Slyke, 1922)). B represents the molar concentration of added NaOH and C the molar concentration of ester in the titration mixture at any given time. (A pK_1 and pK_2 for the pyrophosphoric esters could not be distinguished by this method.) The graphs for the titration are given in Fig. 1, and the pK values in Table 1.

Enzyme hydrolysis. A range of carbonate-veronal buffers covering pH 7.5–10.7, and a potent faecal phosphatase were prepared as described by Delory & King (1943), and their procedure for the determination of the pH optima and Michaelis constants was followed exactly. These results are shown graphically in Figs. 2 and 3.

DISCUSSION

It was expected, when these two substituted phenyl phosphates were made, that they would show a greater rate of hydrolysis than unsubstituted phenyl phosphate, due to the strong positive inductive, acid-strengthening effect of the carboxyl group. At the pH values used for enzymic hydrolysis, however, the group which is influencing the secondary phosphate dissociation is the negatively charged ionized carboxyl group ($-\text{COO}^-$); and the two negative charges on the dissociated primary phosphate and

carboxyl groups have outweighed the positive inductive action which we had expected.

These results, however, agree with those of Delory & King (1943) in showing an increase in the amount of hydrolysis and a shift of the pH optimum to a more alkaline reaction with increasing acidity of the substrate.

SUMMARY

1. *o*-Carbomethoxyphenyl pyrophosphate and *p*-carbethoxyphenyl pyrophosphate have been prepared; they are converted by mild hydrolysis to the orthophosphoric esters *o*-carboxy- and *p*-carboxyphenyl phosphate.

2. These esters are not such strong acids (pK *o*-, 6.11; *p*-, 6.40) as unsubstituted phenyl phosphate (pK 5.73).

3. The optimum pH values for enzymic hydrolysis by faecal phosphatase (*o*-, 9.10; *p*-, 8.00) are not as alkaline as that of phenyl phosphate (9.73). The rates of enzymic hydrolysis are also less.

4. The Michaelis constants (K_m for *o*-, 0.0010; *p*-, 0.0030) are greater than that of phenyl phosphate (K_m 0.0006).

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The Fermentable Form of Fructose

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(Received 15 December 1949)

Hopkins (1931) found that when a rapid fermentation of fructose by brewer's yeast is arrested the residual sugar exhibits mutarotation in the positive sense. From this and other observations it was concluded that one form of fructose was specifically fermented or at least preferred, that this form possessed a specific rotation less negative than that of fructose at equilibrium, and was fructofuranose. It was not possible to furnish clear experimental proof since neither α -fructopyranose nor any form of fructofuranose could be isolated in the crystalline form. However, a preparation of fructose exhibiting substantially less negative specific rotation than that of the solution at equilibrium can be prepared by slowly fusing normal fructose and cooling suddenly. The rates of fermentation of such preparations

have been compared with those of fructose at equilibrium, and of freshly dissolved normal fructose.

MATERIALS AND METHODS

Substrates. D-Fructose recrystallized from ethanol (Harding, 1922), was heated slowly with stirring in a nickel crucible immersed in a glycerine bath until the temperatures stated in Table 1 were attained. The crucible was then plunged into solid CO_2 . It is important to minimize access of moisture to the sugar throughout.

Fermentation. Brewer's top fermentation yeast was used. Rates of fermentation were measured as by Hopkins & Roberts (1935a). In the fermentation vessel the brewer's yeast in suspension was brought to the desired temperature and the sugar, in solid form or in solution at equilibrium, was

contained in a small beaker on a float on the surface. This sugar was discharged into the yeast suspension by starting the shaker.

RESULTS

Table 1 shows the initial specific rotation at 20° of the fructose preparations obtained by heating.

Table 1. *Effect of heat on specific optical rotation of D-fructose*

Temperature to which fructose was heated (°)	Colour of product	$[\alpha]_D^{20}$	
		Before mutarotation (°)	After mutarotation (°)
Unheated	White	—	-88.9
110	White	-59.8	-88.7
120	White	-54.6	-88.7
130	Cream	-56.1	-83.7
140	Buff	-57.0	-76.4

These values were obtained by extrapolation from a succession of polarimetric readings. If $[\alpha]_D^{20}$ of β -fructopyranose is -131° and that of β -fructofuranose is $+17^\circ$ (Bailey & Hopkins, 1933), then $[\alpha]_D^{20} - 54.6^\circ$ would correspond to 51% of β -fructofuranose. It was decided to adopt 120° as the maximum temperature of heating. As the value of $[\alpha]_D^{20}$ before mutarotation was found to increase in a few hours even when the heated sugar was stored in a vacuum desiccator, it was necessary to prepare the specimens immediately before use.

give low induction periods, so that the maximum rates of fermentation were attained before mutarotation could be appreciable. Only the early rates are unaffected by the latter.

The preheated fructose, $[\alpha]_D^{20} = -57$ to 58° , was much more rapidly fermented than the equilibrium solution and the latter than the β -fructopyranose form, especially at 35° . The fermentable component was apparently maintained, when equilibrium solution and β -fructopyranose were used, by continual transformation of β -fructopyranose into fructofuranose with accompanying mutarotation. (Incidentally the temperature coefficients $v_{35^\circ}/v_{25^\circ}$ of the maximum velocities agree with that of the mutarotation of fructose. For fuller treatment, see Hopkins & Roberts, 1935b.) With the heated fructose, however, mutarotation, instead of reinforcing it, acted against the fermentation, which in consequence slowed down markedly after attaining its maximum rate, especially at 35° .

In these experiments an attempt was made to approach as nearly as possible to the use of pure fructofuranose. Gottschalk (1943) employed conditions designed to minimize the quantity of fructofuranose present. He found that if β -fructopyranose was added to a yeast suspension at 0° the rate of fermentation was very small compared with that of glucose under the same conditions, and was independent of yeast concentration. The rate was

Table 2. *Rate of fermentation of forms of fructose*

(Fructose 0.5 g.; yeast 10 g.; pH 4.6, 0.01 N-acetate buffer; 50 ml.)

		Temperature 35°									
Time (min.)	...	2	3	4	5	6	7	8	9	10	15
		CO ₂ (mg./min.)									
β-Fructopyranose		8.1	13.9	16.2	15.7	14.4	12.9	11.6	11.1	10.4	6.4
Equilibrium fructose		16.4	18.4	16.8	15.1	13.5	11.9	10.3	9.5	9.0	6.0
Preheated fructose, [α] _D ^{30°} - 56.5°		22.2	25.2	17.3	10.9	10.7	10.3	9.3	8.5	7.6	4.7
		Temperature 25°									
Time (min.)	...	2	3	4	5	6	7	8	9	10	15
		CO ₂ (mg./min.)									
β-Fructopyranose		2.8	4.3	6.0	7.1	7.4	7.1	6.7	6.2	6.2	4.8
Equilibrium fructose		4.5	7.1	8.9	9.3	8.9	8.4	7.8	6.9	6.4	4.9
Preheated fructose, [α] _D ^{30°} - 58.5°		5.3	8.1	9.7	9.6	9.2	8.9	8.0	6.9	6.4	4.2

Fermentations performed using β -fructopyranose, equilibrium and preheated fructose are summarized in Table 2.

DISCUSSION

The rate of fermentation of fructose is appreciably influenced by concentration of the sugar over the range 0–2%, especially if a large proportion of yeast is used. Yeast and temperatures were selected to

indeed approximately that of the conversion of the pyranose into the furanose form by mutarotation.

The results reported here, like those of Gottschalk (1943, 1945, 1947) and of Suomalainen & Toivonen (1948), support the hypothesis first put forward by Hopkins (1931), and strongly supported by Gottschalk, that fructofuranose is the only form of fructose fermentable by yeast.

SUMMARY

1. Dried crystalline D-fructose, heated to 120° and cooled suddenly, yielded a product which, it is claimed, contained 51 % of fructofuranose.

2. This product was fermented much more rapidly than fructose at equilibrium in a corresponding solution.

3. This observation supports the theory that fructofuranose is the only form of fructose fermentable by yeast.

One of the authors (M. H.) is indebted to the Department of Scientific and Industrial Research for a grant. This work was recorded in a thesis presented to the University of Birmingham on 1 October 1938.

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The Biogenesis of Porphyrins

2. THE ORIGIN OF THE METHYNE CARBON ATOMS

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(Received 23 December 1949)

The method which the organism uses to synthesize protoporphyrin has aroused much speculation (Dobriner & Rhoads, 1928, 1940; Rimington, 1938; Turner, 1940). No experimental evidence for the mechanism of porphyrin synthesis was obtained, however, until Bloch & Rittenberg (1945) reported that deuterium, when supplied to rats in the form of acetate, was found in their blood haem. Shemin & Rittenberg (1946*a, b*) showed that the blood porphyrin from men and rats who had received ¹⁵N-labelled glycine had a relatively high isotope content. Other nitrogen-labelled compounds including ammonia, leucine, proline, glutamic acid, histidine (Tesar & Rittenberg, 1947), aspartic acid (Wu & Rittenberg, 1949), and ethanolamine (Muir & Neuberger, 1949*a*) were relatively ineffective in rats and rabbits respectively.

In a previous paper (Muir & Neuberger, 1949*a*), the partial degradation of mesoporphyrin on a small scale was described. Three products of oxidation were isolated, viz. carbon dioxide, which was derived mainly from the methyne carbon atoms, methyl-ethylmaleic acid imide from the non-acidic rings I and II of the porphyrin, and 1-methyl-2:2'-carboxy-ethylmaleic acid imide (haematinic acid), from the acidic rings III and IV. It was shown that feeding ¹⁵N-labelled glycine to rabbits produced a blood porphyrin in which the isotope content was the same in the acidic and non-acidic fractions. Wittenberg & Shemin (1949) independently carried out an

analogous degradation of the haemin obtained from a human subject and from ducks who had received ¹⁵N-labelled glycine, and obtained the same result.

In the present investigation the method has been used to find the distribution within the protoporphyrin molecule of labelled carbon atoms derived from likely precursors. These included sodium acetate, glycine, serine and sodium formate labelled with ¹⁴C and ¹⁵N as indicated by the following formulae:

For preliminary communication see Muir & Neuberger (1949*b*).

EXPERIMENTAL

Treatment of animals and administration of compounds

Rabbits. Male and female Hollingsworth half-lopped rabbits were used (2.5–3 kg. body weight). They were given the Institute stock diet, except in the experiment in which glycine was administered. In this experiment the animal was kept on a low protein diet of bran, hay and cabbage during the experiment, and for 1 month prior to it. Except in the experiment with ¹⁴CH₃·COONa, to increase the amount of isotope incorporated into the haem, an anaemia was produced by withdrawing a total of 50–70 ml. of blood over 2–4 days. The labelled compound was injected sub-