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The Preparation and Some Properties of α-Aminoadipic-δ-Semialdehyde (Δ¹-Piperideine-6-carboxylic Acid)*

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α-Aminoadipic-δ-semialdehyde (Δ¹-piperideine-6-carboxylic acid), which has been suggested as an intermediate in the degradative metabolism of lysine and in its biosynthesis from α-aminoadipic acid, has been prepared by ozonolysis of 2-amino-6-heptenoic acid. 2-Amino-6-heptenoic acid was prepared by alkylation of ethylacetamidocyanoacetate with 5-bromo-1-pentene, followed by alkaline hydrolysis of the condensation product. *N*-Acetyl-α-aminoadipic-δ-semialdehyde was also prepared, and preparation of α-ketoadipic-δ-semialdehyde was attempted. Nonenzymatic transamination of lysine with glyoxylate at *p*H 5 and 100° gave α-keto-ε-aminocaproic acid rather than α-aminoadipic-δ-semialdehyde; under these conditions, ε-*N*-acetyllysine (but not α-*N*-acetyllysine) transaminated with glyoxylate. Evidence for the enzymatic reduction by liver and kidney preparations of *N*-acetyl-α-aminoadipic-δ-semialdehyde to the corresponding ω-alcohol in the presence of reduced pyridine nucleotides has been obtained. Diphosphopyridine nucleotide–dependent enzymatic oxidation of α-aminoadipic-δ-semialdehyde and of its *N*-acetyl derivative to the corresponding dicarboxylic acids was observed.

The enzymatic steps in the degradative metabolism of lysine and in its biosynthesis by certain yeasts and fungi from α-aminoadipic acid have not been fully elucidated. Although it has been suggested that α-aminoadipic-δ-semialdehyde may be formed as an intermediate in both pathways, this has not yet been experimentally demonstrated. The present work was initiated in an attempt to achieve a synthetic preparation of this compound that would be useful for enzymatic studies. In analogy with glutamic-γ-semialdehyde (Vogel and Davis, 1952; Strecker, 1960) and α-keto-ε-aminocaproic acid (Meister, 1954), α-aminoadipic-δ-semialdehyde would be expected to exist in solution in equilibrium with the corresponding cyclic form Δ¹-piperideine-6-carboxylic acid, as shown in reaction (1). Reduction of the cyclic form would give pipecolic acid, while oxidation of the open-chain form would yield α-aminoadipic acid. Reductive amination or trans-

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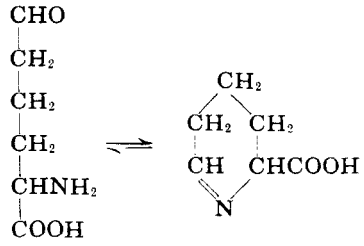

Reaction 1

amination of the open-chain form would be expected to yield lysine.

The present report describes the preparation of 2-amino-6-heptenoic acid, and ozonolysis of this compound to yield α-aminoadipic-δ-semialdehyde. Certain chemical and enzymatic properties of α-aminoadipic-δ-semialdehyde and of its *N*-acetyl derivative are given here. Attempted synthesis of the unstable α-ketoadipic-δ-semialdehyde is also described.

Experimental

Materials.—The amino acids were obtained as described in the accompanying paper (Aspen and Meister, 1962). Sodium glyoxylate was synthesized according to the directions of Radin and Metzler (1955). TPN, DPN, and the corresponding reduced pyridine nucleotides were obtained from the Sigma Chemical Company. Sodium borohydride was purchased from Metal Hydrides, Inc. *o*-Aminobenzaldehyde was prepared from *o*-nitrobenzaldehyde in small quantities as needed by the procedure of Smith and Opie (1955). Ethylacetamidocyanoacetate was obtained from Winthrop Laboratories, and platinum oxide catalyst was purchased from the American Platinum Works. Glutamic-γ-semialdehyde was prepared by acid hydrolysis of γ,γ-dicarbethoxy-γ-acetamidobutyraldehyde (Moe and Warner, 1948; Vogel and Davis, 1952), and Δ^1-piperideine-2-carboxylic acid was prepared as described by Meister (1954).

Enzyme Preparations.—Freshly excised rat and rabbit liver and kidney were homogenized with two volumes of 0.1 M potassium phosphate buffer (*p*H 7.0) in a glass homogenizer, and then centrifuged for 1 hour at 100,000 × *g* at 0°. The supernatant solutions were dialyzed for 4 hours against 100 volumes of 0.05 M potassium phosphate buffer (*p*H 7.0) at 5°. Δ^1-Piperideine-2-carboxylate reductase was obtained from rat kidney as described by Meister *et al.* (1957); the studies reported here were carried out at *p*H 6.1 in 0.1 M potassium phosphate buffer and an enzyme concentration of 10 mg per ml. Glutamic-γ-semialdehyde reductase was prepared from rat liver according to the method of Smith and Greenberg (1957); the system employed by these investigators was used here. Hog kidney acylase was prepared according to Birnbaum *et al.* (1952). Crystalline beef liver catalase was purchased from Worthington Biochemicals, Inc. Rattlesnake venom was obtained from Ross Allen's Reptile Institute.

DL-*2-Amino-6-heptenoic Acid.*–This amino acid was prepared by alkylation of ethylacetamidocyanoacetate with 5-bromo-1-pentene, followed by alkaline hydrolysis of the condensation product according to the general procedure of Albertson (1946). 4-Pentene-1-ol was prepared according to the method of Brooks and Snyder (1955) and converted to 5-bromo-1-pentene essentially as described by Gaubert *et al.* (1937). The latter procedure was modified as described by Goering *et al.* (1948) for the preparation of 5-bromo-2-pentene. 5-Bromo-1-pentene (47.2 g; 0.317 mole) was added drop-wise to a solution of sodium (7.2 g; 0.313 mole) and ethylacetamidocyanoacetate (48.5 g; 0.285 mole) in 150 ml of absolute ethanol. The solution was refluxed during the addition, which took about 1 hour, and for 1 additional hour. After cooling to 26°, the clear supernatant solution was decanted from a small sediment and the ethanol was removed by evaporation *in vacuo*. The residue was mixed with 450 ml of 10% sodium hydroxide and refluxed for 15 hours. The mixture was cooled in ice, acidified by addition of concentrated hydrochloric acid, and concentrated *in vacuo* to low volume. The residue was dissolved in the minimal volume of water and adjusted to *p*H 5 by addition of pyridine. Two volumes of ethanol were added and the mixture was cooled for 18 hours. The crude crystalline amino acid product was recrystallized twice with Norit from hot water (35 ml of water per g). The yield of pure product was 6 g (15% based on ethylacetamidocyanoacetate). Calculated for $C_7H_{13}O_2N$: C, 58.7; H, 9.09; N, 9.79. Found: C, 59.0; H, 9.10; N, 9.59. The product, which decolorized bromine water rapidly, melted with decomposition at 260–263°. Catalytic hydrogenation of 20 μmoles of the product in 0.2 ml of water with PtO_2 catalyst (2 mg) in the Parr apparatus (20 lb. per square inch; 2 hours; 26°) gave a product which exhibited paper chromatographic properties identical to that of 2-aminoheptanoic acid. The R_F values of 2-amino-6-heptenoic acid, 2-aminoheptanoic acid, and related derivatives are given in Table I.

When the condensation product obtained from 0.2 mole of ethylacetamidocyanoacetate and 0.2 mole of 5-bromo-1-pentene was refluxed with 200 ml of concentrated hydrochloric acid for 18 hours, a product was obtained which, after recrystallization from 50% ethanol, exhibited a melting point of 208–211° (with decomposition); yield, 3.5 g. This product did not decolorize bromine water nor did it take up ozone. Elemental analyses are consistent with 2-amino-6-chloroheptanoic acid. Calculated for $C_7H_{14}O_2NCl$: C, 46.8; H, 7.86; N, 7.80; Cl, 19.7. Found: C, 46.7; H, 7.88; N, 8.01; Cl, 19.4.

DL - α - *Aminoadipic* - δ - *semialdehyde.*—Ozonolysis of 1 to 25 ml of 0.1 M solutions of 2-amino-6-heptenoic acid hydrochloride was carried

TABLE I
PAPER CHROMATOGRAPHY OF α-AMINOADIPIC-δ-SEMIALDEHYDE AND RELATED COMPOUNDS

Amino Acid	R_F[a] A	B	C	D
α-Aminoadipic-δ-semialdehyde	0.44	0.85	0.54	0.62
α-Aminoadipic acid	0.29	0.26	0.58	0.31
2-Amino-6-heptenoic acid	0.70	0.81	0.85	0.79
2-Aminoheptanoic acid	0.75	0.84	0.89	0.82
2-Amino-6-chloroheptanoic acid	0.78	0.81	0.85	0.78
α-Amino-ε-hydroxycaproic acid	0.36	0.55	0.63	0.56
Lysine	0.08	0.23	0.30	0.08
α-Keto-ε-aminocaproic acid	0.43	0.46	0.62	0.67
Pipecolic acid	0.45	0.89	0.75	0.64
ε-N-Acetyllysine	0.40	0.83	0.71	0.56
α-N-Acetyllysine	0.42	0.76	0.69	0.49

[a] Solvent systems: A, *n*-Butanol–acetic acid–water (4:1:1); B, phenol–0.1 M potassium phosphate buffer, *p*H 7.2 (80:20); C, *t*-butanol–formic acid–water (70:15:15); D, ethanol-water (77:23).

out at 26° with a Welsbach Laboratory Ozonator (Model T-23). The reaction was complete within 5 to 30 minutes depending upon the volume employed. A stream of air was passed through the solution for several minutes to remove residual ozone. Paper chromatography revealed α-aminoadipic-δ-semialdehyde after treatment with ninhydrin as a bright yellow spot. No 2-amino-6-heptenoic acid was found on the chromatograms, but variable amounts of α-aminoadipic acid, presumably formed by further oxidation of the aldehyde, were present. The maximum conversion to α-aminoadipic acid was estimated to be about 10%. When the solution obtained after ozonolysis was subjected to catalytic hydrogenation (PtO_2, 20 lb., 26°, 3 hours) and then chromatographed, pipecolic acid was found. Elution of the pipecolic acid with water followed by quantitative determination according to Schweet (1954) indicated over-all yields of 77–82% of pipecolic acid, based on the 2-amino-6-heptenoic acid used. Similar yields were obtained when ozonolysis was carried at 0° and when reduction was carried out by addition of an excess of sodium borohydride (25 mg of sodium borohydride per ml; *p*H 7; 26°; 1 hour). When reduction was carried out with sodium borohydride, several minor ninhydrin-positive products were observed on paper chromatography. The data indicate that the minimum yield of α-aminoadipic-δ-semialdehyde was approximately 80%; the actual yield may have been greater than this if the reduction was not quantitative.

Paper electrophoresis of the solution obtained after ozonolysis was carried out in an apparatus similar to that of Markham and Smith (1952) with a buffer consisting of acetic acid, pyridine, and water (0.4:10:90; *p*H 6.4) for 1.5 hours at 20 v per cm. After treatment of the paper with ninhydrin, discrete spots corresponding to α-aminoadipic-δ-semialdehyde and α-aminoadipic acid were observed. A faint ninhydrin-positive spot corresponding in mobility to glutamic acid was also found. The formation of a small amount of glutamic acid after ozonolysis would be consistent with the presence of 2-amino-5-heptenoic acid in the starting material; some isomerization may have occurred in the preparation of 4-pentene-1-ol, 5-bromo-1-pentene, or in the alkaline hydrolysis of ethyl 2-acetamido-2-cyano-6-heptenoate; however, other explanations for the presence of trace amounts of glutamic acid in the solution after ozonolysis are not excluded.

When 0.1 ml of the solution after ozonolysis was added to 0.2 ml of a 1% solution of *o*-aminobenzaldehyde in ethanol, a deep orange color was formed within 2 or 3 minutes. Under these conditions, α-keto-ε-aminocaproic acid gave a similar color, but maximum intensity of color was achieved only after standing for 30–45 minutes. Attempts were made to isolate the dihydroquinazolinium complex formed by reaction of Δ^1-piperideine-6-carboxylate with *o*-aminobenzaldehyde as the picrate according to the procedure of Schöpf and Oechler (1936). When a solution of picric acid was added to the complex at *p*H 4.5, a precipitate was formed which was collected by centrifugation and recrystallized from methanol. Attempts to further purify and characterize this product were unsuccessful because of the instability of the product.

Treatment of the solution after ozonolysis with a 1% solution of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid gave a crystalline yellow precipitate, which gradually increased in quantity over a period of 3 days. The product was evidently a mixture (m.p. 152–165°); paper chromatography indicated that the main component was the 2,4-dinitrophenylhydrazone of formaldehyde. Catalytic hydrogenation of the mixture of hydrazones (Meister and Abendschein, 1956) gave pipecolic acid and lysine; on the other hand, catalytic hydrogenation of the filtrate obtained after removal of the hydrazones gave only pipecolic acid. These findings are consistent with the reaction of some of the aminoadipic-δ-semialdehyde with the reagent to yield the corresponding hydrazone, but it appears that most of the aldehyde remained unchanged, presumably as the corresponding cyclic Δ^1-piperideine-6-carboxylic acid. It is of interest that the 2,4-dinitrophenylhydrazone of the isomeric α-keto-ε-aminocaproic acid also forms relatively slowly (Meister, 1954), and gives both lysine and pipecolic acid on catalytic hydrogenation (Meister and Abendschein, 1956).

Separation of α-aminoadipic-δ-semialdehyde from formaldehyde and α-aminoadipic acid was accomplished by ion-exchange column chromatography. One ml of the solution obtained after

ozonolysis was added to the top of a Dowex-50 column (H^+; 1 × 20 cm) and the column was washed with several column-volumes of water. Elution was carried out with 2.5 N hydrochloric acid; α-aminoadipic acid was eluted separately and ahead of α - aminoadipic - δ - semialdehyde. Separation was also achieved with a Dowex-1-acetate column equilibrated with 0.3 M sodium acetate buffer (*p*H 4.9); the aldehyde was eluted before α-aminoadipic acid under these conditions. Isolation of α-aminoadipic-δ-semialdehyde in solid form was not carried out because of its instability and tendency to undergo oxidation. Acid solutions of the aldehyde were stable over a period of several months, as judged by the reaction with *o*-aminobenzaldehyde and by enzymatic criteria (see below).

N - Acetyl - DL - α - aminoadipic - δ - semialdehyde.—A solution containing 215 mg of DL-2-amino-6-heptenoic acid in 30 ml of water was cooled to 0° and treated with one equilvalent of sodium hydroxide. Three equivalents of acetic anhydride were added in ten portions together with equivalent quantities of sodium hydroxide to maintain the *p*H between 7 and 8. The mixture was shaken vigorously after each addition and the disappearance of free amino acid was followed by the ninhydrin color reaction. After the reaction was complete, the solution was acidified by addition of concentrated hydrochloric acid and evaporated to low volume *in vacuo*. DL-*N*-Acetyl-2-amino-6-heptenoic acid crystallized on cooling. After recrystallization from water, 145 mg (52%) of product was obtained; m.p. 95–96°. Calculated for $C_9H_{15}O_3N$: C, 58.4; H, 8.16; N, 7.56. Found: C, 58.2; H, 8.09; N, 7.81.

The product was treated with ozone as described above. Addition of 0.5% 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid to the solution obtained after ozonolysis led to prompt precipitation of a crystalline derivative, which was recrystallized from water; m.p. 110°. Catalytic hydrogenation of this derivative gave α-*N*-acetyllysine as the only amino acid product.

No reaction was observed when the solution after ozonolysis was treated with *o*-aminobenzaldehyde. However, when solutions, after ozonolysis, were made 6 N with respect to hydrochloric acid and then heated at 100° for 5 minutes, a color reaction was obtained promptly on addition of *o*-aminobenzaldehyde. Paper chromatography of the hydrolyzed material revealed the presence of α-aminoadipic-δ-semialdehyde and a small amount of α-aminoadipic acid. Hydrolysis of the acetyl group was also accomplished enzymatically as follows. A reaction mixture containing the acetyl derivative (30 μmoles) and purified kidney acylase (3 mg) in 0.5 ml of 0.1 M potassium phosphate buffer (*p*H 6.7) was incubated at 37° for 2.5 hours; after incubation an orange-yellow color was formed immediately on addition of *o*-aminobenzaldehyde.

D-*α-Aminoadipic-δ-semialdehyde.*—This compound was prepared by ozonolysis of D-2-amino-6-heptenoic acid, which was prepared from the corresponding racemate by selective enzymatic destruction of the L-isomer with snake venom L-amino acid oxidase (Meister, 1952). DL-2-Amino-6-heptenoic acid (429 mg) was dissolved in 22 ml of water and added to a mixture of 600 units of crystalline beef liver catalase and 600 mg of rattlesnake venom dissolved in 8 ml of water. The enzyme solutions were thoroughly dialyzed against water prior to use. The *p*H of the mixture was adjusted to 7.5 by cautious addition of 0.1 M potassium hydroxide, and oxygen was bubbled into the solution for 3.5 hours, at which time ammonia analyses indicated that the reaction had gone to completion. The mixture was dialyzed against 2 liters of water for 18 hours at 5° and the dialysate was concentrated to approximately 5 ml *in vacuo*. The concentrated solution was added to the top of a Dowex-50 column (H^+; 1.4 × 30 cm) and the column was washed with water. The eluate, which contained 2-keto-6-heptenoic acid, was concentrated *in vacuo* to 15 ml and stored at −10°. The D-amino acid was eluted from the column with 3 N hydrochloric acid, and the eluate was evaporated to dryness *in vacuo* and taken up in a small volume of water. The amino acid was isolated by large-scale paper chromatography with a solvent consisting of *n*-butanol–acetic acid–water (4:1:1). Ozonolysis was carried out as described above for the racemic compound.

α-Ketoadipic-δ-semialdehyde.—Attempts to prepare this compound were made by passing ozone through solutions containing 2-keto-6-heptenoic acid obtained by enzymatic oxidative deamination of L-2-amino-6-heptenoic acid as described above. Evidence was obtained for the formation of the desired product, but the compound was apparently very unstable under these conditions. When solutions of 2-keto-6-heptenoic acid were treated with ozone for 5 minutes at 0°, prompt addition of a 1% solution of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid immediately gave a precipitate, which was collected by centrifugation and washed with water. After catalytic hydrogenation, paper chromatography revealed six ninhydrin-positive spots, of which four were identified as lysine, α-aminoadipic acid, pipecolic acid, and δ-aminovaleric acid. The formation of lysine on reduction of the hydrazone is consistent with the presence of α-ketoadipic-δ-semialdehyde in the original solution. The formation of pipecolic acid would be expected if a mono rather than the di-2,4-dinitrophenylhydrazone derivative of α-ketoadipic-δ-semialdehyde had formed. The finding of δ-aminovaleric acid after catalytic hydrogenation suggests the occurrence of decarboxylation of α-ketoadipic-δ-semialdehyde to glutaric semialdehyde; oxidative decarboxylation of 2-keto-6-heptenoic acid to 5-hexenoic acid, followed by cleavage of the double bond, would also yield

glutaric semialdehyde. When such solutions were stored at −10° for 1 day, they failed to yield a hydrazone derivative that gave lysine on catalytic hydrogenation.

Nonenzymatic Transmination of Lysine.—In the course of this work we investigated nonenzymatic transamination of lysine as a potentially useful reaction for the preparation of α-aminoadipic-δ-semialdehyde. However, under the conditions employed, we obtained evidence only for the formation of α-keto-ε-aminocaproic acid. In these studies, reaction mixtures consisting of L-lysine (5 μmoles), sodium glyoxylate (25 μmoles), cupric sulfate (0.5 μmole), and sodium acetate buffer (20 μmoles, *p*H 5.0) in a final volume of 0.3 ml were heated at 100° for varying periods up to 1 hour. Catalytic hydrogenation of the mixtures after heating gave pipecolic acid; maximum formation of pipecolic acid was obtained after heating for 2.5 minutes. Separation of the pipecolic acid formed, followed by enzymatic test with D-amino acid oxidase as described in the accompanying paper (Aspen and Meister, 1962), revealed that the pipecolic acid was racemic. This result indicates that α-keto-ε-aminocaproic acid was formed in the transamination rather than α-aminoadipic-δ-semialdehyde. Catalytic reduction of D-α-aminoadipic-δ-semialdehyde has been found to give D-pipecolic acid (Aspen and Meister, 1962).

Nonenzymatic transmination of α-*N*-acetyllysine and ε-*N*-acetyllysine with glyoxylate was investigated under similar conditions. Paper chromatography of such reaction mixtures after heating at 100° for 20 minutes revealed considerable quantities of glycine when ε-*N*-acetyllysine was used, but no glycine was formed with α-*N*-acetyllysine. The failure of the ε-amino groups of α-*N*-acetyllysine and of lysine to transaminate under these conditions is consistent with other studies which indicate that β- and γ-amino acids do not undergo nonenzymatic transamination at 100°, although at higher temperatures transamination may take place (Snell, 1958; Kalyankar and Snell, 1957; Heyns and Walter, 1953). It is of interest that in contrast to the apparent reactivity of its α-amino group in transamination with α-keto acids, lysine reacts preferentially with aromatic aldehydes such as benzaldehyde and salicylaldehyde through its ε-amino group to yield Schiff bases (Witkop and Beiler, 1954).

Enzymatic Studies.—Purified rat kidney Δ^1-piperideine-2-carboxylate reductase was not active toward α-aminoadipic-δ-semialdehyde with either DPNH or TPNH, under conditions similar to those previously employed (Meister *et al*, 1957). On the other hand, a preparation of Δ^1-pyrroline-5-carboxylate reductase (Smith and Greenberg, 1957) catalyzed oxidation of DPNH in the presence of α-aminoadipic-δ-semialdehyde at 25–33% of the rate observed with glutamic-γ-semialdehyde; however, the observed oxidation with α-aminoadipic-δ-semialdehyde was only about 1.5 times that of the endogenous rate. Paper chromatography of the reaction mixtures failed to reveal formation of either pipecolic acid or α-amino-ε-hydroxycaproic acid. Although the findings are consistent with enzymatic reduction of α-aminoadipic-δ-semialdehyde, the product of reduction was not identified.

When the rat and rabbit kidney and liver preparations described under Methods (above) were incubated with α-aminoadipic-δ-semialdehyde and either DPNH or TPNH, the rates of oxidation of the pyridine nucleotides were not greater than those found in the absence of substrate. In contrast, these enzyme preparations were significantly active in catalyzing the reduction of Δ^1-piperideine-2-carboxylate to pipecolate with DPNH. They also catalyzed reduction of *N*-acetyl-α-aminoadipic-δ-semiladehyde with either DPNH or TPNH. Oxidation of reduced pyridine nucleotides was followed spectrophotometrically at 340 mμ in reaction mixtures (final volume, 1.05 ml) containing enzyme preparation (0.1 ml), reduced pyridine nucleotide (0.1 μmole), *N*-acetyl-α-aminoadipic - δ - semialdehyde (2 μmoles), and potassium phosphate buffer (100 μmoles, *p*H 6.1); readings were made at intervals against reference mixtures that lacked the pyridine nucleotides. Under these conditions, the rates of oxidation of TPNH and DPNH with the rabbit liver preparation at 26° were 6.7 and 2.2 mμmoles per minute, respectively; these values are corrected for endogenous oxidation (0.3–0.6 mμmole per minute). In order to identify the product of reduction, such reaction mixtures (containing TPNH) were incubated at 37° for 1 hour; they were then heated at 100° for 3 minutes to denature the enzyme. Purified kidney acylase (5 mg) was added, and the mixture was incubated at 37° for 2 hours and then placed again at 100° for 3 minutes; the precipitated protein was removed by centrifugation and the supernatant solution was examined by paper chromatography. Such study revealed the formation of α-amino-ε-hydroxycaproic acid; this amino acid was not found in controls in which the enzyme preparations were separately omitted.

The rabbit liver preparation reduced DPN in the presence of either α-aminoadipic-δ-semialdehyde or *N*-acetyl-α-aminoadipic-δ-semialdehyde (Fig. 1). TPN was not active. Results similar to those given in Figure 1 were also obtained with rabbit kidney and rat kidney and liver preparations. Paper chromatographic study of such reaction mixtures revealed the formation of α-aminoadipic acid from α-aminoadipic-δ-semialdehyde. When reaction mixtures containing *N*-acetyl-α-aminoadipic-δ-semialdehyde were treated with kidney acylase as described above, α-aminoadipic acid was found.

Discussion

The procedure described here for the preparation of α-aminoadipic-δ-semialdehyde is analogous to that employed by Black and Wright (1955) for aspartic-β-semialdehyde, and is applicable also

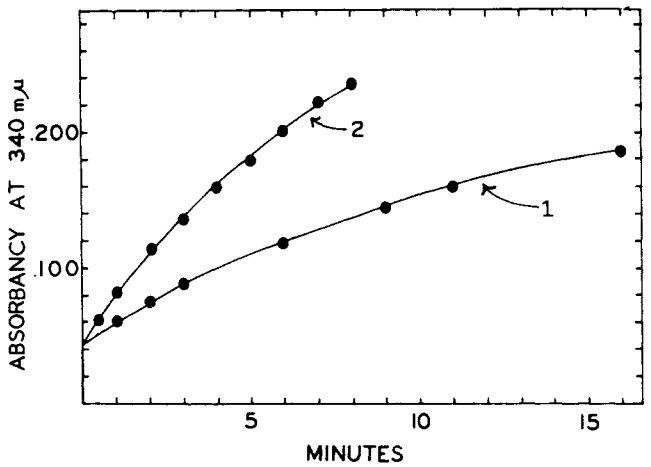

FIG. 1.—Enzymatic reduction of DPN in the presence of α-aminoadipic-δ-semialdehyde (curve 1) and *N*-acetyl-α-aminoadipic-δ-semialdehyde (curve 2). The reaction mixtures contained rabbit liver enzyme preparation (0.04 ml), DPN (0.1 μmole), substrate (4 μmoles), and potassium phosphate buffer (100 μmoles, *p*H 8.1); final volume, 1.05 ml; 26°. Reduction was followed spectrophotometrically at 340 mμ by reading the reaction mixtures against reference mixtures that lacked DPN. No reduction of DPN occurred in the absence of substrates.

to the preparation of the individual optical isomers. It is of interest that aspartic-β-semialdehyde did not yield the usual aldehyde derivatives readily (Black and Wright, 1955); although α-aminoadipic-δ-semialdehyde combined slowly with 2,4-dinitrophenylhydrazine, the corresponding *N*-acetyl derivative reacted rapidly. The available evidence suggests that α-aminoadipic-δ-semialdehyde has a marked tendency to cyclize.

Evidence for the enzymatic formation of α-aminoadipic-δ-semialdehyde from α-amino-ε-hydroxycaproic acid catalyzed by a pyridine nucleotide-dependent enzyme from *Neurospora crassa* that also catalyzes the reversible formation of glutamic - γ - semialdehyde from α - amino - δ-hydroxyvaleric acid has been reported by Yura and Vogel (1959). Hasse *et al.* (1961) have also observed this reaction and have reported that the oxidation of lysine by pea seedling diamine oxidase yields α-aminoadipic-δ-semialdehyde; identification was based on comparisons of the electrophoretic and chromatographic properties of the product (and of its *o*-aminobenzaldehyde derivative) with those of an α-aminoadipic-δ-semialdehyde preparation obtained by chromic acid oxidation of α-amino-ε-hydroxycaproic acid (Hasse and Wieland, 1960).

Although detailed enzymatic studies have not as yet been carried out with mammalian tissues, the present preliminary investigations indicate the presence in rat and rabbit liver and kidney preparations of pyridine nucleotide-linked enzymatic activities capable of oxidizing both α-aminoadipic-δ - semialdehyde and *N* - acetyl - α - aminoadipic-δ-semialdehyde to the corresponding dicarboxylic acids, and of an activity capable of reducing *N*-acetyl-α-aminoadipic-δ-semialdehyde to *N*-acetyl-α-amino-ε-hydroxycaproic acid. α-Aminoadipic-δ-semialdehyde may undergo enzymatically catalyzed reduction to pipecolic acid; such a reaction would be in accord with the studies of Rothstein and Miller (1954) on the conversion of lysine to pipecolic acid, and would be consistent with observations on the formation of pipecolic acid from α-aminoadipic acid (Aspen and Meister, 1962).

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