iodine (0.14 g., 71% yield) was obtained after recrystallization from water–ethanol; m.p. 281–285° (with decomposition), $[\alpha]^{25}D$ +8.1 ± 0.5° (*c* 0.80, water). The reported[16] value is $[\alpha]^{25}D$ +8.12 ± 0.5° (*c* 0.80, water).

Anal. Calcd. for $C_5H_{11}NO_2S$: C, 40.25; H, 7.43; N, 9.39. Found: C, 40.30; H, 7.58; N, 9.30.

Resolution of L-Methionyl-D,L-methionine. (a) **N-Benzylsulfonyl-L-methionyl-L-methionine Phenylhydrazide.**—N-Benzylsulfonyl-L-methionyl-D,L-methionine (3.0 g.) was dissolved in 40 ml. of *N* sodium hydroxide solution. To this solution was added successively 2 g. of phenylhydrazine hydrochloride in 30 ml. of water, 2 g. of L-cysteine hydrochloride in 30 ml. of water, 50 ml. of 1 *M* acetic acid–sodium acetate buffer (*p*H 4.7) and 1.5 g. of papain. This enzymatic reaction was allowed to proceed in the usual manner. After the reaction mixture had been incubated at 40° for two hours, N-benzylsulfonyl-L-methionyl-L-methionine phenylhydrazide was collected by filtration and recrystallized from acetone–water; 1.51 g. (84% based on the weight of L-substrate), m.p. 175°, $[\alpha]^{26}D$ −22.1 ± 0.7° (*c* 1.00, butanone). After another 411 hours of incubation, no appreciable amount of phenylhydrazide was obtained.

Anal. Calcd. for $C_{23}H_{32}N_4O_4S_3$: N, 10.68. Found: N, 10.63.

(b) **Conversion of N-Benzylsulfonyl-L-methionyl-L-methionine Phenylhydrazide to N-Benzylsulfonyl-L-methionyl-L-methionine.**—The phenylhydrazide (1.2 g.) from the above enzymatic reaction was oxidized with 8 g. of ferric chloride ($FeCl_3 \cdot 6H_2O$) in the usual manner as for N-benzylsulfonyl-L-methionine phenylhydrazide. Slightly yellow crystals were obtained after acidification with concentrated hydrochloric acid and dissolved in 50 ml. of ethyl acetate. This solution was extracted with three 30-ml. portions of *N* sodium bicarbonate solution. The aqueous solution was evaporated *in vacuo* until no odor of ethyl acetate remained and acidified (congo red) with concentrated hydrochloric acid. After refrigerating the mixture overnight, white crystals were obtained which were recrystallized from ethanol–water; 0.74 g. (75% yield), m.p. 107–108°, $[\alpha]^{26}D$ −25.2 ± 0.9° (*c* 1.00, *N* sodium hydroxide).

Anal. Calcd. for $C_{17}H_{26}N_2O_5S_3$: C, 46.98; H, 6.03; N, 6.45; neut. equiv., 434.58. Found: C, 47.00; H, 6.17; N, 6.40; neut. equiv., 436.

(c) **L-Methionyl-L-methionine.**—N-Benzylsulfonyl-L-methionyl-L-methionine (0.40 g.) was dissolved in 200 ml. of liquid ammonia. To this mixture was added, with mechanical stirring during one-half hour, 0.08 g. of metallic sodium in 15 approximately equal portions. A blue color persisted for one minute after the last addition of sodium. The mixture was treated with 0.15 ml. of methyl iodide, stirred for a few minutes more, and then allowed to evaporate spontaneously. The residue was dried *in vacuo* and dissolved in 10 ml. of water. The solution was filtered; the filtrate was neutralized with hydrobromic acid (20%) to *p*H 6–7 and then evaporated to dryness under reduced pressure. The residue was extracted into 40 ml. of hot 95% ethanol. The insoluble residue was discarded. The alcoholic solution was evaporated to 10 ml. *in vacuo* and diluted with 30 ml. of ether. The white crystals were recrystallized from 80% ethanol; 0.21 g. (81% yield), m.p. 224–226° (with decomposition), $[\alpha]^{25}D$ +26.1 ± 0.7° (*c* 2.00, water). The reported[12] value is $[\alpha]^{26}D$ +26.5° (*c* 2, water).

Anal. Calcd. for $C_{10}H_{20}N_2O_3S_2$: C, 42.83; H, 7.19; N, 9.99. Found: C, 42.36; H, 7.29; N, 9.75.

(d) **N-Benzylsulfonyl-L-methionyl-D-methionine.**—The filtrate from the enzymatic preparation of N-benzylsulfonyl-L-methionyl-L-methionine phenylhydrazide was acidified (congo red) with concentrated hydrochloric acid and extracted with three 50-ml. portions of ethyl acetate. The ethyl acetate solution was extracted with three 20-ml. portions of *N* sodium bicarbonate solution. The resulting aqueous solution was evaporated *in vacuo* until no odor of ethyl acetate remained and acidified (congo red) with concentrated hydrochloric acid. White crystals were obtained after refrigerating the mixture for a few hours. The product was recrystallized from ethanol–water; 0.54 g. (36% based on the weight of D-substrate), m.p. 136–137°, $[\alpha]^{26}D$ −51.3 ± 0.7° (*c* 1.00, *N* sodium hydroxide).

Anal. Calcd. for $C_{17}H_{26}N_2O_5S_3$: C, 46.98; H, 6.03; N, 6.45; neut. equiv., 434.58. Found: C, 46.80; H, 5.83; N, 6.70; neut. equiv., 431.

(e) **L-Methionyl-D-methionine.**—N-Benzylsulfonyl-L-methionyl-D-methionine (0.35 g.) in 200 ml. of liquid ammonia was treated with 0.07 g. of metallic sodium in the usual manner. L-Methionyl-D-methionine was recrystallized from 80% ethanol; 0.20 g. (80% yield), m.p. 236–238° (with decomposition), $[\alpha]^{25}D$ +75.8 ± 1.2° (*c* 1.00, water).

Anal. Calcd. for $C_{10}H_{20}N_2O_3S_2$: N, 9.99. Found: N, 10.26.

PULLMAN, WASHINGTON

[CONTRIBUTION FROM THE LABORATORY OF BIOCHEMISTRY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Preparation and Properties of the Isomeric Forms of α-Amino- and α,ε-Diaminopimelic Acid

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Resolution of α-aminopimelic acid, as its N-acetyl derivative, has been effected *via* the asymmetric action of hog renal acylase I at *p*H 7.0. The yield of the optically pure L- and D-isomers, which showed $[\alpha]D$ +21.5° and $[\alpha]D$ −21.0° (1% in 5 *N* HCl) values, was 77 and 48%, respectively. In addition, a modified procedure, whereby the three isomeric forms of α,ε-diaminopimelic acid may be secured, is described. Such modification consists in a fractional separation of the carbobenzoxy derivatives of the *meso*- and DL-forms from the inactive synthetic epimeric mixture. Conversion of the DL-form to the corresponding amino acid amide proceeded through hydrogenolysis of carbobenzoxy-DL-α,ε-diaminopimelic acid amide. Resolution of the racemic amino acid amide with a purified hog kidney amidase preparation subsequently resulted in the optically pure L- and D-isomers, which showed $[\alpha]D$ values of +45.0° and −45.5° (1% in 1 *N* HCl), respectively, in agreement with previous results. Infrared spectra for each of the stereoisomeric forms of α-amino- and α,ε-diaminopimelic acid, as well as the apparent dissociation constants of the latter, are presented.

The natural occurrence of α-aminopimelic acid as a component of green plants has been reported recently by Virtanen and Berg.[1] This compound, isolated in milligram quantities, was characterized *via* chromatographic, melting point and titration data. Paucity of the natural material unfortunately permitted neither determination of its optical rotation nor configuration. It is with such identification and characterization of the L- and D-antipodes of α-aminopimelic acid with which the present communication is, in part, concerned. In addition, a modification and improvement of the procedure for the preparation of the three isomeric forms of α,ε-diaminopimelic acid, previously de-

(1) A. I. Virtanen and A. M. Berg, *Acta Chem. Scand.*, **8**, 1725, 1085 (1954).

scribed,[2] is reported herein. This was deemed of importance in view of the recent finding by Hoare and Work[3] that not only the *meso* but also the L,L-form occurs in nature.

Results and Discussion

Resolution of DL-α-Aminopimelic Acid.—The general enzymic resolution procedure developed in this Laboratory over the past seven years has been concerned primarily with the preparation of amino acid derivatives which would be asymmetrically susceptible to cleavage by purified hog kidney amidase or acylase fractions.[4] Substrates were therefore confined to either the amide or N-acyl derivatives. In the instance of α-aminopimelic acid, choice of the action of the renal acylase I system[5] on the N-acetyl derivative was dictated, to large extent, by previous knowledge that the next lower homologs, N-acylated α-aminoadipic and glutamic acids, respectively, were hydrolyzed by this same enzyme system at a rate of sufficient magnitude to ensure their satisfactory resolution.[5-7] A preliminary determination indicated that although the enzymic cleavage of acetyl-DL-α-aminopimelic acid proceeded at a rate appreciably slower than that of the corresponding glutamic acid derivative, its rate of hydrolysis was nonetheless of sufficient magnitude to permit its resolution to be successfully achieved.

The DL-α-aminopimelic acid was prepared by modifications of various reactions which have appeared in the literature.[8-13] The over-all yield for the five stages from pimelic acid was 15%.

The N-acetyl derivative was subjected to the hydrolytic action of the L-directed renal acylase I. Preparation of this enzyme from hog kidney homogenates has been described previously.[5] Digestion of a 0.1 *M* solution of the racemic substrate and the lyophilized enzyme, at *p*H 7.0, was effected at 39°. The rate of hydrolysis of the susceptible L-form, as followed by the Van Slyke manometric ninhydrin-CO_2 procedure, was 10 micromoles per hr. per mg. of protein nitrogen. Optical rotation values of the enantiomorphic amino acids, subsequently secured, were $[\alpha]_D$ +21.5° and −21.0° (1% in 5 *N* HCl) for the L- and D-forms, respectively. Such data provide the basis for assigning a configurational designation to natural α-aminopimelic acid.

Preparation of the Isomeric α,ε-Diaminopimelic Acids.—A recent communication from this Laboratory[2] reported the preparation of the three isomeric forms (L, D and *meso*) of α,ε-diaminopimelic acid through the action of hog kidney amidase[14] on an epimeric mixture of its amide derivative. The separation of the L-diaminopimelic acid, L-diaminopimelic acid-D-monoamide and D-diaminopimelic acid diamide, so derived, could be effected on an Amberlite XE-64 cation exchange column. During such separation, some overlap of the mono- and di-amide fractions occurred which resulted in reduced yields of the corresponding amino acid isomers subsequently obtained therefrom. Since the separation of the *meso* from the racemic form prior to resolution presumably would lead to higher yields of final material, as well as to less tedious manipulations, a search for chemical methods whereby such could be accomplished was undertaken.

Since the carbobenzoxy derivative of the epimeric mixture of diaminopimelic acid had been prepared previously,[2] fractionation of this derivative was investigated. The twice-recrystallized mixture from ethyl acetate yielded a product whose melting point of 164–165° remained unchanged upon additional recrystallizations from this same solvent. Concentration of the mother liquors to dryness resulted in a residue which, upon crystallization from chloroform, exhibited a melting point of 123–125° which, too, remained unaltered upon further recrystallization. Identification of the high melting form as the racemic and the low melting form as the *meso* modification of diaminopimelic acid was effected by hydrogenolysis of their respective carbobenzoxy derivatives to the free amino acid, followed by chromatographic identification on paper[15] according to the procedure of Rhuland, Work, Denman and Hoare.[16] The amino acid derived from the high melting material exhibited two ninhydrin-sensitive spots of equal intensity corresponding to the D- and L-forms, whereas that derived from the low melting isomer showed only a single spot corresponding to the *meso* form.

Conversion of the high melting carbobenzoxy derivative of diaminopimelic acid to the diamide derivative, *via* the mixed anhydride method of Vaughan and Osato,[17] was succeeded by its subsequent hydrogenolysis to DL-diaminopimelic acid diamide diacetate. Asymmetric cleavage of this latter compound, followed by separation of products with an Amberlite XE-64 resin, was effected according to essentially the same procedure as employed previously.[2] Optical rotation values for the enantiomorphs so secured were equal and opposite, within the limits of experimental error, and were in complete agreement with those reported earlier.[2] The yields of optically pure L- and D-forms were 88 and 73%, respectively.[18]

(2) E. Work, S. M. Birnbaum, M. Winitz and J. P. Greenstein, THIS JOURNAL, **77**, 1916 (1955).

(3) D. S. Hoare and E. Work, *Biochem. J.*, **61**, 562 (1955).

(4) J. P. Greenstein, *Advances in Protein Chem.*, **9**, 121 (1954).

(5) S. M. Birnbaum, L. Levintow, R. B. Kingsley and J. P. Greenstein, *J. Biol. Chem.*, **194**, 455 (1952).

(6) L. Levintow, J. P. Greenstein and R. B. Kingsley, *Arch. Biochem. and Biophys.*, **31**, 77 (1951).

(7) J. P. Greenstein, S. M. Birnbaum and M. C. Otey, THIS JOURNAL, **75**, 1994 (1953).

(8) A. I. Vogel, *J. Chem. Soc.*, 333 (1934).

(9) M. E. Dobson, J. Ferris and W. H. Perkin, *ibid.*, **95**, 2015 (1909).

(10) W. Dieckmann, *Ann.*, **317**, 94 (1901).

(11) R. W. Jackson and R. H. Manske, THIS JOURNAL, **52**, 5029 (1930).

(12) F. Adickes, *Ber.*, **58**, 211 (1925).

(13) R. P. Linstead and A. B.-L. Wang, *J. Chem. Soc.*, 807 (1937).

(14) D. Hamer and J. P. Greenstein, *J. Biol. Chem.*, **193**, 81 (1951); *cf.* S. M. Birnbaum in "Methods in Enzymology," Vol. II, Academic Press, Inc., New York, N. Y., 1955, p. 397.

(15) A pyridine–methanol–water system (10–77–20) was employed.

(16) L. E. Rhuland, E. Work, R. F. Denman and D. S. Hoare, THIS JOURNAL, **77**, 4844 (1955).

(17) J. R. Vaughan, Jr., and R. Osato, *ibid.*, **73**, 5553 (1951).

(18) An earlier communication (Y. Izumi, *J. Chem. Soc. Japan*, **75**, 1152 (1954), has reported a papain-catalyzed separation of a mixture of the dibenzoyl derivative of DL- and *meso*-α,ε-diaminopimelic acid *via* selective anilide formation. However, the properties of the individual stereoisomeric amino acids subsequently secured from such reaction mixture are so divergent from those described herein, that a comparison of the two materials would serve no useful purpose.

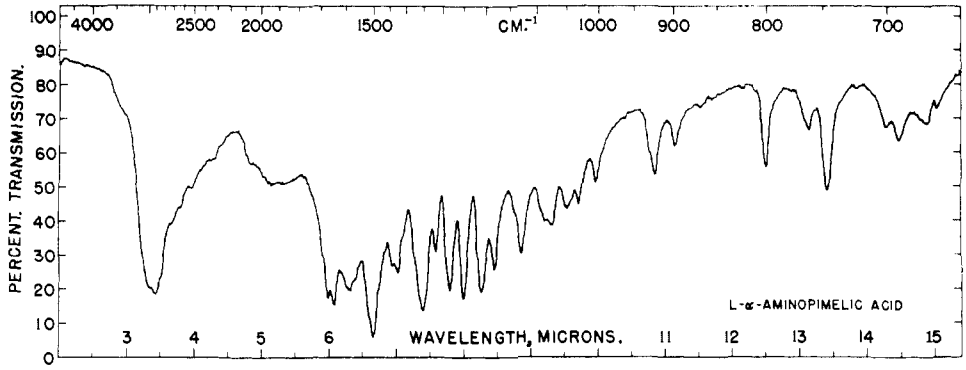

Fig. 1.—Infrared spectrum of L-α-aminopimelic acid in the solid state.

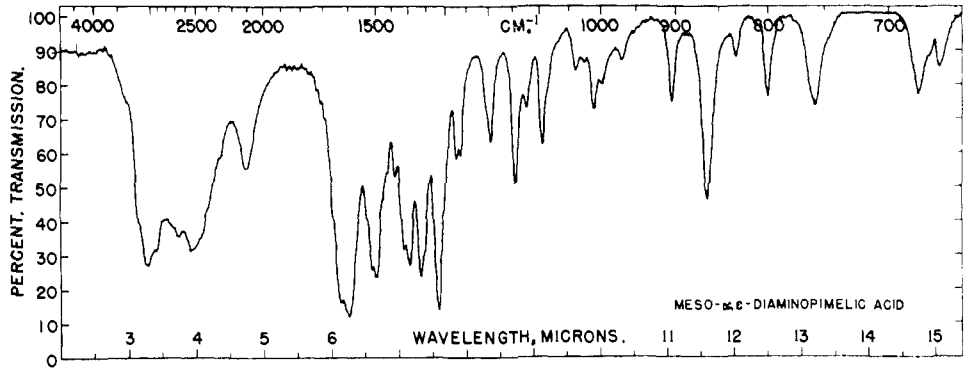

Fig. 2.—Infrared spectrum of *meso*-α,ε-diaminopimelic acid in the solid state.

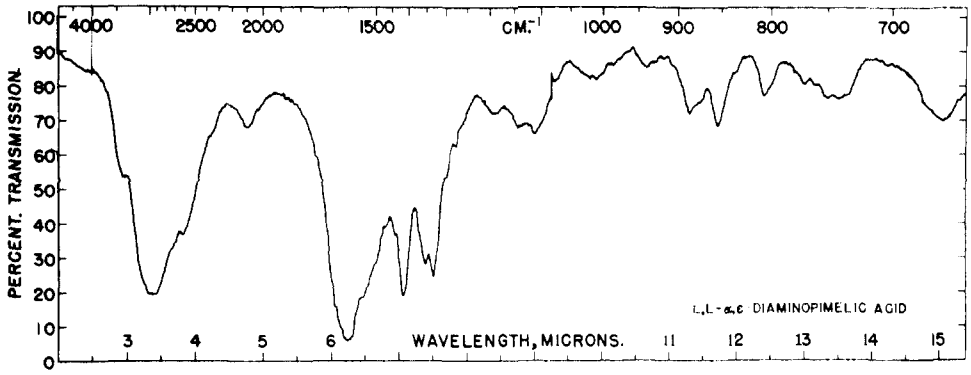

Fig. 3.—Infrared spectrum of L-α,ε-diaminopimelic acid in the solid state.

Apparent Dissociation Constants of L- and *meso*-Diaminopimelic Acid.—Solutions of these amino acids were made up at 0.05 *M* in 0.10 *N* HCl containing 0.1 *M* KCl. Five-ml. aliquots were removed, titrated with 0.20 *N* NaOH and the solution brought to 10 ml. by addition of water. The *p*H of each such solution was measured at 25° and the apparent dissociation constants calculated in the usual manner.[19] Both forms of diaminopimelic acid yielded identical constants, which were pK_1' 1.8, pK_2' 2.2, pK_3' 8.8 and pK_4' 9.9. The calculated isoelectric point, pI, was close to 5.5.

Infrared Spectra.—The solid state spectra of α-aminopimelic acid and of the isomeric α,ε-diaminopimelic acids were determined in the range of 3 to 15 μ as described before.[20] The spectra of the L- and D-forms of α-aminopimelic acid were identical, as were likewise the spectra of L- and D-diaminopimelic acids. The possession of a sharp band at 7.80 μ in the spectrum of α-aminopimelic acid is the principal source of difference with the spectrum of glutamic acid. The spectrum of *meso*-diaminopimelic acid possesses a number of sharp bands in the region of 6 to 8 μ, in contrast with the more diffuse spectrum of L-diaminopimelic acid within this range. The spectra of the compounds herein studied are given in Figs. 1–3.

Experimental

Diethyl Pimelate.—The ester was prepared in 80% yield from pimelic acid by the general method of Vogel.[8]

(19) *Cf.* J. P. Greenstein, *J. Biol. Chem.*, **93**, 479 (1931).

(20) R. J. Koegel, J. P. Greenstein, M. Winitz, S. M. Birnbaum and R. A. McCallum, THIS JOURNAL, **77**, 5708 (1955).

α-Carbethoxycyclohexanone.—Cyclization of the above compound was effected by Dieckmann condensation under the conditions employed by Dobson, Ferris and Perkin[9] for the ring-closure of diethyl adipate to 2-carbethoxycyclopentanone; yield, 76%; b.p., 70–72° (0.4 mm.) (lit., 107–108° (12 mm.), 106–107° (11 mm.)[10]).

Ethyl Hydrogen α-Ketopimelate Phenylhydrazone.—The method of coupling the cyclic ketone with benzenediazonium chloride has been described by Jackson and Manske[11]; yield, 60%; m.p., 141–142° dec. (lit., 142–143°).

α-Ketopimelic Acid Phenylhydrazone.—Saponification of the ester group was effected by the use of 1.1 *N* sodium hydroxide in 50% aqueous dioxane. After acidification and condensation of the solution, the residue was recrystallized from ethyl acetate–petroleum ether to give α-ketopimelic acid phenylhydrazone as prisms, m.p., 141–143° dec. (lit., 143–144° dec.,[12] 153–154° dec.[13]); yield, 80%.

α-Aminopimelic Acid.—Ten grams of phenylhydrazone[11–13] was treated with 15 g. of zinc dust and 150 ml. of 75% acetic acid and the mixture boiled under reflux for 6 hr. After removal of the excess of zinc by filtration, the solution was evaporated to dryness, the residue dissolved in 50 ml. of water and hydrogen sulfide passed through the solution for 3 hr. The mixture was filtered hot, the filtrate evaporated to dryness and the crystalline residue shaken with a little ethanol. The white solid was filtered off and recrystallized from aqueous alcohol to give plates, m.p. 216° dec. (lit. 225° dec.,[21] 215–216°,[22]) yield 50%.

Resolution and Derivatives of α-Aminopimelic Acid. Acetyl-DL-α-aminopimelic Acid.—Three and one-half grams of α-aminopimelic acid was dissolved in 25 ml. of 2 *N* NaOH and the solution cooled to 5° in an ice-bath. To the solution was added, in alternate portions, 2.2 ml. of acetic anhydride and 20 ml. of 2 *N* NaOH, with intermittent shaking and cooling. The reaction mixture was then allowed to stand at room temperature for a period of 1 hr. after which time it was acidified to about *p*H 1.7 with 4 *N* HCl and evaporated to dryness at 40° under reduced pressure. About 20 ml. of water was added to the residue and the evaporation repeated. The residual crystalline mass was extracted several times with hot acetone and the extract filtered, evaporated to small bulk and subsequently treated with ether to incipient turbidity. Crystallization was induced by scratching the side of the vessel and the product then filtered off over suction. Recrystallization from acetone–ether gave prisms; yield 2.5 g. (60%), m.p. 111–112°.

Anal. Calcd. for $C_9H_{15}O_5N$: C, 49.8; H, 6.9; N, 6.5. Found: C, 50.0; H, 6.9; N, 6.4.

Enzymic Resolution.—Two and one-half grams of acetyl-DL-α-aminopimelic acid was dissolved in 100 ml. of water and the solution adjusted to *p*H 7.0–7.5 with 2 *N* LiOH. After the addition of 1 g. of renal acylase I,[5] the solution was diluted to 130 ml. (0.1 *M* in substrate). The reaction mixture was incubated at 39° and the enzymic hydrolysis of the substrate followed by the usual manometric ninhydrin–CO_2 procedure. After a digestion period of about 4 hr., analyses on an aliquot of the digest revealed that hydrolysis of the compound had proceeded to 50%. The turbid solution was concentrated to 50 ml. under reduced pressure and then dialyzed four times against 750 ml. of distilled water. The pooled dialysates (3 l.) were concentrated to 15 ml. *in vacuo* and the *p*H of the residual solution adjusted to 3.4 with 6 *N* HCl. Further evaporation resulted in the separation of crystals, at which point 50 ml. of absolute ethanol was added and the solution set aside at room temperature for 24 hr. The precipitate was subsequently filtered off, washed with alcohol and recrystallized from water–alcohol. An 800-mg. yield (77%) of optically pure L-α-aminopimelic acid was so secured; $[\alpha]^{26}D$ +21.5° (1% in 5 *N* HCl).

Anal. Calcd. for $C_7H_{13}O_4N$: C, 48.0; H, 7.4; N, 8.0. Found: C, 47.6; H, 7.3; N, 7.9.

The filtrate from the above, which contained the soluble lithium salt of acetyl-D-α-aminopimelic acid, was acidified to *p*H 1.7, taken to dryness *in vacuo* and the residual material extracted several times with boiling acetone. The combined acetone extracts were then concentrated, under a jet of dry air, to an oil which was dissolved in 125 ml. of 2 *N* HCl and subsequently refluxed for 2 hr. After concentration of the hydrolysate to dryness under reduced pressure, the residue was taken up in a little water, the *p*H adjusted to 3.4 with 2 *N* LiOH and the solution concentrated to incipient crystallization and treated with absolute alcohol as for the L-isomer. The D-amino acid, so secured, was recrystallized twice from water-alcohol; yield 500 mg. (48%), $[\alpha]^{26}D$ −21.0° (1% in 5 *N* HCl).

Anal. Calcd. for $C_7H_{13}O_4N$: C, 48.0; H, 7.4; N, 8.0. Found: C, 47.6; H, 7.3; N, 7.8.

The chromatographic behavior of each of the isomers of α-aminopimelic acid was identical in three solvent pairs, showing the following R_f values: (a) phenol–ammonia: 0.44 on Whatman No. 4 paper; (b) 1-butanol–acetic acid–water (4:1:5 by volume): 0.43 on Whatman No. 4 paper; (c) pyridine–methanol–water (10:77:20 by volume): 0.73 on Whatman No. 1 paper.

Resolution and Derivatives of α,ε-Diaminopimelic Acid. Diaminopimelic Acid (Epimeric Mixture).—A synthetic mixture of the three isomers of α,ε-diaminopimelic acid was prepared in essentially the same manner as described previously,[2] a yield of 66% being secured over the four stages. The product was lithium and halide free and showed, on paper chromatography employing the pyridine–methanol–water system,[15] two ninhydrin-sensitive spots with R_f values of 0.46 and 0.57 corresponding to the *meso*- and D- and to the L-isomers, respectively.[16]

Dicarbobenzoxy-DL-diaminopimelic Acid.—Nine and one-half grams of synthetic α,ε-diaminopimelic acid was dissolved in 125 ml. of 2 *N* NaOH and 19.5 ml. of carbobenzoxy chloride added in portions, the reaction mixture being cooled in an ice-bath and stirred vigorously during this time. When all of the acid chloride had been added (about 30 minutes), the reaction flask was transferred to a mechanical shaker and the mixture shaken vigorously at room temperature for 2 hr. After the unreacted carbobenzoxy chloride had been removed by extraction with ethyl acetate, the aqueous layer was acidified to *p*H 1.7 with 4 *N* HCl. The precipitated oil was extracted into ethyl acetate and the organic layer dried over anhydrous sodium sulfate and concentrated to 50 ml. *in vacuo*. The concentrate was allowed to stand at 4° overnight and the precipitated dicarbobenzoxy-DL-α,ε-diaminopimelic acid filtered over suction and washed with a little cold ethyl acetate. Recrystallization from ethyl acetate gave prisms. All of the above ethyl acetate filtrates were pooled and saved for recovery of the soluble *meso* form. The melting point of 164–165°, with shrinking at 155°, was unchanged by further recrystallization; yield 6.0 g.

Anal. Calcd. for $C_{23}H_{26}O_8N_2$: C, 60.3; H, 5.7; N, 6.1. Found: C, 60.5; H, 5.6; N, 6.1.

Dicarbobenzoxy-*meso*-α,ε-diaminopimelic Acid.—The pooled ethyl acetate mother liquors secured above were evaporated to a gummy residue which was dissolved in the minimal volume of hot chloroform and set aside to crystallize. Although the crystallization process was slow, the precipitated *meso* form could be collected in a 6.2-g. yield after 3 days. The melting point of 123–125° was unchanged on further recrystallization.

Anal. Calcd. for $C_{23}H_{26}O_8N_2$: C, 60.3; H, 5.7; N, 6.1. Found: C, 60.1; H, 5.6; N, 6.1.

The combined yield of *meso* and racemic forms was 12.2 g. (53% of theory).

***meso*-Diaminopimelic Acid.**—Hydrogenolysis of a solution of 30 g. of the dicarbobenzoxyamino acid (m.p. 123–125°), in a mixture of 300 ml. of acetic acid and 100 ml. of water, was effected in the presence of palladium black catalyst. Upon termination of the hydrogenolysis, the catalyst was filtered off and the filtrate concentrated to dryness *in vacuo*. Water was added (50 ml.) and the evaporation repeated. The crystalline residue was recrystallized twice from 35% aqueous alcohol; yield 7.5 g. (60%).

Anal. Calcd. for $C_7H_{14}O_4N_2$: C, 44.2; H, 7.4; N, 14.7. Found: C, 44.1; H, 7.5; N, 14.5.

Chromatography on paper[15] revealed the presence of only a single ninhydrin-positive component (R_f 0.45) under conditions wherein the L-isomer (R_f 0.57) would have appeared if present.

Dicarbobenzoxy-DL-diaminopimelic Acid Diamide.—A solution of 45.8 g. of dicarbobenzoxy-DL-diaminopimelic acid (m.p. 164–165°) and 27.8 ml. of triethylamine in 600 ml. of dioxane was cooled to the freezing point. The addition thereto of 24.4 ml. of isovaleryl chloride was slowly

(21) W. Dieckmann, *Ber.*, **38**, 1660 (1905).

(22) V. V. Feofilaktov, *Bull. acad. sci. U.R.S.S. Classe sci. chim.*, 521 (1941).

effected (temperature kept to below 12°) and the solution then kept at 10° for 1 hr. After this time, the dropwise addition of 26 ml. of aqueous ammonia (sp. gr. 0.90) resulted in the separation of a white solid which, after 16 hr., was filtered over suction and washed successively with dilute ammonia and water. Recrystallization from dimethylformamide–water gave a mass of needles; yield 18.0 g. (40%), m.p. 223–224°.

Anal. Calcd. for $C_{23}H_{28}O_6N_4$: C, 60.5; H, 6.1; N, 12.3. Found: C, 60.4; H, 6.1; N, 12.3.

DL-**Diaminopimelic Acid Diamide Diacetate.**—The dicarbobenzoxylated diamide (21.5 g.) secured above was dissolved in 400 ml. of acetic acid and hydrogenated in the presence of palladium black catalyst. At the completion of hydrogenolysis, the catalyst was removed by filtration and the filtrate concentrated to dryness. After the addition of 25 ml. of water, the evaporation was repeated. The residual oil was used directly in the resolution step, below, without prior crystallization.

Enzymic Resolution.—The hydrogenolysis product, secured directly above, was dissolved in 300 ml. of water containing 1.15 g. of manganous acetate tetrahydrate, the *p*H adjusted to 6.5 with 2 *N* LiOH, 1.8 g. of lyophilized amidase powder[14] added, the *p*H adjusted to 8.0 with 2 *N* LiOH and the final volume brought to 470 ml., corresponding to 0.1 *M* in amide and 0.01 *M* in Mn^{++} concentration. The mixture was subsequently digested at 38° for 5 hr., at which point the manometric ninhydrin–CO_2 procedure revealed 50% hydrolysis of the substrate. Concentration of the digest to about 50 ml. was followed by its dialysis against 4 changes of water (about 900 ml. each) at 5°. The combined dialysates were concentrated to 50 ml. *in vacuo* and the concentrate (at *p*H 8.0) then run onto a column of Amberlite XE-64 (49 × 4 cm.) in the Li^+ form which had been equilibrated previously with lithium acetate (*p*H 6.5) and washed with water. Fractions were collected every 20 ml. and examined for ninhydrin-positive material. Tubes 19 to 31 contained L-diaminopimelic acid (R_f 0.57)[15] whereas tubes 19 and 20 contained, in addition, a small amount of a faster moving compound (R_f 0.63)[15] which, although not identified, was separable from the diamide (R_f 0.59),[15] the monoamide (R_f 0.53)[15] and lysine (R_f 0.55)[15] and which presumably could have arisen by autolysis of the enzyme. As no further ninhydrin positive material could be eluted with water, the eluent was changed to 1% acetic acid at tube 57. Emergence of ninhydrin-sensitive material began at tube 176 and was identified as the diamide (R_f 0.94).[23] This came off as an extended band (overlapping the end of the lithium) and was apparently uncontaminated with other detectable ninhydrin-positive compounds.

L-**Diaminopimelic Acid.**—The combined eluate of tubes 19–31 was concentrated to dryness, the residual material taken up in the minimal amount of hot water, treated with charcoal, filtered, adjusted to *p*H 6.5 with 2 *N* LiOH and 4 volumes of absolute ethanol subsequently added. A gelatinous precipitate formed which was filtered over suction and sucked dry to a white amorphous powder. Repetition of such precipitation, twice, finally yielded a product[24] which was ash and chloride free and which exhibited a single spot (R_f 0.57)[2] upon chromatographic examination; yield 3.5 g. (88%), $[\alpha]^{26}D$ +45.0° (1% in 1 *N* HCl).[25]

Anal. Calcd. for $C_7H_{14}O_4N_2$: C, 44.2; H, 7.4; N, 14.7. Found: C, 44.1; H, 7.5; N, 14.5.

D-**Diaminopimelic Acid.**—The eluate from the column which contained the diamide was concentrated *in vacuo* to a sirup which, in turn, was dissolved in 1 l. of 3 *N* HCl and boiled for 6 hr. under reflux. Chromatographic analysis indicated complete hydrolysis at this point. The hydrolysate was concentrated to dryness and the residual material taken up in 1.5 *N* HCl and run onto a Dowex 50 column (40 × 4 cm.) which had been equilibrated previously with 1.5 *N* HCl. The acid strength of the eluent was increased to 2.5 *N* and the amino acid thereby eluted was uncontaminated by other ninhydrin-positive material or by lithium. After the combined eluate was evaporated to dryness, the D-form of the amino acid[24] was recovered and purified as given for the L-antipode, above; yield 2.9 g. (73%), $[\alpha]^{26}D$ −45.5° (1% in 1 *N* HCl).[25]

Anal. Calcd. for $C_7H_{14}O_4N_2$: C, 44.2; H, 7.4; N, 14.7. Found: C, 44.1; H, 7.5; N, 14.6.

(23) A phenol–ammonia system on Whatman No. 1 paper was here employed.

(24) On drying *in vacuo* at 100° for 3–5 days, the compound lost 9% in weight, equivalent to one molecule of water of crystallization.

(25) Specific rotation of the monohydrate.

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[CONTRIBUTION FROM THE LABORATORY OF BIOCHEMISTRY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Studies on Diastereoisomeric α-Amino Acids and Corresponding α-Hydroxy Acids. VIII. Configuration of the Isomeric Octopines

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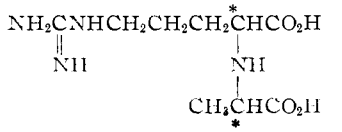
Preparation of the four stereoisomers of octopine was effected *via* a modification of previous methods involving the action of DL-α-bromopropionic acid, in alkaline solution, on L- or D-arginine. The epimeric mixture of (+)-octopine (natural form) and (+)-isoöctopine, secured with L-arginine, or (−)-octopine and (−)-isoöctopine, obtained when D-arginine was employed, was separated into the pure isomers through utilization of the picrate and flavianate derivatives. Optical rotation values of (+)-octopine and (+)-isoöctopine were $[\alpha]^{24}D$ +20.8° and +26.8° (2% in water), respectively, while their respective levorotatory antipodes exhibited values of −20.6 and −26.6° under the same conditions. (−)-Isoöctopine was alternatively prepared by the action of L-α-bromopropionic acid on D-arginine. Utilization of L- and DL-α-bromopropionyl-L-valine *in lieu* of α-bromopropionic acid in the above reactions resulted in the formation of the corresponding "octopinyl" peptides. Infrared spectra, apparent dissociation constants and optical rotatory dispersion data at 589, 578, 546, 435, 405 and 365 mμ, of the diastereomeric optical antipodes of octopine, are presented. From the latter data, calculations of the contribution (partial rotation) of the α- and α′-asymmetric centers to the observed rotation were effected and the empirical rule of Patterson and Brode then employed to assign tentatively a configuration to the alanine portion of each of the stereoisomeric octopines. Such assignment was confirmed unequivocally through the use of kinetic measurements which established that the reaction of L-α-bromopropionic acid with L-arginine, in alkaline solution, yielded (+)-octopine through a bimolecular substitution (SN2) mechanism.

Octopine was first isolated from octopus muscle by Morizawa,[1] in 1927, but it remained for Moore and Wilson[2] and for Akasi,[3] some ten years later, to establish independently the constitution of this compound as the guanidine derivative, α,α′-imino-(δ-guanidovaleric acid)-propionic acid



(1) K. Morizawa, *Acta Schol. Med. Univ. Imp. Kioto*, **9**, 285 (1927).

(2) E. Moore and D. W. Wilson, *J. Biol. Chem.*, **119**, 573, 585 (1937).

(3) S. Akasi, *J. Biochem. (Japan)*, **25**, 261, 281, 291 (1937).