reducing phosphates had $[\alpha]_{5461} + 34.4^\circ$ and the Ba salt of the difficultly hydrolysable ester $[\alpha]_{5461} - 3.4^\circ$. From these values it was calculated that the natural anhydrous Ba galactose-1-phosphate has $[\alpha]_{5461} + 113^\circ$, while the synthetic product has $[\alpha]_{5461} + 109.5^\circ$.

3. The velocity constant (k) of hydrolysis of the acid-labile fraction of the ester mixture, freed from reducing phosphates, in $0.25N$ HCl at 25° was 0.79×10^{-3} . From this value the k of the natural

galactose-1-phosphoric acid was calculated to be 0.91×10^{-3} , while the k of the synthetic product is 0.89×10^{-3} .

4. These results strongly suggest that the natural and synthetic galactose-1-phosphoric acid are identical. The ester probably is an α -pyranoside as is the natural glucose-1-phosphoric acid.

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The Apparent Dissociation Constants of Galactose-1-Phosphoric Acid

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Meyerhof & Suranyi [1926], Meyerhof & Lohmann [1927], and Cori, Colowick & Cori [1937] found that the dissociation constants (pK' values) of hexose-diphosphoric, hexose-6-monophosphoric, fructose-6-phosphoric, and glucose-1-phosphoric acids were smaller than those of orthophosphoric acid. In the present paper α (?) galactose-1-phosphoric acid is shown to have dissociation constants similar to those of the other hexosemonophosphates. The knowledge of the acidic properties of galactose-1-phosphoric acid is necessary for the assessment of the changes in pH which may be brought about by the phosphorylation of galactose.

EXPERIMENTAL

The pH values were measured by means of a Cambridge pH meter with dip glass electrode (portable pattern). A buffer consisting of equal parts of Na acetate and acetic acid ($0.1N$) and having pH 4.62 was used for standardizing the glass electrode. The galactose-1-phosphoric acid was prepared by treating the Ba salt (from the K_2 salt [Kosterlitz, 1939]) with the exact quantity of H_2SO_4 . The concentration of galactose-1-phosphoric acid in the final solution was determined by estimation of phosphorus. 10 ml. of $0.1015M$ (Exp. 1) and $0.1038M$ (Exp. 2) galactose-1-phosphoric acid were titrated with CO_2 -free $0.0999N$ NaOH (temp. = $18-20^\circ$). For comparison, 10 ml. of $0.1001M$ H_3PO_4 were

similarly titrated. The results were plotted after correction for the deviations from 0.1 molarity

Fig. 1. 10 ml. $0.1M$ orthophosphoric acid $\bullet-\bullet$ 10 ml. $0.1M$ galactose-1-phosphoric acid: Exp. 1 $---\times$; Exp. 2 $\times-\times$.

(Fig. 1). The dissociation constants were calculated from 3 to 4 values each; pK'_1 by means of

$$K'_a = \frac{[H]^+ (B + [H]^+)}{C - (B + [H]^+)}$$

and pK'_2 by means of Henderson-Hasselbalch's equation

$$pK'_a = pH - \log \frac{B}{C - B}$$

[Van Slyke, 1922]. In these equations B stands for molar concentration of total alkali added and C for molar concentration of galactose-1-phosphate in the titration mixture at any given time.

The values found for orthophosphoric acid, pK'_1 1.95 and pK'_2 6.83, agree well with those recorded in the literature [Van Slyke, 1922; Meyerhof & Suranyi, 1926; Cori *et al.*, 1937]. Galactose-1-phosphoric acid has pK'_1 1.00 and pK'_2 6.17 while hexose-6-phosphoric acid (Robison ester) has pK'_1 0.94 and pK'_2 6.11, fructose-6-phosphoric acid pK'_1 0.97 and pK'_2 6.11 [Meyerhof & Lohmann, 1927] and glucose-1-phosphoric acid pK'_1 1.10 and pK'_2 6.13 [Cori *et al.*, 1937]. There is no significant difference between the biologically important pK'_2 values of galactose-1-phosphoric acid and those of the other hexosemonophosphates.

Since Meyerhof & Lohmann [1927] found that the pK_2 values of fructose-6-phosphoric and hexose-6-phosphoric (Robison) acids were decreased by about 0.14 by the addition of 0.1 M neutral salt solution, 10 ml. 0.1 M K_2 galactose-1-phosphate solution were titrated with N HCl. pK'_2 determined in this way was 6.06, as compared with 6.17, the value obtained by titrating the ester with NaOH.

SUMMARY

α (?)-Galactose-1-phosphoric acid, like other hexosemonophosphates, is a stronger acid than is orthophosphoric acid. α (?)-Galactose-1-phosphoric acid has pK'_1 1.00 and pK'_2 6.17, while orthophosphoric acid has pK'_1 1.95 and pK'_2 6.83.

An expenses grant by the Medical Research Council which partly defrayed the cost of this investigation is gratefully acknowledged.

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The Fermentation of Galactose and Galactose-1-phosphate

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Galactose-1-phosphate is present in the livers of rabbits and rats during galactose assimilation [Kosterlitz, 1937; Kosterlitz & Ritchie, 1943b]. No information as to the fate of this ester has so far been obtained except that it is more resistant to the phosphatase of liver than is glucose-1-phosphate [Cori, Colowick & Cori, 1938; Kosterlitz & Ritchie, 1941]. Since the carbohydrate metabolism of yeast cells is strikingly similar to that of mammalian tissue, it was decided to examine the fermentation of galactose and galactose-1-phosphate and compare the results with those obtained in the fermentation of glucose and glucose-1-phosphate.

The ability of some yeasts to ferment galactose is due to the formation of 'galactozymase' during adaptation. This process takes place in aqueous solutions of galactose and can occur without cell multiplication [Stephenson & Yudkin, 1936; for the older literature see that paper, and Harden, 1932]. Yeast juice [Harden & Norris, 1910; Grant, 1935], dried yeast and maceration extract [Abderhalden,

1925] prepared from adapted yeast, will ferment galactose. In the products of fermentation Nilsson [1930] found, besides Harden-Young ester, a monophosphate with a specific rotation higher than that of Robison ester. No data as to the reducing power of this ester and the nature of its constituent hexose were given. Grant [1935] confirmed the presence of 1:6-fructose diphosphate, and found trehalosemonophosphate and Robison ester. Using the methylphenylhydrazine test, he could not find any evidence for the accumulation of a galactose phosphoric ester. He further could demonstrate that galactose-6-phosphate was not fermented by galactose-adapted yeast.

EXPERIMENTAL

Materials and methods

Maceration extracts. These were prepared in the usual manner from a pure strain of *Saccharomyces cerevisiae* (*S. cerevisiae*) Froberg, which had been propagated in this laboratory since 1935, when it was generously supplied by