necessary to dry the solution before addition of the 2,5-dimethoxyaniline.

Experimental

5,8-Dimethoxy-4-quinolinol (I). Method A.—2,5-Dimethoxyaniline (0.75 mole) was condensed with ethyl ethoxalylacetate (from one mole of the sodium salt) essentially according to the procedure of Surrey and Hammer.[10] The crude ethyl 5,8-dimethoxy-4-hydroxy-2-quinolinecarboxylate (190 g., m. p. 139–143°) was saponified in dilute sodium hydroxide and upon acidification gave 165 g. of crude 5,8-dimethoxy-4-hydroxy-2-quinolinecarboxylic acid (m. p. 215–216° dec.). The crude substance was decarboxylated by heating in phenyl ether giving an 88% yield of crude I (m. p. 216–219°). I was recrystallized from isopropyl alcohol giving short tan-colored crystals which melted at 220–221°.

Anal. Calcd. for $C_{11}H_{11}NO_3$: N, 6.82. Found: N, 6.76.

Method B.—Following the general procedure of Price and Roberts,[11] an 86% yield of crude ethyl 5,8-dimethoxy-4-hydroxy-3-quinolinecarboxylate (m. p. 190–194°) was obtained by condensation of 0.35 mole of diethyl ethoxymethylenemalonate[12] with 0.3 mole 2,5-dimethoxyaniline. The crude ester (69.2 g.) was saponified, yielding 61.7 g. of crude 5,8-dimethoxy-4-hydroxy-3-quinolinecarboxylic acid (m. p. 254–256° dec.) which gave a 93% yield of crude I (m. p. 217–220°) when decarboxylated in boiling phenyl ether.

5,8-Dimethoxy-4-chloroquinoline (II).—Two-tenths mole (41 g.) of I was heated with 150 ml. of phosphoryl trichloride until the solid was dissolved, then most of the excess phosphoryl trichloride was removed in vacuum and the residue poured into a flask containing 200–300 ml. of chipped ice and water. The cold solution was made alkaline with concentrated ammonia water, allowed to stand 2–3 hours and the solid removed by filtration. The crude II was recrystallized from dilute (25–50%) ethyl alcohol giving long white needles; the yield was 35 g. (78%), m. p. 110–111°.

Anal. Calcd. for $C_{11}H_{10}ClNO_2$: Cl, 15.84. Found: Cl, 16.08.

5,8-Dimethoxyquinoline (III).—One-tenth mole (22.4 g.) of II was reduced with hydrogen and palladium–charcoal in accordance with the generally accepted procedure.[13] After isolation of the 5,8-dimethoxyquinoline, the solid was recrystallized from low boiling ligroin (60–80°) as short white needles. The yield was 15.2 g. (80%), m. p. 75–76°.

Anal. Calcd. for $C_{11}H_{11}NO_2$: N, 7.39. Found: N, 7.28.

(12) Fuson, Parham and Reed, *J. Org. Chem.*, **11**, 194 (1946); Parham and Reed, "Organic Syntheses," Vol. 28, John Wiley and Sons, Inc., New York, N. Y., 1948, p. 60.

(13) Neumann, Sommer, Kaslow and Shriner, "Organic Syntheses," Vol. 26, John Wiley and Sons, Inc., New York, N. Y., 1946, p. 45.

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The Utilization of Formaldehyde by Propionic Acid Bacteria[1]

BY FREDERICK W. LEAVER

During studies on the fermentation of various substrates by *Propionibacterium arabinosum* C^{14} formaldehyde (0.001 *M*) was included in the medium to test the possibility that it could be utilized. Glycerol, *i*-erythritol, pyruvate and glucose served as substrates in separate experiments, and in each case a substantial amount of C^{14} was incorporated in the resulting propionic acid. This acid was extracted from the medium by ether and purified by partition chromatography.[2] The propionic acid from the glycerol fermentation was diluted approximately 1:1 with known acid as carrier and the S-benzylthiouronium salt was made and recrystallized from hot water. The melting point (148–149° uncor.) was the same as that of an authentic sample, and the mixed melting point was not depressed. The specific activity of the acid regenerated from the derivative was the same as that determined prior to making the derivative. The activity was determined by counting as barium carbonate the carbon dioxide resulting from the wet combustion[3] of the free propionic acid. The propionic acid was degraded to carbon dioxide and acetic acid by oxidation with chromic acid.[4] The resulting acetate which represents the α- and β-carbons of the original propionic acid was separated from any residual propionic acid by partition chromatography, converted to carbon dioxide by wet combustion, and the activity determined. The activity of the carboxyl group of the propionic acid was calculated from the values for the total propionate and the acetate derived therefrom. An aliquot of this acetate from the glycerol fermentation was further degraded by pyrolyzing the barium salt to carbon dioxide and acetone. The carbon dioxide represents the α carbon of the original propionic acid. The acetone was degraded by NaOI to CHI_3 and acetate, and the CHI_3, which represents the β-carbon of the propionic acid was oxidized to CO by $AgNO_3$, and then to CO_2 by I_2O_5. The activity expressed as counts per minute per mM. of carbon was distributed in the propionic acid as shown.

$$\underset{CH_3}{916}\text{—}\underset{CH_2}{858}\text{—}\underset{COOH}{1570}$$

A summary of the activities found in the propionic acid produced from the fermentation of various substrates is given in the table.

Since it has previously been shown that carbon dioxide is fixed only in the carboxyl group of propionic acid,[5] it is clear that the formaldehyde was not utilized exclusively via conversion to carbon dioxide. Furthermore, since the specific activity of the carboxyl group is appreciably higher than that of the carbon dioxide in every case it seems apparent that formaldehyde carbon was incorporated into the carboxyl position of propionate by some mechanism in addition to carbon

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(2) F. A. Isherwood, *Biochem. J.*, **40**, 688 (1946).

(3) D. D. Van Slyke and J. Folch, *J. Biol. Chem.*, **136**, 509 (1940).

(4) P. Nahinsky and S. Ruben, THIS JOURNAL, **63**, 2275 (1941).

(5) H. G. Wood, C. H. Werkman, A. Hemingway and A. O. Nier, *Proc. Soc. of Expt. Biol. Med.*, **46**, 313 (1941).

TABLE I

5% washed wet cells was used per cup in 0.3 *M* phosphate buffer of *p*H of 7.0 and 0.6% $NaHCO_3$ under 400 mm. pressure of CO_2. Substrate was added to approximate 20 mM. of carbon in all experiments except of pyruvic where 10 mM. was added. Glycerol and pyruvic was fermented for 18 hr., glucose for 6 hr. and erythritol for 42 hr. After 0.5 hr. of fermentation 50 millimoles of formaldehyde containing 67,000 counts was added from a side arm. Total volume was 50 ml.

Substrate fermented	Fermented, mM.	Propionic acid produced, mM.	CO_2[a]	Propionic acid: Average[a] activity	Carboxyl[a] group	α and β[a] carbon	Total activity
Glycerol	3.63	3.36	274	2180	3110	1750	22,000
i-Erythritol	3.96	4.08	604	2300	3650	1630	28,200
Pyruvic acid	2.64	0.76	1230	2500	3200	2150	5,700
Glucose	1.62	2.32	308	2320	..	..	16,100

[a] Activity expressed as counts/minute/mM. of carbon.

dioxide fixation. In relation to these observations it is of interest that Wood and Werkman[6] identified formaldehyde in the fermentation medium of *Propionibacterium* when dimedon was used as a trapping agent.

It is clear from the table that the distribution of formaldehyde carbon in the propionic acid from the various fermentations is quite similar, although different substrates have been employed. These data indicate that formaldehyde participates in the formation of propionic acid in a manner common to all the fermentations with *Propionibacterium arabinosum* thus far investigated, and that it may be an essential intermediate in the reactions involved. This problem is being investigated further.

(6) H. G. Wood and C. H. Werkman, *J. of Bacteriology*, **30**, 652 (1935).

DEPARTMENT OF BIOCHEMISTRY FREDRICK W. LEAVER
WESTERN RESERVE UNIVERSITY SCHOOL OF MEDICINE
CLEVELAND 6, OHIO
RECEIVED JUNE 28, 1950

An Improved Method for the Hydrolysis of Diazonium Salts

BY JOHN P. LAMBOOY

The great importance of the replacement of a diazonium group by a hydroxyl group is well known in the laboratory and in the chemical manufacturing industry. In the majority of cases the yields from the reaction are discouragingly small as a result of the coupling of the phenol with the undecomposed diazonium compound and the formation of tars.

Our recent need for a variety of phenols led us to investigate procedures by which the yield of the reaction could be increased. The improved procedure is based simply on the provisions that the phenol is removed immediately after its formation by the hydrolysis being performed in an actively steam distilling system and that the diazonium salt solution be dilute so that the chances of side reactions occurring are reduced. Not only does the procedure appear to be one of general usefulness in cases where the phenol is volatile with steam but the yields are considerably increased and the quality of the product is unusually high.

Experimental

A three-liter, round-bottom flask is equipped for ordinary steam distillation except that in addition to the steam inlet tube and the vapor outlet tube a cold finger type addition tube (Fig. 1) is also passed through the stopper. This tube reaches to within 1 cm. of the surface of the hydrolysis mixture. To the flask is added a solution of 200 ml. of water and 150 ml. of concentrated sulfuric acid. This solution is brought to the boiling point by a burner and then steam injection is begun. Any time after vapors begin to condense in the condenser the addition of the diazonium solution may be begun.

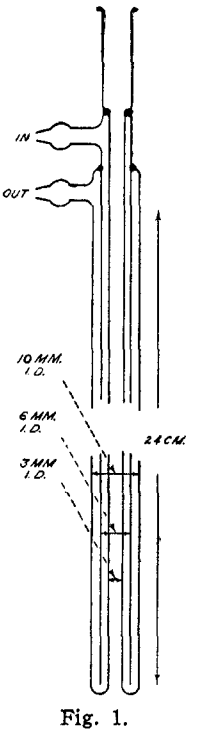

Fig. 1.

Just preceding and during the addition of from 80 to 100 ml. of the diazonium solution, the addition tube is cooled by the rapid flow of cold water. The rate of addition must be controlled or the rate of steam injection reduced to prevent excessive frothing. The distillation is continued until droplets of phenol are no longer seen in the condensate and until the volume of the hydrolyzing solution has returned almost to its starting level, before another addition is made. By controlled application of heat from the burner, the volume of the hydrolyzing solution can be maintained fairly constant.

Using the cold diazonium salt solution prepared from *o*-toluidine with 20% sulfuric acid and sodium nitrite, this modification has been used on 0.5-, 1.5-, 2.0- and 3.5-mole batches. In all cases the amine sulfate solutions were prepared in 0.5-mole lots and in all but the 0.5-mole batch, kept in the refrigerator until needed. Once started, each successive 0.5-mole lot was diazotized during the hydrolysis of the preceding one. Due to the accumulation of sulfuric acid in the boiling flask it was necessary to permit a gradual increase in volume from the original 350 ml. to approximately 1700 ml. in the case of the 3.5-mole batch. Corresponding volume increases were also permitted in the 1.5- and 2.0-mole batches. Hydrolysis of the 1.5-mole batch was done by slow and continuous addition of the diazonium solution. This was found to be less convenient than the periodic addition of portions because of the need to keep large volumes of diazonium solution cold in the vicinity of the hot distillation apparatus. The time required was somewhat increased because of the refluxing caused by the continuous flow of cold water through the addition tube.

The *o*-cresol was extracted with ether and processed in the usual fashion. After removal of the ether the material was fractionated through a saddle packed column 50 cm. long and 18 mm. i. d. equipped with a cutter head. The *o*-cresol, boiling at 190–191° (746–747 mm.), produced a pure white crystalline material melting 33–34° (cor.).

The procedure has been tried on a number of substituted anilines, and while the quantities of materials used in the