Quaternary Derivation of Pyridyl Ethers.   Onium Compounds.   XVI

BY R. R. RENSHAW AND R. C. CONN[1]

In previous publications the synthesis[2] and the pharmacological effects, determined by Reid Hunt,[3] of certain onium derivatives of pyridine, piperidine and pyrrolidine have been described. From these data and other work it was found that many of these quaternary derivatives without substitution on the cyclic carbon atoms acted on the autonomic nervous system producing the acetylcholine effect or the nicotine actions or both. Thus the carbethoxypyridinium ion was 75 times as active as choline in producing the acetylcholine effect and the beta-phenoxyethyl-methyl-pyrrolidinium ion half as active as nicotine in causing a rise of blood pressure.[3b]

A limited number of quaternary compounds having substituents on the cyclic carbon have been synthesized and studied. With the exception of quaternary derivatives of nicotine acid ester[3a,4] and its hydrogenated product[3a,4a] most of these showed little or no effect on the autonomic system even though the groups present[3b] (amide and acetamino) would produce these effects when introduced in the choline structure. Since the number of compounds of this latter type which have been investigated was so small we began in this Laboratory the preparation of a series of ethers, of choline type esters and of carbaminoyl derivatives of these heterocycles. The latter two fields will be the subject of papers to follow.

2-Pyridyl Ethers.—A number of 2-pyridyl ethers were prepared by heating 2-bromopyridine with the alkali salts of alcohols and phenols. Yields of 90% and better of 2-aryloxypyridines were obtained with no indication of any N-aryl-pyridines being formed. This is a more satisfactory method than that reported by Chichibabin[5] for the preparation of 2-phenoxypyridine, the only alpha-substituted pyridine reported in the literature. He obtained a mixture of ortho- and para-pyridyl phenols together with some 2-phenoxypyridine by warming 2-pyridyl diazotate with phenol.

The reaction was extended to include the use of alkylated phenols, resorcinol as a typical dihydric phenol and benzyl alcohol. With resorcinol, although the quantities of reactants were such as to give the mono-ether, only the dipyridoxy ether and an unidentified, very difficultly soluble, red crystalline product were obtained.

These 2-pyridyl ethers are very weak bases. This is probably due to the proximity of the aryloxy grouping to the ring nitrogen. They fail to deposit picrates from either ether or benzene solution and may be steam distilled from solutions of mineral acids. This behavior is in contrast to that of the corresponding 3- and 4-substituted ethers of pyridine which form stable picrates and salts of mineral acids. Alkyl halides add much more slowly to the 2-substituted ethers than to the corresponding 3- and 4-substituted derivatives although the final yields of pyridinium halides are essentially the same. 2-Pyridyl-benzyl ether did not yield a methiodide.

4-Pyridyl Ethers.—4-Pyridyl ethers were prepared by the interesting method of Koenigs and Greiner[6] whereby 4-pyridylpyridinium dichloride is heated with an alcohol or phenol in the optional presence of an acid binding agent. A number of previously reported ethers were prepared in this way and, in addition, one new alkoxy ether, 4-n-butoxypyridine, was prepared.

Methyl and ethyl iodide were also added to N-4'-pyridyl-4-pyridone (I) first prepared by Arndt

I

and Kalischek[7] by warming 4-hydroxypyridine with acetic anhydride. Although first considered by them to be 4,4'-dipyridyl ether, Arndt[8] later showed it to have the N-pyridyl-pyridone structure (I). We have found that, whereas I forms dibasic salts with hydrochloric, chloroplatinic and chloroauric acids, it will only add one mole of alkyl halide. This may be due to a steric effect or to the decreased basicity of the pyridone nitrogen.[9] Further confirmation for the pyridone structure

(1) This is the first paper constructed from a portion of a thesis presented by R. C. Conn, June, 1935, for the degree of Doctor of Philosophy at New York University.

(2) Renshaw and Shand, THIS JOURNAL, **54**, 1474 (1932).

(3) Hunt and Renshaw, (a) *J. Pharmacol.*, **35**, 75 (1929); (b) **37**, 177 (1929).

(4) (a) Loewy and Wolffenstein, *Therap. Gegenwart*, **61**, 287 (1920); (b) Haramaki, *Biochem. Z.*, **130**, 267 (1922).
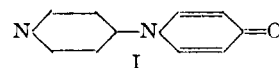
(5) A. E. Chichibabin, *J. Russ. Phys.-Chem. Soc.*, **50**, 502 (1918).

(6) Koenigs and Greiner, *Ber.*, **64**, 1049 (1931); German Patent 536,891.

(7) Arndt and Kalischek, *Ber.*, **63**, 592 (1930).

(8) Arndt, *ibid.*, **65**, 92 (1932).

(9) Weitz, Werner and Schwechter, *ibid.*, **59**, 2307 (1926).

lies in the fact that both methiodide and ethiodide are colored.

3-Pyridyl Ethers.—No 3-substituted aryl ethers are reported in the literature and only two (3-methoxy[10,11] and 3-ethoxy[12,13]) alkyl ethers have been prepared.

We have prepared 3-phenoxypyridine in the two following ways:

(1) Pyridine ⟶ 3-pyridyl sulfonic acid ⟶ 3-hydroxy-pyridine ⟶ 3-phenoxypyridine

(2) 2-Chloro-5-nitropyridine ⟶ 3-aminopyridine ⟶ 3-iodopyridine ⟶ 3-phenoxypyridine

The final step (phenylation) in both these syntheses was carried out according to the method of Ullmann.[14] Method 2 proved to be the more satisfactory because of the better yields and fewer experimental difficulties in the intermediate steps and because of the considerably greater yield in the final phenylation.

Reduction of Ethers.—The catalytic reduction of the methiodides of the three isomeric phenoxy-pyridines prepared in this investigation was attempted with the hope of obtaining the corresponding phenoxypiperidines for the purpose of physiological study. It was found that both 2- and 4-phenoxymethylpyridinium iodide were reduced with cleavage of the phenoxy grouping, phenol and N-methylpiperidine being formed. This behavior is consistent with results obtained by other investigators. Graves[15] found that 2-methoxypyridine underwent a similar reductive cleavage with the formation of methyl alcohol and piperidine. Koenigs and Neumann[16] reduced 4-pyridyl ethers with sodium–alcohol and obtained 90% yields of piperidine.

In contrast to the behavior of the 2- and 4-phenoxypyridines, the catalytic reduction of 3-phenoxymethylpyridinium iodide proceeded rapidly and in good yields without loss of the 3-phenoxy group. In one experiment, however, where a comparatively large amount of catalyst was used, a cleavage did take place to an extent of almost 50%. In all cases the reduction product was converted directly into the dimethylpiperidinium compound by warming in alcohol solution with methyl iodide and excess barium hydroxide. The stability of substituents in the 3-position

(10) H. Meyer, *Monatsh.*, **26**, 1311 (1905).
(11) Koenigs, Gredes and Serf, *Ber.*, **61**, 1022 (1928).
(12) O. Fischer and Renouf, *ibid.*, **17**, 1896 (1884).
(13) Weidel and Blau, *Monatsh.*, **6**, 651 (1885).
(14) Ullmann and Sponagel, *Ann.*, **350**, 86 (1906).
(15) T. B. Graves, THIS JOURNAL, **46**, 1460 (1924).
(16) Koenigs and Neumann, *Ber.*, **48**, 960 (1915).

as contrasted to the lability of 2- and 4-substituted groups is well illustrated by the behavior of these three phenoxy ethers on reduction. Similarly, the reactivity of 2-bromopyridine, which is essentially that of an aliphatic bromide, as contrasted to the inactivity of 3-iodopyridine is to be noted. This behavior is general, the stability and aromatic nature of 3-substituted pyridines being quite marked whereas 2- and 4-pyridyl derivatives are characterized by the lability and often by the anomalous behavior of the substituted groups.

Experimental Part

2-Pyridyl Ethers.—The method of preparation employed for the aryl ethers of this series was as follows: 0.05 mole of 2-bromopyridine,[17] 0.1 mole of phenol and 0.05 mole of anhydrous potassium carbonate were placed in a flask fitted with a long, upright air condenser and heated in a Wood's metal bath at 200–210° for three hours. The contents of the flask were then diluted with water, made strongly alkaline and steam distilled. The distillate was extracted with ether and the extract dried. After removing the ether, the residual oil was vacuum distilled. The yields and boiling points under reduced pressures obtained for these ethers are recorded in Table I.

The reaction with resorcinol was carried out in the same way except that only 0.025 mole of potassium carbonate was used. The reaction initially was quite vigorous, a deep red color developing in the melt. Resorcinol was removed from the reaction mixture by repeatedly washing with hot water until the aqueous extract no longer gave a color test with ferric chloride. The remaining tar was extracted with ether and the ethereal extract dried. A red, solid product remained which could only be dissolved in hot glacial acetic and concentrated sulfuric acids. No identification of this material has as yet been made. On working up the ether extract a solid residue was obtained which was purified by vacuum distillation and recrystallized from a benzene–ligroin mixture from which it separated as pale yellow plates, m. p. 154–156° (corr.). Analysis showed it to be resorcinol di-2-pyridyl ether.

2-Pyridylbenzyl Ether.—A solution of 1.15 g. (0.05 mole) of sodium in 22 g. (0.2 mole) of benzyl alcohol was heated with 7.9 g. (0.05 mole) of 2-bromopyridine for three hours at 210°. Since it was found that ether extraction of a hydrochloric acid solution of the product removed not only benzyl alcohol but 2-pyridylbenzyl ether as well, the two were separated by fractional vacuum distillation.

4-Pyridyl Ethers.—Yields and boiling points of the 4-pyridyl ethers prepared are listed in Table I. The four previously prepared aryl ethers were prepared from 4-pyridylpyridinium dichloride by the method of Koenigs and Greiner.[6] In each case 15 g. of recrystallized 4-pyridylpyridinium dichloride, 40 g. (excess) of phenol and 1.1 equivalents of the sodium salt of the phenol were used. The 70% yield obtained for 4-phenoxypyridine is somewhat better than that (53%) reported by the authors for the same compound. The sodium phenolates used here were

(17) Craig, THIS JOURNAL, **56**, 281 (1934).

TABLE I

2- AND 4-PYRIDYL ETHERS

Ether	Yield	°C.[a]	B. p. Mm.	Formula	Nitrogen, % Calcd.	Found
2-Phenoxypyridine[b]	92	134–135	11			
2-o-Cresoxypyridine	90	156–158	21	$C_{12}H_{11}ON$	7.56	7.71
2-p-Cresoxypyridine	92	171.5–172.5	22	$C_{12}H_{11}ON$	7.56	7.76
2-m-Cresoxypyridine	83	164–166	20	$C_{12}H_{11}ON$	7.56	7.83
2-Carvacroxypyridine	78	133–134	2	$C_{15}H_{17}ON$	6.16	6.13
Resorcinol di-2-pyridyl ether[c]	38	183–185	3	$C_{16}H_{12}O_2N_2$	10.6	10.5
2-Pyridyl benzyl ether	65	162–164	20	$C_{12}H_{11}ON$	7.56	7.20
4-n-Butoxypyridine	37	129–131	25	$C_9H_{13}ON$	9.27	9.7[d]
4-Methoxypyridine[e]	15	95–96[f]	31			
4-Phenoxypyridine	70	157–158[g]	21			
4-o-Cresoxypyridine	66	161–162[h]	19			
4-p-Cresoxypyridine	67	166–167[i]	22			
4-m-Cresoxypyridine	67	124–126[j]	4			

[a] All boiling points are corrected for stem emergence. [b] Chichibabin[4] gives 277–277.5°. [c] Yellow plates, m. p. 154–156° (corr.). [d] Converted directly into the methiodide without redistillation for further purification. [e] Prepared by heating 20 g. of purified 4-pyridylpyridinium dichloride and 20 g. of methyl alcohol in a sealed tube for five hours at 150°. Picrate, m. p. 171–172°. [f] Haitinger and Lieben [Monatsh., 6, 320 (1885)] give 190.5–191.0° at 738.3 mm. [g] Koenigs and Neumann (Ber., 48, 959 (1915)) give 134–136° at 10 mm. [h] Koenigs and Greiner[6] give 276–280°. [i] Koenigs and Greiner[6] give 288–290°. [j] Koenigs and Greiner[6] give 284–288°.

TABLE II

ONIUM DERIVATIVES OF PYRIDYL ETHERS

Pyridinium iodide	Crystal form	Yield, %	M. p., °C. (corr.)	Formula	I, %[a] Calcd.	Found	
2-Phenoxymethyl-	Four pointed starlike clusters	96	174–175	$C_{12}H_{12}ONI$	40.54	40.55	40.47
2-Phenoxyethyl-	Thin, irregular plates	57	150.5–151.5	$C_{13}H_{14}ONI$	38.81	38.83	38.77
2-o-Cresoxymethyl-	Needles	92	186–186.2 (dec.)	$C_{13}H_{14}ONI$	38.81	38.79	38.83
2-o-Cresoxyethyl-	Fine, narrow rectangles	69	122–124	$C_{14}H_{16}ONI$	37.21	37.16	37.12
2-p-Cresoxymethyl-	Thin, rectangular plates	93	149–150	$C_{13}H_{14}ONI$	38.81	38.75	38.72
2-m-Cresoxymethyl-	....	90	145–146.5	$C_{13}H_{14}ONI$	38.81	38.93	38.90
2-m-Cresoxyethyl-	....	70	126–126.5	$C_{14}H_{16}ONI$	37.21	37.22	37.19
2-Carvacroxymethyl-	Flat, transparent plates	85	134–135	$C_{16}H_{20}ONI$	34.38	34.20	34.20
3-Phenoxymethyl-	Narrow rectangular plates (yellow)	96	82.5–84	$C_{12}H_{12}ONI$	40.54	40.49	40.56
3-Phenoxyethyl-	Transparent plates	98	136–137	$C_{13}H_{14}ONI$	38.81	38.73	38.79
4-Phenoxymethyl-	Irregular clusters of short, stout plates	68	227.5–228.5	$C_{12}H_{12}ONI$	40.54	40.36	40.52
4-Phenoxyethyl-	Narrow rectangular plates	70	110.5–111	$C_{13}H_{14}ONI$	38.81	38.92	38.93
4-o-Cresoxymethyl	Thick needles	100	163–164	$C_{13}H_{14}ONI$	38.81	39.05	39.02
4-o-Cresoxyethyl-	Narrow plates	79	148	$C_{14}H_{16}ONI$	37.21	37.10	37.21
4-p-Cresoxymethyl-	Irregular masses of short, stout plates	81	163	$C_{13}H_{14}ONI$	38.81	38.88	38.84
4-p-Cresoxyethyl-	Long spear-like needles	93	126–126.5	$C_{14}H_{16}ONI$	37.21	37.25	37.24
4-p-Cresoxy-β-phenoxy-ethyl-[b]	Plates	64	129–130	$C_{20}H_{20}O_2NBr$			
4-m-Cresoxymethyl-	Irregular clusters of short, thick plates	92	157–158	$C_{13}H_{14}ONI$	38.81	38.69	38.76
4-m-Cresoxyethyl-	Irregular clusters of plates	85	128	$C_{14}H_{16}ONI$	37.21	37.21	37.00
4-m-Methoxymethyl-	Narrow, rectangular plates	100	145 (dec.)	$C_7H_{10}ONI$	50.60	50.76	50.57
4-n-Butoxymethyl-	Plates	100	74–75	$C_{10}H_{16}ONI$	43.32	43.28	43.29

[a] All halogen analyses were carried out by the Fajans method of titration using 0.01 N silver nitrate. [b] Obtained as the pyridinium bromide. Calcd. for $C_{20}H_{20}O_2NBr$: Br, 20.70. Found: Br, 20.63, 20.61.

prepared by adding sodium wire to the phenol in benzene, filtering and washing with ether.

4-n-Butoxypyridine was prepared by refluxing a suspension of 22 g. of purified 4-pyridylpyridinium dichloride in 400 cc. of n-butyl alcohol containing 6.8 g. of sodium. 4-

n-Butoxypyridine is insoluble in water and possesses an ethereal odor.

Preparation of Onium Derivatives.—The onium derivatives listed in Table II were prepared by allowing a solution of the pyridyl ether in 10 cc. of dry ether and an excess of

alkyl iodide to stand at room or slightly elevated temperatures for periods varying from one to six days. Addition of methyl iodide to the 3- and 4-pyridyl ethers proceeded rapidly at room temperature but ethyl iodide added more slowly and the reaction was carried out at 40–50°. Addition to the 2-pyridyl ethers was still less rapid but by heating to 70° satisfactory yields were obtained.

The methiodides of the 4-pyridyl ethers were recrystallized from a hot mixture of Cellosolve and ethyl acetate and the other onium derivatives were recrystallized from hot amyl alcohol–amyl acetate. All were well-defined, crystalline products and were not generally hygroscopic. The low melting point of 3-phenoxymethylpyridinium iodide is interesting and the higher melting point of the corresponding ethiodide (3-phenoxyethylpyridinium iodide) is particularly surprising. Whereas the methiodide tended to form an oil on recrystallization and was appreciably hygroscopic, the ethiodide showed no tendency to form oils, crystallized without seeding and was not appreciably hygroscopic.

4-p-Cresoxy-β-phenoxyethylpyridinium bromide was prepared by heating 2 g. (0.001 mole) of 4-cresoxypyridine and 2.2 g. (0.001 mole) of β-phenoxyethyl bromide in dry toluene for three days at 70–80°. The toluene was decanted and the separated oil dissolved in water, the aqueous solution extracted with ether and evaporated to dryness on the water-bath. The residue was dried thoroughly and washed several times with dry benzene. Crystallization was effected by dissolving in absolute alcohol and slowly removing the solvent under reduced pressure. The product was recrystallized from hot Cellosolve–ethyl acetate.

N-4'-Pyridyl-4-pyridone.—This product was prepared by heating 4-hydroxypyridine with acetic anhydride following the directions of Arndt and Kalischek.[7] Considerable difficulty was encountered in obtaining a product melting above 168°, although a sample melting in the range (m. p. 177–178°) given by these authors was obtained. The lower melting product gave a chloroplatinate and chloroaurate which analysis without recrystallization showed to be quite pure. It was therefore used directly for the preparation of onium compounds.

The chloroaurate of the pyridone was obtained as a yellow crystalline powder, m. p. 218–219° (corr.).

Anal. Calcd. for $C_{10}H_9ON_2Cl_4Au\cdot2H_2O$: Au, 44.39. Found: Au, 44.36.

The chloroplatinate was obtained as a buff powder melting above 300°.

Anal. Calcd. for $C_{20}H_{18}O_2N_4Cl_6Pt\cdot2H_2O$: Pt, 31.59. Found: Pt, 31.55

This pyridyl-pyridone forms a dihydrochloride which was obtained both by passing dry hydrogen chloride into an alcoholic solution of the base and by evaporating its solution in dilute hydrochloric acid. It was recrystallized from alcohol in which it was only sparingly soluble; m. p. 238° (dec.).

Anal. Calcd. for $C_{10}H_{10}ON_2Cl_2$: Cl, 28.95. Found: Cl, 28.90.

N-(4'-Methylpyridinium Iodide)-4-pyridone.—Methyl iodide was added to N-4'-pyridyl-4-pyridone, both in alcohol and benzene solution. The product obtained by adding ether to the alcoholic solution after twelve hours of

heating at 70° was quite impure and it was found that better results could be obtained by carrying out the reaction in dry benzene. A suspension in benzene, in which it is only moderately soluble, was heated with an excess of methyl iodide at 80° for twelve hours. The crystalline methiodide was filtered and the precipitate extracted with hot benzene to remove the non-alkylated product. The yield was 95% based on the quantity of the pyridone used. The methiodide was recrystallized from hot absolute alcohol from which it separated as yellowish-brown, rectangular plates, m. p. 238–238.5° (corr.). Repeated recrystallization and treatment with mercury and Norite failed to change the color. Analysis showed both the product obtained from alcohol and that obtained from benzene to be the monomethiodide.

Anal. Calcd. for $C_{11}H_{11}ON_2I$: I, 40.41. Found: I, 40.25, 40.20.

N - (4' - Ethylpyridinium Iodide) - 4 - pyridone.—Ethyl iodide was added to N-4'-pyridyl-4-pyridone in benzene solution in the same manner as methyl iodide. The yield of ethiodide was 91% based on the quantity of pyridone used. Recrystallization from hot absolute alcohol gave buff colored rectangular plates, m. p. 134–135° (corr.), which analyzed as the. monohydrate. Repeating the preparation and recrystallizing from hot Cellosolve–ethyl acetate gave a product with the same melting point and analysis Arndt and Kalischek[7] noted the curious tendency of the original pyridyl-pyridone to deposit a crystalline dihydrate from benzene solution when exposed to air.

Anal. Calcd. for $C_{12}H_{13}ON_2I\cdot H_2O$: I, 36.68. Found: I, 36.68, 36.73

3-Phenoxypyridine. Method 1.—Nine and five-tenths grams (0.1 mole) of 3-hydroxypyridine and 5.6 g. (0.1 mole) of potassium hydroxide were heated at 150° to drive off water and a 6-g. excess of 3-hydroxypyridine was then added together with 15.7 g. (0.1 mole) of bromobenzene and 0.2 g. of copper bronze. The mixture was heated at 200° in a flask fitted with a long, upright air condenser. After six hours heating was discontinued and the reaction mixture worked up in the manner given for the 2-pyridyl ethers. The 3-phenoxypyridine obtained was purified by vacuum distillation, b. p. 147–149 at 17 mm. The yield was 4.7 g. or 27%. 3-Phenoxypyridine has the same pleasant ethereal odor possessed by 2- and 4-phenoxypyridine. It, unlike them, is a liquid at room temperature. No appreciable reaction was obtained when anhydrous potassium carbonate was substituted for potassium hydroxide.

Anal. Calcd. for $C_{11}H_9ON$: N, 8.18. Found: N, 8.01.

Method 2.—Ten and two-tenths grams (0.05 mole) of 3-iodopyridine[18] and 6.1 g. (0.05 mole) of potassium phenolate in an excess of phenol were heated with 0.2 g. of copper bronze as before. A yield of 59% of 3-phenoxypyridine was obtained. Using anhydrous potassium carbonate, the yield was 46%.

Reduction of Ethers.—2-(II), 3-(III) and 4-(IV) phenoxymethylpyridinium iodides were reduced catalytically using Adams platinum oxide catalyst and an initial hydrogen pressure of 3 atm. II and III were reduced in alcohol solution but IV, which was quite difficultly soluble in alcohol, was dissolved in water and reduced in this solvent.

(18) C. Räth, *Ann.*, **486**, 101 (1931).

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.

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

BY L. A. UNDERKOFLER AND ELLIS I. FULMER

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

(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).