dilute hydrochloric acid (1:1) and the mother liquor and washings were evaporated again. This process was repeated until nearly all of the solvent was gone. The crude prehnitic acid weighed 19 g. (33%), was white, and melted at 225–230°. One recrystallization from a little dilute hydrochloric acid (1:1) gave a product melting at 236–238°.

A specimen of the tetramethyl ester was prepared from the acid and diazomethane in ether. The crude ester melted at 122–127°; when recyrstallized once or twice from methanol, it melted at 131–133°.[16]

On exposure to light, this ester acquires a purple color, which is lost on fusing or dissolving the substance and which reappears when the solid is exposed to light.

(16) *Cf.* ref. 12, where the m. p. of this ester is given as 135°.

Anal. Calcd. for $C_{14}H_{14}O_8$: C, 54.17; H, 4.55. Found: C, 54.19; H, 4.70.

Summary

1. A convenient, rapid method for preparation of moderate amounts of prehnitic acid (benzenetetracarboxylic acid-1,2,3,4) from easily accessible materials, is described.

2. The results—all negative—of several attempts to bring about a diene reaction between muconic acid (or its esters) and acetylenedicarboxylic acid (or its esters) are described.

MINNEAPOLIS, MINNESOTA RECEIVED DECEMBER 2, 1938

[CONTRIBUTION FROM THE FLEISCHMANN LABORATORIES, STANDARD BRANDS INCORPORATED]

Fermentation of Maltose

BY ALFRED S. SCHULTZ AND LAWRENCE ATKIN

Fermentation of maltose by yeast has been the subject of considerable study. It has been claimed by Willstätter and Bamann[1] and Sobotka and Holzman[2] that yeast is capable of directly fermenting maltose. They claim that direct fermentation is likely even when the yeast employed contains demonstrable amounts of maltase. Direct fermentation is inferred by the disparity between the rate of maltose fermentation and the maltase content. This would be a convincing argument if it could be shown that maltase can be recovered completely from yeast and tested at maximum activity. Krieble, Skau and Lovering[3] have studied maltase extraction and have shown that Willstätter's method of extraction is by no means quantitative. Thus it can be seen that the theory of direct fermentation of maltose rests on uncertain ground. More recently Blish and Sandstedt[4] have undertaken the study of maltose fermentation. They have called attention to the fact that it is possible to stimulate the fermentation of maltose by the addition of various substances. They conclude that their activators are not connected with maltase activity and therefore must be considered as activating the direct fermentation of maltose.

The most active of Blish's activators is a dried baker's yeast. The addition of this material to a mixture of pure maltose and baker's yeast markedly reduces the induction period. Blish, however, reports no test for maltase in this activator. The senior author of the present communication had found (unpublished data obtained in 1928) that an extract of dried yeast would reduce the maltose induction period. His extract was made according to the method of Krieble, Skau and Lovering[3] for making a maltase extract. If maltase is responsible for the remarkable decrease in the induction period, we might then expect the fermentation technique to provide a very sensitive test for the enzyme. To test this hypothesis a sample of baker's yeast was dried and an extract of it made. This extract was very active in promoting the fermentation of maltose. A series of experiments was then undertaken to prove that the responsible agent or activator was maltase. Other points of interest were discovered as a result of this work and are herein reported.

(1) R. Willstätter and E. Bamann, *Z. physiol. Chem.*, **152**, 202 (1926).

(2) H. Sobotka and M. Holzman, *Biochem. J.*, **28**, 734 (1934).

(3) V. K. Krieble, E. L. Skau and E. W. Lovering, THIS JOURNAL, **49**, 1728 (1927).

(4) M. J. Blish and R. M. Sandstedt, *J. Biol. Chem.*, **118**, 765 (1937).

Experimental

Maltase in Dried Yeast.—Baker's yeast was passed through a coarse sieve and then dried before a fan at room temperature. Following the procedure recommended by Krieble, *et al.*,[3] 50 g. of the dried yeast was thoroughly suspended in 300 cc. of 5% $Na_2HPO_4 \cdot 12H_2O$. After storing at 18° for twenty hours the supernatant fluid was separated by means of the centrifuge and filtered. This yielded an opalescent solution which was neutralized to *p*H 6.5–6.7 by means of a 4% solution of potassium dihydrogen phosphate. Forty ml. of this extract represented 3 g. of the original dried yeast.

The maltase activity of this extract was first measured polarimetrically. Table I shows the results obtained at 30°. The reaction mixture contained 3 g. of maltose and 40 ml. of extract to a volume of 100 ml.

TABLE I

Enzyme	Rotation in 2-dm. tube, °V at 0 min.	90 min.	145 min.	1200 min.
40 ml. (unboiled)	+8.18	+7.76	+7.55	+6.27
40 ml. (boiled)	+8.18			+8.18

Influence of Dried Yeast Extract on Maltose Fermentation.—The fermentation tests were made with apparatus previously described by Schultz and Landis.[5] The reaction mixture was shaken by machine at 30° and the gas evolved measured at atmospheric pressure by displacement of a 10% calcium chloride solution. A typical control reaction mixture consisted of 3 g. of maltose, 15 ml. of buffer, 25 ml. of yeast suspension (3 g. of fresh yeast), 10 gammas of thiamin and water to make 100 ml. We add the thiamin because, as we have shown,[6,7] it is a powerful accelerator of dextrose fermentation and by including it here in all cases we eliminate its effect as variable. Unless otherwise noted, the buffer salts contained in 15 cc. were 0.9 g. of ammonium dihydrogen phosphate and 0.36 g. of diammonium hydrogen phosphate. The *p*H of this mixture when dissolved in 100 ml. is 6.2 (glass electrode). When an extract is to be added to the fermentation mixture it replaces a portion of the water.

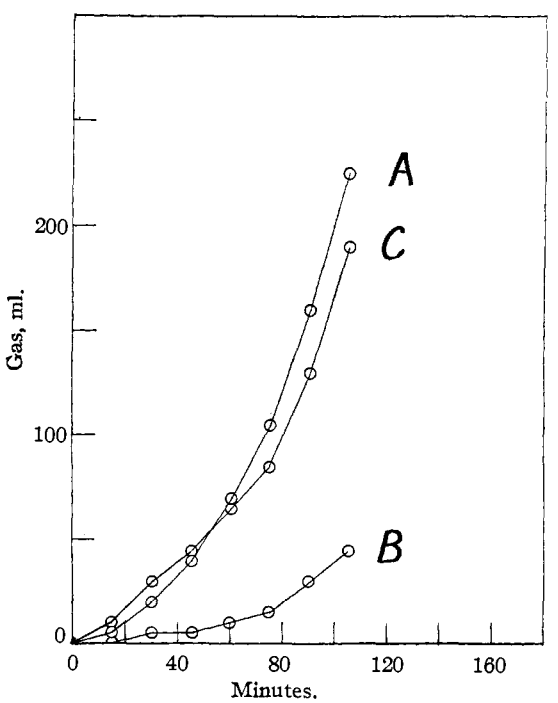

Fig. 1.—Influence of dried yeast extract on maltose fermentation. Control reaction mixture (maltose III) plus A, 40 ml. maltase extract; B, 40 ml. boiled extract; C, 40 ml. boiled extract, ninety minutes prior action on maltose.

(5) A. S. Schultz and Q. Landis, THIS JOURNAL, **54**, 211 (1932).
(6) A. S. Schultz, L. Atkin and C. N. Frey, *ibid.*, **59**, 948 (1937).
(7) A. S. Schultz, L. Atkin and C. N. Frey, *ibid.*, **59**, 2457 (1937).

The results shown graphically in Fig. 1 demonstrate the influence of the extract on the induction period. The difference between Curve A and Curve B is a measure of the activity of the extract. It may be noted here that this active extract was made from the same yeast with which it was tested. In order to see whether the extract acts on the yeast or the substrate the experiment described in Curve C was made. In this case the extract was allowed to act on the maltose for ninety minutes in the absence of any yeast. Before adding the yeast the mixture was brought to a boil and then cooled. It may be concluded from this result that the extract works on the substrate.

Fermentation with Mycoderma.—For this purpose use was made of the method described by Schultz and Kirby.[8] They used a culture of a mycoderma which is quite incapable of fermenting maltose. We have employed the same culture, merely substituting it for the yeast used in

TABLE II

FERMENTATION WITH MYCODERMA

Control reaction mixture modified as noted below. Each with 3 g. of mycoderma.

No.		Cc. gas 75 min.	150 min.
A	3 g. maltose + 40 ml. maltase extract	92	245
B	3 g. maltose + 40 ml. boiled maltase extract	10	0
C	2.5 g. maltose + 0.5 g. dextrose + 40 ml. boiled extract	70	70
D	0.5 g. dextrose + 40 ml. boiled extract	73	73

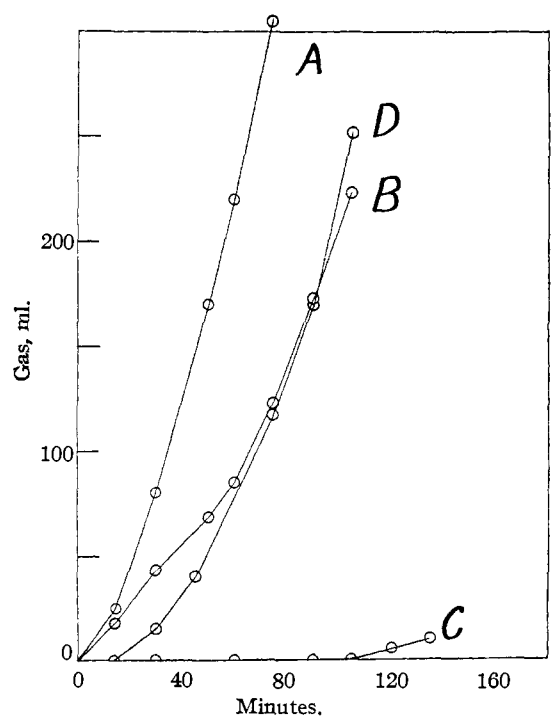

Fig. 2.—Influence of dextrose on maltose fermentation. Control reaction mixture except as noted. A, 3.0 g. dextrose; B, 2.8 g. maltose + 0.2 g. dextrose; C, 3.0 g. maltose; D, 3.0 g. maltose + 40 ml. maltase extract.

(8) A. S. Schultz and G. W. Kirby, *Cereal Chem.*, **10**, 149 (1932).

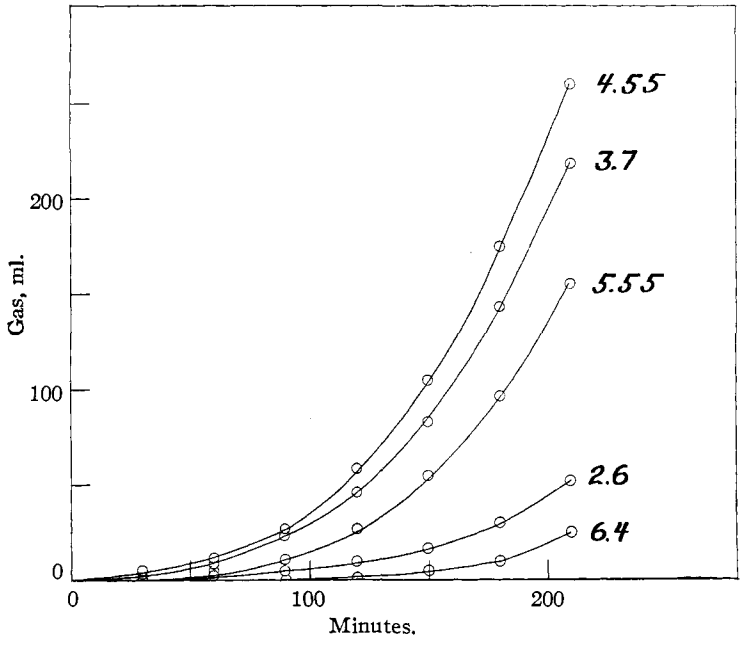

Fig. 3.—The effect of *p*H on maltose fermentation. Control reaction mixture except for citrate-phosphate buffer at *p*H's indicated.

TABLE III

MIXTURES OF MALTOSE AND DEXTROSE—CONTROL REACTION MIXTURE WITH DIFFERENT SUGARS

No.		Cc. gas 75 min.	Cc. gas 150 min.
A	3 g. maltose I	5	25
B	3 g. maltose II	10	50
C	3 g. maltose III	25	140
D	3 g. maltose I + 10 mg. dextrose	10	65
E	3 g. maltose I + 50 mg. dextrose	55	262

our other tests. Table II shows the influence of the extract. The parallel nature of tests C and D shows that maltose is, for this organism, an inert substance.

Influence of Dextrose on Maltose Fermentation.—We have investigated the influence of dextrose itself on the fermentation of maltose. Figure 2 shows the striking accelerating effect of dextrose. Included in this graph are curves showing the rate of fermentation of pure dextrose and also of pure maltose.

Mixtures of Maltose and Dextrose.—The effect of dextrose is so pronounced that it may be employed to detect the admixture of relatively small percentages of dextrose in maltose preparations. Table III shows the effect of different amounts of dextrose upon the rate of fermentation of maltose. Tests B and C refer to special maltose preparations. Tests D and E may be used to estimate the differences between them.

The Effect of Fermentation Products and Intermediates.—In an attempt to locate the specific cause of the dextrose effect we tried various substances known to occur in the fermentation reaction. The results were negative with acetaldehyde, pyruvic acid and virtually so with ethyl alcohol. Alcohol showed a slight improvement over the control, but it could not be compared with the dextrose effect. Carbon dioxide showed a definite stimulation. To add carbon dioxide to the reaction mixture distilled water was saturated with carbon dioxide at atmospheric pressure and added to the mixture in different amounts. Care was taken to eliminate the first discharge of gas (on shaking). We recognized that the effect of carbon dioxide might be due merely to a change in *p*H and therefore the effect of *p*H was investigated.

The Effect of *p*H on Maltose Fermentation.—A series of buffers was made to cover the range from *p*H 2.6 to 6.4 by taking 20 g. of ammonium dihydrogen phosphate, 45 g. of dipotassium hydrogen phosphate and 71 g. of citric acid, adding different amounts of sodium hydroxide and diluting to 1 liter; 10 ml. of this solution was used to buffer the 100 ml. of reaction mixture. The variation in the rate of maltose fermentation as a function of *p*H may be seen in Fig. 3. This effect was described by Willstätter and Bamann[1] and may be seen to hold under present conditions. Carbon dioxide was now added to a reaction mixture buffered at 4.55. The stimulation, if any, was greatly reduced and must for the present be considered questionable. To see whether the dextrose effect also could be an indirect result of *p*H shift, the series given in Fig. 4 was made. Here it can be seen that dextrose stimulates the fermentation of maltose over a wide range of *p*H values.

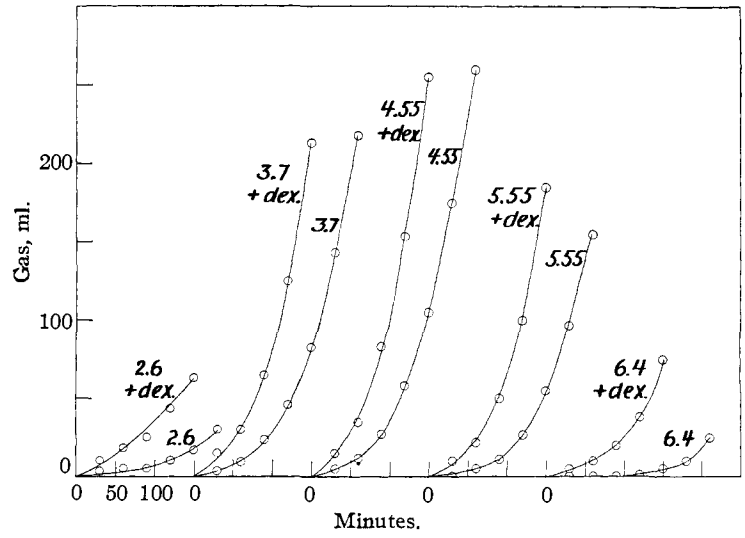

Fig. 4.—Effect of dextrose at different *p*H's. Reaction mixture the same as in Fig. 3 except for 50 mg. added dextrose in curves marked dextrose.

Discussion

How the presence and subsequent fermentation of a very small quantity of dextrose can hasten the fermentation of maltose is rather difficult to explain. One supposition involves a change in the permeability of the cell wall. This seems logical since, it may be recalled, a maltase-active extract can be made from a yeast which is slow to ferment maltose. Since maltase was present in the interior of the cell an increased permeability to the disaccharide maltose should produce a corresponding increase in fermentation rate.

The results we have here reported indicate that one might easily be misled in a search for fermentation activators of maltose fermentation. The seemingly dual nature of the accelerators, one heat labile and one heat stable, as well as the known influence of thiamin, produces a rather complex system. The action of maltase upon maltose fermentation is obvious and there is no reason to consider maltase a fermentation activator or accelerator according to the present usage of these terms. On the other hand, the action of dextrose may be considered to activate a fermentation system contained in the cell.

Summary

Dried yeast prepared from baker's yeast contains maltase and the addition of such dried yeast or an extract made from it to a yeast–maltose system will cause a sharp increase in the initial rate of fermentation.

Small quantities of dextrose when added to a yeast-maltose system greatly hasten the appearance of active fermentation.

The influence of small quantities of dextrose may be used to estimate dextrose when present as a minor constituent of maltose preparations.

The initial rate of fermentation of maltose is dependent upon the *p*H and shows an optimum in the region of *p*H 4.5.

Small quantities of maltase and dextrose, either separately or together, may be responsible for some of the action of the various accelerators of maltose fermentation.

NEW YORK, N. Y. RECEIVED NOVEMBER 12, 1938

[CONTRIBUTION FROM THE RESEARCH LABORATORY OF THE GENERAL ELECTRIC CO.]

Conductance of Some Salts in Tricresyl Phosphate at 40°

BY MYRON A. ELLIOTT WITH RAYMOND M. FUOSS

I. Introduction

A large number of electrolytic systems in non-aqueous solvents have been studied, but data are lacking on systems where the solvent molecules are large compared to those of the solute. Furthermore, a systematic general study of temperature coefficients is not yet available. Experimental data on these problems are necessary for a clear understanding of the dynamics of electrolytic conductance; Stokes' law, as applied in the form of Walden's rule is, at best, a first approximation. In this paper will be presented the results of a study of a group of electrolytes in tricresyl phosphate $[(CH_3C_6H_4O)_3PO]$ at 40°. This solvent has a high viscosity at room temperatures, with a large temperature–viscosity coefficient, and a fairly low dielectric constant (6.92 at 40° and 60 cycles). These properties make it a very useful solvent medium for an experimental investigation of the problems under consideration.

Some aromatic amine picrates were included among the salts studied; they all gave unusual conductance curves in that the shape of the conductance–concentration curve was that of a curve for a strong electrolyte, while the magnitude of the conductance was that of a very weak electrolyte. This result suggests that two modifications of amine picrates exist, a non-electrolytic molecular addition compound, and an electrolytic compound which is a true salt, formed by the migration of a proton from the picric acid to the amine, to form an ammonium salt.

II. Materials, Apparatus and Method

Tricresyl phosphate was purified by heating for two hours at 50°, with constant stirring, with previously dried fuller's earth (about 300 g. of adsorbent to 2.5 l. of solvent). After pumping the dissolved air out of the hot tricresyl phosphate, it was filtered to remove the fuller's earth. The following procedure was used. The degassed mixture of fuller's earth and tricresyl phosphate was allowed to settle overnight in a long vertical tube 10 cm. in diameter, containing an inverted sintered glass funnel attached