manganate. M. p.: 105°C, subst.: 0.0493g., CO$_2$: 0.1058g., H$_2$O: 0.0271g.,
C: 58.52%, H: 6.10% [C: 58.40% as C$_{11}$H$_4$O$_4$. CH$_3$].

This substance is quite stable against any oxidizing agent alkaline per-
manganate showing no trace of oxidation.

Dihydro dibromo methyl γ-acid. Dihydro methyl γ-acid was dissolved in
glacial acetic acid and was brominated. M. p.: 122°C, subst.: 0.1005g.,
AgBr: 0.0984g., Br: 41.67% [Br: 41.63% as C$_{11}$H$_4$O$_4$.Br$_2$].

Oxime. 3g. of γ-acid, 2.5g. of hydroxylamine hydrochloride and 3.5g.
of sodium acetate were dissolved in 80c.c. of 60% alcohol and the solution
was heated for 3 hours on a water bath. Colorless plates separated out.
The substance had no melting point, decomposing at 199–201°C. N: 5.95%
[6.22% as C$_{11}$H$_4$O$_4$.N].

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ON THE REVERSIBILITY OF ENZYME ACTION.

PART I.

By

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Armstrong$^{(1)}$ found that, by the action of yeast maltase on glucose,
isomaltose mainly was formed and has taken the view that an enzyme
synthesizes exactly those bodies which it does not hydrolyse. To accept
this view, it is a matter of primary importance to ascertain whether the
maltase preparation used by Armstrong really did not contain any β-glucosi-
dase. On reading Armstrong's papers, one cannot get any information about
this matter either positively or negatively.

The observation of the presence of β-glucosidase in yeast cells (brewer's
yeast), made by Henry and Auld$^{(2)}$ is well founded and Pringsheim$^{(3)}$ noticed
that maltose and a β-glucoside are formed by the action of yeast enzymes.
It is very likely that these experiments present an argument against Arm-
strong's view.
If it were possible to have in hand a maltase preparation free from β-glucosidase the matter would be settled, once for all. Unhappily, however, maltase is weaker than β-glucosidase against acids which are to be produced during the autolysis of yeast cells, so that it is quite difficult to obtain such an enzyme preparation. It is an easy task, on the contrary, to prepare, by taking advantage of this property, β-glucosidase solution free from maltase.

Using the latter preparation we were able to prove that isomaltose is formed by its action upon a concentrated glucose solution. Isomaltose was identified as its osazone, comparing it with that of isomaltose synthesized, following Fischer’s method. When the synthesis was completed, that is when the change of rotatory and reducing power was at a standstill, the solution was diluted and the recovery to their original state was observed.

Since maltase was not contained in the enzyme preparation which we used, maltase, without doubt, does not play a role in the reaction. Nevertheless isomaltose was formed. It is to be expected naturally that our enzyme preparation may contain other carbohydrases than β-glucosidase, for instance, invertase. It is contrary, however, to the general conception of the nature of enzyme action to suspect them to be responsible for the synthesis of isomaltose. It has been thought, therefore, that isomaltose was formed, in our experiments, by the action of β-glucosidase upon glucose.

**Experimental**

A dry preparation of yeast was prepared by treating beer yeast, supplied to us from Dai Nihon Brewering Co., with alcohol and ether successively after it was well centrifuged to drive water. To 10g. of the dry preparation, 100c.c. of water and 5c.c. of toluene were added and the mixture was well macerated in a mortar. Standing over night at room temperature (18-20°C), the mixture was filtered with Chamberland’s filter and the filtrate was used for experiments after being neutralized with a 1% ammonia solution.

1. **Action on maltose.** 1.26g. of maltose were dissolved in 70c.c. of water and to the solution 7c.c. of enzyme preparation and 10c.c. of buffer solution were added. Secondary sodium phosphate and primary potassium phosphate were used as buffers. For observation 1c.c. of the mixed solution was used. pH: 6.8, temp.: 37°C. No change of rotatory and reducing power was observed during the course of 168 hours.

2. **Action on α-methyl glucoside.** All conditions, except the concentration of the substrate, were the same as those of case 1. Concentration of glucoside was 1.5g. in 35c.c. of reaction mixture. No change of rotatory and reducing power was observed.

3. **Action on β-methyl glucoside.** The reaction mixture consisted of the
following solution: 15c.c. of the glucoside solution (0.5g.), 5c.c. of enzyme preparation, 1.18c.c. of 0.2N acetic acid and 2.82c.c. of 0.2N sodium acetate solution. pH: 5.0, temp.: 37°C. After 58 hours, 1c.c. of the solution precipitated cuprous oxide to the amount of 61.37mg. as copper.

4. Action on isomaltose. An isomaltose solution was prepared after Fischer's method. pH: 5.1, temp.: 37°C. 1c.c. of the reacting solution precipitated 51.81mg. of Cu in the beginning and after 90 hours the precipitates increased to 61.37mg. of Cu.

As the isomaltose solution might contain other di-or polysaccharides than isomaltose, isomaltosazone was formed and the action of the enzyme preparation on the osazone was observed. 0.01g. of the osazone, which naturally did not show any reducing power, precipitated 4.02mg. of Cu 3 days after the enzyme solution had been added. Control experiments which, however, showed no change were duly carried out, side by side.

5. Action on glucose. Concentration of glucose: 52.8%, pH: 6.4, temp.: 37°C. The decrease of reducing power was observed and when the same solution was diluted as much as five times the original reducing power was recovered.

One part of the synthesized solution was taken out, diluted with water to make the concentration of sugar 10%, pasteurized as usual, and impregnated with S. marxianus. When fermentation ceased, an osazone was formed by the ordinary method. M. p.: 155°C. C: 55.75% (Calc.: 55.35), N: 10.57% (Calc.: 10.77). A mixed sample of the osazone and isomaltosazone, obtained after Fischer's method, melted also at 155°C.

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