45 g. of aluminum chloride and 200 cc. of carbon disulfide. The mixture was then refluxed on the steam-bath for sixteen hours, after which the carbon disulfide was removed by distillation. The residue was decomposed with iced dilute hydrochloric acid, the oil layer separated, washed, and distilled *in vacuo*. The product distilled over at 170–185° (9 mm.) as a colorless oil.

*Anal.* Calcd. for $C_{14}H_{21}O_2Cl$: C, 65.45; H, 8.27; Cl, 13.83. Found: C, 66.44; H, 8.02; Cl, 13.35.

(XII) **β-(p-(α,α,γ,γ-Tetramethylbutylphenoxy))-β'-chlorodiethyl Ether.**—To a solution of 100 g. of β-phenoxy-β'-chlorodiethyl ether and 56 g. of diisobutylene (b. p. 100–104°) there was added dropwise during forty-five minutes while stirring and occasionally cooling, 20 g. of 98% sulfuric acid. The mixture was then stirred for seven hours at room temperature and allowed to stand for eighteen hours. The thick oil obtained was warmed with 145 cc. of 10% sodium hydroxide solution, the oil layer separated, washed and distilled *in vacuo*. The fraction of b. p. 157–167° (1 mm.) was the desired product; yield 107.5 g. or 68%.

*Anal.* Calcd. for $C_{18}H_{29}O_2Cl$: Cl, 11.34. Found: Cl, 11.68.

(XIII) **β-(s-Octylphenoxy)-β'-chlorodiethyl Ether.**—A mixture consisting of 180 g. of β-phenoxy-β'-chlorodiethyl ether, 117 g. of octanol-2 and 20 g. of "Tonsil" clay was heated with stirring for four hours at 160–175° under reflux attached to a water trap. The clay was then filtered off and the *s*-octylphenoxyethoxyethyl chloride, b. p. 175–185° (3 mm.), isolated by vacuum distillation; yield 60%.

*Anal.* Calcd. for $C_{18}H_{29}O_2Cl$: Cl, 11.34. Found: Cl, 10.78.

(XIV) **β-Dodecylphenoxyethyl Chloride.**—A mixture consisting of 56 g. of β-phenoxyethyl chloride, 66.5 g. of lauryl alcohol and 15 g. of "Tonsil" clay was boiled with rapid stirring for three and one-half hours under a reflux condenser attached to a water trap. The temperature gradually rose from 165 to 195° during this period. Heating was continued for sixteen hours at 180–195°. The mixture was then cooled, diluted with toluene and filtered hot by means of a Büchner funnel. The filtrate was fractionated under reduced pressure. Dodecylphenoxyethyl chloride distilled over at about 185–195° (1 mm.) as a colorless oil, the chlorine analysis of which indicated a purity of 97.5%.

## Summary

Aromatic alkylene ether chlorides or polyalkylene ether chlorides of the type R–(O-alkylene)$_n$Cl readily undergo Friedel–Crafts nuclear alkylation or acylation reactions without splitting the ether links, loss of the terminal chlorine atom, or appreciable polymeric intercondensation. A number of acylations with phthalic, succinic, maleic and acetic anhydrides and alkylations with butyl chloride, capryl alcohol, diisobutylene and lauryl alcohol are described.

PHILADELPHIA, PENNA. RECEIVED AUGUST 5, 1938

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

# The Effect of the Composition of the Medium upon the Growth of Yeast in the Presence of Bios Preparations. II. The Response of Several Strains of Saccharomyces Cerevisiae[1]

BY JAMES B. LESH, L. A. UNDERKOFLER AND ELLIS I. FULMER

In a previous communication from these Laboratories[2] data were presented showing that, for the one strain of yeast employed, the presence of magnesium sulfate markedly increased the growth of the yeast in the presence of Bios II or a mixture of Bios II and Bios I (*i*-inositol). The present paper deals with the response of several strains of *Saccharomyces cerevisiae* to the above-mentioned reagents. While 23 strains of yeast were studied during the present investigation, data are presented here for only the 13 of these which can be obtained readily from standard sources: these strains are listed in Table I.

Lucas,[3] Williams, Warner and Roehm,[4] Williams and Saunders,[5] Stantial[6] and Farrell[7] have noted significant differences among various strains of yeast as to their response to various Bios preparations, but the effect of magnesium sulfate had not been considered previously. Magnesium sulfate is a customary ingredient of most media used for growing yeast. However, the media developed in these Laboratories by Fulmer, Nelson and Sherwood[8] for the growth of yeast in the

(1) This work was supported in part by a grant from the Industrial Science research funds of the Iowa State College for studies on the fermentative utilization of agricultural products.

(2) E. I. Fulmer, L. A. Underkofler and J. B. Lesh, THIS JOURNAL, **58**, 1356 (1936).

(3) G. H. W. Lucas, *J. Phys. Chem.*, **28**, 1180 (1924).

(4) R. J. Williams, M. E. Warner and R. R. Roehm, THIS JOURNAL, **51**, 2764 (1929).

(5) R. J. Williams and D. H. Saunders, *Biochem. J.*, **28**, 1887 (1934).

(6) Helen Stantial, *Trans. Roy. Soc. Can.*, **26**, Sec. III, 163 (1932).

(7) Leone N. Farrell, *ibid.*, **29**, Sec. III, 167 (1935).

(8) E. I. Fulmer, V. E. Nelson and F. F. Sherwood, THIS JOURNAL, **43**, 191 (1921).

TABLE I

KEY TO STRAINS OF *Saccharomyces cerevisiae*
(ATCC = American Type Culture Collection)

| Strain | | |
|---|---|---|
| 5 | (Hansen) | ATCC No. 4360 |
| 6 | (Type Frohberg) | ATCC No. 2334 |
| 7 | (Type Saaz) | ATCC No. 2352 |
| 9 | (I Hansen) | ATCC No. 2368 |
| 10 | | ATCC No. 765 |
| 11 | (Berlin RII) | ATCC No. 4099 |
| 16 | | ATCC No. 764 |
| 18 | | ATCC No. 4132 |
| 19 | | ATCC No. 4109 |
| 22 | (Hansen) | Univ. Ill. No. 2235 |
| 26 | (Hansen) | ATCC No. 4923 |
| 41 | | ATCC No. 4226 |
| 42 | (Gebrüde Mayer) | Fleischmann No. 21.4–40 |

absence of bios do not contain this salt since it was found to be of no advantage. The marked difference in the response of yeast to bios preparations added to Clark's medium (used by Miller and co-workers) which contains magnesium sulfate, and to Medium C or Medium D, which do not, led to the study reported in the previous paper.[2]

The basal medium used in the present investigation was Medium C, developed by Fulmer, Nelson and Sherwood,[8] and contained per 100 cc., 0.188 g. of ammonium chloride, 0.100 g. of dipotassium phosphate and 5 g. of sucrose. Preliminary tests showed the optimum *p*H (after sterilization) to be 6.2. In order to assure the proper *p*H, the medium was made 0.0025 *N* with hydrochloric acid before sterilization. All media were sterilized at 15 lb. (1 atm.) pressure for fifteen minutes. In all experiments, 25 cc. of medium was used in 125-cc. Erlenmeyer flasks. The basal medium was made up in concentrations five-fourths of that given above. Twenty cc. was placed in each flask and made up to 25 cc. by the addition of solutions to be tested or of distilled water. The concentration of *i*-inositol (Bios I) was 0.032 mg., and that of magnesium sulfate 100 mg. (0.008 *M*) per 100 cc. of medium. The Bios II, per 100 cc. of medium, was equivalent to 4.0 cc. of the original extract which had been prepared from malt sprouts by the method of Lucas.[3]

In order to make direct comparisons of the cultures it was necessary to standardize the method of preparing the inoculum. Since several of the strains grew slowly, or not at all, in Medium C, the technique of Miller, Eastcott and Maconachie[9] was adopted. The cultures were transferred daily, for four days, in a medium containing 2% dextrose and 0.5% peptone. The twenty-four-hour culture, from the fourth transfer, was filtered rapidly on a small sterile filter. The yeast was then washed three or four times with sterile distilled water and a portion suspended in sufficient sterile Medium C so that 1 cc. contained the proper number of cells for inoculation of each experimental flask. The yeast count in the various media was determined by means of a Thoma–Zeiss counting chamber after twenty-four hours of incubation at 30°. When the count = 1 there are 250,000 cells per cc. Inoculations were made such that the initial count was one.

In Table II are given the actual counts in the Control (Medium C), together with the relative counts in the control medium and the control to which had been added (a) Bios II, (b) Bios II and *i*-inositol, (c) Bios II and magnesium sulfate and (d) Bios II, inositol and magnesium sulfate. Growth was also determined in the control to which had been added magnesium sulfate, inositol and both magnesium sulfate and inositol; since no increased growth was obtained over the controls the values are not included in the table.

TABLE II

Relative growth of various strains of *Saccharomyces cerevisiae* in Medium C (control) and in the control to which Bios II, inositol and magnesium sulfate had been added. (When the count = 1 there are 250,000 cells per cc.).

| Medium strain | Actual count in control | Control | Bios II control | Inositol Bios II control | $MgSO_4$ Bios II control | $MgSO_4$ inositol Bios II control |
|---|---|---|---|---|---|---|
| 5 | 3 | 1 | 15 | 22 | 10 | 14 |
| 6 | 2 | 1 | 3 | 5 | 1 | 10 |
| 7 | 1 | 1 | 11 | 15 | 14 | 19 |
| 9 | 2 | 1 | 2 | 2 | 4 | 4 |
| 10 | 5 | 1 | 14 | 21 | 19 | 88 |
| 11 | 6 | 1 | 4 | 4 | 8 | 19 |
| 16 | 4 | 1 | 21 | 21 | 25 | 38 |
| 18 | 10 | 1 | 1 | 2 | 2 | 13 |
| 19 | 3 | 1 | 1 | 4 | 22 | 27 |
| 22 | 1 | 1 | 21 | 40 | 46 | 100 |
| 26 | 12 | 1 | 2 | 2 | 15 | 25 |
| 41 | 5 | 1 | 3 | 4 | 6 | 14 |
| 42 | 7 | 1 | 5 | 7 | 4 | 51 |

It is at once evident that the various strains of yeast show wide differences in response to the different media. For convenient comparison the various strains may be divided into the following three groups:

*Group I.* The addition of magnesium sulfate with Bios II does not give increased growth. This group includes strains Nos. 5, 6 and 42. With these strains the counts were actually de-

(9) W. L. Miller, Edna V. Eastcott and J. E. Maconachie, THIS JOURNAL, 55, 1502 (1933).

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.


AMES, IOWA RECEIVED JULY 15, 1938

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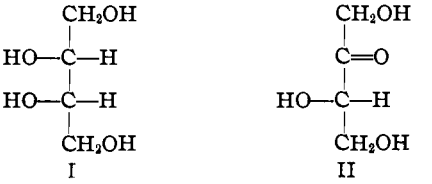
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

# 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.




I II

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).