodium metaperiodate solution (1 ml) overnight, ethylene glycol (2 drops) was added and the formic acid produced was titrated with 0.01 N sodium hydroxide solution using phenol red as an indicator. Found: 1.93 moles of formic acid/mole of glycoside A.

Enzymatic degradation. a) Enzyme activity. The activity of the enzyme preparations was determined approximately by incubation with 5 % solutions of melibiose or lactose in 0.1 M sodium acetate buffer (pH 4.6) at 25°: the hydrolysis was followed polarimetrically over periods of 15—170 h. The activities were calculated as mg of disaccharide hydrolysed in 1 h by 1 ml of enzyme solution. Alternatively a mixture of enzyme solution (13.3 μl) and a 2 % solution (1 ml) of *o*-cresyl α- or β-D-galactopyranoside [7,8] in 0.1 M acetate buffer of pH 4.6 was kept at 25° for 5 min (β-galactoside) or 1 h (α-galactoside). The hydrolysis was stopped by the addition of 0.5 M sodium carbonate buffer of pH 10.6 (2 ml), 0.05 N iodine solution (1.00 ml) was added and after 20 min the mixture was acidified with acetic acid (2 ml) and the excess of iodine was titrated with 0.01 N sodium thiosulphate solution. A blank was run in a similar way but the carbonate buffer was added before the enzyme solution and oxidation with iodine was done immediately. The activities were calculated as mg of cresyl galactoside hydrolysed by 1 ml of enzyme solution in 1 h.

b) α-Galactosidase. Emulsin was extracted from de-fatted sweet almond meal essentially according to the procedure described by Sumner and Somers [9]. The meal (10 g), which had been stored in a refrigerator for about a year, was stirred with 1/2-saturated aqueous ammonium sulphate (100 ml) for 5 min. After filtration the filter cake was extracted with 1/4-saturated aqueous ammonium sulphate (100 ml), ammonium sulphate (2 g/10 ml of filtrate) was added to the extract which was centrifuged and the precipitate was dialysed against distilled water. The dialysed solution was centrifuged and the supernatant solution concentrated to a small volume (0.75 ml). Acetone (0.25 ml) was added in the cold, the solution was clarified by centrifuging, a further quantity of acetone (0.5 ml) added and after 1 h the precipitated emulsin was isolated by centrifuging. It was dissolved in water (1 ml). α-Galactosidase activity: 2.1 mg of melibiose/ml/h; 11 $\pm$ 2 mg of *o*-cresyl α-D-galactopyranoside/ml/h. β-Galactosidase activity: 2.2 mg of lactose/ml/h; 90 $\pm$ 20 mg of *o*-cresyl β-D-galactopyranoside/ml/h.

c) β-Galactosidase was prepared by the method of Edman and Jorpes [10], omitting the final steps of purification. Sweet almonds (1 000 g), with the cuticle, were ground and de-fatted, the meal was extracted with dilute aqueous ammonia, impurities were precipitated from the extract by adding dilute acetic acid, the emulsin fraction was precipitated by adding ammonium sulphate and the precipitate after centrifuging dialysed against distilled water and clarified. The final solution (100 ml) contained 52 mg of dry material/ml. α-Galactosidase activity: 0.1 mg of melibiose/ml/h; 2 $\pm$ 2 mg of *o*-cresyl α-D-galactopyranoside/ml/h. β-Galactosidase activity: 18 mg of lactose/ml/h; 940 $\pm$ 20 mg of *o*-cresyl β-D-galactopyranoside/ml/h.

d) Enzymatic hydrolysis. A mixture of the substrate (2 mg) in 0.1 M sodium acetate buffer (pH 4.6, 0.1 ml) and enzyme solution (13.3 μl) was kept at 25°. At intervals samples were withdrawn and run on paper chromatograms in ethyl acetate — acetic acid — water (3:1:1). The spots were detected by spraying with periodate — benzidine [11], silver nitrate — sodium hydroxide [12] or anisidine hydrochloride reagents. The β-galactosidase preparation gave almost complete hydrolysis of O-β-D-galactopyranosyl-(1 → 6)-D-galactose in 24 h, and after 16 h synthetic O-β-D-galactopyranosyl-(1 → 1)-D,L-glyceritol gave strong spots for D-galactose and glyceritol. However, after 21 h a sample of glycoside A, which had been incubated with the β-galactosidase, gave only a faint spot for D-galactose with the silver nitrate and anisidine reagents, and even after 36 h no hydrolysis products were

detected with the periodate spray. With the α-galactosidase preparation glycoside A gave spots for D-galactose with all spray reagents after 4.5 h, and after 12 h the periodate spray revealed a faint spot from glyceritol. With the same spray the galactose spot acquired a yellowish tinge which has been observed with 1-glyceritol galactopyranosides in general. These compounds would not separate from D-galactose on the chromatograms. The experiments strongly indicate that glycoside A is first attacked by α-galactosidase to yield D-galactose and a glyceritol β-D-galactopyranoside, which is later split by the β-galactosidase present to give D-galactose and glyceritol.

Identification of glycoside C with O-β-D-galactopyranosyl-(1 → 1)-D-glyceritol. The synthesis of O-β-D-galactopyranosyl-(1 → 1)-D-glyceritol, m. p. 140.5—141.5°, $[\alpha]_D^{20}$ −7° (water, c = 2.0), and the corresponding L-glyceritol derivative, m. p. 104—107°, $[\alpha]_D^{20}$ +2° (water, c = 2.1) is described in a later paper [4]. The compounds are isomorphous and both of them can be induced to crystallise from an ethanolic solution upon seeding with glycoside C. Though the specific rotation reported [3] for glycoside C agrees best with that of the L-glyceritol derivative, the corrected m. p. of C, 139.5—142°, and the infrared spectra [4] prove its identity with the D-glyceritol derivative.

Infrared spectra were recorded with a *Perkin-Elmer* double-beam spectrometer Model 21 with a rock-salt prism, using the potassium bromide disc technique.

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