was processed as described for small scale preparation of oximes. Gas chromatography indicated no carbonyl components in the distillate.

(*b*) A sample of 0.25 mmole of 3-methyl-2-butanone was distilled as described under (*a*). Gas chromatography indicated a recovery in the distillate of 0.2 mmole of the ketone.

(*c*) A solution of 1 mmole of acetaldehyde and 0.5 mmole of 3-methyl-2-butanone in 10 ml. of water was added beneath the surface of 50 ml. of a distilling *N* solution of aqueous alkali. Rate of addition was about equal the rate of distillation, and the procedure was as in (*a*) except for the gradual addition to the distilling solution. Gas chromatography indicated a recovery in the first 10 ml. of distillate of 75% of the acetaldehyde and 86% of the ketone.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF CHAS. PFIZER AND CO.]

(+)-2,3-Diaminosuccinic Acid

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(+)-2,3-Diaminosuccinic acid has been found in the fermentation beers of *Streptomyces rimosus*. This is the first reported isolation of a diaminosuccinic acid from a natural source.

Diaminosuccinic acid has long been known as a synthetic substance. The first preparations of *dl*- and *meso*- forms were reported as early as 1893, and many syntheses have been effected since that time.[1,2] There is no record of the isolation of this material from natural sources, though it has been reported to be a product of enzymatic synthesis from hydrazine and fumaric acid by pig liver and *E. coli* preparations.[3]

We wish to report here the isolation of an optically active form of diaminosuccinic acid from the fermentation beers of *Streptomyces rimosus*, an actinomycete better known as the producer of the antibiotic Terramycin.[4] When the acidified filtered beers were adjusted to *p*H 2–4, the crude acid in some cases precipitated directly as white crystals of the dihydrate, which decomposes without melting at 240–260°. The pure anhydrous compound shows $[\alpha]_D^{25}$ +28° in 5% sodium hydroxide.

The structure of the new compound follows from its elemental analysis, $C_4H_8N_2O_4$, and from its chemical and physical properties. Titration shows it to be a dibasic acid, pK_a 6.7 and 9.1. The amine nature of the two nitrogen atoms is evident from the ready formation of the dibenzoyl and di-2-naphthalenesulfonyl derivatives. The compound shows only end absorption in the ultraviolet spectrum. The infrared spectrum, (KBr) which shows strong bonded NH and OH absorption at 3–3.5μ, and broad bonded carbonyl absorption at 6.1μ, is entirely consistent with this structure. Our product is identical to synthetic *dl*-2,3-diaminosuccinic acid on two paper chromatography systems.

We were unable to detect diaminosuccinic acid in either the raw broth prior to fermentation, or in acid hydrolysates of the protein source (soy bean meal). It seems evident that diaminosuccinic acid is therefore an elaboration product of *S. rimosus*, and is not merely liberated from the vegetable protein. This conclusion is strengthened by the observation that the yields of acid (1–2 g./l.) would account for 10% of the total protein nitrogen introduced in the fermentation media.

Qualitative (paper chromatographic) examination of the beers from several strains of *S. rimosus* all showed the presence of diaminosuccinic acid. However, two strains of *S. aureofaciens* yielded no detectable quantities of diaminosuccinic acid.

EXPERIMENTAL

Isolation of (+)-2,3-diaminosuccinic acid. Streptomyces rimosus was grown for 96 hr. at 28° with aeration in a medium containing only 4% soybean meal, and 0.5% sodium nitrate. (Other media may also be used.)

Two l. of the broth were adjusted to *p*H 2 with HCl, filtered from mycelia, and readjusted to *p*H 4. After refrigerated storage (2–5°) for one week, 1.36 g. of crude (+) diaminosuccinic acid was separated by filtration. Twenty g. of the crude product isolated in a similar manner was purified by solution in 400 ml. of 5% aqueous hydrochloric acid at 80°, treatment with 2 g. "Darco G-60" charcoal, and filtration. On addition of 10*N* sodium hydroxide, precipitation started at *p*H 1, and appeared to be substantially complete at *p*H 4. The suspension was cooled to 5° overnight and 18 g. of white crystals were recovered by filtration. Paper chromatographic examination on a methyl ethyl ketone-acetic acid-water 3:1:1 system showed substantially one component, which gave an abnormal gray-violet ninhydrin color on spraying with detection reagent and heating the paper. A methanol-pyridine-water 8:2:1 system showed a single component with R_f between that of *meso*- and of (+)-diaminopimelic acid.

Two grams of once recrystallized diaminosuccinic acid was dissolved in 300 ml. of boiling water and cooled to 5° to yield 1.8 g. of a pure hydrated diaminosuccinic acid. Drying overnight at 20 mm. and room temperature over calcium

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(1) J. M. Farchy and J. Tafel, *Ber.*, **26**, 1980 (1893) and J. Tafel and H. Stern, *Ber.*, **38**, 1589 (1905).

(2) H. McKennis, Jr., and A. S. Yard, *J. Org. Chem.*, **23**, 980 (1958) have reported a recent study. They give an excellent series of references to chemical and biochemical work through 1957.

(3) K. P. Jacobsohn and M. Soares, *Enzymologia*, **1**, 183 (1936).

(4) Terramycin is the registered trade name of Charles Pfizer and Co. for the antibiotic oxytetracycline.

chloride yielded the dihydrate (18% volatile found, calcd. 19.0%) which was further dried at 100°, 0.1 mm. to constant weight (6 hr.) for analysis.

The anhydrous compound has $[\alpha]_D^{25}$ +28° (*c*, 2 in 5% NaOH) $[\alpha]_D^{25}$ +59° (*c*,2 in 5% HCl). It decomposes without melting over a broad range, 240–290°, depending on rate of heating. Titration of an aqueous solution indicates a dibasic acid, pK_a 6.7, and 9.1, eq. wt. 72 and 156. (Calcd., eq. wt. 74 and 148.1.)

Anal. Calcd. for $C_4H_8N_2O_4$: C, 32.43; H, 5.44; N, 18.91. Found C, 32.42; H, 5.23; N, 18.89.

The ultraviolet absorption spectrum in water solution shows only end absorption, ϵ = 38 at 220 mμ. The infrared absorption spectrum (KBr pellet) shows bonded OH and NH absorption at 3–3.5μ, and a very broad carbonyl absorption band at 6–6.2μ.

A quantitative analysis (paper chromatography–ninhydrin) of the broth indicates the presence of about 1–2 g. of (+)-2,3-diaminosuccinic acid per liter of the broth examined. Unfermented broths showed no diaminosuccinic acid.

Acid stability of diaminosuccinic acid. A solution containing 2% diaminosuccinic acid in 10% aqueous HCl was heated under reflux for 24 hr. Aliquots were withdrawn at 2, 6, and 24 hr. Neutralization to *p*H 3 yielded crystalline acid in each case.[5] Paper chromatographic examination of the samples indicated that slight decomposition to other ninhydrin positive substances had occurred at 6 hr.; definite decomposition was detectable at 24 hr. A sample of the soybean meal used in the fermentation was hydrolyzed in 10% hydrochloric acid at reflux for 24 hr. Paper chromatographic examination of the hydrolysates showed no diaminosuccinic acid.

N,N′-Dibenzoyl-(+)-2,3-diaminosuccinic acid. One-half g. of (+)-diaminosuccinic acid, was dissolved in 9 ml. of 5% sodium hydroxide, cooled to 0°, and 1 ml. of benzoyl chloride added to the stirred solution. After standing at room temperature overnight, the solution was acidified to *p*H 2 with HCl, and the resulting suspension was extracted twice with an equal volume of ether to yield 0.8 g. of crude dibenzoyldiaminosuccinic acid. This crude product was twice crystallized from 5 ml. ethanol by addition of water. The pure product, 0.35 g., melts at 164–166° with softening and darkening from 158°. It was dried for 2 hr. at 80°, 0.05 mm. for analysis. $[\alpha]_D^{25}$ +109° (*c*, 1 in MeOH).

Anal. Calcd. for $C_{18}H_{16}N_2O_6 \cdot H_2O$: C, 57.75; H, 4.85; N, 7.48. Found: C, 57.51; H, 4.89; N, 7.71.

N,N′-di-2-Napthalenesulfonyl-(+)-2,3-diaminosuccinic acid. Two hundred mg. of (+)-diaminosuccinic acid in 27 ml. 1*N* sodium hydroxide was stirred with 1.2 g. 2-naphthalenesulfonyl chloride in 5 ml. ether. Three 2.7-ml. portions of *N* NaOH were added at 1, 3, 4 hr. The aqueous phase was separated, acidified, and the crude product, 0.55 g. was crystallized twice from ethanol-water and dried at 26°, 0.01 mm. for analysis. The product obtained in this way melted at 190–195° with much prior decomposition. This m.p. is dependent on rate of heating. This substance did not analyze satisfactorily.

Anal. Calcd. for $C_{24}H_{20}N_2S_2O_8 \cdot {}^1/_2\, H_2O$. C, 53.70; H, 3.95; N, 5.21. Found: C, 53.78; H, 4.38; N, 5.90.

The corresponding *dl* derivative melts at 200°, the *meso* derivative at 234°.[1]

Examination of S. aureofaciens beers. Beers from the fermentations of two strains of *S. aureofaciens* in media containing corn steep liquor as the proteinacious ingredient were filtered and examined directly by chromatography on a methyl isobutyl ketone-acetic acid-water system. No (+)-2,3-diaminosuccinic acid could be detected. A standard containing 0.1% diaminosuccinic acid gave a strong positive test in the same run.

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(5) R. Kuhn and F. Zumstein, *Ber.*, **59**, 479 (1926), state that *dl*-diaminosuccinic acid is converted to the *meso* form after 3 hrs. reflux in 15% hydrochloric acid. A similar conversion may have occurred here, but was not evident from the paper chromatograms.

[CONTRIBUTION FROM THE ROCKEFELLER INSTITUTE]

Reaction of Ethylene Dibromide with Triethylamine and the Restoring Action of Some Alkanebis(triethylammonium) Ions upon Sodium-Deficient Nerve Fibers

FREDERICK VIDAL

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It is shown that the reaction of ethylene dibromide with triethylamine yields no ethylenebis(triethylammonium bromide-contrary to the reports in the literature. The only crystalline products obtained are triethylamine hydrobromide and 1) bromoethane-2-triethylammonium bromide.

The synthesis and properties of the ethylenebis(triethylammonium halides), prepared in the course of studies on neurophysiological problems, are reported. The bisquaternary ammonium salts were formed by quaternization of *N,N,N′,N′*-tetraethylethylenediamine with ethyl halide. In addition to the bisquaternary ammonium compounds mixed tertiary-quaternary ammonium salts, ethane-1-diethylamino-2-triethylammonium halides, were also isolated, whose properties are described.

For many years this laboratory has been engaged[1] in testing the neurophysiological effects of different nitrogenous basic compounds on nerve fibers. Some of these onium ions, of which the tetraethylammonium ion may be regarded as one of the prototypes, restore the ability to conduct impulses in sodium-deficient nerve fibers.

In order to study the biological activities of the