The reduction products were not isolated directly but converted first into the corresponding dimethylpiperidinium iodides by the addition of methyl iodide and excess barium hydroxide to the alcoholic solution. In the case of IV, the aqueous solution was first evaporated to dryness and the solid residue taken up in alcohol. The alcoholic solution was dried and methyl iodide added as above.

After warming for twelve hours, the solution was filtered and dry hydrogen chloride passed in to precipitate barium chloride. This was filtered and, after shaking the filtrate with anhydrous sodium carbonate, the alcohol was removed and the last bits of solid product precipitated by the addition of ethyl acetate and ether. The products obtained from the reduction of II and IV were recrystallized from ethyl alcohol-ethyl acetate. They melted at 333–335° with decomposition. Analysis and comparison with an authentic sample showed them to be dimethylpiperidinium iodide, m. p. 334° (dec.).[19]

(19) Wedekind and Oechslen, *Ber.*, **35**, 1076 (1902).

3-Phenoxydimethylpiperidinium Iodide.—The reduction product of III gave a derivative melting, after recrystallization from ethyl alcohol-ethyl acetate, at 177–178° (corr.). It was obtained in the form of small blunt needles which were only moderately soluble in alcohol. Analysis showed it to be the 3-phenoxy derivative. The yield was 87%.

Anal. Calcd. for $C_{13}H_{20}ONI$: I, 38.10. Found: I, 37.97, 38.05.

Summary

1. The preparation of a number of new pyridyl ethers is described.

2. These ethers along with several previously known have been converted into their methyl and ethyl pyridinium salts for pharmacological examination.

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

The Production of Dihydroxyacetone by the Action of Acetobacter Suboxydans upon Glycerol*

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Since the discovery by Bertrand[1–3] that his "sorbose" bacterium (*Acetobacter xylinum*) transformed many polyhydric alcohols into the corresponding ketose sugars, several other species of the genus *Acetobacter* have been found to behave similarly. This type of conversion has been studied particularly with reference to the production of *l*-sorbose by the action of the appropriate organism upon sorbitol; the sorbose then serves as the starting point for the synthesis of ascorbic acid. The influence of the concentration of sorbitol upon the production of sorbose by the action of *Acetobacter suboxydans* has been studied quantitatively by Fulmer, Dunning, Guymon and Underkofler.[4] The authors give a brief literature survey and state the reasons for the use of *Acetobacter suboxydans* in the fermentation. The present communication deals with the optimum conditions for the production and isolation of dihydroxyacetone produced by the action of *Acetobacter suboxydans* upon glycerol.

Bertrand[1–3] was the first to report this conversion by *Acetobacter xylinum;* he isolated and identified the material. This reaction by the above organism was also studied by Fischer and Mildbrand[5] and by Bernhauer and Schoen.[6] Other organisms employed for this purpose include *Acetobacter dioxyacetonicum* by Virtanen and Bärlund[7] and *Acetobacter suboxydans* by visser't Hooft,[8] Virtanen and Nordlund,[9] and by Neuberg and Hofmann.[10] The latter authors considerably improved the methods of crystallization of the dihydroxyacetone and recovered 77% of the theoretical yield.

Experimental

Methods.—The culture of *Acetobacter suboxydans* was obtained from the American Type Culture Collection and is listed as No. 621. The stock cultures were carried on malt-extract agar slants. The cultures used for inoculation in these studies were kept active by transfer each forty-eight hours into a medium containing per 100 cc. 0.5 g. of yeast extract (Difco powdered product) and 6 g. of glycerol. Preliminary experiments showed temperatures of 28–30° to be optimum and all subsequent incubations

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(1) G. Bertrand, *Compt. rend.*, **122**, 900 (1896); *Bull. soc. chim.*, [3] **15**, 627 (1896).

(2) G. Bertrand, *Compt. rend.*, **126**, 984 (1898); *Bull. soc. chim.*, [3] **19**, 502 (1898).

(3) G. Bertrand, *Ann. chim. phys.*, [8] **3**, 181 (1904).

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

(5) H. O. L. Fischer and H. Mildbrand, *Ber.*, **57**, 707 (1924).

(6) K. Bernhauer and K. Schoen, *Z. physiol. Chem.*, **177**, 107 (1928).

(7) A. I. Virtanen and B. Bärlund, *Biochem. Z.*, **169**, 169 (1926).

(8) F. visser't Hooft "Biochemische onderzoekingen over het Geslacht Acetobacter," Thesis, Delft, 1925.

(9) A. I. Virtanen and M. Nordlund, *Biochem. J.*, **27**, 442 (1933).

(10) C. Neuberg and E. Hofmann, *Biochem. Z.*, **279**, 318 (1935).

were at 28°. It was also found that concentrations of glycerol above 6% gave decreased yields of dihydroxyacetone. There was no appreciable difference between the action on C. P. glycerol and on glycerol which had been distilled in a vacuum. In each case the inoculum consisted of 3 cc. of active (twenty-four to seventy-two hour) culture per 100 cc. of fresh medium; 100 cc. of medium was used in 300-cc. Erlenmeyer flasks.

It has been noted[4] in studies on the production of sorbose by the action of *Acetobacter suboxydans* that disturbing the culture materially reduced the yield. A similar phenomenon was observed with reference to the production of dihydroxyacetone. Hence, each datum presented represents an analysis on a separate flask.

The course of the conversion of the glycerol into dihydroxyacetone was followed by use of the Shaffer–Hartmann[11] method. The dextrose value was multiplied by 1.5 to obtain the value for dihydroxyacetone. This factor was verified on the pure compound and is accurate only if the amount of the dihydroxyacetone in the sample is in the neighborhood of 75 mg. This finding agrees with that of Bernhauer and Schoen.[6]

The Development of the Medium.—In Table I are given the data for the effect of varying concentrations of yeast extract and of potassium dihydrogen phosphate and of various *p*H values upon the yields of dihydroyxacetone. Although analyses were obtained for periods of two to fourteen days, only the results for the seven-day period are given. In all cases incubation beyond the seven-day period did not give increased yields.

TABLE I

THE INFLUENCE OF THE CONCENTRATION OF YEAST EXTRACT AND OF POTASSIUM DIHYDROGEN PHOSPHATE AND OF *p*H VALUE UPON THE PRODUCTION OF DIHYDROXYACETONE BY THE ACTION OF *Acetobacter suboxydans* ON GLYCEROL

I Yeast extract		II KH_2PO_4		III *p*H	
G. per 100 cc.	Yield, %	G. per 100 cc.	Yield, %	*p*H	Yield, %
0.05	57	0.00	87	9.0	0
.10	67	.30	91	8.0	49
.20	72	.60	90	7.0	91
.30	75	.80	89	6.0	91
.40	76	1.00	87	5.5	89
.50	80	1.50	86	5.0	87
1.00	80	2.00	82	4.0	87
				3.0	87
				2.0	0

I. Medium contained per 100 cc.: 6.0 g. glycerol, yeast extract vary. II. Medium contained per 100 cc.: 6.0 g. glycerol, 0.50 g. yeast extract, KH_2PO_4 vary. III. Medium contained per 100 cc.: 6.0 g. glycerol, 0.50 g. yeast extract, 0.30 g. KH_2PO_4, *p*H vary.

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

The following conclusions may be drawn from the data presented. The medium must contain at least 0.5% of yeast extract. Optimum yields are obtained with concentrations of potassium dihydrogen phosphate of 0.10–0.30%; further addition of the phosphate decreases the yields. The optimum *p*H range is from 5.5 to 7.0.

The Recovery of Crystalline Dihydroxyacetone.—The method of Bertrand[3] did not give significant yields of crystals. The procedure of Fischer and Mildbrand[5] gave beautiful white crystals but the yield was small. The method finally adopted is a modification of that developed by Neuberg and Hofmann.[10] This modified procedure is illustrated by the example given below.

To 1000 cc. of the fermented medium are added 10 g. each of Norite, calcium carbonate and diatomaceous earth, the medium shaken and filtered. The clear filtrate is evaporated below 35°, *in vacuo*, to about 150 cc., 3–4 volumes of absolute alcohol are added, with stirring, and the mixture filtered. Most of the alcohol is evaporated from the filtrate at 30°, *in vacuo*, and the sirup poured into 10 volumes of acetone, stirring vigorously. The turbid solution is allowed to stand overnight, shaken with Norite and filtered. The clear filtrate is evaporated at 30°, *in vacuo*, to a very thick sirup. The remaining water and acetone are removed in a vacuum desiccator over sulfuric acid. The sirup crystallizes spontaneously into an almost solid mass. Seeding the sirup with a few crystals of dihydroxyacetone accelerates the crystallization somewhat but is not required. The crystalline mass is triturated with *cold* absolute alcohol, filtered with suction, and washed with *cold* absolute alcohol until the pure white crystals are obtained. The crystals are dried in a vacuum desiccator. A further yield may be obtained by combining mother liquors and wash liquids from several batches and proceeding as before by evaporating *in vacuo*, pouring into acetone, etc. The first crop is practically pure but it is readily purified further by recrystallization from acetone. By the above procedure over 80% of the dihydroxyacetone, as determined by the Shaffer–Hartmann method, is readily recovered as crystalline dihydroxyacetone.

Summary

A simple procedure has been given for the production of dihydroxyacetone by the action of *Acetobacter suboxydans* upon glycerol and for the preparation of the crystalline product from the fermented medium.

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