CLIX. TREHALOSEMONOPHOSPHORIC ESTER ISOLATED FROM THE PRODUCTS OF FERMENTATION OF SUGARS WITH DRIED YEAST.

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SINCE the original work of Harden and Young on the function of phosphates in alcoholic fermentation, the idea that the formation of phosphoric esters may be an essential stage not only in the biochemical degradation of hexoses but also in the condensation of these sugars to the polysaccharides has taken firm hold of biochemical imagination. The experimental evidence, which consists in (a) the stimulating effect of phosphates on certain processes involving carbohydrates in living tissues or tissue extracts, (b) the occurrence of phosphoric esters among the reaction products, is admittedly insufficient to prove such a general conception, but this remains a stimulating basis for research. As to the manner in which the introduction of the phosphoric acid group furthers such reactions we have, as yet, very little knowledge.

Of the two phosphoric esters hitherto isolated from the products of alcoholic fermentation, hexosediphosphoric acid is probably the 1:6-diphosphoryl derivative of γ -fructose [Young, 1909, 1911; Morgan, 1927; Morgan and Robison, 1928], although the proof of this structure is not yet complete. The constitution of the hexosemonophosphoric acid is still unknown but certain facts set out in the account of its isolation and investigation [Robison, 1922] point to it being a mixture of monophosphoric esters of glucose and fructose. This view is supported by the evidence [Meyerhof and Lohmann, 1927] of its reducing power towards sodium hypoiodite, a reagent which distinguishes between aldoses and ketoses. It is possible that one of the components may prove to be identical with the monophosphoric ester obtained by Neuberg [1918] by the partial hydrolysis of hexosediphosphoric ester by acids and by the enzymes of *Aspergillus oryzae* and of yeast [Neuberg and Leibowitz, 1927, 1, 2].

During the isolation of hexosemonophosphoric acid from the products of the action of yeast juice on hexoses indications were obtained of the presence of other esters, in particular of a compound with properties intermediate between those of the mono- and di-phosphates, but the results of experiments

carried out at that time were not sufficiently conclusive to warrant publication. During the past two years this and other points of interest arising out of the early work have been reinvestigated, employing micro-methods of analysis which have made it possible to follow the progress of the fractionations at every stage. The full account of these experiments will be given in a later communication. The present paper is confined to the description of a new ester, entirely distinct from the compound referred to above, which has been isolated from the products of the fermentation of hexoses by dried yeast in presence of phosphates. This ester has proved to be a monophosphoric ester of the non-reducing disaccharide trehalose, a glucose glucoside found in trehala manna (excreted by the insect *Larinus maculatus*), in certain fungi, seaweeds and yeasts.

A series of fermentation experiments was carried out with various samples of yeast juice, dried yeast and zymin, using both fructose and glucose but otherwise keeping the conditions as similar as possible. The products were worked up according to a definite scheme, somewhat modified from the original method [Robison, 1922] as further experience had suggested. The separate experiments were carried out using 50 g. of dried yeast or zymin, or 300 cc. of yeast juice, to which were added 100 g. glucose or fructose, and sodium phosphate equivalent to about 7 g. P. The phosphate solution was added in six equal portions over a period of 5 to 6 hours, as indicated by the rate of evolution of carbon dioxide. When, after the last addition, the rate had again fallen, trichloroacetic acid was added to the fermentation mixture in amount equal to 4 % of the total volume. The precipitated proteins were filtered off and the filtrate treated with barium acetate equal in weight to the crystalline sodium phosphate originally added, followed by baryta in hot aqueous solution until pink with phenolphthalein. The precipitate consisting of crude barium hexosediphosphate was removed and a further precipitate, obtained by adding one-tenth volume of alcohol to the filtrate, was likewise filtered off. The solution containing the soluble barium salts was treated with basic lead acetate, and the precipitated lead salts decomposed with hydrogen sulphide. After removal of the excess of hydrogen sulphide by aeration, the acid solution was neutralised with baryta and poured into 3 volumes of alcohol. By extracting the fraction with 10 parts of 10 % aqueous alcohol the readily soluble barium salts were freed from a further quantity of sparingly soluble compounds.

All fractions obtained at each step in the separation were quantitatively examined, the total and inorganic phosphorus, the reducing power by the Hagedorn and Jensen and by the sodium hypiodite methods, and the specific rotation being measured.

On comparison of the results obtained for the whole series of experiments a very striking difference was seen between the products of fermentation with yeast juice on the one hand and with dried yeast and zymin on the other. In the experiments with yeast juice the sparingly soluble barium hexosediphosphate represented less than half the total esterified phosphorus, while

the properties of the large, readily soluble fraction were similar to those of the barium hexosemonophosphate previously described. As in the earlier experiments fractions of intermediate solubility were also obtained and these are being investigated. In the experiments with dried yeast and zymin a much higher proportion of diphosphate was obtained, this fraction representing between 80 and 90 % of the total esterified phosphorus. Further, the small fraction of readily soluble salt differed markedly in its properties from barium hexosemonophosphate, as is shown in Table I, in which the results of a number of experiments with dried yeast and zymin are summarised. One yeast juice experiment is included for purposes of comparison.

Table I. *Readily soluble fraction of barium salts.*

Exp.	Type of yeast preparation*	Sugar	Total P in fraction as percentage of total esterified P†	P %	Reducing power as glucose		[α] ₅₄₆₁	Reduction	
					H. & J %	Iodine %		P	P
6	Dried yeast	Fructose	5.9	5.5	8.3	12.5	+56.7°	1.5	10.4
7	"	"	11.7	6.3	13.6	15.6	+41.4°	2.2	6.6
8	"	Glucose	10.7	5.9	11.0	14.1	+56.4°	1.9	9.6
9	"	Fructose	6.4	5.3	5.5	10.8	+63.8°	1.0	12.0
15	Zymin	"	7.5	6.7	18.1	20.0	+28.0°	2.7	4.2
16	"	Glucose	4.3	6.2	16.2	19.0	+30.0°	2.6	4.8
13	Yeast juice	Fructose	47.3	7.4	27.4	23.4	+11.4°	3.7	1.6

* Exps. 6, 7 and 8 were carried out with English brewery top yeast. In Exp. 9 a bottom yeast obtained from the Standard Yeast Co. was used.

† A small quantity of organic phosphorus remained in the final filtrates from the basic lead and barium salts. Some of this could be recovered in a very impure condition by treatment of these filtrates with basic lead acetate and ammonia. These amounts, if credited to the most soluble fraction, would increase the figures shown in the above table by several per cent.

Isolation of the new phosphoric ester.

The values shown in Table I indicated that the soluble fractions from the dried yeast and zymin fermentations contained a compound possessing a much higher dextro-rotation and lower reducing power than those of barium hexosemonophosphate. It was found that on fractional precipitation of the aqueous solution by alcohol the specific rotation of the more soluble portion was still further raised and the reducing power lowered. Accordingly a quantity of the crude soluble barium salts (about 50 g.) obtained from 5 experiments (Nos. 1-5), in all of which dried English top yeast and fructose had been used, was dissolved in 10 parts of water and treated with alcohol, raising the concentration of the latter successively to 10, 17, 23, 30 and 70 %. The precipitates obtained between 10 and 30 % alcohol were oily in nature and as the solubility of these salts increased with the temperature it was found convenient to heat the solution to about 50° before adding the alcohol, whereby the separation of an oily phase took place slowly during cooling to room temperature. After standing for some hours the clear supernatant solution could be readily poured off, again warmed and treated with a further quantity of alcohol. No advantage was gained by cooling the solution to 0° or by separating the fraction at 37°.

The small flocculent precipitate obtained on raising the concentration of alcohol from 30 to 70 % possessed a high dextro-rotation and very low reducing power. After many repetitions of this process a quantity of barium salt was obtained which possessed a reducing power (H. and J.) of only 1 % in terms of glucose whilst the specific rotation had risen to over + 100°. Since continued fractionation by alcohol did not further lower the reducing power the barium salt was converted into the brucine salt which unexpectedly proved to be very sparingly soluble in cold water and readily crystallisable from hot aqueous solution. After several recrystallisations it was reconverted into the barium salt which now showed no reducing power either by the Hagedorn and Jensen or the sodium hypiodite method. The analytical figures for this salt agreed with those required for a disaccharide monophosphate, while the properties of the ester, its resistance to acid hydrolysis, negative Selivanov's reaction and the very high specific rotation ($[\alpha]_{5461} + 132^\circ$) suggested that it was a derivative of trehalose. This supposition was confirmed by investigation of the products of hydrolysis of the ester by acids and by the bone phosphatase, the former yielding glucose and glucosemonophosphoric ester, while the latter yielded trehalose itself which was isolated in pure crystalline form.

Brucine salt of trehalosemonophosphoric ester.

The barium salt obtained from the crude soluble fraction by repeated precipitation with alcohol (30 to 70 % fractions) was dissolved in five parts of water and treated with the theoretical quantity of sulphuric acid. After removal of the barium sulphate by centrifuging, brucine (2 mols.) was added and the solution treated with an equal volume of acetone. The brucine salt crystallised out in the course of a few hours. It was recrystallised several times from hot water, from which it separated in large clusters of needles. It is very slightly soluble in hot ethyl alcohol, somewhat more soluble in methyl alcohol, insoluble in chloroform and acetone.

Analysis. For analysis the salt was dried over sulphuric acid at room temperature. The water of crystallisation was estimated by heating over P_2O_5 at 100° and 15 mm. until the weight was constant (2 hours). P was estimated by the Briggs method; C and H by Pregl's micro-method.

	H ₂ O	C	H	P
Found	11.8 11.7	50.9	6.8	2.31
Calculated for $C_{12}H_{21}O_{10}PO_4H_2(C_{23}H_{35}O_4N_2)_2 \cdot 9H_2O$	11.66	50.73	6.82	2.26
$[\alpha]_{5461}^{20^\circ}$ in water ($c=0.77$ %) + 31.3°				
$[\alpha]_{5461}^{20^\circ}$ for the anhydrous salt + 35.4°				

Barium trehalosemonophosphate.

The pure brucine salt was dissolved in warm water and treated with a cold aqueous solution of baryta until pink with phenolphthalein. The barium salt was precipitated by the addition of 3 volumes of alcohol, filtered, washed with absolute alcohol and dried quickly in a vacuum desiccator. To remove traces

of brucine the salt was dissolved in 5 parts of water, reprecipitated with alcohol, filtered and dried as before, and the process repeated. The salt so prepared is a white amorphous powder, stable in the air, but like hexosemonophosphate turning syrupy with small quantities of water, in which it is very soluble. It is insoluble in absolute alcohol but in aqueous alcohol it is decidedly more soluble than barium hexosemonophosphate, the solubility increasing with the temperature. At 22°, 100 cc. of 30 % (by vol.) alcohol dissolves about 1 g. while the 75 % alcohol filtrates still contain appreciable traces of the salt. Unlike the hexosephosphates it can be heated to above 100° in the air or *in vacuo* without becoming discoloured, this stability being probably connected with absence of a reducing group. At 130° it slowly turns yellow. It does not reduce Hagedorn and Jensen's solution and gives no coloration with Selivanov's reagent for fructose.

Analysis. For analysis the salt was dried at 100° and 15 mm. over P_2O_5 , or in Pregl's micro-desiccator at 120°, until the weight remained constant. *a*, *b* and *c* were different specimens, but were all derived from the same 50 g. of crude soluble salts. The results do not at present justify a definite conclusion as to the presence of a molecule of water in the salt.

	C	H	P	Ba
Found (<i>a</i>)	25.5	4.0	5.43	23.6
	25.5	4.0	—	—
(<i>b</i>)	25.9	4.3	5.35	24.4
(<i>c</i>)	25.7	4.2	5.51	23.4
	25.6	4.1	5.49	23.4
	25.5	4.1	—	23.5
	25.6	4.1	—	—
Calculated for $C_{12}H_{21}O_{14}P\text{Ba}$	25.82	3.80	5.57	24.63
Calculated for $C_{12}H_{21}O_{14}P\text{Ba} + H_2O$	25.01	4.03	5.39	23.84
$[\alpha]_{5461}^{20^\circ}$ of the salt in water ($c=3.2\%$)	+132°			
$[\alpha]_{5461}^{20^\circ}$ of the free acid in water ($c=2.4\%$)	+185°			
$[M]_{5461}^{20^\circ}$ of the free acid ($M=422$)	+781°			

Hydrolysis of trehalosemonophosphoric ester by bone phosphatase.

A preliminary experiment showed that bone phosphatase removed the phosphoric acid group of the new ester but was without action on the liberated disaccharide. Accordingly 380 mg. of the dry barium salt dissolved in 10 cc. water was treated with purified bone phosphatase (to be described shortly in a paper by Martland and Robison) and kept at 40°, the p_H being maintained at 8.6–8.8 by the addition of baryta at frequent intervals. Hydrolysis was complete in about 10 hours and alcohol (3 vols.) was then added to precipitate the enzyme and any barium salts remaining in solution. The alcoholic filtrate was evaporated in a vacuum desiccator and the syrupy residue taken up in methyl alcohol. The solution was filtered and evaporated to dryness, the residue dissolved in a few cc. of water and the solution again filtered and evaporated.

Weight of residue, 169 mg. representing 73 % of the theoretical yield of trehalose. It contained no phosphorus, was non-reducing, and had $[\alpha]_{5461}^{20} + 196^\circ$.

The product was further purified by recrystallisation from hot alcohol from which it separated in lustrous rhombic prisms, m.p. 96° – 98° (uncorr.). This figure is in agreement with the m.p. of hydrated trehalose given by Schukoff [1900]. Koch and Koch [1925] give a somewhat higher m.p., 102.5° . The solubilities of the crystals in water and alcohol also agreed with those of trehalose.

Analysis. For analysis the air-dried crystalline substance was used.

	C	H
Found	38.1	6.8
	38.0	6.8
Calculated for $C_{12}H_{22}O_{11} \cdot 2H_2O$	38.1	6.9
$[\alpha]_{5461}^{19}$ in water ($c=0.2\%$) $+222^\circ$		

The values of $[\alpha]_D$ of trehalose recorded in the literature vary between $+173^\circ$ (for the hydrated sugar) [Mitscherlich, 1858] and $+184^\circ$ [Koch and Koch, 1925]. No figures for $[\alpha]_{5461}$ have been found, but Koch's figure for $[\alpha]_D$ multiplied by the factor 1.18 gives $[\alpha]_{5461} + 217^\circ$. Calculated from this figure the molecular rotation of trehalose is $[M]_{5461} + 820^\circ$, while that found for the free trehalose-monophosphoric ester was $+781^\circ$. The effect of the introduction of the phosphoric acid group on the rotatory power of the molecule is thus relatively small.

Hydrolysis by acids. The disaccharide was very resistant to acid hydrolysis. 16 mg. of the crystalline sugar were dissolved in 10 cc. 2 N H_2SO_4 and heated at 100° until the rotation became approximately constant (5 hours). The initial and final values observed for rotation and reducing power are shown below:

Time (hours)	α_{5461} ($l=1$)	Reducing power (mg. glucose in 10 cc.)	
		H. and J.	Iodine
0	$+0.35^\circ$	0	0
5	$+0.13^\circ$	13.6	13.2
Percentage of total change calculated for conversion to two molecules of glucose	86	88	86

An osazonewas prepared from the hydrolysis product, which, after recrystallisation from dilute pyridine, melted at 202° – 204° and did not lower the melting-point when mixed with a specimen of glucosazone. The properties of the disaccharide obtained from the ester by enzymic hydrolysis thus agreed in all respects with those of trehalose.

Hydrolysis of trehalosemonophosphoric ester by acid.

Trehalosemonophosphoric ester is slowly hydrolysed by dilute mineral acids at 100° , the ultimate products being glucose and phosphoric acid. Since

the rate of hydrolysis of the phosphoryl linkage "b" is very much slower than that of the glucosidic linkage "a"

glucose and glucosemonophosphoric ester at first accumulate and the latter can be isolated by stopping the hydrolysis at a suitable stage. Owing to the small quantity of material available, the glucosemonophosphate has not yet been obtained in absolutely pure condition but it will be interesting to discover whether, as appears likely, it is identical with one constituent of hexosemonophosphate (Robison).

Table II.

Hydrolysis of trehalosemonophosphoric ester (*M*/20 sol.) by 0.2 *N* H₂SO₄ at 100°.
Degree of hydrolysis as measured by

Time (hours)	Inorganic P set free %	Reducing power		Fall in rotation %
		H. and J. %	Iodine %	
5	3	18	22	19
10	9	34	40	39
21	18	64	71	73
40	36	82	93	93
58	51	86	97	100
67	53	84	100	101

Table II shows the progress of one hydrolysis experiment with 0.2 *N* acid. Even after 67 hours only 53 % of the phosphoric acid had been set free. The reducing power towards an alkaline solution of iodine is a measure of the free aldose group and the figures in this column show that hydrolysis of the glucosidic linkage was complete. The lower values obtained by the Hagedorn and Jensen method are due to the presence of glucosemonophosphate which has only two-thirds the reducing power of the equivalent weight of glucose. The fall in rotation, also calculated as a percentage of the total change for complete hydrolysis to glucose, is seen to follow very closely the figures for the reducing power towards iodine, showing that the molecular rotation of the glucosemonophosphoric ester is very similar to that of glucose.

The results of another acid hydrolysis with *N* H₂SO₄ are shown in Fig. 1, the parallelism between the increase in reducing power towards iodine (curve *C*) and fall in rotation (curve *D*) being again very marked. The divergence of the two curves towards the end of the experiment is due to the destruction of a little glucose by the acid which increases the apparent hydrolysis as measured by the fall in rotation. This also explains the fall in the reducing power by the Hagedorn and Jensen method. In this experiment the hydrolysis was stopped after 21 hours and the acid solution neutralised with baryta. After removal of the barium sulphate the solution was concentrated and poured into twice its volume of alcohol to precipitate the barium glucosemonophosphate. The

alcoholic filtrate on evaporation yielded a reducing sugar having all the properties of glucose.

Weight of product	...	...	...	...	...	...	0.524 g.
Reducing power as glucose (Hagedorn and Jensen)	...	...	...	...	...	...	0.393 g.
Reducing power as glucose (Iodine)	...	...	...	...	...	...	0.384 g.
[α] ₅₄₆₁ calculated on the glucose content as estimated by Hagedorn and Jensen's method							
	...	...	...	...	...	...	+ 61°
For glucose [α] ₅₄₆₁	...	...	...	...	...	...	+ 62°
Osazone: M.P. 204°, not lowered by admixture with pure glucosazone.

Fig. 1. Hydrolysis of trehalosemonophosphoric ester by $N H_2SO_4$ at 100°.

Curve A. Inorganic P liberated.

Curve B. Reducing power by Hagedorn and Jensen method.

Curve C. Reducing power by the sodium hypoiodite method.

Curve D. Change in rotation.

The points on the curves represent in each case percentages of the total values calculated for complete hydrolysis of the ester to glucose (2 mols.) and phosphoric acid.

The alcohol precipitates obtained from this and other hydrolysis experiments were separately purified by solution in 10 % alcohol, and precipitation by addition of twice the volume of alcohol. Even after several repetitions of this process the salt still contained a small proportion of sparingly soluble substance. It was difficult to account for the persistence of this impurity which may have been due to oxidation or other change of the monophosphate. The figures for this compound must therefore be given subject to future correction.

Barium glucosemonophosphate.

Preparation	Total P %	Reducing power as glucose		[α] ₅₄₆₁
		H. and J. %	Iodine %	
<i>a</i>	7.63	27.9	35.6	+20°
<i>b</i>	7.40	32.0	42.0	+23.5°
<i>c</i>	7.33	31.0	42.4	+21°
Calculated for barium glucosemonophosphate	7.85	(?32)	45.5	—

Fermentation of trehalosemonophosphate.

Experiments were made to determine whether trehalosemonophosphoric ester is fermented by yeast juice, dried yeast and zymin. A solution of the sodium salt (sol. *a*) was used, of such strength that 1 cc. was equivalent to 30 mg. glucose, and control tests were also made to determine (*b*) the rate of fermentation of sucrose (sol. *b*, 1 cc. = 40 mg. sucrose), and (*c*) the auto-fermentation rate of the yeast preparation.

1 cc. sol. *a* should give about 8 cc. carbon dioxide on complete fermentation. 1 cc. sol. *b* should give about 11.2 cc. carbon dioxide on complete fermentation.

The results show that the ester was readily fermented by dried yeast, the rate of decomposition being even more rapid than in the case of sucrose. It was also fermented, although more slowly, by zymin and by yeast juice.

Exp. 1. (*a*) 0.2 g. dried yeast + 1 cc. water + 1 cc. sol. *a* + 1 drop toluene.
 (*b*) 0.2 g. dried yeast + 1 cc. water + 1 cc. sol. *b* + 1 drop toluene.
 (*c*) 0.2 g. dried yeast + 2 cc. water + 1 drop toluene.

Time (hours)	cc. carbon dioxide evolved		
	(<i>a</i>)	(<i>b</i>)	(<i>c</i>)
0	0.0	0.0	0.0
$\frac{1}{2}$	1.70	0.71	0.30
1	3.45	1.70	0.71
$1\frac{1}{2}$	4.40	2.30	0.80
3	5.69	3.59	0.90
5	5.91	5.35	0.95
22	6.47	6.66	0.95
Total corrected for autofermentation (<i>c</i>)	5.52	5.71	—

Exp. 2. (*a*) 0.2 g. zymin + 1 cc. water + 1 cc. sol. *a* + 1 drop toluene.
 (*b*) 0.2 g. zymin + 1 cc. water + 1 cc. sol. *b* + 1 drop toluene.
 (*c*) 0.2 g. zymin + 2 cc. water + 1 drop toluene.

Time (hours)	cc. carbon dioxide evolved		
	(<i>a</i>)	(<i>b</i>)	(<i>c</i>)
0	0.0	0.0	0.0
1	3.75	4.36	3.15
$2\frac{3}{4}$	6.90	11.36	6.30
18	11.97	17.86	7.42
Total corrected for autofermentation (<i>c</i>)	4.55	10.44	—

Exp. 3. (a_1) and (a_2) 1 cc. yeast juice + 1 cc. sol. a + 1 drop toluene.
 (c) 1 cc. yeast juice + 1 cc. water + 1 drop toluene.

Time (hours)	cc. carbon dioxide evolved		
	(a_1)	(a_2)	(c)
0	0.0	0.0	0.0
$\frac{1}{2}$	0.63	0.68	0.37
1	1.37	1.39	0.98
$1\frac{1}{2}$	2.07	2.17	1.50
15	6.65	7.01	2.37
Total corrected for autofermentation (c)	4.28	4.64	—

Examination of dried yeast for trehalose and trehalosemonophosphate.

Trehalose has been isolated from dried yeast by Koch and Koch [1925]. These workers obtained the sugar by extracting 40 lb. of air-dried yeast with 90–95 % alcohol but give no information as to the yield. From the data of our experiments it may be reckoned that the amount of trehalosemonophosphoric ester present in the fermentation products from 50 g. dried yeast, corresponded with at least 100–300 mg. P, *i.e.* about 1–3 g. of trehalose. It was not impossible that such amounts of the ester were originally present in the yeast or were produced during the fermentation by simple esterification of preformed trehalose. Experiments were carried out to test these possibilities.

(a) *Presence of trehalose in dried yeast.* 10 g. dried yeast was extracted for 8 hours with boiling ether to remove fat. It was then extracted with three successive quantities of boiling 96 % alcohol for 24 hours in all. The residues obtained on evaporating the three alcoholic extracts weighed 445 mg., 59 mg. and 38 mg., making a total of 542 mg. The rotation and reducing power of this residue before and after acid hydrolysis (2 *N* HCl, 100°, 6 hours) are shown below:

542 mg. in 30 cc. 2 <i>N</i> HCl	Before hydrolysis	After hydrolysis
$\alpha_{5461} (l=1)$	–0.02°	–0.04°
Total reducing power as glucose	18 mg.	37 mg.

Even assuming that the increase in reducing power on hydrolysis was entirely due to trehalose, the amount of this sugar present in the yeast could not be more than about 0.2 %, equivalent to 100 mg. trehalose in 50 g. dried yeast. Other samples of dried yeast used in the fermentation experiments gave similar results.

(b) *Presence of trehalosemonophosphoric ester in dried yeast.* 50 g. of the dried yeast used in the fermentation experiments Nos. 1–5 were extracted with 250 cc. of 4 % trichloroacetic acid for 3 hours at room temperature. The extract, which contained 198 mg. inorganic P and 552 mg. organic P, was filtered and neutralised with baryta. The precipitated barium salts contained the whole of the inorganic P and over 92 % of the organic P, a large proportion of the latter being present as nucleic acid. Some hexosediphosphate was also present. The readily soluble fraction, in which barium trehalosemonophosphate would be found, contained only 15 mg. phosphorus and gave a strongly

laevorotatory solution. Two similar extractions of other dried yeast preparations gave very similar figures showing that trehalosemonophosphoric ester, if present in the original dried yeast, can only occur in very small amounts.

DISCUSSION.

From the experimental results given above it must be concluded that the trehalosemonophosphoric ester isolated from the products of the fermentation of fructose by dried yeast was not originally present in the yeast, nor could it have been derived to any great extent from preformed trehalose. The occurrence of a trehalase in various types of yeast has been observed [Fischer, 1895; Kalanthar, 1898; Bau, 1899; Lindner, 1911] and by the synthetic action of this enzyme, trehalose could be formed from glucose derived from the yeast glycogen. It is unlikely, however, that the dried yeast contained sufficient glycogen to account for more than a small fraction of the trehalose-phosphoric ester, nor is this necessary since there is sufficient other evidence of the formation of glucose derivatives during the fermentation of fructose.

Trehalosemonophosphoric ester has not so far been found in the products of fermentation of yeast juice. It appears to be formed equally in the fermentation of fructose or glucose by dried yeast and also by zymin. (The investigation of the zymin products is not yet complete.) Using the same dried yeast preparation but varying the conditions of fermentation we have been able to modify the amount and nature of the soluble fraction of barium salts and are continuing the investigation on these lines.

It would be premature to venture an opinion as to whether the formation of this disaccharide phosphoric ester has any great significance in the carbohydrate metabolism of the yeast, but it is not impossible that the introduction of the phosphoric acid group may facilitate the building up of the complex reserve carbohydrates from the simple sugars.

Neuberg and Leibowitz [1928] have recently reported the preparation of another disaccharide monophosphoric ester by the action of *B. delbrücki* on hexosediphosphate. This ester, which was obtained in a yield of 30 %, possessed reducing properties and $[\alpha]_D + 38^\circ$ for the barium salt. It is therefore not identical with the ester described in this paper. A synthetic trehalosemonophosphate has been prepared by Helferich, Löwa, Nippe and Riedel [1923] by the action of phosphoryl chloride on trehalose in pyridine solution. No properties are given other than the specific rotation of the acid barium salt, $[\alpha]_D + 135.5^\circ$, and it is therefore impossible to decide whether their compound was identical with the ester which has now been isolated from the products of fermentation.

SUMMARY.

1. A monophosphoric ester of the disaccharide trehalose has been isolated from the products of fermentation of fructose by dried yeast, and has been purified by means of its barium salt, which is very soluble in water, and its crystalline brucine salt.
2. The ester is non-reducing and is strongly dextrorotatory: $[\alpha]_{5461}$ of the free acid $+185^\circ$, of the barium salt $+132^\circ$, and of the brucine salt $+31^\circ$.
3. By the action of the bone phosphatase on the ester pure crystalline trehalose has been obtained.
4. Hydrolysis with boiling mineral acid slowly converted the ester into glucose and glucosemonophosphoric ester, the latter being still more slowly hydrolysed to glucose and phosphoric acid. Barium glucosemonophosphate was isolated but in a slightly impure condition.
5. Trehalosemonophosphoric ester is readily fermented by dried yeast and more slowly by yeast juice and zymen.
6. Examination of the dried yeast used for the fermentation showed that neither trehalosemonophosphoric ester nor free trehalose was present in quantities sufficient to account for the amount of the ester found in the fermentation products.
7. No evidence has been obtained of the presence of this ester in the products of fermentation by yeast juice.

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