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Carbohydrase and Sulphatase Activities of *Porphyra umbilicalis*

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The carbohydrate constituents of the red seaweeds (Rhodophyceae) are characterized by the frequent occurrence of galactose, its derivatives and polymers. The mucilaginous substance of the algae, often present in very large quantities (up to 80 % of the dry weight), is usually a polysaccharide composed of residues of D-galactose and certain of its derivatives, such as galactose sulphate, 3:6-anhydrogalactose, 6-*O*-methylgalactose, as well as L-galactose. Sucrose, a key substance in higher plants, is absent here and its functions are probably taken over by floridoside (Bean & Hassid, 1955; Bidwell, 1958), which is a glycerol galactoside (2-*O*-α-D-galactopyranosylglycerol), commonly found in the red seaweeds. Compounds related to floridoside have also been reported from some members of this class. Such compounds are 1-*O*-α-D-galactopyranosyl-D-glycerol and 1-*O*-α-D-galactopyranosyl-L-glycerol from *Porphyra umbilicalis* (Lindberg, 1955*b*; Wickberg, 1958*b*); 2-*O*-α-D-mannopyranosyl-D-glyceric acid from *Polysiphonia lanosa* and *Ceramium rubrum* (Colin & Augier, 1939; Bouveng, Lindberg & Wickberg, 1955); *O*-α-D-galactopyranosyl-(1 → 6)-*O*-β-D-galactopyranosyl-(1 → 1)-D-glycerol from *Polysiphonia lanosa* and *Corallina officinalis* (Wickberg, 1958*a*); and *O*-α-D-mannopyranosyl-(1 → 3)-*O*-α-D-galactopyranosyl-(1 → 2)-glycerol from *Furcellaria fastigiata* (Lindberg, 1955*a*).

A common reserve polysaccharide of the red seaweeds is floridean starch, which contains no amylose component and is a polysaccharide of the glycogen–amylopectin type (Fleming, Hirst & Manners, 1956; Peat, Turvey & Evans, 1959).

The synthesis and transformation of carbohydrates in these seaweeds has as yet received little attention. Some of the enzyme activities present in extracts of *Rhodymenia palmata* and *Iridophycus flaccidum* have been described (Duncan, Manners & Ross, 1956; Bean & Hassid, 1956), and the fate of $^{14}CO_2$ after incorporation into certain species has also been studied (Bean & Hassid, 1955; Bidwell, 1958).

Seaweeds of the genus *Porphyra* belong to this class. *Porphyra umbilicalis* grows on the Welsh coast and is collected and marketed in quantity for human consumption. It is a relatively primitive organism, belonging to the subclass Bangioideae (Fritsch, 1945). The carbohydrates present have been investigated by a number of workers. The major polysaccharide (which sometimes represents over 30 % of the dry weight), to which the name of porphyran is given, contains residues of D-galactose, L-galactose, 3:6-anhydro-L-galactose, 6-*O*-methyl-D-galactose and galactose ester sulphate (Turvey & Rees, 1958), much of the ester sulphate being carried at the 6-position of L-galactose residues (Turvey & Rees, 1961). A similar polysaccharide has been isolated from *Porphyra capensis* (Nunn & von Holdt, 1957). The presence of floridean starch has been demonstrated in *Porphyra umbilicalis* (Turvey & Rees, 1958), and the principal low-molecular-weight carbohydrates have been shown to be floridoside and isofloridoside (Lindberg, 1955*b*). The latter is stated to be a mixture of 1-*O*-α-D-galactopyranosyl-D-glycerol and 1-*O*-α-D-galactopyranosyl-L-glycerol (Wickberg, 1958*b*). *Porphyra* is unusual in the nature of the skeletal polysaccharide of the cell wall, which is not cellulose but a β-1:4-linked polymannose (Jones, 1950; Cronshaw, Myers & Preston, 1958).

The purpose of this paper is to describe the results of a preliminary survey of the enzymes present in extracts of *Porphyra umbilicalis*, particular

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attention being paid to the enzyme-catalysed reactions involving derivatives or polymers of galactose.

EXPERIMENTAL

Materials

Substrates. The preparation and characterization of porphyran and floridean starch are described in a separate communication (Peat, Turvey & Rees, 1961). Analysis of porphyran gave the values shown in Table 1. It showed $[\alpha]_D$ −79°. Oligosaccharide A is a mixture of related substances with a degree of polymerization (D.P.) of approximately 20, precipitated by the addition of 2–3 vol. of ethanol to a partial hydrolysate of porphyran. Oligosaccharide B was obtained similarly by the addition of a further 8 vol. and has D.P. 5–7. The conditions used for the partial hydrolysis (0·5 N-sulphuric acid at 50° for 10 hr.) were such that no sulphate was released from ester linkages, although the polysaccharide was extensively depolymerized. The two fractions, oligosaccharide A and oligosaccharide B, were the main sulphated components isolated, and contained between them most of the esterified sulphuric acid originally present. The floridean starch used in these experiments was contaminated with galactan. Glucose 6-sulphate and glucose 3-sulphate were prepared in this laboratory by Mr T. P. Williams, galactose 6-sulphate by Dr K. O. Lloyd, nigerose by Dr J. Mon Evans, and the cellodextrins by Dr J. G. Roberts by precipitation from a partial hydrolysate of cellulose, by the addition of ethanol to a concentration of 20%. Yeast mannan was prepared by Dr D. Doyle (1959); the dextran was supplied by Dextran Ltd., Aycliffe, Darlington, Co. Durham (see Turvey & Whelan, 1957), and inulin by Thomas Kerfoot Ltd., Vale of Bardsley, Lancs.

Seaweed. Samples of *Porphyra umbilicalis* (L.) Kutz. f. *umbilicalis* (Parke, 1953) were gathered, except where otherwise stated, at Aberdesach near Caernarvon on the North Wales coast, in the period November 1959 to May 1960.

Methods

Unless otherwise stated, the evaporation of all solutions was carried out under diminished pressure, at pH 5–7 and at 35–40°.

Incubations of enzymes with substrates were carried out in a thermostat at 35° in the presence of a preservative (usually toluene).

Paper chromatography. This was effected by downward development with the following solvent systems (proportions by volume): (i) butanol–pyridine–water (6:4:3); (ii) butanol–ethanol–water (5:1:4); (iii) butanol–acetic acid–water (4:1:5); (iv) propanol–ethyl acetate–water (7:1:2). Sugar zones were located with alkaline silver nitrate (Trevelyan, Procter & Harrison, 1950), *p*-anisidine hydrochloride (Hough, Jones & Wadman, 1950) or periodate–benzidine (Cifonelli & Smith, 1954). Whatman no. 1 paper was normally used.

Viscosity measurements. These were performed in an Ostwald viscometer with a flow time of 10·7 sec. for water (4 ml.). Experiments were carried out in a thermostat at 35°.

Reducing power. This was determined with the Somogyi (1945*a*) reagents after deproteinization (Somogyi, 1945*b*), or with the Shaffer & Hartmann (1921) reagent. With the latter, deproteinization was unnecessary with 1 ml. samples of mixtures containing 0·5% (w/v) of protein.

Detection of sulphatase activity. The incubation mixture (1·5 ml.) was treated with trichloroacetic acid (40%, w/v, 0·1 ml.); the precipitated protein was coagulated by warming in a water bath at 70–85° and removed on the centrifuge. A portion of the supernatant solution (1 ml.) was withdrawn and tested with acidic barium chloride [2% (w/v) barium chloride in 0·1 N-hydrochloric acid]. The presence of liberated sulphate ions was indicated by the development of a turbidity within 5 min.

The desulphation of monosaccharide derivatives was detected by the appearance of the free monosaccharide on a paper chromatogram.

Isolation of low-molecular-weight carbohydrates of the seaweed

The method was similar to that of Lindberg (1955*b*). Pressed *Porphyra umbilicalis* (7 kg. wet wt.), collected in October 1959, was minced into ethanol (9 l.) soon after harvesting. The mixture was brought to the boil, then left for 24 hr., and filtered successively through a nylon stocking, a pad of Celite and a pad of charcoal (British Drug Houses Ltd.; 'activated'). The solution was adjusted to pH 6, and carefully evaporated to 1 l. The precipitate was removed on the centrifuge, the solution de-ionized by treatment with Zeo-Karb 225 (H^+) and De-Acidite FF (CO_3^{2-}) and evaporated to a brown immobile syrup (125 g.). Part of this product (8·5 g.) was adsorbed on a charcoal–Celite column (130 cm. × 5 cm.; equal parts by weight of 'activated' charcoal and Celite). The column was eluted with aqueous ethanol, the composition of which was changed continuously in a linear manner from 1·5 to 16·5% (v/v) over a total volume of 20 l. The column was finally eluted with aq. 10% (v/v) butan-2-one. Fractions (150 ml. each) were collected and analysed for carbohydrate by using the phenol–sulphuric acid reagent (Dubois, Gilles, Hamilton, Rebers & Smith, 1956). Two major peaks were observed but these were incompletely separated. The fractions containing carbohydrate were therefore examined by paper chromatography, and those containing a mixture of two substances as indicated by this method were rejected. The remaining fractions were combined to give two solutions, each containing a chromatographically pure component. These solutions were each evaporated to dryness and the residues crystallized from ethanol.

Identification of floridoside. The slower-moving substance on charcoal, and the faster-moving on paper [rate of flow relative to galactose ($R_{Gal.}$) in solvent (ii) on Whatman no. 3

Table 1. *Composition of porphyran (sodium salt)*

	Percentage*	Molar ratio
D-Galactose	16·0	1·00
L-Galactose	17·6	1·10
6-*O*-Methyl-D-galactose	20·6	1·18
3:6-Anhydro-L-galactose	10·3	0·73
Esterified sulphate	11·1	1·40
Nitrogenous material (calc. as protein)	1·8	—
Associated inorganic material other than esterified sulphate	13·9	—
Other sugar residues	0·8	—

* Sugars are calculated as anhydro units.

paper, was 1·10] was non-reducing and on acid hydrolysis gave galactose and glycerol as indicated by paper chromatography. Weight 0·58 g.; m.p. 127–128·5° (capillary, uncorr.); $[\alpha]_D$ +163° in water (*c*, 0·36). These properties are in good agreement with those previously reported for floridoside (Lindberg, 1955*a*, *b*, Putman & Hassid, 1954; Nunn & von Holdt, 1955).

Identification of isofloridoside. The faster-moving substance on charcoal and the slower-moving on paper ($R_{Gal.}$, with the same conditions as above, was 0·90), resembled the first component in that it was non-reducing and on acid hydrolysis it gave galactose and glycerol as indicated by paper chromatography. Weight 1·50 g.; m.p. 149·5–151·5°. A sample of isofloridoside was melted on the block of the Kofler apparatus, allowed to cool, seeded and left to crystallize, and the m.p. was retaken (found 147–150°), $[\alpha]_D$ +155°. The chromatographic behaviour and the specific rotation were as expected for isofloridoside (Lindberg, 1955*b*; Wickberg, 1958*b*).

Isolation and examination of isofloridoside from another sample of Porphyra. This sample of *Porphyra* was collected by Dr E. Conway from the splash zone on the island of Colonsay, Scotland, in September 1959. The air-dried seaweed (10·5 g.) was cut into small pieces and extracted briefly with boiling water (4 × 100 ml.). Polysaccharide was precipitated by the addition of ethanol (4 vol.) and removed on the centrifuge. The solution was evaporated to dryness, the residue redissolved in water and the resulting solution de-ionized [Zeo-Karb 225 (H^+) and De-Acidite FF (CO_3^{2-})] and concentrated to a small volume. The isofloridoside was separated from the mixture by thick-paper chromatography [multiple development on Whatman no. 3 paper and solvent (ii)], dissolved in ethanol, decolorized with charcoal and crystallized from ethanol. Weight 0·059 g.; m.p. 148·5–151·5° (Kofler hot-stage, corr.).

Extraction of the enzymes

Fresh pressed seaweed (5 kg.) was minced (Kenwood mixer) into aq. 0·25% sodium carbonate (7–8 l.) precooled to 4°. The mixture was left at this temperature overnight, pressed through muslin and clarified on the centrifuge. To the resulting solution (pH 8·2) was added solid ammonium sulphate to 0·8 saturation; the precipitate was removed on the centrifuge, dissolved in water and the solution dialysed against running tap water at 5–10° until a negative test for sulphate was obtained (the test being that described above for the detection of sulphatase activity). The dialysis bag was changed daily, otherwise it became so weakened that it burst after 2–4 days. This phenomenon was probably due to the presence of a cellulase. The sediment which appeared during dialysis was removed on the centrifuge and the clear orange to red supernatant solution was freeze-dried, giving a red to purple powder (about 5 g.). Several other extraction procedures were used in early investigations, but this method was preferred because it gave the greatest yield of sulphatase activity.

RESULTS

Carbohydrase activity. The freeze-dried extract (5 mg.) was incubated in potassium acetate buffer (0·05 M; pH 6·5; 1 ml.) with each of a number of carbohydrates (5 mg.), and the extent of the hydrolysis of the substrates was followed by paper chromatography. The summarized results are given in Table 2, in which 'marked hydrolysis' means the release of appreciable quantities of hydrolysis products within 48 hr.

Transgalactosylation activity. When the freeze-dried extract (10 mg.) was incubated with floridoside (150 mg.) in potassium acetate buffer (0·1 M; pH 6·5; 1 ml.) for periods up to 3 weeks, there was only doubtful evidence for the formation of a higher saccharide [$R_{Gal.}$ in solvent (i) 0·28?]. It is therefore concluded that if transgalactosylation reactions are catalysed by the α-galactosidase, the activity is very weak when floridoside is the substrate.

Sulphatase activity. When the freeze-dried extract [1% (w/v) in 0·1 M-potassium acetate buffer, pH 6·5; 0·5 ml.] was incubated with porphyran [1% (w/v) in water; 1 ml.], free sulphate

Table 2. *Enzyme activities found in extracts of* Porphyra umbilicalis

Type of activity detected	Marked hydrolysis of substrate	Slight hydrolysis of substrate	Weak, doubtful or no hydrolysis of substrate
α-Galactosidase	Floridoside	Isofloridoside, melibiose	Raffinose, α-methyl galactoside
β-Galactosidase	—	Lactose	β-Methyl galactoside
α-Glucosidase	Sucrose, maltose, nigerose	α-Methyl glucoside	—
β-Glucosidase	—	Cellobiose, cellodextrins	—
Amylase	Amylose, amylopectin, glycogen, floridean starch	—	—
β-Glucanase	Laminarin, cellophan	—	—
Mannanase	Ivory-nut mannan	—	Yeast mannan
Miscellaneous polysaccharases	—	—	Dextran, inulin
Sulphatase	Porphyran	—	Oligosaccharide A, oligosaccharide B, galactose 6-sulphate, galactose disulphate, glucose 3-sulphate, glucose 6-sulphate

was found in the solution within 3 hr. Certain other carbohydrate sulphates, however, were hydrolysed very much less readily (Table 2).

Other enzymes acting on porphyran. The incubation mixture of the enzyme extract and porphyran showed no increase in reducing power after 48 hr., and no low-molecular-weight hydrolysis products could be detected in it by paper chromatography, even after prolonged periods. The only change in the polysaccharide which could be detected concomitant with sulphate release was a fall in viscosity. This fall in viscosity was shown to be due to an enzyme other than the sulphatase by the following experiment. Two digests were prepared by mixing porphyran [1 % (w/v) in water; 3 ml.] with freeze-dried extract [1 % (w/v) in 0·1 M-buffer, pH 6·5; 1·5 ml.]. One of the digests contained sodium citrate buffer and the other potassium acetate. Inhibition of the sulphatase occurred in the mixture containing citrate (a barely detectable opalescence was observed after 48 hr.), whereas a relatively large amount of sulphate was released in the other (an almost opaque turbidity in 24 hr.). In contrast, the two digests showed similar decreases in viscosity (acetate-containing digest showed $\eta_{sp.}$ 1·06 → 0·51 in 16 hr., citrate-containing digest, $\eta_{sp.}$ 1·06 → 0·58). In control experiments, neither the polysaccharide nor the extract separately showed any change in viscosity, even when incubated for prolonged periods.

DISCUSSION

The low-molecular-weight carbohydrates were investigated since they were possible natural substrates for the enzyme system. The two major components of this group have properties similar to those of the glycosides isolated by Lindberg (1955*b*) from a *Porphyra umbilicalis* of different origin. One component was in fact proved to be floridoside (2-*O*-α-D-galactopyranosylglycerol), but the properties of our second component were not entirely consistent with the description given by Wickberg (1958*b*) of Lindberg's sample of isofloridoside. The substance was non-reducing, gave galactose and glycerol on hydrolysis, the specific rotation was close to that quoted for isofloridoside, and its behaviour, when chromatographed on charcoal, was similar to that described by Lindberg. According to Wickberg, however, isofloridoside is a mixture of two diastereomers, 1-*O*-α-D-galactopyranosyl-D-glycerol and 1-*O*-α-D-galactopyranosyl-L-glycerol, which are isomorphous and give no melting-point depression when mechanically mixed, but which melt over a very wide range (130–150°), corresponding to the difference between the melting points of the two components (131·5–133° and 149–151°), although a low-melting zone (120°) was observed when the substance was examined by the mixed fusion technique. We have, however, been unable to detect any product melting below 149·5° in the sample isolated in the present study. Moreover, the melting point of our isofloridoside was virtually unchanged after crystallization from the molten state (147–150°).

It would seem, therefore, that this specimen of isofloridoside consists only of 1-*O*-α-D-galactopyranosyl-D-glycerol, that is, of the isomer with the higher melting point (Wickberg, 1958*b*). The values for the specific rotations of the diastereomers are too close to permit any conclusions about homogeneity to be drawn from the measured value. The infrared-absorption curves obtained by Wickberg for the two synthetic diastereomers showed that this technique was not suitable to determine whether small quantities of the L-glycerol derivative were present in our preparation.

It is difficult to account for the disparity of Wickberg's and our results in regard to the structure of isofloridoside. It is not possible that the missing diastereomer was present in the discarded charcoal chromatography fractions, because the L-glycerol derivative has the greater mobility (Wickberg, 1958*b*), and only the slower-moving fractions were rejected. The disparity may be due to differences between the seaweed sources used. Some confusion still surrounds the botanical classification of the genus *Porphyra*, and it is possible that the seaweed examined by the Swedish workers differs ecologically or seasonally from that taken for the present study (Dr E. Conway, personal communication). The sample which we examined originally was gathered low down in the intertidal belt of a shore that is comparatively sheltered. A second sample was therefore examined, taken from a very exposed coast where the plant is found above the high-tide level so that it is probably under conditions much less favourable for metabolism and growth. However, the melting-point characteristics of the isofloridoside thus isolated again gave no indication of the presence of a second component.

Although floridoside is present in a large number of red algae, isofloridoside has been reported as present only in *Porphyra umbilicalis*, and Lindberg has suggested that it may be peculiar to the subclass Bangioideae. It is reasonable to suppose that floridoside performs the same biological function in the Bangioideae as in the Florideae, namely that it provides a food reserve. The *Porphyra* extracts showed a marked hydrolytic activity towards floridoside, but a much less pronounced activity on isofloridoside and other α-galactosides (Table 2). The only observation that can be made at this stage concerning the role of isofloridoside is that if the α-galactosidase is responsible for the break-

down of floridoside *in vivo*, then isofloridoside cannot be regarded as interchangeable with the floridoside as a reserve substance, although both are α-glyceryl galactosides. It is tempting to suggest that the floridoside–α-galactosidase system here is analogous to the sucrose–invertase system of higher plants. An important difference, however, is that, whereas invertase shows a fairly marked ability to catalyse transglycosylation reactions (Allen & Bacon, 1956), such reactions occur in the α-galactosidase system only to a slight extent, if at all. In accordance with this view is the fact that some of the oligosaccharides which are formed by the mediation of invertase *in vitro* are often found associated with sucrose *in vivo* (cf. Pridham, 1960), but in contrast no oligosaccharides have yet been reported as present in the red seaweeds which could have arisen by trans-α-galactosylation with floridoside as acceptor. In the study of the low-molecular-weight carbohydrates of *Porphyra* reported in this paper, there is indeed no evidence for the presence of any oligosaccharide material at all. There is not yet sufficient information available to enable a useful comparison to be made between the α-galactosidase of *Porphyra* and similar enzymes from other sources (cf. Gottschalk, 1958; French, 1954).

The other carbohydrases of *Porphyra umbilicalis* were as anticipated on the basis of the survey made by Duncan *et al.* (1956) of the carbohydrase activities of extracts of *Cladophora rupestris*, *Ulva lactuca*, *Laminaria digitata* and *Rhodymenia palmata*, with the difference that the β-glucosidase activity towards cellobiose and cellodextrins was much weaker in the *Porphyra* extracts. It should be noted that *Porphyra*, apparently alone amongst seaweeds, is devoid of cellulose (Cronshaw *et al.* 1958). There is thus further reason to believe that the enzyme pattern of α- and β-glucosidase, amylase, β-1:3- and β-1:4-glucanase and mannanase activities found by Duncan *et al.* is a general one present in most seaweeds.

The pronounced carbohydrate-sulphatase activity which was detected in the extracts is of interest because of the occurrence of very substantial deposits of galactan sulphate in the intercellular regions of living *Porphyra*. Although this is the first recorded instance of the extraction of an enzyme of this type from a seaweed, there have been repeated reports of sulphatases from various other marine organisms (for references see Dodgson & Spencer, 1956), and K. S. Dodgson (personal communication) has detected the liberation of sulphate in seaweed extracts, presumably from the interaction of an enzyme with a coextracted substrate.

The chondrosulphatase of *Proteus vulgaris* (Dodgson & Lloyd, 1957) has been shown to require extensive depolymerization of the polysaccharide before desulphation can occur, i.e. the true substrate of this enzyme is not chondroitin sulphate but the sulphated oligosaccharides produced from it by the action of the associated chondroitinase. The sulphatase present in *Porphyra* extracts does not appear to function in this way, its substrate being undegraded porphyran. This follows because there is no increase in the reducing power of the polysaccharide concomitant with desulphation, and there is only a very slight activity exhibited by the enzyme towards low-molecular-weight carbohydrate sulphates, including certain oligosaccharide sulphates derived from porphyran (Table 2).

One qualification attaches, however, to this interpretation. By inhibition of the sulphatase with citrate it was shown that another enzyme having action on porphyran is present in the crude preparation. The only information at present available concerning the mode of action of this enzyme is that it produces a fall in the viscosity of the polysaccharide when incubated with it. Nevertheless, it is quite possible that, even though no low-molecular-weight fragments are produced (since none were detected on paper chromatography, and since there was no increase in reducing power), this activity is of the carbohydrase type. It might, for example, have a debranching action resulting in the production of high-molecular-weight dextrins, which have a negligible copper-reducing power. The conclusion is reached, therefore, that extensive depolymerization is not a necessary preliminary to desulphation of porphyran by enzymes present in *Porphyra* extracts, although fragmentation of the macromolecule into units which are still relatively large may be required. With respect to its molecular-weight specificity, this enzyme thus resembles the cellulose polysulphatase found in liver extracts of a marine mollusc by Takahashi & Egami (1960) rather than the chondrosulphatase of *Proteus vulgaris*.

SUMMARY

1. A qualitative investigation has been made of some enzymes present in extracts of the red seaweed *Porphyra umbilicalis*, particular attention being paid to those which catalyse reactions involving derivatives and polymers of galactose.

2. The principal low-molecular-weight carbohydrates of the alga have been isolated since they were potential substrates for the enzyme system. These were floridoside and isofloridoside. It is concluded that in the seaweed samples examined in the present study, the isofloridoside component is 1-*O*-α-D-galactopyranosyl-D-glycerol.

3. *Porphyra umbilicalis* extracts exhibit many of the carbohydrase activities previously reported

for other algal extracts, namely α- and β-glucosidase, amylase, β-glucanase and mannanase.

4. An α-galactosidase activity is also present for which floridoside is possibly the natural substrate. Some implications of this, in terms of the carbohydrate metabolism of the seaweed, are discussed.

5. At least two enzymes having action on porphyran, the principal component of the plant, are present. One of these is a sulphatase which is inhibited by citrate, and the other catalyses a reaction causing a fall in the viscosity of the polysaccharide solution.

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Equilibrium Constants of Phosphoryl Transfer from $C_{(1)}$ to $C_{(6)}$ of α-D-Glucose 1-Phosphate and from Glucose 6-Phosphate to Water

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The calculation of the free energy of hydrolysis of adenosine triphosphate (Atkinson, Johnson & Morton, 1959; Atkinson & Morton, 1960) from the equilibrium constant of the reaction catalysed by galactokinase (Atkinson, Burton & Morton, 1961) required the estimation of the free energy of hydrolysis of α-D-galactose 1-phosphate. This hexose phosphate only differs from α-D-glucose 1-phosphate in the configuration of the hydroxyl group at $C_{(4)}$, which is *para* and *trans* to the phosphate; the difference between the free energies of hydrolysis of these compounds is probably less than 0·1 kcal./mole (Angyal & McHugh, 1956). The free energy of hydrolysis of α-D-glucose 1-phos-