CCLX. HEXOSEMONOPHOSPHORIC ESTERS: MANNOSEMONOPHOSPHATE.

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It has been established that the hexosemonophosphate formed during alcoholic fermentation of sugars by yeast enzymes is not a single compound but consists of a mixture of aldose and ketose esters.

One, and probably the chief, component of this product was isolated by Robison and King [1931], who concluded from their own experiments and from those of King, McLaughlin and Morgan [1931] that this pure compound most probably had the structure of glucose-6-phosphate. Its specific rotation differed from that first recorded by Levene and Raymond [1930; 1931, 1] for a synthetic ester, which they considered to be glucose-6-phosphate; but the discrepancy largely disappeared as the result of further purification of the synthetic ester [Levene and Raymond, 1931, 2].

Robison and King also isolated a ketose ester similar in most respects to the Neuberg fructosemonophosphate, but having a somewhat lower reducing power (Hagedorn and Jensen); and their further analytical results pointed definitely to the presence of still other esters in the fermentation product. The isolation of one of these esters and the further investigation of the fructosemonophosphate and the residual fractions are described in the present communication.

Isolation of mannosemonophosphate.

During the separation of glucosemonophosphate, many intermediate fractions which no longer contained significant amounts of fructose esters, as judged by the Selivanoff reaction, still gave low values for the iodimetric reducing power. The full value theoretically required for an aldosemonophosphate was obtained only after repeated recrystallisation of the brucine salt from methyl alcohol. The residual fractions obtained in these final stages of the purification were recrystallised 5 times from boiling absolute methyl alcohol and yielded clusters of short needles, very different in appearance from the soft, opaque, silky crystals of pure brucine glucosemonophosphate. These needle fractions were converted into the barium salt, which was purified and analysed by the methods described in previous papers. The results are shown in Table I (salt No. 4), the corresponding values for a typical fermentation hexosemonophosphate (No. 1) and for pure glucosemonophosphate

Table I. *Analyses of barium salts of hexosemonophosphoric acids obtained from the products of fermentation by yeast juice.*

No. of salt	Source of the barium salt	P %	Fructose (Selivanoff) %	Reducing power as glucose (%) H. and J.*	Reducing power as glucose (%) Iodine	Aldose-monophosphate %	$[\alpha]_{5461}^{20°}$	$[\alpha]_{5461}^{20°}$ of free acid
1	Hexosemonophosphate	7·85	6	30·0	25·2	55	+14·4°	+29·5°
2	Glucosemonophosphate	7·86	0·5	35·5	45·7	100	+20·6° (c=0·84 %)	+41·4° (c=0·74 %)
3	Fructosemonophosphate (Neuberg) prepared from highly purified hexosediphosphate	7·80	22	36·2	3·0	7	+ 0·7°	—
4	Brucine salt of new ester recrystallised 5 times from absolute methyl alcohol	7·82	3	36·3	27·5	60	+ 3·1° (c=1·4 %)	— —
5	Residual salt after 17 hours' hydrolysis (28 %) of No. 4 with N HCl at 100°	7·20	1	34·0	25·6	56	+ 3·4° (c=0·4 %)	—
6	Brucine salt from No. 4, recrystallised 5 times from 95 % methyl alcohol	7·82	2	36·9	27·0	59	+ 3·6° (c=0·7 %)	+15·1° (c=1·7 %) +17·0° in 0·1 N H_2SO_4
7	Second fractions from No. 6 recrystallised 4 times from 90 % methyl alcohol and once from water	7·69	1	35·5	30·5	67	+ 3·4° (c=0·7 %)	
8	Another preparation similarly purified	7·65	1·5	36·2	28·0	61	+ 3·1° (c=0·5 %)	
9	Fructosemonophosphate isolated from fermentation hexosemonophosphate	7·73	22	34·9	1·6	3	+ 2·3° (c=0·8 %)	—
10 and 11	Brucine salts obtained in the fractionation of hexosemonophosphate	7·78 7·29	7 4	23·9 20·1	12·6 14·7	28 32	+10·8° +12·7°	—

$C_6H_{11}O_5PO_4Ba$ requires P, 7·85 %; $C_6H_{12}O_6$, 45·6 %.

* Determined with addition of 0·5 cc. of 0·5 N NaOH.

and fructosemonophosphate (Neuberg) being given in the same table for comparison. While the phosphorus content and H. and J. reducing power were the same as for glucose- and fructose-monophosphates, the new compound differed markedly from both these esters in other respects. The iodimetric value was only 60 % of that required for an aldosemonophosphate, but the low Selivanoff value precluded the presence of fructose derivatives in large amount. The low specific rotation also distinguished the new ester sharply from glucosemonophosphate.

Examination by fractional acid hydrolysis. 0·3 g. of this salt, No. 4, was heated with N H_2SO_4 at 100° for 17 hours, the hydrolysis amounting to 28 %; the residual ester was freed from inorganic phosphate and analysed (salt No. 5). The Selivanoff value was still lower (1 %), but the remaining values were not significantly changed from those of the original salt. (The quantity recovered was inadequate for thorough purification and the phosphorus content was somewhat low.)

Further recrystallisation of the brucine salt. The remainder (1·5 g.) of salt No. 4 was reconverted into brucine salt, which was again recrystallised 5 times from 95 % methyl alcohol. Second crops obtained from the mother-liquors were recrystallised 4 times from 90 % methyl alcohol and once from

water at 40°. Analyses of the barium salts prepared from these two fractions are given in Table I (Nos. 6 and 7). No. 8 was a barium salt obtained by similar methods from another hexosemonophosphate preparation.

The properties of these salts suggested that they consisted, wholly or to a very large extent, of a new ester, possibly a mannosemonophosphate.

Hydrolysis of the ester by 0·1 N H_2SO_4.

A quantity equivalent to 1 milligram mol. of the free ester was dissolved in 0·1 *N* H_2SO_4 (17 cc.) and heated in a boiling water-bath, the amount of hydrolysis being estimated from time to time. The hydrolysis was stopped after 93 hours, when 79·6 % of the total phosphate had been set free. The solution, which was then dark brown in colour, was neutralised with baryta, treated with twice its volume of alcohol to precipitate the free and combined phosphates and filtered. The filtrate containing the free sugar was evaporated rapidly on a water-bath to a small volume ($p_H < 7·0$), after which the drying was completed in an evacuated desiccator. The residue was extracted repeatedly with boiling 80 % alcohol and the filtered solution evaporated to dryness as before. The rate of hydrolysis and the properties of the sugar produced are shown in Table II.

Table II.

Time (hrs.)	Hydrolysis %	$k \times 10^3$	The syrupy product gave	
4	6·9	0·13	Total P	0·4 mg.
31	43·5	0·13	Hexose (H. and J. reduction)	85 ,,
93	79·6	0·12	Aldose (I reduction)	89 ,,
			$[\alpha]_{5461}$ calculated on hexose	+15°

The rate of hydrolysis was exactly the same as that for the hydrolysis of pure glucosemonophosphate under similar conditions ($k = 0·13 \times 10^{-3}$). Some decomposition of sugar had evidently occurred, but assuming that the decomposition products were optically inactive the specific rotation, calculated on the reducing hexose, was in fair agreement with that of mannose (+ 17·1°).

The sugar solution on treatment with pure phenylhydrazine dissolved in acetic acid gave at once, in the cold, a crystalline derivative; this was recrystallised twice from 80 % ethyl alcohol. The crystals closely resembled those of mannosephenylhydrazone, which was prepared for comparison. They melted[1] at 200–201°; the specimen of mannosephenylhydrazone also melted at 200–201°, as did an intimate mixture of the two substances. Beilstein, Supplement I, gives the M.P. of mannosephenylhydrazone as 197–201°.

Phenylhydrazine derivatives of the new ester.

Phenylhydrazone. A solution (6 cc.) containing 100 mg. of the free ester was treated at room temperature with 0·2 cc. phenylhydrazine dissolved in acetic acid. A pale yellow crystalline precipitate formed within a few minutes;

[1] These melting-points were determined in the way described by Robison and King [1931, p. 329].

but the solution was allowed to remain at 0° overnight before filtration. The product was recrystallised twice from warm 75 % ethyl alcohol, from which it separated quickly at 0° in very pale yellow crystals, M.P. 144–145°.

Analysis. After being dried over sulphuric acid the substance did not lose weight in 1½ hours at 79° in the Pregl drier.

It gave P, 6·82 %.

Calculated for $C_{18}H_{27}O_8N_4P$, P, 6·78 %.

The phosphorus content corresponded with that of the phenylhydrazine salt of the phenylhydrazone of hexosemonophosphoric acid.

Phenylosazone. A solution (6 cc.) containing 100 mg. of the free ester was treated with 0·5 cc. phenylhydrazine dissolved in acetic acid, and heated in a boiling water-bath for 1 hour. After cooling to 0° the precipitate was filtered off and the solution again heated for 1 hour, cooled and filtered. The combined precipitates were recrystallised twice from boiling ethyl alcohol to which an equal volume of boiling chloroform was added, the solution being then left at 0° for 24 hours. It separated in fine yellow needles; M.P. 154–155°.

Analysis. The substance, dried over sulphuric acid, gave P, 5·69 %.

Calculated for $C_{24}H_{31}O_7N_6P$, P, 5·68 %.

The phosphorus content corresponded with that of the phenylhydrazine salt of the osazone of hexosemonophosphoric acid.

A specimen of osazone prepared from fructosediphosphate and similarly purified also melted at 154–155°, as did an intimate mixture of the two substances. It was, therefore, probable that this derivative of the new ester was identical with the osazone obtained from fructosediphosphate, *i.e.* the phenylhydrazine salt of the osazone of fructosemonophosphate (and of glucosemonophosphate).

The properties of the sugar obtained from the ester by acid hydrolysis confirmed the supposition that the compound was a monophosphoric ester of mannose; and the formation of a sparingly soluble phosphohydrazone also accorded well with this view. The identification of osazones by their melting-points is not, perhaps, conclusive; but such evidence pointed strongly to the identity of the osazone of the mannose ester with that obtained from hexosediphosphate. This would imply that the position of the phosphate group is the same in both compounds and that the new ester is probably mannose-6-phosphate.

Comparison of the rates of hydrolysis of mannosemonophosphate and glucosemonophosphate.

It has been shown that the hydrolysis of the new ester by 0·1 *N* H_2SO_4 proceeded at the same rate as that of glucosemonophosphate under similar conditions. The rates of hydrolysis of the two esters were further compared in the following experiments.

Hydrolysis by N *HCl at 100°.* The ester solutions were prepared by dissolving 85 mg. of the air-dry barium salts together with 40 mg. of K_2SO_4 in 20 cc. of

N HCl and centrifuging at once, to remove barium sulphate. Quantities of about 1·3 cc. of the solutions were introduced into a number of small, thin-walled, glass tubes, previously drawn out, which were then sealed up and dropped into a bath of vigorously boiling water. The bath was kept loosely covered so that the temperature was maintained at 100°. Two tubes were removed at each of the stated intervals and the inorganic phosphate was determined colorimetrically in 1 cc. or 0·5 cc. of each of the solutions. The total P was estimated in the original solution and again at the end of the hydrolysis. The concentration of the ester in each case was approximately 0·01 *M*.

The results, set out in Table III, show that the mannose ester was hydrolysed at a slightly faster rate than glucosemonophosphate and that this rate was maintained during the entire period in which 94·5 % of the phosphate was set free. The values of k are calculated for a unimolecular reaction,

$$k = \frac{1}{t_2 - t_1} \log \frac{a - x_1}{a - x_2},$$

t being the time in minutes.

Table III. *Hydrolysis in* N *HCl at 100°.*

	Glucosemonophosphoric acid (0·301 mg. P per cc.)		Mannosemonophosphoric acid (0·287 mg. P per cc.)	
Time hrs.	Hydrolysis %	$k \times 10^3$	Hydrolysis %	$k \times 10^3$
4	12·7	0·25	16·8	0·33
8	21·6	0·20	28·7	0·28
20	44·9	0·21	53·9	0·26
45	75·0	0·23	82·5	0·28
70	88·9	0·24	94·5	0·33

Autolysis of the free esters at 100°. Ester solutions of similar concentration (0·01 *M*) were prepared by dissolving the barium salts in water containing the exact equivalent of sulphuric acid and centrifuging. The method of heating was the same as that described above.

The results, given in Table IV, show that the hydrolysis of both esters occurred in these solutions at exactly the same rate, and, further, that this rate was nearly the same as that for the hydrolysis in *N* HCl. In the case of glucosemonophosphate the hydrolysis was actually more rapid in the ester solution containing no additional acid than in *N* HCl, which is in agreement

Table IV. *Autolysis in solutions of the free esters at 100°.*

	Glucosemono-phosphoric acid (0·285 mg. P per cc.)		Mannosemono-phosphoric acid (0·306 mg. P per cc.)		Fructosemono-phosphoric acid (0·294 mg. P per cc.)	
Time hrs.	Hydrolysis %	$k \times 10^3$	Hydrolysis %	$k \times 10^3$	Hydrolysis %	$k \times 10^3$
4	14·1	0·28	14·0	0·27	18·8	0·37
24	60·6	0·28	61·2	0·29	77·8	0·46
48	85·3	0·29	85·0	0·29	96·2	0·53

with the previous findings of Robison and King [1931]. The value of k for the autolysis of glucosemonophosphate is somewhat higher than that found previously for more concentrated solutions (0·03 M) by Robison and King, who observed that the value rose as the hydrolysis proceeded.

Isolation of fructosemonophosphate from the hexosemonophosphate of fermentation.

Fractional crystallisation of the brucine salts from further preparations of the fermentation hexosemonophosphate again yielded salts approximating in their properties to the Neuberg fructosemonophosphate. As these still contained a small proportion of aldose esters the barium salts were submitted to oxidation by bromine in the presence of baryta, followed by partial precipitation from their aqueous solutions by alcohol, to remove the sparingly soluble phosphohexonates.

The analytical values obtained for the purified salt agreed closely with those for the Neuberg fructosemonophosphate, the H. and J. reducing power being now within the limits found for the pure ester. It should be noted that somewhat variable values have been recorded for the specific rotation of the Neuberg ester, which is obtained by partial hydrolysis of hexosediphosphate; and this may possibly be accounted for by the presence of traces of the second hydrolysis product, which is strongly laevorotatory [Macleod and Robison, 1932].

Hydrolysis of fructosemonophosphate by acid.

The hydrolysis of the fructosemonophosphate isolated from the fermentation product was investigated under similar conditions to those employed for the aldose ester.

Hydrolysis in N *HCl at 100°.* The ester solution, of approximately 0·01 M concentration in N HCl was prepared, and the hydrolysis carried out in the manner described, except that the time intervals were much shorter. The results are set out in Table V and show that hydrolysis proceeded at a constant rate during 3 hours while over 83 % of the phosphate was liberated. The final value (92·5 %) gives a slightly lower rate; but it should be noted that the

Table V.

	Fructosemonophosphate (0·287 mg. P per cc.)		Lohmann's values for the Neuberg ester	
Time hrs.	Hydrolysis %	$k \times 10^3$	Hydrolysis %	$k \times 10^3$
0·5	24·4	4·05	20·1	3·23
1	45·3	4·68	36·9	3·23
1·5	59·6	4·39	—	—
2	70·3	4·45	62·5	3·76
3	83·6	4·30	75·0	2·93
5	92·5	2·83	84·9	1·81
8	—	—	87·4	0·44
13	—	—	90·4	0·39
33	—	—	93·0	0·14

possible errors in the P determinations have a very marked effect on the value of k when hydrolysis is approaching completion.

In the same table are given Lohmann's [1928] values for the hydrolysis of the Neuberg ester by N HCl under conditions similar to those here described. Lohmann, who applied this method to the quantitative examination of the hexosephosphates, concluded that the Neuberg monophosphate, like the parent hexosediphosphate, consisted of a mixture of esters; and his figures certainly justify this conclusion for the preparations which he examined. It is obvious that, if the hexosediphosphate contains any esters only slowly hydrolysable by acids, these would accumulate in the monophosphate fraction, obtained from it by partial hydrolysis, which represents the Neuberg ester. The question, therefore, resolves itself into the degree of purification of the hexosediphosphate or what shall be considered to constitute this fraction. Preparations of this ester, which have been very highly purified in this laboratory, show little indication, from their hydrolysis curves, of the presence of other compounds; but some evidence has emerged that other esters, forming barium salts of similar solubility to barium hexosediphosphate, may occur in the fermentation products.

It is clear from the rates of hydrolysis that the fructosemonophosphate, whose isolation from hexosemonophosphate is here described, contained a much higher proportion of the main constituent than Lohmann's ester. This is shown both by the higher initial value found for k and by the persistence of this value until a larger proportion of the total ester had been hydrolysed. It may be inferred that the substance contained less than 5 % of other esters[1].

Autolysis of the free ester at 100°. The rate of hydrolysis of the free fructosemonophosphoric acid in approximately 0·01 M solution containing no additional acid was also determined. The results, which are given in Table IV, show a quite different behaviour of this ester from that of the two aldosemonophosphates. The rate of hydrolysis in absence of HCl is, initially, less than one-tenth of the rate in N HCl, but increases somewhat as the hydrolysis proceeds. One must conclude that, in the case of the aldose esters, two essentially different compounds are being hydrolysed in the solutions of different acidity, while this is not so in the case of the fructose ester. The similarity in the values of k for the three esters, in 0·01 M solutions containing no additional acid, suggests that the compounds present in these solutions are of similar type, while, in presence of N HCl, the aldose esters pass over into another type, which is hydrolysed much less rapidly. The fructose ester cannot, or does not so readily, change into the second type and its rate of hydrolysis consequently increases with increasing hydrogen ion concentration.

Since the phosphate group is probably in the same position, 6, in the three esters, the formation of the stable 1:5 oxygen bridge is possible in glucose-

[1] Subsequent fractional precipitation of this product from aqueous solution by alcohol removed further traces of a sparingly soluble salt, probably phosphohexonate. The soluble portion was now hydrolysed by N HCl to the extent of 96·3 % in 5 hours and 98·3 % in 7 hours.

and mannose-monophosphates, but not in fructosemonophosphate. It is conceivable, therefore, that the easily hydrolysable forms present in the aqueous solutions of all three esters may represent either the furanose derivatives or possibly the free aldehydes or ketone, while the less readily hydrolysable compounds formed in N HCl or H_2SO_4 may be pyranose derivatives containing the stable oxygen ring.

It may be recalled that the phospho-osazone common to all three esters which contains no oxygen bridge is hydrolysed by N H_2SO_4 even more rapidly than fructosemonophosphate [Robison and King, 1931]. The investigation of the hydrolysis of these esters and their derivatives is being continued and may throw more light on their structure and on the causes of their reactivity.

Other facts which may be related to the existence of these dual forms are (*a*) the marked increase in the specific rotation of the glucose and mannose esters, especially the latter, on liberation from their salts, and the further increase shown in presence of 0·1 N and N H_2SO_4, (*b*) the very low iodimetric reducing power of the mannose ester. This has not yet been satisfactorily accounted for. Slightly higher values than those shown in Table I were obtained by varying the conditions of the oxidation, particularly by increasing the concentration of potassium iodide in the iodine solution; but the highest figure corresponded with only 70 % of that theoretically demanded.

Other constituents of the fermentation hexosemonophosphate.

Some progress has been made towards the isolation of the fourth ester, which is responsible for the low H. and J. reducing power of the fermentation hexosemonophosphate, compared with that of the three known components.

This ester becomes concentrated in the final mother-liquors during the crystallisation of the brucine salts. These mother-liquors were evaporated to dryness, the residues recrystallised from methyl alcohol, the mother-liquors again evaporated and these operations repeated. A crystalline fraction, obtained by adding ether and light petroleum to some of these alcoholic mother-liquors, gave the barium salt No. 10, whose analysis is shown in Table I, while No. 11 was obtained from mother-liquors at a similar stage.

Attempts to purify the brucine salt of this ester by repeated crystallisation from various solvents gave products of higher reducing power. These salts are not yet homogeneous but certainly contain a high proportion of a new ester whose reducing power is very much lower than that of the three known hexosemonophosphates.

Fractional precipitation of the barium salt by alcohol or of the lead and basic lead salts gave products differing in little except the $[\alpha]_{5461}$, which was higher in the more soluble fractions. This may have been due to traces of trehalosephosphate.

Investigation by fractional acid hydrolysis, by bromine oxidation and other methods is being continued. One property common to these fractions may be mentioned. When heated with orcinol and hydrochloric acid a green colour is

developed, but the absorption band of the amyl alcohol solution is not identical with that given by pentoses, but is situated further towards the red end of the spectrum. The salts contain only traces of nitrogen as determined by a micro-Kjeldahl method.

Discussion.

The isolation of a mannosemonophosphate from the products of alcoholic fermentation fulfils an expectation long held in these laboratories. It was shown by Harden and Young [1909] that the fermentation of mannose as well as that of glucose and fructose by yeast juice responded to the addition of phosphate and that the same hexosediphosphate was formed from all these sugars [Young, 1909]. The deduction that at some stage in the esterification the three hexoses pass through a form which is common to them all leads to the further conclusion that, since monophosphoric esters of glucose and fructose are simultaneously formed from both these sugars, a corresponding ester of mannose should also occur among the products of their fermentation.

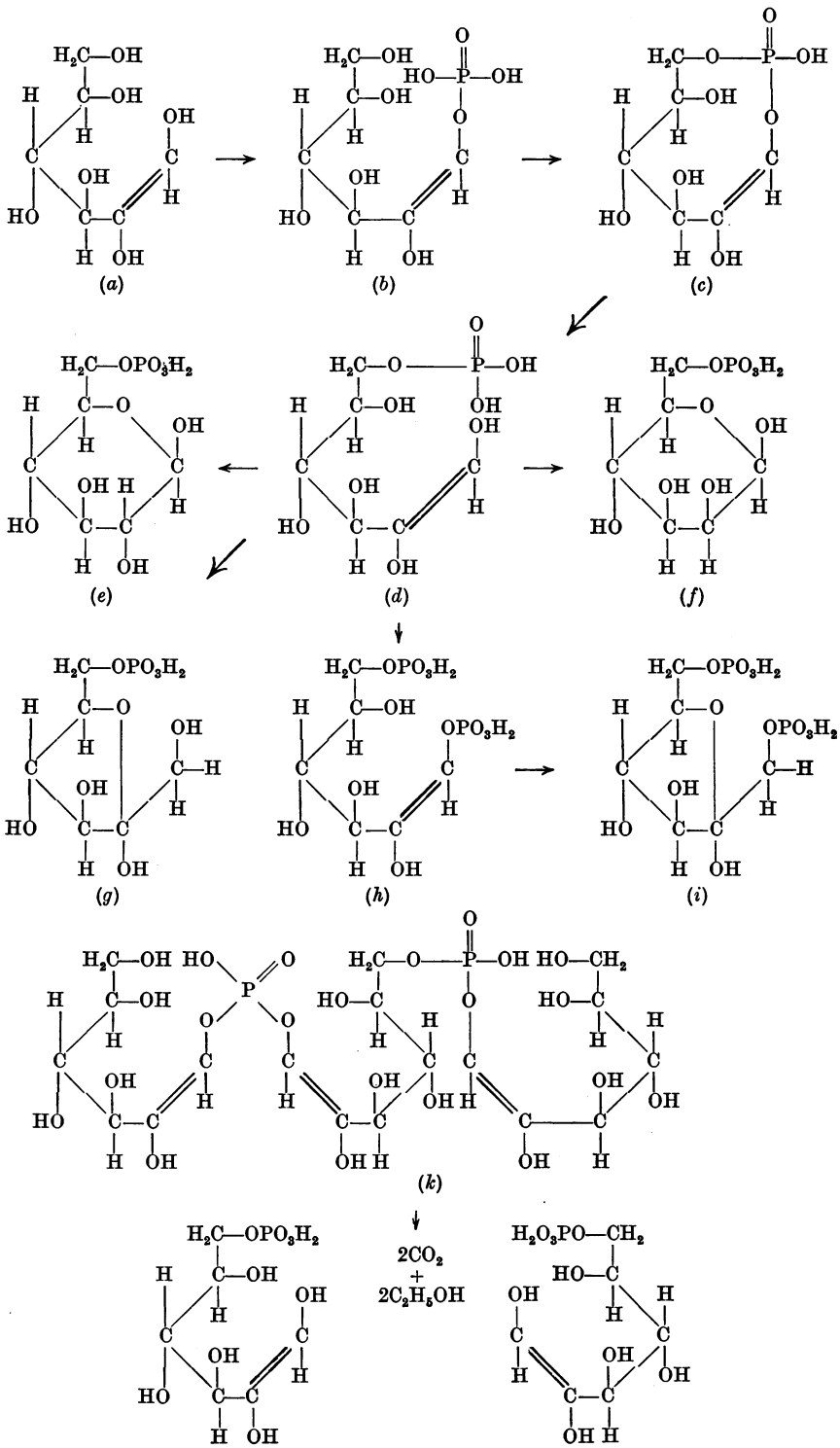
A scheme which has been suggested by the author [Robison, 1932] to account for the formation of these esters may be briefly recapitulated here. It is based on Armstrong's [1904] suggestion that the substance actually fermented by yeast is the enolic compound common to glucose, fructose and mannose. It is supposed that this enolic compound (*a*) (p. 2200) reacts rapidly with phosphoric acid, one molecule of which becomes attached to carbon atom 1 (*b*). This phosphoric group then wanders to carbon atom 6 *via* the intermediate compound (*c*) (*cf.* the wandering of the acetyl group in 1:2:3:4-tetra-acetyl-β-glucose under the influence of alkali [Fischer, 1920; Haworth, Hirst and Teece, 1930]). The resulting ester (*d*) by the addition of water to carbon atoms 1 and 2 would yield hydrated forms of glucose-, mannose-, and fructose-phosphates. Subsequent removal of water from the hydrated glucose and mannose esters could give 6-phosphoglucopyranose (*e*) and 6-phosphomannopyranose (*f*) while the hydrated fructose ester could yield only the γ-derivative, 6-phospho-fructofuranose (*g*).

The enolic group of ester (*d*) could, however, react with a further molecule of phosphoric acid, giving the diphosphoric ester (*h*) which would yield 1:6-diphosphofructofuranose (*i*), the hexosediphosphate of Harden and Young.

During the hydrolysis of these esters by phosphatases (of yeast or of bone) this series of changes may be supposed to take place in the reverse direction.

This scheme although it explains the formation of the phosphoric esters of fermentation does not account for the decomposition of half a molecule of hexose which accompanies the introduction of each phosphoric group during the fermentation.

It is conceivable that three molecules of hexose become linked together through two molecules of phosphoric acid and that the complex ester (*k*) so formed then breaks down, yielding two molecules each of hexosemonophosphate, carbon dioxide, and alcohol.


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(k)
2CO2
+
2C2H5OH

Two molecules of monophosphate could similarly become linked with another molecule of hexose through two additional molecules of phosphoric acid, and this new complex ester, on disruption, would yield two molecules each of hexosediphosphate, carbon dioxide, and alcohol. The ratio

$$\frac{CO_2 \text{ produced}}{PO_4 \text{ esterified}}$$

would then be equal to unity for both stages of esterification.

We have, however, at present no evidence to prove that these assumptions are correct. The isolation and identification of the still unknown esters in the fermentation products may help us to answer this question.

The mannosemonophosphate described in this paper was obtained from the mixed products of fermentations of glucose and of fructose, so that further evidence is required to prove that it is formed from each of these sugars. The investigation of the ester was strictly limited by the small amount available and more work and more analyses are necessary to substantiate the conclusions arrived at. It is probable that the purest products were almost free from fructosemonophosphate. The absence of small amounts of glucosemonophosphate is more difficult to prove[1].

The purification of the ester by means of its phenylhydrazone is being attempted and should yield a homogeneous product. Fortunately, a much easier method has been found for the preparation of the ester than the very laborious fractionation hitherto carried out. Experiments by Dr Jephcott and the author on the fermentation of mannose by dried yeast in presence of phosphate have shown that at high temperatures (37°) esterification proceeds in a somewhat abnormal manner; a very large proportion of the total phosphate is converted into hexosemonophosphate which consists almost entirely of the mannose ester.

An account of these experiments will shortly be published and the investigation of the relationship between this abnormal esterification and production of CO_2 is being continued. It is apparent that such abnormal esterification would require some modification in the scheme outlined above.

The fermentation and esterification of mannose at 37° by fresh yeast in presence of toluene has been investigated by I. Neuberg and Ostendorf [1930], who also observed a greatly increased production of the monophosphate fraction compared with the amount formed from glucose. The products isolated by these authors, however, showed a high dextrorotation, $[\alpha]_D$, + 45° and + 34°, for the free acid, and a low P content (7·19 %). It may be suggested that this compound possibly contained mannosemonophosphate mixed with trehalosemonophosphate. The particular sample of dried yeast used by Jephcott and Robison produced much less trehalose from glucose and fructose than did those previously examined and this may have accounted, to some extent, for the purity of the mannose ester which they obtained.

[1] The synthesis of mannose-6-phosphate is at present being attempted by Mr V. N. Patwardhan and the author.

SUMMARY.

1. A third component has been isolated from the hexosemonophosphate produced by fermentation of hexoses with yeast juice. This compound has been shown to be a monophosphoric ester of mannose.

2. The $[\alpha]_{5461}^{20°}$ of the free ester is + 15·1°, and of the barium salt + 3·5°. The H. and J. reducing power of the ester is the same as that of glucose- and fructose-monophosphate. The iodimetric reducing power is only 60–70 % of the value theoretically required for an aldosemonophosphate.

3. The ester yields a sparingly soluble phenylhydrazine salt of a phenylhydrazone (M.P. 144–145°), and a phenylhydrazine salt of an osazone (M.P. 154–155°), which appears to be identical with that obtained from fructose-diphosphate and from glucose- and fructose-monophosphate.

4. From this it is inferred that the phosphate group is in the same position in all these esters, probably position 6.

5. The ester is very slowly hydrolysed by *N* HCl at 100° ($k = 0{\cdot}29 \times 10^{-3}$); the rate is slightly higher than that for glucosemonophosphate in *N* HCl at 100°, and is practically the same as the rate of hydrolysis of both the free esters in 0·01 *M* solution, containing no additional acid. The sugar produced by acid hydrolysis has the character of *d*-mannose.

6. The presence of the Neuberg fructosemonophosphate as a component of the fermentation hexosemonophosphate has been confirmed; and this ester has been isolated in purer form than that previously reported by Robison and King.

7. The hydrolysis of this fructosemonophosphate by acids at 100° has been examined. In *N* HCl the value of k was found to be $4{\cdot}36 \times 10^{-3}$, but in 0·01 *M* solution of the free ester the initial value of k was $0{\cdot}37 \times 10^{-3}$, only slightly higher than that of glucose- and mannose-monophosphate.

8. The bearing of these rates of hydrolysis on the structure of the three esters is discussed.

9. The reactions leading to the formation of fructosediphosphate and of the three monophosphates during the fermentation of hexoses are discussed, and a scheme is suggested to explain the facts at present known.

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