215. *Red-seaweed Polysaccharides. Part II.** Porphyra capensis *and the Separation of* D- *and* L-*Galactose by Crystallisation.*

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A polysaccharide, isolated from the red seaweed *Porphyra capensis*, was found to consist of D- and L-galactose, 6-*O*-methyl-D-galactose, 3 : 6-anhydro-L-galactose, and a hydrogen sulphate in the approximate molecular ratio 1 : 1 : 2 : 1. D- and L-Galactose were separated by recrystallisation.

THE polysaccharides of the *Porphyra* species of the Rhodophyceae have received little attention and only in the case of *P. umbilicalis*,[2] which yielded a mannan on extraction with sodium hydroxide solution, has any detailed investigation been made. Apart from this there have been reports of the isolation of DL-galactose from *P. laciniata*,[3] *P. tenera*,[3] and *P. crispata*,[4] and of galactose, glucose, pentose, and methylpentose from *P. tenera*.[5]

Several of the *Porphyra* species are extensively used as human foods in various parts of the world, especially in the Far East, and the " laver bread " of Wales is chiefly made from *P. umbilicalis*. Their value as a food presumably lies in their high protein content, which (by nitrogen estimation) lies between 25 and 30% on a dry weight basis.

P. capensis, which grows in profusion on the Atlantic side of the Cape Peninsula and along several other parts of the South African coast, afforded a sulphated polysaccharide, as its mixed sodium–magnesium salt, in about 30% yield by extraction with hot water. The polysaccharide was hydrolysed and chromatography of the neutralised hydrolysate revealed the presence of two sugars, namely, galactose and another almost coincident on paper with fucose. This mixture was separated on a cellulose column, and the second sugar proved to be 6-*O*-methyl-D-galactose by oxidation of the sugar with periodate: this sugar gave 4 mol. of formic acid and consumed 4 mol. of periodate, and methoxyacetaldehyde was isolated from the reaction mixture. As additional evidence 6-*O*-methyl-D-galactose was synthesised for comparison by methylation of 1 : 2-3 : 4-di-*O*-*iso*propylidene-D-galactose with Purdie's reagents, followed by acid hydrolysis. This is the first recorded instance of 6-*O*-methyl-D-galactose in Nature.

Crystallisation of the galactose fraction from methanol yielded L-galactose, which required a large number of recrystallisations before it had a constant melting point. A part of the mother-liquor from the first crystallisation afforded 2-DL-galactobenziminazole when treated according to Moore and Link's procedure, indicating the presence of D-galactose [6] in the solution. Concentration of the remainder of the mother-liquor afforded another crop of crystals, which had a positive rotation. Repeated recrystallisation of this product from methanol afforded pure D-galactose. The separation of D- and L-galactose from synthetic mixtures containing an excess of the L-enantiomorph was repeated several times, and although it appears to be the first recorded separation by crystallisation of a pair of enantiomorphs in the sugar series, it is by no means unique as other separations, such as that of D- and L-threonine,[7] are on record.

Methanolysis of the polysaccharide and separation of the products on a cellulose column afforded, in the first fraction, methyl 3 : 6-anhydro-α-L-galactopyranoside, indicating that 3 : 6-anhydro-L-galactose also occurred in the polysaccharide. The

* Part I, *J.*, 1957, 197. A preliminary note on Part II has appeared.[1]

[1] Nunn and von Holdt, *Chem. and Ind.*, 1956, 467.
[2] Jones, *J.*, 1950, 3292.
[3] Oshima and Tollens, *Ber.*, 1901, **34**, 1422.
[4] Hayashi, *J. Soc. Trop. Agr.* (*Taikoku Imp. Univ.*), 1941, **13**, 193.
[5] Hibino, *J. Chem. Soc. Japan*, 1942, **63**, 1078.
[6] Bell and Baldwin, *J.*, 1941, 125.
[7] Velluz and Amiard, *Bull. Soc. chim. France*, 1953, 903.

following fraction contained more 3 : 6-anhydro-α-L-galactopyranoside, but it was contaminated with the methyl glycosides of galactose and 6-*O*-methyl-D-galactose, and no attempt was made to separate the mixture.

3 : 6-Anhydro-sugars are extremely labile and are not amenable to the ordinary methods of sugar analysis. One method has been published [8] but it gave unsatisfactory results in our hands, and we have been unable to obtain direct evidence for the amount of 3 : 6-anhydro-sugar in the polysaccharide. However, if it is assumed that the polysaccharide consists only of the above three sugars and ester sulphate it appears, from analyses of galactose, 6-*O*-methyl-D-galactose, and sulphate on the purified sodium salt of the polysaccharide, that the molecular ratio of galactose : 6-*O*-methyl-D-galactose : 3 : 6-anhydro-L-galactose : sulphate is approximately 1 : 1 : 2 : 1. Such a repeating unit would have an equivalent weight (for the sodium salt) of 729 (Found : 740). The methoxyl content of such a repeating unit should be 4·2% (Found : 5·6%); this discrepancy could possibly be accounted for by adsorbed ethanol on the polysaccharide, which is often difficult to remove from polysaccharides prepared by precipitation in ethanol.[9]

Experimental

Unless otherwise stated, concentration of solutions was carried out at 40°/20 mm. and specific rotations were measured in H_2O. Paper chromatograms were run in (*a*) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4), (*b*) butanol–pyridine–water (9 : 2 : 2), or (*c*) butanol–ethanol–water (40 : 11 : 19). M. p. are corrected.

Isolation and Purification of the Polysaccharide.—Extraction was carried out on fresh material owing to the difficulty experienced in extracting material previously dried at 45°. Fresh (wet) *Porphyra capensis* (5·5 kg., *i.e.*, *ca.* 0·5 kg. dry wt.) was mixed with water and acetic acid added to pH 2. The acid caused rapid disintegration of the weed and did not appear to degrade the polysaccharide. Steam was passed into the mixture for ½ hr. after disintegration of the weed had begun. The pH rose to between 6 and 7 during this operation. The extract was strained through a wire sieve and then introduced into a continuous centrifuge (23,000 r.p.m.) while still hot, yielding a clear pale brown liquid. A colloidal precipitate, which appeared on cooling, was separated by passing the mixture through a continuous centrifuge at 50,000 r.p.m. Precipitation into ethanol (5 vols.) and washing with ethanol and acetone afforded a white product (164 g., *ca.* 32% on a dry wt. basis) [Found (on material dried at 60°/0·5 mm.) : N, 1·6; SO_4^{2-} (after hydrolysis), 13·0; ash (carbonated), 15·5%]. Analysis of the ash indicated the presence of iron (trace), magnesium, and sodium. Attempts to remove the protein [10] by shaking a solution with chloroform were ineffective. The polysaccharide was further purified for analysis by passing a solution through Amberlite IR-120 and IRA-400 resins. The acid eluate was exactly neutralised with sodium hydroxide solution, and the polysaccharide precipitated in excess of ethanol. The precipitate was collected in a centrifuge, washed with ethanol, and dried, then having $[\alpha]_D^{18}$ −60° [Found (on material dried at 60°/0·5 mm.) : N, 0·2; OMe, 5·6; SO_4^{2-} (after hydrolysis), 12·9; ash (sulphated), 12·6%. Equiv. (from SO_4^{2-} detn.), 740]. Galactose and 6-*O*-methylgalactose were estimated in a hydrolysate of the polysaccharide, after separation on a paper chromatogram, with maltose as reference sugar,[11] by Somogyi's micro-method [12] [Found : galactose (calc. as $C_6H_{10}O_5$), 23·1, 23·0; 6-*O*-methyl-D-galactose (calc. as $C_7H_{12}O_5$), 29·4, 30·1%]. The value for 6-*O*-methyl-D-galactose is probably too high since, although 3 : 6-anhydro-L-galactose is largely destroyed during prolonged acid hydrolysis, some might have remained and both sugars run to nearly the same position on a chromatogram.

Hydrolysis of the Polysaccharide.—Hydrolysis of the polysaccharide in N-sulphuric acid for 16 hr. at 100° and chromatography of the neutralised (barium carbonate) hydrolysate revealed the presence of two spots, one of which corresponded in position to galactose. The second spot R_R (relative to rhamnose), 0·79 in (*a*), 0·65 in (*b*), 0·84 in (*c*), almost exactly coincided with fucose, 0·79 in (*a*), 0·70 in (*b*), 0·87 in (*c*). However, the colour of the spot when sprayed with *p*-anisidine hydrochloride was different from that of fucose, especially under ultraviolet light.

[8] Smith, O'Neill, and Perlin, *Canad. J. Chem.*, 1955, **33**, 1353.
[9] Percival and Ross, *J.*, 1950, 717.
[10] Sevag, Smolens, and Lackman, *J. Biol. Chem.*, 1938, **124**, 425.
[11] Flood, Hirst, and Jones, *J.*, 1948, 1679.
[12] Somogyi, *J. Biol. Chem.*, 1952, **195**, 19.

For the isolation of these sugars the polysaccharide (45 g.) was hydrolysed with N-sulphuric acid (400 c.c.), and the mixture neutralised and evaporated to a syrup (36·6 g.), which was separated on a cellulose column (7·6 × 48 cm.) with butanol–water (9 : 1), giving fractions (1) 8·6 g., and (2) 11·9 g.

6-O-*Methyl*-D-*galactose*.—Fraction (1) was decolorised with charcoal in water, the solution was filtered and evaporated to dryness, and the residue recrystallised from ethanol, yielding colourless needles, m. p. 122—123°, $[\alpha]_D^{17}$ +117° ⟶ + 77·3° in 4·5 hr. (*c* 2·02) (Found: C, 43·2; H, 7·45; OMe, 16·2. Calc. for $C_7H_{14}O_6$: C, 43·3; H, 7·3; OMe, 16·0%). The osazone, recrystallised from methanol, had m. p. 196—197°, $[\alpha]_D^{19}$ +137° (*c* 0·42 in pyridine).

This sugar (*ca.* 10 mg.) was demethylated by 48% hydrobromic acid in a sealed tube in a water-bath for 5 min.[13] The solution was neutralised (Amberlite IR-4B resin) and filtered. Chromatography of the filtrate revealed spots corresponding to galactose and to the original material.

When the above sugar was oxidised in the usual manner with 0·04M-sodium metaperiodate, 3·79 mol. of formic acid per mol. of substance were liberated, and 4·0 mol. of periodate per mol. of substance were consumed. A large-scale oxidation of the sugar (0·5 g.) was effected with sodium metaperiodate (3 g.) in water (5·0 c.c.) for 24 hr. After the addition of N-hydrochloric acid (30 c.c.) and 20% sodium arsenite solution (30 c.c.) to remove the iodine, 8% sodium acetate solution (20 c.c.) and 8% ethanolic dimedone solution (10 c.c.) were added to the mixture, which was heated on a boiling-water bath for 10 min. When cool the crystals were filtered off and recrystallised from aqueous ethanol; they had m. p. and mixed m. p. with the dimedone complex of methoxyacetaldehyde, 169—170°. The latter was prepared by periodate oxidation of glycerol 1-methyl ether.[14]

For the preparation of 6-*O*-methyl-D-galactose, 1 : 2-3 : 4-di*iso*propylidene-D-galactose was methylated with methyl iodide and silver oxide, and, after filtration and evaporation of the filtrate, the residue was hydrolysed with N-sulphuric acid. This solution was neutralised (barium carbonate), filtered, and evaporated, and the residue fractionated on a cellulose column. The fraction containing 6-*O*-methyl-D-galactose, recrystallised from ethanol or methanol, had m. p. and mixed m. p. with the 6-*O*-methyl-D-galactose isolated from the polysaccharide, 122—123°.

The m. p. and optical rotation of 6-*O*-methyl-D-galactose have been recorded as: m. p. 128°, $[\alpha]_{578}^{20}$ +114° ⟶ +77°;[15] m. p. 118°, $[\alpha]_D^{20}$ +120° ⟶ +70°;[16] m. p. 122—123°, $[\alpha]_D^{18}$ +112° ⟶ +66°;[16] m. p. 113—114°, $[\alpha]_D^{18}$ +137° ⟶ +77°;[17] m. p. 119·5—120·5°.[18] Chromatographic fractionation enabled us to prepare a very pure specimen in the present investigation, and it seems possible that some of the earlier preparations were contaminated with other material. 6-*O*-Methyl-D-galactosazone has similarly been recorded on several occasions, as having: m. p. 204—206°, $[\alpha]_{578}^{17}$ +135 in pyridine;[15] m. p. 200—201°, $[\alpha]_D^{20}$ +141° in pyridine;[16] m. p. 200°, $[\alpha]_D^{26}$ +141 ⟶ +92° (after 24 hr., const.) in pyridine;[17] m. p. 201—203°.[19]

D- *and* L-*Galactose*.—Fraction (2) (11·9 g.), when recrystallised several times from methanol, had m. p. and mixed m. p. with authentic L-galatose 161—162°, $[\alpha]_D^{17}$ −83·3° (*c* 1·1). The m. p. was depressed to 155° on admixture with D-galactose. The sugar gave mucic acid, m. p. and mixed m. p. 215—217°, on oxidation with nitric acid (*d* 1·15). The methylphenylhydrazone, crystallised from methanol, had m. p. and mixed m. p. 174°. When this L-galactose was mixed in equimolecular proportions with D-galactose it had zero rotation (*c* 1·6).

The mother-liquor from the first recrystallisation of fraction (2) was used to prepare a benziminazole according to Moore and Link's procedure.[20] The product, on recrystallisation from water, had m. p. 227° and zero rotation in 5% citric acid. Bell and Baldwin [6] report that 2-DL-galactobenziminazole is less soluble than either the D- or the L-form and that it has m. p. 233° and zero rotation in 5% citric acid solution. The mother-liquor from the first recrystallisation of the L-galactose yielded more crystals on concentration. This material had a positive

[13] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[14] Hatch and Nesbitt, *J. Amer. Chem. Soc.*, 1945, **67**, 39.
[15] Freudenberg and Smeykal, *Ber.*, 1926, **59**, 100.
[16] Munro and Percival, *J.*, 1936, 640.
[17] Pacsu and Trister, *J. Amer. Chem. Soc.*, 1940, **62**, 2301.
[18] Hough, Jones, and Magson, *J.*, 1952, 1525.
[19] Dewar and Percival, *J.*, 1947, 1622.
[20] Moore and Link, *J. Biol. Chem.*, 1940, **133**, 293.

rotation and on repeated recrystallisation from methanol was shown to be D-galactose, m. p. 161—162°, $[\alpha]_D^{18}$ +84° (*c* 0·6). It gave mucic acid, m. p. and mixed m. p. 215—217°, on oxidation with nitric acid (*d* 1·15). It had zero rotation when mixed with an equal weight of the L-isomer. The methylphenylhydrazone had m. p. and mixed m. p. 174—175° with authentic material prepared from D-galactose and it depressed the m. p. (168—171°) of the material prepared from L-galactose.

When a mixture of L-and D-galactose in the ratio of 4 : 1 was recrystallised from methanol, L-galactose, $[\alpha]_D^{18}$ −83° (*c* 0·9), was recovered from the first recrystallisation. Partial evaporation of the mother-liquors afforded D-galactose which, after further recrystallisation, had $[\alpha]_D^{17}$ +90°. The mother-liquor left after the D-galactose had crystallised had $[\alpha]_D^{18}$ +28°. This separation was repeated several times.

Methanolysis of the Polysaccharide.—To the crude polysaccharide (21 g.), dried at 60°/1 mm., was added 2·5% anhydrous methanolic hydrogen chloride (500 c.c.). This was refluxed for 15 hr., neutralised with silver carbonate and the residue removed in a centrifuge. Evaporation of the clear solution afforded a syrup (21 g.), which was mixed with cellulose powder packed on top of a cellulose column (41 × 5·5 cm.), and was eluted with butanol–water (9 : 1). The first fraction (2·87 g.) was a syrup. It gave a positive Molisch reaction and a faint spot R_R 2·2, R_F 0·55, when chromatographed in solvent (*a*) and sprayed with periodate–benzidine [21] or the periodate–starch spray of Metzenberg and Mitchell.[22] On distillation it yielded a main fraction (2·32 g.), b. p. 165—175°/0·1 mm., as a pale yellow oil which partly solidified, $[\alpha]_D^{18}$ −38° (*c* 2·23). The mixture of oil and solid was hydrolysed on a boiling-water bath with 0·1N-acetic acid, and this solution, which restored the colour to Schiff's reagent, was used for the preparation of the phenylosazone, which was obtained as yellow crystals (from methanol), m. p. and mixed m. p. 203—205° with 3 : 6-anhydro-L-galactose phenylosazone. Trituration of the mixture of oil and crystals with ethyl acetate afforded colourless needles, which after recrystallisation from the same solvent, had m. p. and mixed m. p. 138·5—140° with methyl 3 : 6-anhydro-α-L-galactopyranoside, $[\alpha]_D^{19}$ −77° (*c* 0·9).

The 3 : 6-anhydro-L-galactose isolated did not represent the total amount in the methanolysate. The remaining fractions obtained from the cellulose column after the first were contaminated with methyl galactofuranosides and 6-*O*-methylgalactofuranosides, which run very close to it on a chromatogram.

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PRETORIA, S. AFRICA. [*Received, September 21st*, 1956.]

[21] Cifonelli and Smith, *Analyt. Chem.*, 1954, **26**, 1132.
[22] Metzenberg and Mitchell, *J. Amer. Chem. Soc.*, 1954, **76**, 4187.