creased by the addition of the magnesium sulfate.

Group II. The addition of inositol with Bios II does not give increased growth. This group includes strains Nos. 9, 11, 16 and 26.

Group III. Growth is increased under the conditions given for Groups I and II. These include Nos. 7, 10, 18, 19, 22 and 41.

The above data and groupings show that discrepancies in published results of bios studies may be due largely to differences in the strains of yeast employed and in the composition of the medium. For example, if a strain of yeast belonging to Group I were grown in a medium containing magnesium sulfate, Bios II would give increased counts in the presence of Bios I (inositol); that is, the complementary effect of Bios I and Bios II, as described by Miller and co-workers, would be demonstrated. If, however, a strain of Group II or Group III were employed under the same conditions, the Bios II would be potent in the absence of Bios I and their complementary effect would not be apparent.

Summary

The effect of Bios II, inositol (Bios I) and magnesium sulfate alone and in combinations on the growth of thirteen strains of *Saccharomyces cerevisiae* is reported. On the basis of the marked differences in response to the different media by the various yeast strains they are separated into three distinct groups.

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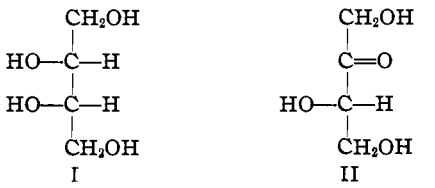
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

The Production of *l*-Erythrulose by the Action of Acetobacter suboxydans upon Erythritol

BY ROY L. WHISTLER AND L. A. UNDERKOFLER

The use of microörganisms for the production of ketose sugars has made possible the extension of carbohydrate investigations to include several of the rare ketoses which are now easily obtainable in pure form. *l*-Erythrulose was first produced from *meso*-erythritol by Bertrand[1] who employed the "sorbose bacterium" or *Acetobacter xylinum*. Müller, Montigel and Reichstein,[2] using the same organism, recently have converted erythritol to *l*-erythrulose in approximately 60% yield, and have isolated in pure form 50% of the sugar produced.

The present communication deals with the optimum conditions for the production of *l*-erythrulose by the action of *Acetobacter suboxydans* upon *meso*-erythritol, and for the isolation of the erythrulose produced. This organism has been found to effect practically quantitative conversion of the alcohol to the sugar in a short time. It is of interest to note that, although the configuration of the *meso*-erythritol, I, is such that the formation of both *d*- and *l*-erythrulose might be expected, only the *l*-erythrulose, II, is produced by the organism. No organism yet studied has been found to oxidize *meso*-erythritol to *d*-erythrulose.



It was found that Bertrand's[1] method of purification of the *l*-erythrulose by passage through the bisulfite addition compound was not accomplished easily since complete removal of the sodium bisulfite and recovery of pure unaltered sugar was very difficult. Purification by precipitating the sugar from alcoholic solution through the addition of ether and then distilling the sirup in a molecular still was found most suitable.

Experimental

Methods.—The culture of *Acetobacter suboxydans*, listed as No. 621, was secured from the American Type Culture Collection, and is the same culture previously used in studies reported from these Laboratories.[3,4] The stock cultures are carried on yeast extract–glycerol–agar slants. The cultures used for inoculations in the present experi-

(1) G. Bertrand, *Compt. rend.*, **130**, 1330 (1900); *Bull. soc. chim.*, [3] **23**, 681 (1900); *Ann. chim. phys.*, [8] **3**, 181 (1904).

(2) H. Müller, C. Montigel and T. Reichstein, *Helv. Chim. Acta*, **20**, 1468 (1937).

(3) E. I. Fulmer, J. W. Dunning, J. F. Guymon and L. A. Underkofler, THIS JOURNAL, **58**, 1012 (1936).

(4) L. A. Underkofler and E. I. Fulmer, *ibid.*, **59**, 301 (1937).

ments were kept active by transfer each twenty-four hours into a medium containing 0.5 g. of yeast extract (Difco powdered product) and 3 g. of erythritol per 100 ml. All media were sterilized in the autoclave for twenty minutes at fifteen pounds (1 atm.) steam pressure, and all cultures were incubated at the optimum temperature of 28°. Preliminary studies were made using 10 ml. of medium in 50-ml. Erlenmeyer flasks. In each case the inoculum consisted of 3 ml. of the twenty-four hour culture of the organism per 100 ml. of fresh medium, measured from a sterile pipet. All fermentations were run in duplicate, and the values reported are the averages for the duplicate determinations. The course of the conversion of the erythritol into erythrulose was followed by determination of copper reduction values by means of the Shaffer–Hartmann[5] sugar titration method.

After isolation of erythrulose had been accomplished, an equation was derived experimentally which permitted calculation of the yields of erythrulose from the copper reduction values previously obtained. This equation was obtained as follows: weighed samples of the sirupy erythrulose were diluted and aliquots of 50 ml. heated with 50 ml. of mixed Fehling's solutions in 500-ml. uncovered Erlenmeyer flasks. The flames were so adjusted as to bring the mixtures to a boil in exactly four minutes, boiling was continued for exactly two minutes and the flasks were then cooled promptly in running water. The cuprous oxide was titrated by the Shaffer–Hartmann method. All reagents, including the Fehling's solutions, were made up exactly as directed by Shaffer and Hartmann.[5] The copper reduction values so obtained were plotted against weight of erythrulose, giving a straight line for amounts of the sugar between 20 and 100 mg. The equation for this line is

$$E = 0.831\ R + 4.380$$

where E represents mg. of erythrulose corresponding to R mg. of copper. This equation provides a convenient method for the calculation of erythrulose from analytical data. Since the erythrulose was recovered as a sirup which did not crystallize, the purity was based on the optical rotation given by Müller, Montigel and Reichstein.[2] Any inaccuracy involved here would mean a corresponding error in the above equation, which must therefore be considered subject to revision if a purer product be secured.

The Development of the Medium.—Employing the proportion of yeast extract which previously had been found optimum for the production of sorbose from sorbitol and of dihydroxyacetone from glycerol,[3,4] *i. e.*, 0.5 g. per 100 ml., media were prepared in which the concentrations of erythritol were varied. After inoculation and incubation, duplicate flasks of each medium were analyzed and the yields ascertained after intervals of three, four, seven and thirty days. The maximum yields were obtained after four to seven days, a slight decrease being sometimes observed after thirty days. The yields after seven days with 3, 4.5, 6, 9, 12, 15 and 18 g. of erythritol per 100 ml. were, respectively, 96, 99, 92, 61, 42, 30 and 18% of erythrulose.

An investigation on the influence of surface–volume ratio on the yield of erythrulose was made, employing different volumes of medium containing 4.5 g. of erythritol and 0.5 g. of yeast extract per 100 ml. in 125-ml. Erlenmeyer flasks. Incubation was for four days. When 10, 20, 30 and 40 ml. of medium were used, the respective surface–volume ratios (sq. cm. area per 1 cc. volume) were 3.18, 1.59, 1.06 and 0.80, and the yields were 98, 97, 93 and 92% of erythrulose.

From the data it may be concluded that the optimum concentration of erythritol in the medium is about 4.5 g. per 100 ml., and that maximum yields are obtained when the ratio of surface to volume of medium is high. Since the conversion of the erythritol to erythrulose was found to be practically quantitative in four days under these conditions when the medium contained 0.5 g. of yeast extract per 100 ml., no attempt was made to improve the medium by variation of the concentration of yeast extract, by addition of salts or by adjustment of the *p*H of the medium (*p*H 6.1 for the medium as prepared).

The Recovery of *l*-Erythrulose.—Thirty-five grams of yeast extract and 335 g. of *meso*-erythritol were dissolved in distilled water to make 7000 ml. of medium which was distributed in quantities of 200 ml. in 2-l. Erlenmeyer flasks. The sterilized medium in each flask was inoculated with 6 ml. of a twenty-four hour culture of *Acetobacter suboxydans* in similar medium, and incubated for nine days at 28°. According to the Shaffer–Hartmann titration, 95% of the erythritol had been converted to *l*-erythrulose.

To each 2000 ml. of the fermented medium were added 15 g. each of Norite, calcium carbonate and infusorial earth and the mixture was well shaken. The liquid was then separated by means of a supercentrifuge. To the recovered liquid was added 10 g. of Norite, the mixture passed through the supercentrifuge and the recovered liquid again passed through the centrifuge for the third time. The light yellow liquid, which was almost free of bacteria or other suspended material, was evaporated under reduced pressure at 35° to a volume of 200 ml. Six hundred ml. of absolute alcohol was added with stirring and the mixture filtered. The filtrate was evaporated under reduced pressure at 30° to a thick sirup of light yellow color, $[\alpha]^{30}D$ +10.0 (*c*, 2.4 in water). The sirup was taken up in alcohol and a small quantity of ether added. On standing in the refrigerator 2% of the original erythritol separated. On the addition of a little more ether a small quantity of a sirup having a low positive optical rotation separated. Further addition of ether precipitated erythrulose as a light yellow sirup. This sirup was taken up repeatedly in alcohol and precipitated with ether. The resulting sirup was distilled in a molecular still at 90–95° to produce a colorless sirup, $[\alpha]^{30}D$ +11.31 (*c*, 4.0 in water). By this procedure 87.4% of the erythrulose present in the original fermented liquid, as determined by the Shaffer–Hartmann titration, could be recovered as a sirup.

Summary

A procedure has been given for the production of *l*-erythrulose by the action of *Acetobacter suboxydans* upon *meso*-erythritol and for the isolation of the sugar from the fermented medium.

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(5) P. A. Shaffer and A. F. Hartmann, *J. Biol. Chem.*, **45**, 365 (1920).