122. A MICRO-DETERMINATION OF SUCCINIC ACID

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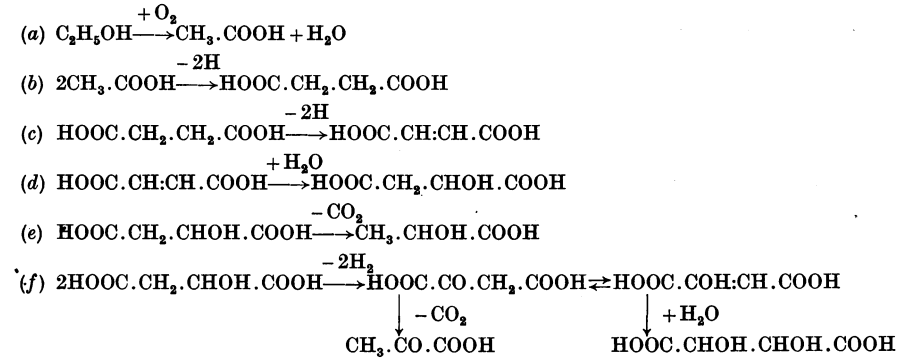
One of the main differences in the alcoholic fermentation brought about by living yeasts or by living *Fusaria* is the fact that the phase sequence inherent to the former ends with the production of carbon dioxide and an incompletely degraded substance, namely, ethyl alcohol, whereas, in the case of the latter, the enzymic dissimilation of the carbohydrates to carbon dioxide and ethyl alcohol is continued and accompanied by dehydrogenation of the alcohol formed.

The complete phase sequence of the dehydrogenation of alcohols by means of *Fusarium lini* Bolley up to the stage of carbon dioxide can, at present [Nord, 1940], be illustrated as follows:



With the exception of fumaric acid and oxaloacetic acid, all the compounds appearing in this scheme, in which malic acid possesses a central position following acetic and succinic acids, were experimentally demonstrated. However, no actual mechanism is so far known for the transient formation of one of the most important C_4 compounds, viz. succinic acid, from acetic acid, which contrary to earlier convictions [Meyerhof, 1936] can not only be accumulated but also isolated without the introduction of fixatives, etc. [Rotini *et al.* 1936].

A rapid and accurate method for the routine determination of succinic acid was therefore deemed necessary, all the more since (*a*) the method of Denigès [1936] used in earlier work for qualitative detection could not be developed as a quantitative one and (*b*) the procedures suggested, e.g. by Rau [1893], Bordas *et al.* [1898], Moyle [1924], Clutterbuck [1929] and Bernhauer [1939], were limited in application.

I have therefore undertaken, at the suggestion of Mr Nord, the determination of minute quantities of succinic acid in nutrient media and in the presence of other organic acids.

Experimental

1. *Removal of volatile acids and acids oxidized by potassium permanganate. Extraction of succinic acid*

The solution containing 2 ml. 0·1 *N* succinic acid, volatile acids and nutrient media containing NO_3^-, $SO_4^=$ and $PO_4^{\equiv}$, was acidified with conc. H_2SO_4 until acid to Congo red. It was then heated on the water bath and treated with 0·1 *N* $KMnO_4$ until a brown precipitate was obtained. This was then dissolved by addition of Na_2SO_3 and the solution evaporated to dryness. The residue was dissolved in 15 ml. of chloride-free distilled water; 2 ml. conc. H_2SO_4 were added and the solution was saturated with K_2SO_4. The whole was transferred quantitatively to an extractor [McNair, 1933] and the casserole washed with three successive portions of saturated K_2SO_4 (4 ml., 3 ml., 3 ml.).

After extracting for 3–4 hr. with ether, the latter was transferred to a flask and 5 ml. of chloride-free distilled water added. The ether was removed by distillation on a water bath and the aqueous residue boiled over an open flame for 30 sec. The flask was then cooled under the tap and one drop of *m*-nitrophenol indicator added. The analysis was then carried out as in the control titration. The recovery determined so far on 25 samples amounts to $98 \pm 2\%$.

2. *Control titration*

2–4 ml. of 0·1 *N* succinic acid were placed in a flask and diluted to 5 ml. with chloride-free distilled water or a known weight of succinic acid was dissolved in 5 ml. of chloride-free distilled water. As an indicator one drop of *m*-nitrophenol (0·3 % in water) was used; 0·05 *N* NaOH was added until one drop caused the solution to turn yellow. It was decolored by means of one drop of 0·1 *N* HNO_3 and a measured excess of 0·02 *M* $AgNO_3$ added immediately. The solution was brought back to neutral by the addition of one drop of 0·05 *N* NH_4OH. The precipitate was kept for 1·5–2 hr. in the dark. The solution was filtered through a Gooch crucible or a fritted glass filter and washed quantitatively from the flask on to the filter by three successive portions of 1 % NH_4NO_3 (3 ml., 3 ml., 2 ml.). Two drops of dichlorofluorescein indicator (0·1 % in 70 % ethanol) and 7–9 drops of 1 % soluble starch (chloride-free) were added to the filtrate. The excess silver was then titrated with 0·02 *M* KBr until the pink colour disappeared. One ml. of 0·02 *M* $AgNO_3$ is equivalent to 1·18 mg. succinic acid. Recovery based on 40 samples was $99{\cdot}5 \pm 0{\cdot}5\%$.

Summary

1. An accurate and rapid method is presented for the estimation of quantities of succinic acid as low as 10 mg. This method has the advantage that it can be carried out without difficulty with the usual laboratory equipment.

2. The reproducibility of the actual titration has been established by using *m*-nitrophenol and dichlorofluorescein as indicators along with potassium bromide.

3. The time of extraction of succinic acid from the solution has been decreased to 3–4 hr.

To the Rev. Dr F. W. Power, I wish to express my thanks for helpful suggestions.

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