α- and γ-Picolinic Acid Hydrochlorides.—Oxidation of the corresponding picolines yielded the acids which were then isolated as the hydrochlorides.[8]

Ethyl Esters.—The ethyl esters of nicotinic, α-picolinic and γ-picolinic acids were prepared in yields ranging from 78–85% according to a procedure described by LaForge[9] for the preparation of ethyl nicotinate.

Ethyl quinoline-3-carboxylate was prepared by a series of reactions consisting of the bromination of quinoline hydrobromide,[10] formation of the nitrile with cuprous cyanide,[11] subsequent hydrolysis with 70% sulfuric acid[10] and esterification according to the method used by LaForge.[9] A 55% yield of the ethyl ester based on 3-bromoquinoline was obtained.

Pyrazine-2,3-dicarboxylic Acid.—The acid was prepared by the oxidation of quinoxaline according to directions given by Gabriel and Sonn.[12]

Quinoxaline.—In the preparation of quinoxaline, it was found necessary to use a dilute solution of *o*-phenylenediamine and glyoxal bisulfite; otherwise, polymeric substances formed and little if any of the product was obtained. Since the method[12] for the preparation of quinoxaline is vague, we are including the procedure we used.

In a one-liter erlenmeyer flask containing 400 ml. of water at 70°, was placed 27.0 g. (0.25 mole) of *o*-phenylenediamine. A solution of 68.8 g. (0.26 mole) of glyoxal bisulfite in 300 ml. of water heated to 70° was then added with shaking. The shaking was continued for five minutes after completion of the mixing. The solution was allowed to cool to approximately 40° and 100 g. of potassium carbonate added. The quinoxaline separated and was extracted with 100 ml. and three 50-ml. portions of ether. The ether extracts were combined, dried over anhydrous sodium sulfate and distilled. Yields from 29.6 to 30.5 g. of product (90.8–93.5% of theoretical) boiling at 112–115° (17 mm.) were obtained.

Benzyl Nicotinamide.—In a 200-ml. erlenmeyer flask, connected by ground glass joints to a calibrated water trap, in turn attached to a reflux condenser, were placed 12.3 g. (0.10 mole) of nicotinic acid and 10.7 g. (0.10 mole) of benzylamine. The mixture was heated gently until it became homogeneous. Seventy-five ml. of anhydrous xylene was added and the mixture refluxed for one hundred and fifty hours in an oil-bath kept at 165–170°. At the end of this time, 1.95 ml. of water was found in the trap (1.80 ml.—theoretical amount). On cooling, the product formed pure white crystals. A yield of 19.7 g. (93.0% of theoretical) of product, m. p. 72–73°, was obtained.

***n*-Amyl-α-picolinamide.**—In a 250-ml. round-bottomed flask fitted with a reflux condenser were placed 15.1 g. (0.10 mole) of ethyl α-picolinate and 17.4 g. (0.20 mole) of *n*-amylamine. The mixture was heated in an oil-bath maintained at 125° for twenty-five hours. Distillation *in vacuo* yielded 1.8 g. (11.9% recovery) of ethyl α-picolinate, 16.0 g. (83.3%) of product boiling at 135–136° (2 mm.) and 1.0 g. of residue.

(8) Weidel, *Ber.*, **12**, 1992 (1879); *Org. Syn.*, **20**, 79 (1940).
(9) LaForge, THIS JOURNAL, **50**, 2479 (1928).
(10) Claus and Collischonn, *Ber.*, **19**, 2763 (1886).
(11) Gilman and Spatz, THIS JOURNAL, **63**, 1553 (1941).
(12) Gabriel and Sonn, *Ber.*, **40**, 4850–4860 (1907).

Summary

1. Benzyl nicotinamide has been prepared and found to possess antispasmodic activity.

2. A series of fifteen other amides of nicotinic acid and related acids has been prepared and submitted for pharmacological testing.

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[CONTRIBUTION FROM THE FERMENTATION DIVISION, NORTHERN REGIONAL RESEARCH LABORATORY[1]]

Optical Isomers of 2,3-Butanediol Produced by Fermentation

BY GEORGE E. WARD, O. GLENN PETTIJOHN, LEWIS B. LOCKWOOD AND ROBERT D. COGHILL

In connection with fermentation studies conducted at this Laboratory on the production of 2,3-butanediol, some of the properties of the isolated products have been determined. *levo*-2,3-Butanediol, previously unknown in pure form and not recognized heretofore as a fermentation product, has been identified as the chief substance formed by the action of *Bacillus polymyxa* on grain mash substrates.

Previous reports concerning fermentation butanediol have stated it to be essentially optically inactive, the slight *dextro* rotation sometimes observed being attributed to the presence of a relatively small quantity of the *dextro* isomer. The butanediol produced by Harden and Walpole,[2] the first investigators in the field, was examined by Walpole[3] and was reported to be essentially a mixture of two optically inactive glycols. This product had resulted from the action of *Bacterium lactis aerogenes* (*Aerobacter aerogenes*) on glucose. The butanediol prepared by Kluyver's process,[4] presumably using *Aerobacter aerogenes*, was investigated by Böeseken and Cohen[5] and found to have a specific rotation of +2.42° and a melting point of +25°. Attempts at resolution were unsuccessful. These data suggested that the principal constituent was *meso*-2,3-butanediol and that a small quantity of the *dextro*-rotatory isomer was also present.

During the past three years we have conducted numerous fermentations with *Aerobacter aerogenes*. The 2,3-butanediol isolated *en masse* from such liquors has consistently had a specific rotation of approximately +1.0° and has shown a tendency to crystallize at room temperatures. These observations are in essential agreement with the data presented by Böeseken and Cohen. The discrepancy in specific rotation is not especially significant, since we have observed that appreciable fractionation occurs during distillation of the glycol. Unless a quantity of glycol is distilled *in toto*, the rotation exhibited by any

(1) The Northern Regional Research Laboratory is one of four regional laboratories operated by the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.
(2) Harden and Walpole, *Proc. Roy. Soc.* (London), Series B, **77**, 399 (1906).
(3) Walpole, *ibid.*, Series B, **83**, 272 (1911).
(4) Kluyver and Scheffer, U. S. Patent 1,899,156.
(5) Böeseken and Cohen, *Rec. trav. chim.*, **47**, 839 (1928).

given fraction may not be typical of that of the total product, the asymmetric isomers concentrating in the first fractions and the *meso* form appearing in the distillate in higher amounts toward the end of the distillation.

Besides studying the *Aerobacter* fermentation, we have investigated in some detail the fermentation of grain mashes by strains of *Bacillus polymyxa*. Although this species has long been known to produce 2,3-butanediol and acetylmethylcarbinol from starch, previous investigators have not reported differences between the butanediol of this origin and that formed by *Aerobacter* species. We have recently observed that the 2,3-butanediol produced by our preferred strain of *B. polymyxa* (N. R. R. L. No. B-510) differs markedly from the 2,3-butanediol produced by *Aerobacter aerogenes*, as shown in Table I.

TABLE I

	2,3-Butanediol isolated *en masse* from fermentation by	
	Bacillus polymyxa	*Aerobacter aerogenes*
Specific rotation (25°C.)	−13.0°	+1.0°
Melting point, °C.	+19	+25
Refractive index (25°C.)	1.4307	1.4384
Viscosity (25°C.), c. p.	41.0	118.0
Hydrate	None formed	Pentahydrate, m. p. +16.8°C.

Fractional distillation at 5 mm. pressure of butanediol possessing a specific rotation of approximately −13° has not yielded fractions of greater specific rotation than −13.19°, although such a procedure is effective in partially separating the *meso–dextro* mixture. It therefore seems probable that pure *levo*-2,3-butanediol has a specific rotation slightly in excess of −13.0°. It should be noted that we have not attempted resolution by procedures other than distillation, which would not separate the *dextro* and *levo* forms.

The highest specific rotation previously reported for optically active 2,3-butanediol was +6.9° for a sample of *dextro*-2,3-butanediol prepared from *dextro*-2,3-diaminobutane by Chappell.[6] There have been very few investigations of *levo*-2,3-butanediol. Neuberg and Nord[7] obtained small quantities of a substance possessing a specific rotation of −2.4° by the reducing action of yeast on diacetyl. Neuberg and Kobel[8] obtained an analogous preparation with a specific rotation of −5.51° by the action of yeast on acetylmethylcarbinol. In the light of our findings, it is apparent that these two products were composed principally of material other than *levo*-2,3-butanediol.

The fact that *levo*-2,3-butanediol does not form a hydrate is of importance in at least one of its possible applications, namely, as a constituent of antifreeze preparations. Freezing-point measurements of aqueous solutions show that whereas pure *meso*-butanediol with an equal weight of water forms a hydrate melting at +16.8°, the levorotatory isomer does not form such a hydrate, the freezing point depression decreasing regularly with increasing glycol concentration. *levo*-2,3-Butanediol appears to be approximately the equal of ethylene glycol in this respect.

Results are not yet at hand regarding the ability of other strains of *Bacillus polymyxa* to produce *levo*-2,3-butanediol. In view of the fact that the production of the *meso–dextro* mixture is characteristic of numerous strains of *Aerobacter*, it would not be surprising if many strains of *Bacillus polymyxa* will eventually be shown to be capable of producing the *levo* isomer.

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Summary

levo-2,3-Butanediol, possessing a specific rotation slightly in excess of −13.0°, has been isolated and identified as the chief substance formed by the action of *Bacillus polymyxa* (N. R. R. L. No. B-510) on grain mash substrates. This optical activity is far in excess of values previously ascribed to the asymmetric 2,3-butanediols.

In contrast to the *meso–dextro*-2,3-butanediol mixture produced in *Aerobacter aerogenes* fermentations, *levo*-2,3-butanediol possesses a relatively low viscosity and does not form a hydrate.

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(6) C. H. Chappell, "A Study of 2,3-Butylene Glycol and Its Derivatives," Thesis, 1935, Iowa State College, Ames, Iowa.

(7) Neuberg and Nord, *Ber.*, **52**, 2252 (1919).

(8) Neuberg and Kobel, *Biochem. Z.*, **160**, 250 (1925).