rived from liquid sulfides or mixed melting points of the solid sulfides were taken in all cases.

DEPARTMENT OF CHEMISTRY
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The Synthesis of New β-Diketones

BY ROBERT LEVINE AND JAMES K. SNEED

For other work, which is now in progress in this Laboratory, the following four β-diketones were required.

Nicotinoyltrifluoroacetone.—Commercial sodium methoxide (0.2 mole, 11.4 g. of commercial 95% material[1]) was placed in a 1000-ml. three-neck, round-bottom flask, equipped with ground glass joints and carrying a mercury-sealed stirrer, a reflux condenser and dropping funnel (drying tubes). The sodium methoxide was suspended in 250 ml. of anhydrous ether and to the rapidly stirred mixture, cooled in an ice-bath, 0.2 mole (28.4 g.) of ethyl trifluoroacetate, diluted to 125 ml. with anhydrous ether, was added. The ice-bath was removed and then 0.2 mole (24.2 g.) of 3-acetylpyridine, diluted to 125 ml. with anhydrous ether, was added dropwise. After the addition of the ketone was complete, the mixture was stirred and refluxed for two hours on a water-bath. The water bath was removed and water (*ca.* 100 ml., exothermic reaction) was added slowly to dissolve the solid which was present and the mixture extracted with several 100-ml. portions of ether to remove unreacted ester and ketone. The aqueous phase was acidified with 0.2 mole of glacial acetic acid and extracted with ether until the extracts no longer gave a positive test with alcoholic iron(III) chloride solution. The combined extracts were dried over Drierite, the solvent distilled and the residue crystallized to give 38.2 g. (88.0%) of nicotinoyltrifluoroacetone, m.p. 173.5–174°. *Anal.* Calcd. for $C_9H_6O_2NF_3$: C, 49.78; H, 2.79. Found: C, 49.87; H, 2.86. The β-diketone gave a green copper chelate, m.p. 262–262.5°. *Anal.* Calcd. for $C_{18}H_{10}O_4N_2F_6Cu$: C, 43.60; H, 2.02. Found: C, 43.34; H, 1.91.

Isonicotinoyltrifluoroacetone.—Using the apparatus described above, a mixture of 0.345 mole (47.3 g.) of 4-acetylpyridine, 0.345 mole (19.6 g.) of 95% sodium methoxide and 0.345 mole (49.0 g.) of ethyl trifluoroacetate was refluxed for six hours. Then, the addition of 0.345 mole (20.5 g.) of glacial acetic acid caused the precipitation of a mixture of sodium acetate and condensation product. Water (*ca.* 250 ml.) was added to the vigorously stirred reaction mixture to dissolve the sodium acetate and the mixture filtered. The precipitate was washed with several portions of water and dried in a vacuum desiccator. In this manner, there was obtained 71.4 g. (95.2%) of isonicotinoyltrifluoroacetone, m.p. 213–214.5° (sealed tube). *Anal.* Calcd. for $C_9H_6O_2NF_3$: C, 49.78; H, 2.79. Found: C, 49.81; H, 2.75. The β-diketone gave a green copper chelate, which decomposed without melting when heated above 280°. *Anal.* Calcd. for $C_{18}H_{10}O_4N_2F_6Cu$: C, 43.60; H, 2.02. Found: C, 43.40; H, 1.77.

Nicotinoyl-2-thenoylmethane.—When 0.25 mole (31.5 g.) of 2-acetylthiophene, 0.3 mole (41.1 g.) of methyl nicotinate and 0.50 mole of sodium amide[2] (prepared from 11.5 g. of sodium) were allowed to react for two hours, as described previously for other heterocyclic β-diketones,[2] and the mixture worked up as described above for the preparation of nicotinoyltrifluoroacetone, there was obtained 34.1 g. (59%) of nicotinoyl-2-thenoylmethane, m.p. 133–134°. *Anal.* Calcd. for $C_{12}H_9O_2NS$: C, 62.32; H, 3.92. Found: C, 62.46; H, 3.68. The β-diketone formed a picrate, m.p. 208–209°. *Anal.* Calcd. for $C_{18}H_{12}O_9N_4S$: N, 12.17. Found: N, 12.29.

Isonicotinoyl-2-thenoylmethane.—A mixture of 0.50 mole (63.0 g.) of 2-acetylthiophene, 0.25 mole (34.3 g.) of methyl isonicotinate and 0.25 mole (14.5 g.) of 95% sodium methoxide was refluxed for six hours and the reaction stopped by the addition of glacial acetic acid and water as described above in the preparation of isonicotinoyltrifluoroacetone. The mixture was extracted with ether until a negative test was obtained with alcoholic iron(III) chloride solution, the extracts dried over Drierite and the solvent and unreacted reactants distilled. The residue was recrystallized from 95% ethanol to give 37.6 g. (64.8%) of isonicotinoyl-2-thenoylmethane; m.p. 152–152.5°. *Anal.* Calcd. for $C_{12}H_9O_2NS$: C, 62.32; H, 3.92. Found: C, 62.25; H, 3.68. The β-diketone gave a picrate, m.p. 211–212.5°. *Anal.* Calcd. for $C_{18}H_{12}O_9N_4S$: N, 12.17. Found: N, 12.42.

(1) Purchased from the Mathieson Chemical Corporation, Niagara Falls, N. Y.

(2) Harris and Levine, THIS JOURNAL, **70**, 3360 (1948).

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Preparation and Microbiological Activity of an Homolog of Lysine

BY A. D. MCLAREN AND C. A. KNIGHT

Considerable attention has been directed recently to the investigation of compounds which are structurally similar to metabolically important substances.[1] In the present communication we are reporting the synthesis of a lysine homolog, ε-C-methyllysine (2,6-diaminoheptanoic acid), and some effects of this compound on the growth of 2 lactobacilli, *Leuconostoc mesenteroides* P-60 and *Streptococcus faecalis*, American Type Culture Collection No. 9790.

Experimental

The 2,6-diaminoheptanoic acid dihydrochloride was prepared by a several step synthesis according to the procedures of Eck and Marvel[2] from 2-methylcyclohexanone oxime.[3] The intermediates, 2-keto-7-methylhexamethylenimine and 6-aminoheptanoic acid, have been reported elsewhere.[3]

6-Benzoylaminoheptanoic Acid.—2-Methylcyclohexanone oxime, 630 g., was converted to the benzoylamino acid.[2] The product, m.p.[4] 87–89°, weighed 749 g.

Anal. Calcd. for $C_{14}H_{19}O_3N$: C, 67.42; H, 7.70. Found: C, 67.24; H, 7.70.

6-Benzoylamino-2-bromoheptanoic Acid.—6-Benzoylaminoheptanoic acid, 720 g., was brominated in the usual way.[2] The 2-bromo acid, m.p. 152–153.5°, crystallizes very slowly from acetone or chloroform over a period of months. Consequently some of the product was withdrawn and recrystallized for analyses and further syntheses.

Anal. Calcd. for $C_{14}H_{18}O_3NBr$: C, 51.23; H, 5.53; N, 4.27. Found: C, 51.57; H, 5.55; N, 4.51.

6-Benzoylamino-2-aminoheptanoic Acid.—The bromo-acid, 16 g., was allowed to react with ammonia according to the procedures of Eck and Marvel.[2] The product, m.p. 247–250°, weighed 9 g.

Anal. Calcd. for $C_{14}H_{20}O_3N_2$: C, 63.60; H, 7.62; N, 10.62. Found: C, 63.40; H, 7.72; N, 10.70.

2,6-Diaminoheptanoic Acid Dihydrochloride.—6-Benzoylamino-2-aminoheptanoic acid, 1 g., was hydrolyzed[2] to give 0.42 g. of ε-C-methyllysine dihydrochloride. The product, m.p. 190.5–192°, sinters at *ca.* 185° and releases bubbles at *ca.* 206°.

Anal. Calcd. for $C_7H_{18}O_2N_2Cl_2$: C, 36.02; H, 7.78; N, 12.02. Found: C, 36.21; H, 7.58; N, 11.89.

Tests for Lysine Activity and Lysine Inhibition.—Tests were set up for the microbiological assay of lysine using either *Streptococcus faecalis* or *Leuconostoc mesenteroides* and employing the conditions previously described[5] with the exceptions that the tests were run in a total volume of 2 ml.

(1) D. W. Woolley, *Physiol. Rev.*, **27**, 308 (1947).

(2) J. C. Eck and C. S. Marvel, "Organic Syntheses," Coll. Vol. 2, A. H. Blatt, Ed., John Wiley and Sons, Inc., New York, N. Y., 1943, pp. 74, 76, 374.

(3) H. E. Ungnade and A. D. McLaren, *J. Org. Chem.*, **10**, 29 (1945).

(4) All melting points are corrected.

(5) C. A. Knight, *J. Biol. Chem.*, **171**, 297 (1947).

rather than 5 ml., that the Henderson *basal* medium[6] was employed, and that the acid produced by growth of the organisms was titrated to a *p*H of 6.8 using a Canon titrator[7] and recording the results in counts rather than in milliliters of alkali. In the inhibition tests, 8.0 micrograms of L-lysine was added to the basal medium in each tube and then from 0 to 16 micrograms of ε-C-methyllysine were added, testing each amount in triplicate. At the end of the appropriate incubation period, the amount of growth in each tube was determined by titration of the acid produced in the manner described above. Similarly, the ability of L-lysine to overcome the inhibition of the homolog was tested by adding increments of L-lysine to tubes containing, in addition to the basal medium, 8 micrograms of homolog.

Results and Discussion

It should be noted that there are four possible isomers of ε-C-methyllysine and that the preparation tested may have contained all of them. Hence the results observed may represent the net effect of a mixture of compounds each of which possesses a characteristic inhibitory (or even stimulatory) influence upon growth. In any case, the preparation, hereafter referred to as the homolog, failed to support growth of *L. mesenteroides* or of *S. faecalis* when amounts of the homolog up to 70 micrograms were substituted for lysine. In parallel tests, definite growth of both organisms was observed with as little as 1 microgram of L-lysine.

Tests were next made to see if the homolog would inhibit growth of the test organisms. Pronounced inhibition of growth was observed as illustrated for *S. faecalis* in curve 2 of Fig. 1. Similar results were obtained with *L. mesenteroides*.[8] In the tubes which received approximately equivalent amounts of lysine and the homolog, and in those which received more homolog than lysine, growth was reduced to about one-quarter of that obtained in the absence of homolog. If molar inhibition ratios[9] are calculated on the basis of the amount of homolog required to reduce the growth level obtained with 8 micrograms of L-lysine to that obtained with one-half that amount, it appears that 1 molecule of homolog can inhibit approximately 3 of L-lysine. However, in view of the finding by Ferger and du Vigneaud[10] that only one of the isomers of β-2-thienylalanine inhibited microbial growth, it is possible that one or more of the isomers of the homolog possesses even greater inhibitory capacity than the preparation tested.

The inhibition of growth of the two test organisms by the homolog could be overcome by addition of L-lysine. This is illustrated for *S. faecalis* in curve 3 of Fig. 1. Inhibition of growth by the homolog was more readily and completely overcome in the case of *L. mesenteroides* than with *S. faecalis*.

Numerous investigations have been made of analogs of metabolites and comparatively fewer tests have been made with homologs. The results of the present tests suggest that it might be profitable to explore further the potentialities of the present and other amino acid homologs in the inhibition of microbial growth.

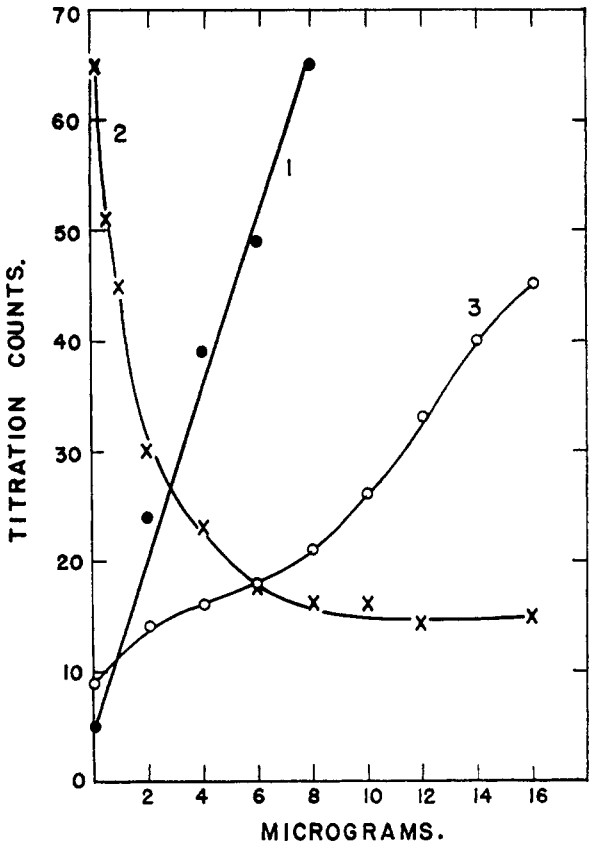

Fig. 1.—Growth of *Streptococcus faecalis* upon addition to basal medium of L-lysine, and various proportions of L-lysine and ε-C-methyllysine: curve 1, growth obtained with 0 to 8 micrograms of L-lysine; curve 2, growth obtained with 8 micrograms of L-lysine and 0 to 16 micrograms of ε-C-methyllysine; curve 3, growth obtained with 8 micrograms of ε-C-methyllysine and 0 to 16 micrograms of L-lysine.

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(6) L. M. Henderson and E. E. Snell, *ibid.*, **172**, 15 (1947).

(7) L. M. Henderson, W. L. Brickson and E. E. Snell, *ibid.*, **172**, 31 (1948).

(8) In the case of *S. faecalis*, inhibition of growth in the presence of 8 micrograms of lysine was apparent with 0.5 microgram of homolog or less, whereas inhibition first appeared with *L. mesenteroides* with approximately 4 micrograms of homolog, and up to this level there was definite indication that the homolog was causing an enhancement of growth. However, the ultimate inhibitions at 16 micrograms of homolog were comparable for the two organisms.

(9) K. Dittmer and V. du Vigneaud, *J. Biol. Chem.*, **169**, 63 (1947).

(10) M. F. Ferger and V. du Vigneaud, *ibid.*, **174**, 241 (1948).

Millicoulometry. III. The Polarographic Reduction of Copper(II) from Thiocyanate Solutions

BY LOUIS MEITES

The polarographic characteristics of copper(II) in thiocyanate solutions were first studied by Lingane and Kerlinger,[1] who found a double wave, corresponding to stepwise reduction to copper(I) and copper amalgam, in 0.1 *F* thiocyanate. They reported the half-wave potentials to be −0.02 and −0.39 v., which, although the first is slightly more positive than the depolarization potential of the

(1) J. J. Lingane and H. Kerlinger, *Ind. Eng. Chem., Anal. Ed.*, **13**, 77 (1941).