acetoacetate level is markedly raised it does not contribute to any increase in the third spot. The absence of any detectable acetoacetate spot is probably due to decarboxylation, both during the extraction process and during the run on the paper, and it was noticed that an acetone 2:4-dinitrophenylhydrazone spot was regularly seen on the chromatograms from the alloxan-treated animals.

In view of these findings, together with the resemblance between the absorption curve of the substance giving the third spot in blood filtrates with that of the second pyruvate spot (Cavallini & Frontali, 1954), which finding we have also confirmed, we conclude that this third blood component does not result from acetoacetic acid but corresponds to the more rapidly moving pyruvate component. It would seem therefore that a safe estimate of the pyruvate content of blood can be obtained by summing the two more rapidly moving spots, even when high levels of acetoacetate are present.

SUMMARY

1. A chromatographic study has been made of the keto acids present in the blood of normal rats and of rats poisoned with sodium arsenite and alloxan respectively.

2. It has been shown that a rise in the blood pyruvate level is accompanied by a rise in the 'third spot' visible in chromatograms of dinitrophenyl-

hydrazones from blood, and that no increase in this occurs when the acetoacetate level is high.

3. It is concluded that this third spot is due to the more rapidly running component of the pyruvic acid 2:4-dinitrophenylhydrazone, and that high acetoacetate levels are not likely to interfere in the chromatographic estimation of the blood pyruvate level.

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Analogues of Diaminopimelic Acid as Inhibitors of Bacterial Growth

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Many analogues of amino acids have been synthesized and tested as potential bactericides. β -2-Thienylalanine inhibits growth of *Escherichia coli*, *Streptococcus faecalis* and *Lactobacillus arabinosus*, but it has no chemotherapeutic value, since it is also toxic to animals (du Vigneaud, McKennis, Simmonds, Dittmer & Brown, 1945; Dittmer, Ellis, McKennis & du Vigneaud, 1946; Ferger & du Vigneaud, 1948). This, and much other work, has made it evident that chemical analogues of the amino acids which are normal constituents of animal proteins are unlikely to display sufficient

selective toxicity to make them valuable chemotherapeutic drugs.

The demonstration (Work, 1951; Work & Dewey, 1952, 1953) that $\alpha\alpha'$ -diaminopimelic acid was confined to the bacterial kingdom, suggested that analogues of this compound might inhibit bacterial metabolism without being toxic to animal cells. The present communication records the synthesis of a series of such analogues and their derivatives, together with tests on these compounds as inhibitors of bacterial growth. Their behaviour as competitive inhibitors for the specific diaminopimelic acid decarboxylase of bacterial cells is recorded in the following paper (Dewey, Hoare & Work, 1954).

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SYNTHETIC WORK

$\alpha\alpha'$ -Diaminoadipic acid. This was prepared by condensation of dimethyl $\alpha\alpha'$ -dibromoadipate (Guha & Sankaran, 1946), with potassium phthalimide in dimethylformamide, followed by hydrolysis of the resulting dimethyl $\alpha\alpha'$ -diphthalimidoadipate with hydrazine hydrate and 12N-HCl (Sheehan & Bolhofer, 1950). After the filtrate had been neutralized with 2N-NH₃, the product crystallized from water and melted at 286–298° with decomposition. (Found: C, 41.1; H, 6.7; N, 15.7. Calc. for C₈H₁₂O₄N₂: C, 40.9; H, 6.8; N, 15.9%.)

$\alpha\alpha'$ -Diaminopimelic acid. This was prepared as above by condensation of diethyl $\alpha\alpha'$ -dibromopimelate with potassium phthalimide to yield diethyl $\alpha\alpha'$ -diphthalimidopimelate as a syrup. This was hydrolysed with hydrazine hydrate and 12N-HCl. The product did not precipitate after neutralization of the filtered hydrolysate with 3N-NH₃. Ammonium chloride was therefore removed in an electrolysis cell, and $\alpha\alpha'$ -diaminopimelic acid precipitated with ethanol from the solution after concentration (yield 30%). It charred at 312° and decomposed at 345–351°. (Found: C, 43.8; H, 7.4; N, 14.9. Calc. for C₇H₁₀O₄N₂: C, 44.2; H, 7.4; N, 14.8%.)

$\alpha\alpha'$ -Diaminosuberic acid. The tetraethyl ester of 1:6-diacetamidohexane-1:1:6:6-tetracarboxylic acid was prepared by refluxing 1.3 moles of redistilled tetramethylene dibromide with 2 moles of ethyl acetamidomalonate and 2 g. atoms of Na in dry ethanol for 6 hr. Yield, 52% on ethyl acetamidomalonate; from ethylacetate–light petroleum (b.p. 60–80°), m.p. 136–137°. (Found: C, 54.2; H, 7.6; N, 5.8. C₂₂H₃₈O₁₀N₄ requires C, 54.0; H, 7.4; N, 5.7%.)

Hydrolysis of this material was carried out with 48% (w/w) aqueous HBr at 160° for 7 hr. The reaction mixture was evaporated to remove HBr and the dry residue was taken up in ethanol. Excess 50% (v/v) ethanolic pyridine was added to precipitate the $\alpha\alpha'$ -diaminosuberic acid (85%). The product darkened slightly at 280–360° but did not melt below 360° (cf. Abderhalden & Zeisset, 1928). (Found: C, 47.0; H, 7.6; N, 13.7. Calc. for C₈H₁₀O₄N₂: C, 47.2; H, 7.8; N, 13.8%.)

$\alpha\alpha'$ -Diaminoazelaic acid. This was prepared by amination of $\alpha\alpha'$ -dibromoazelaic acid with (NH₄)₂CO₃ and aqueous NH₃ (sp.gr. 0.880). The product (84% yield) charred at 310° but did not melt below 360° (Neuberg & Niemann, 1905). (Found: C, 48.9; H, 8.4; N, 11.4. Calc. for C₉H₁₂O₄N₂: C, 49.5; H, 8.3; N, 12.9%.)

It was also prepared by condensation of ethyl acetamidomalonate (2 moles) with pentamethylene dibromide (1.3 moles) in the presence of sodium ethoxide (2 moles) as above to yield the tetraethyl ester of 1:7-diacetamidohexane-1:1:7:7-tetracarboxylic acid (42% on acetamidomalonate ester), m.p. 148.5–149.5°. (Found: C, 55.4; H, 7.5; N, 5.8. C₂₃H₃₈O₁₀N₄ requires C, 54.9; H, 7.6; N, 5.6%.) This was hydrolysed with 48% HBr (12 moles) as described for $\alpha\alpha'$ -diaminosuberic acid. $\alpha\alpha'$ -Diaminoazelaic acid (92% yield) did not melt below 360°. (Found: C, 48.9; H, 8.0; N, 12.6. Calc. for C₉H₁₂O₄N₂: C, 49.5; H, 8.3; N, 12.9%.)

$\alpha\alpha'$ -Diaminosebacic acid. Prepared by the bromination of sebacic acid and amination of the resulting $\alpha\alpha'$ -dibromosebacic acid (Neuberg & Niemann, 1905). Free $\alpha\alpha'$ -diaminosebacic acid was prepared from its hydrochloride by precipitation with 2N-NH₃. It charred at 330–340° but did not melt below 360° (yield 85%). (Found: C, 49.7; H, 8.7; N, 11.9. Calc. for C₁₀H₂₀O₄N₂: C, 51.7; H, 8.6; N, 12.1%.)

$\alpha\alpha'$ -Diaminosebacic acid diamide. Diethyl $\alpha\alpha'$ -dibromosebacate was treated with (NH₄)₂CO₃ and liquid NH₃ in a sealed tube at room temperature for 1 week. The NH₃ was allowed to evaporate spontaneously, and recrystallization of the amide was attempted from water and ethanol, in both of which it was exceedingly soluble. The highest m.p. recorded (142–147° with decomposition) was obtained with a sample which had been recrystallized from ethanol. Analytical figures were unsatisfactory.

$\alpha\alpha'$ -Bis(dimethylamino)sebacic acid. $\alpha\alpha'$ -Dibromosebacic acid was treated with 33% (w/v) ethanolic dimethylamine (5 moles) at 37° in a sealed tube for 3 days. The contents were evaporated and the residue recrystallized from aqueous ethanol. The product melted at 268–269° with decomposition, varying slightly with rate of heating (yield 85%). (Found: C, 57.7; H, 9.6; N, 9.5. C₁₄H₂₈O₄N₂ requires C, 58.3; H, 9.7; N, 9.7%.)

1:10-Diaminodecane-1:10-dicarboxylic acid. Decane-1:10-dicarboxylic acid was prepared by a method based on that described by Ruhoff (1936) for the preparation of tridecanoic acid. Decamethylene dibromide (1 mole), KCN (3 moles) and 95% (v/v) ethanol were refluxed for 20 hr. on the water bath. KCN (3 moles) was added at the end of this time and the mixture was refluxed for a further 9 hr. After adding 85% (w/v) aqueous KOH (4 moles) and refluxing for 24 hr. the mixture was steam-distilled, acidified, steam-distilled again, and the precipitated decane-1:10-dicarboxylic acid was filtered off (yield 75%). M.p. 118–123°. It was converted into 1:10-dibromodecane-1:10-dicarboxylic acid with SOCl₂ (2.4 moles) and Br₂ (2.1 moles) (Guha & Sankaran, 1946). The product (57%) was recrystallized from benzene–light petroleum (b.p. 60–80°) and melted at 115–118°. Mixed with decane-1:10-dicarboxylic acid (m.p. 118°) it melted at 85–100°. (Found: C, 37.0; H, 5.0; Br, 41.2. C₁₂H₂₀O₄Br₂ requires C, 37.1; H, 5.2; Br, 41.3%.) This bromo acid was aminated with powdered (NH₄)₂CO₃ and aqueous NH₃ (sp.gr. 0.880). The product melted at 295–297° (yield 70%). (Found: C, 54.7; H, 9.3; N, 10.7. C₁₂H₂₄O₄N₂ requires C, 55.3; H, 9.2; N, 10.8%.)

1:10-Bis(dimethylamino)decane-1:10-dicarboxylic acid was prepared by treating the same bromo acid with 33% (w/v) ethanolic dimethylamine (12 moles) in a sealed tube at 37° for 5 days. M.p. of product was 248–250° with decomposition (yield 49%). (Found: C, 60.4; H, 10.0; N, 8.6. C₁₆H₂₂O₄N₂ requires C, 60.7; H, 10.1; N, 8.9%.)

1:12-Diaminododecane-1:12-dicarboxylic acid. The tetraethyl ester of 1:12-diacetamidododecane-1:1:12:12-tetracarboxylic acid was prepared in 33% yield from acetamidomalonate ester (2 moles) by refluxing with decamethylene dibromide (1.3 moles) and sodium ethoxide (2 moles) in anhydrous ethanol for 6 hr. The product had m.p. 93–94°. Mixed with acetamidomalonate ester (m.p. 95–97°) it softened at 81° and melted at 87–93°. (Found: C, 58.7; H, 8.6; N, 5.0. C₂₆H₄₈O₁₀N₄ requires C, 58.7; H, 8.9; N, 4.9%.) This material was hydrolysed with 48% (w/v) HBr for 6 hr. The resulting free amino acid (66% yield) was precipitated from an aqueous solution of its hydrobromide by the addition of excess 2N-NH₃. Since the free amino acid could not be recrystallized because of its insolubility, it was purified through its hydrochloride. This melted at 291–293° with decomposition. (Found: C, 45.9; H, 8.3; N, 8.0. C₁₄H₂₈O₄N₂ · 2HCl requires C, 46.5; H, 8.3; N, 7.8%.)

NN'-Diglycyl- $\alpha\alpha'$ -diaminosuberic acid. This was prepared by bromoacetylation of $\alpha\alpha'$ -diaminosuberic acid, followed by amination of the product. Although the tripeptide gave

a single spot on paper chromatography and yielded only glycine and $\alpha\alpha'$ -diaminosuberic acid on hydrolysis, insufficient was available to give satisfactory analytical figures. On heating, it started to char at 205° and melted with decomposition at 335–360°.

NN'-Diglycyl- $\alpha\alpha'$ -diaminosebacic acid. This was prepared by coupling *N-p*-toluenesulphonylglycyl chloride (2.05 moles) with diethyl $\alpha\alpha'$ -diaminosebacate (2 moles) in anhydrous ether. The ester was obtained by treatment of diaminosebacic acid with ethanol and HCl. Diethyl *NN'-p*-toluenesulphonylglycyl- $\alpha\alpha'$ -diaminosebacate, was recrystallized from ethyl acetate–light petroleum (b.p. 60–80°) to constant m.p. 146–147°, and hydrolysed with *n*-NaOH (2 moles), to yield *NN'-p*-toluenesulphonylglycyl- $\alpha\alpha'$ -diaminosebacic acid, which was recrystallized from methanol (m.p. 161–162°, yield 20%). (Found: N, 8.5. $C_{28}H_{38}O_{10}N_4S_2$ requires N, 8.6%.)

The *N-p*-toluenesulphonyl tripeptide was treated with Na (12 atoms) in liquid NH_3 , to yield *NN'*-diglycyl- $\alpha\alpha'$ -diaminosebacic acid which was precipitated by the addition of 25% mercuric acetate and 10% Na_2CO_3 according to the method of Neuberg & Kerb (1912), to free it from contaminating ammonium salts. The basic Hg complex was decomposed with H_2S . The tripeptide (yield 50%) charred at 250° but, did not melt below 360°. (Found: N, 16.2. $C_{14}H_{28}O_8N_4$ requires N, 16.2%.)

Chromatography of compounds

The identity and purity of all the amino acids and peptides prepared above were checked by chromatography. The R_F values are given in Table 1.

Table 1. *Chromatographic properties of $\alpha\alpha'$ -diaminopimelic acid and its homologues*

The phenol and collidine solvents are the mixtures described by Dent (1948). Whatman no. 4 paper was used with descending flow and substances were located with ninhydrin.

Substance	R_F	
	Phenol–water– NH_3	Collidine
$\alpha\alpha'$ -Diaminoadipic acid	0.22	0.03
$\alpha\alpha'$ -Diaminopimelic acid	0.33	0.03
$\alpha\alpha'$ -Diaminosuberic acid	0.44	0.03
$\alpha\alpha'$ -Diaminoazelaic acid	0.55	0.05
$\alpha\alpha'$ -Diaminosebacic acid	0.65	0.07
1:10-Diaminodecane-1:10-dicarboxylic acid	0.76	0.13
1:12-Diaminododecane-1:12-dicarboxylic acid	0.81	0.21
<i>NN'</i> -Diglycyl- $\alpha\alpha'$ -diaminosuberic acid	0.67	0.03
<i>NN'</i> -Diglycyl- $\alpha\alpha'$ -diaminosebacic acid	0.78	0.05

Table 2. *Concentrations of synthetic amino acids required to prevent visible growth in Hedley Wright broth overnight at 37°*

Compound	mg./100 ml.			
	<i>Strep. aureus</i>	<i>Staph. aureus</i>	<i>Esch. coli</i>	<i>C. dipht.</i>
$\alpha\alpha'$ -Diaminoadipic acid	> 50	> 50	> 50	—
$\alpha\alpha'$ -Diaminopimelic acid	> 500	> 500	> 500	—
$\alpha\alpha'$ -Diaminosuberic acid	50	> 50	> 50	25
$\alpha\alpha'$ -Diaminoazelaic acid	> 500	> 500	> 500	500
$\alpha\alpha'$ -Diaminosebacic acid	Inactive in sat. solution	Inactive in sat. solution	Inactive in sat. solution	~ 10
1:10-Diaminodecane-1:10-dicarboxylic acid	Inactive in sat. solution	Inactive in sat. solution	Inactive in sat. solution	—
1:12-Diaminododecane-1:12-dicarboxylic acid	Inactive in sat. solution	Inactive in sat. solution	Inactive in sat. solution	—
$\alpha\alpha'$ -Bis(dimethylamino)sebacic acid	> 500	> 500	> 500	> 500
1:10-Bis(dimethylamino)decane-1:10-dicarboxylic acid	> 500	> 500	> 500	300
$\alpha\alpha'$ -Diaminosebacamide	500	> 500	> 500	500
<i>NN'</i> -Diglycyl- $\alpha\alpha'$ -diaminosebacic acid	> 200	> 200	> 200	—

bis(dimethylamino)sebacic acid, *NN'*-diglycyldiaminosuberic acid and *NN'*-diglycyldiaminosebacic acid were all readily soluble in water, but were less active than diaminosuberic acid. It is also possible that permeability considerations play an important part in the assimilation of these compounds.

SUMMARY

1. Methods are described for the synthesis of six $\alpha\alpha'$ -diaminodicarboxylic acids, homologues of $\alpha\alpha'$ -diaminopimelic acid.

2. These amino acids and some of their derivatives have been tested as antibacterial agents.

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Diaminopimelic Acid Decarboxylase in Cells and extracts of *Escherichia coli* and *Aerobacter aerogenes*

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$\alpha\alpha'$ -Diaminopimelic acid is a constituent of many bacteria and blue-green algae, but is absent from all other organisms so far examined. It has been found in certain cellular proteins, in insoluble cell-wall fractions, in the free state and in an extracellular peptide (Work, 1951; Work & Dewey, 1953; Gendre & Lederer, 1952; Holdsworth, 1952; Salton, 1953; Blass, Lecomte & Macheboeuf, 1951; Work, 1949; Pauletta & DeFranceschi, 1952; Powell & Strange, 1953). In order to gain some knowledge of the metabolism of diaminopimelic acid, a search was made for a bacterial enzyme which would attack it. The possibility of decarboxylation was first considered, and *Escherichia coli* was the organ-

ism originally chosen since it was known to contain a large number of amino acid decarboxylases (Gale, 1946). Using the acidic conditions of growth and enzyme assay found by Gale to produce optimum decarboxylase activity, no decarboxylation of diaminopimelic acid was obtained. Attention was then directed to the transamination reaction which occurs in an alkaline medium (Feldman & Gunsalus, 1950). Under alkaline conditions, freeze-dried cell suspensions of *Esch. coli* converted diaminopimelic acid into lysine and cadaverine, and it was apparent that decarboxylation, and not transamination, had taken place.