The Stereoisomers of αε-Diaminopimelic Acid: their Distribution in Nature and Behaviour towards certain Enzyme Preparations

By D. S. HOARE and ELIZABETH WORK
Department of Chemical Pathology, University College Hospital Medical School, London, W.C. 1

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αε-Diaminopimelic acid (1:5-diaminopentane-1:5-dicarboxylic acid) is symmetrical about the γ-carbon atom and contains two asymmetric centres; it has three stereoisomers, namely the LL, DD and *meso* (internally compensated) compounds. It appears to be confined to bacterial organisms and is widely distributed among them (Work & Dewey, 1953). Diaminopimelic acid was originally isolated from hydrolysates of *Corynebacterium diphtheriae* and *Mycobacterium tuberculosis*, and the suggestion was made that it was the *meso* isomer because of its optical inactivity and its behaviour in the presence of the L-amino acid oxidase of *Neurospora* (Work, 1951). Subsequent evidence obtained with diaminopimelic acid decarboxylase supported this view (Work, 1953; Dewey, Hoare & Work, 1954), but rigorous proof could not be obtained without a supply of the authentic *meso* isomer.

Diaminopimelic acid has also been isolated from the culture filtrate of the lysine auxotroph *Escherichia coli* 26–26 (Work & Denman, 1953; Wright & Cresson, 1953). Both groups of workers found that this diaminopimelic acid had a slight positive rotation when first crystallized, but after several recrystallizations it was optically inactive. Wright & Cresson suggested that some LL isomer was present in addition to the *meso* isomer.

This paper describes the isolation from the mutant culture filtrate of a highly dextrorotatory compound which proved to be LL-diaminopimelic acid. The isomeric forms of all the naturally occurring diaminopimelic acids were established by direct comparison with the authentic isomers, made available by the resolution of synthetic diaminopimelic acid (Work, Birnbaum, Winitz & Greenstein, 1955). The enzymic decarboxylation, growth-promoting properties and natural distribution of the isomers have also been investigated.

Preliminary reports of these results have already been given (Hoare, 1955; Hoare & Work, 1955*a*).

METHODS

Diaminopimelic acid. Reference stereoisomers of diaminopimelic acid were prepared by enzymic resolution of the synthetic amino acid: $[\alpha]_D$ for LL-diaminopimelic acid was +45·1 (Work *et al.* 1955). For convenience these stereoisomers are described as 'resolved' throughout the present paper.

'Diphtheria' diaminopimelic acid was prepared from hydrolysed toxin-extracted *C. diphtheriae* as described by Work (1951).

'Mutant' diaminopimelic acid was prepared from the culture filtrate of *Esch. coli* 26/26 by the method of Work & Denman (1953), with the following quantitative modifications. Batches (8 l.) of culture filtrate were prepared and concentrated as described; half of the product was then chromatographed at laboratory temperature on a column (4·8 cm. × 50 cm.) of Zeocarb 225 (8%-cross-linked, 60–200 mesh). 1·5N-HCl (10 l.) and 2·5N-HCl (2 l.) were run through the column and discarded; the next 1·2–1·5 l. of 2·5N-HCl contained diaminopimelic acid, and, unless otherwise stated, this fraction was combined with the equivalent fraction from the other half of the culture filtrate and worked up as previously described, except that chloride was removed by electrodialysis.

Free diaminopimelic acid was crystallized from boiling water or from aqueous ethanol. Diaminopimelic acid monohydrochloride was crystallized from a highly concentrated solution of amino acid made slightly acid with HCl, by addition of acetone until a permanent visible turbidity was produced. The crystalline hydrochloride was dried *in vacuo* over $CaCl_2$ at room temperature.

Diaminopimelic acid decarboxylase. The crude enzyme was a preparation of acetone-dried *Aerobacter aerogenes* grown for 24 hr. at 25°, with compressed-air aeration, on the minimal medium of Davis & Mingioli (1950) modified to contain 2% (w/v) glucose. *Purified decarboxylase* was prepared as follows, all operations being carried out at 0–2° unless otherwise stated. Dried *Aero. aerogenes* cells (5 g.), suspended in 50 ml. of 0·1M potassium phosphate buffer pH 6·8 containing 10^{-4}M dimercaptopropanol, were disintegrated by passage at 12 000 lb./sq.in. through an apparatus described by Milner, Lawrence & French (1950). The cell residues, which sedimented at 20 000 *g* after 20 min. centrifuging, were resuspended in 25 ml. of buffer, passed again through the disintegrator and centrifuged. The combined supernatants, after dialysis overnight against 10^{-4}M dimercaptopropanol, were treated with ⅓ vol. of 1% (w/v) protamine sulphate and the precipitate was removed by centrifuging at 20 000 *g*. The supernatant was again dialysed overnight against dimercaptopropanol and fractionated with acetone at −5°. Each batch of cells behaved slightly differently with respect to the volume of acetone required to precipitate diaminopimelic acid decarboxylase, but as a rule the enzyme was present in the fraction precipitated at acetone concentrations between 50 and 70% (v/v). This fraction was collected by centrifuging, lyophilized and stored *in vacuo* at −10°.

Decarboxylation of diaminopimelic acid was carried out quantitatively in the Warburg apparatus as described by Dewey *et al.* (1954), except that the reaction was carried

out in N_2, and M citric acid (0·1 ml.) was used for the 'acid tip'. Small samples (e.g. 0·1 ml.) were qualitatively examined for decarboxylation after incubation overnight at 37° with enzyme (e.g. 10 mg. cells in 0·5 ml. of buffer); deproteinization was carried out with 2 vol. of ethanol, and a suitable aliquot (e.g. 200 μl.) of the supernatant was examined by paper chromatography for diaminopimelic acid or its decarboxylation product.

Other techniques. Paper chromatography was carried out either with phenol (NH_3 atmosphere) on Whatman no. 4 paper, or (when separation of isomers was required) with methanol–water–10N-HCl–pyridine (80:17·5:2·5:10) on no. 1 paper (Rhuland, Work, Denman & Hoare, 1955). The latter solvent system separates DD- and LL-diaminopimelic acid; the *meso* and DD isomers behave identically. All the other naturally occurring amino acids, with the exception of cystine, travel faster than diaminopimelic acid.

Micro-organisms were extracted with alcohol as described by Lindan & Work (1951) or hydrolysed as described by Work & Dewey (1953). Neutral amino acid fractions of hydrolysed organisms were usually prepared for enzymic or chromatographic examination by electrophoresis of 200 μl. of hydrolysate on Whatman no. 3 paper, using 0·5M ammonium acetate buffer, pH 5·0, the neutral fraction being eluted with water (Salton, 1953). When larger amounts were required, or when preparative desalting of diaminopimelic acid was carried out, the appropriate size of electrodialysis apparatus was used (Work, 1950).

Optical rotations were measured on 5% (w/v) solutions in 5N-HCl in 2 dm. tubes at 24°.

Investigation of conditions for the separation of LL and *meso* isomers by elution with HCl from Zeocarb 225 resin was carried out by using a water-jacketed column 0·9 cm. in diameter and, unless otherwise stated, 50 cm. in length. A mixture (12 mg.) containing approx. equal amounts of LL- and *meso*diaminopimelic acid, dissolved in the eluting acid, was applied to the column. Ninhydrin colour reaction with the reagent of Cocking & Yemm (1954) was carried out on 0·1 ml. of effluent fractions added to 5M acetate buffer, pH 5·6 (0·4 ml.); to this was added ninhydrin (5%, w/v) in redistilled methylcellosolve (0·2 ml.) and 2×10^{-4}M-KCN in methylcellosolve (1 ml.). The tubes were immersed in boiling water for 15 min. and cooled under the cold tap for 5 min.; 50% (v/v) aqueous ethanol (3 ml.) was added and the optical density at 570 mμ. was measured.

RESULTS

Diaminopimelic acid from the culture filtrate of Escherichia coli 26–26

Various batches of free diaminopimelic acid, when first crystallized from the electrodialysed column eluate, showed values for $[\alpha]_D$ varying between +7·6 and +9·0; the values for the material in the mother liquors varied from +11·8 to +12·5. No change in rotation was produced by acid hydrolysis, either of the concentrated culture filtrate before Zeocarb fractionation or of the crystalline diaminopimelic acid; cold bromination of the culture filtrate also had no effect on the final rotation. Therefore the rotation was not due to contamination by peptides or carbohydrates. Recrystallization as the free amino acid reduced the rotation; after three or four recrystallizations it was zero. Recrystallization as the hydrochloride raised the rotation. Two 400 mg. samples of twice crystallized amino acid, with $[\alpha]_D$ +3·4, were each recrystallized twice, one as free amino acid, the other as hydrochloride; 100 mg. of free amino acid were obtained with $[\alpha]_D$ +2·2, and 180 mg. of hydrochloride with $[\alpha]_D$ +4·7.

The dextrorotatory samples on paper chromatography gave two spots (see Fig. 1, spot 4), the one corresponding with LL-diaminopimelic acid being the weaker. The optically inactive material gave only one spot, corresponding with either *meso*- or DD-diaminopimelic acid (spot 5).

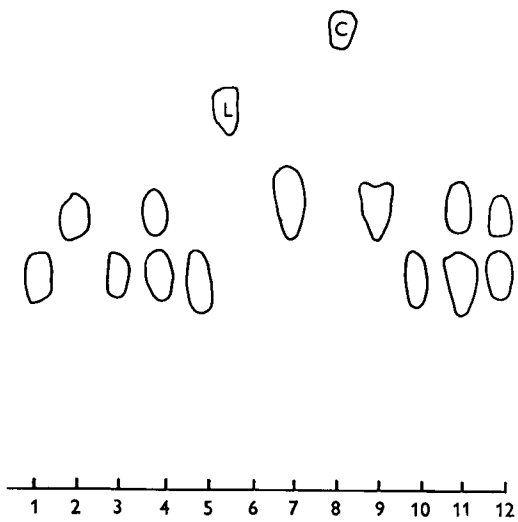

Fig. 1. Descending chromatogram in methanol–water–5N-HCl–pyridine (80:17·5:2·5:10); Whatman no. 1 paper. Development was by dipping in ninhydrin in acetone (0·1%, w/v) and heating for 2 min. at 100°. Portions (20 μg.) of the following diaminopimelic acid preparations were applied: 1, resolved *meso*; 2, resolved LL; 3, resolved DD; 4, crude 'mutant', once recrystallized; 5, 'mutant' *meso*; 6, 'mutant' *meso* treated with purified decarboxylase; 7, 'mutant' LL; 8, 'mutant' LL treated with crude decarboxylase; 9, 'mutant' LL treated with purified decarboxylase; 10, 'diphtheria' preparation; 11, untreated 'mutant' (26–26) culture filtrate (10 μl.); 12, alcoholic extract of mutant (26–26) cells. Spot L is lysine, spot C is cadaverine.

The pooled mother liquors which accumulated from successive recrystallizations of the free amino acid were subjected to fractional ethanol precipitation; paper chromatography showed that the concentration of LL isomer increased in each successive precipitation. A stiff gel was obtained from the more soluble fractions; this resembled the gelatinous ethanol precipitate obtained from LL- or

DD-diaminopimelic acid (Work *et al.* 1955). The material from the gel crystallized easily from acetone–dilute hydrochloric acid; after one recrystallization it was shown by paper chromatography, rotation and analysis to be LL-diaminopimelic acid monohydrochloride (see Fig. 1, spot 7, and Table 1).

During the preparation of diaminopimelic acid by chromatography on Zeocarb 225, in some cases the diaminopimelic acid did not come off the column as a single symmetrical peak. In one preparation from a single batch of culture filtrate, both the column runs produced a late peak of about 300 ml. at the end of the main diaminopimelic acid peak of 960 ml. The materials in the two peaks were worked up separately as the hydrochlorides without preliminary desalting; the first precipitation with acetone resulted in an oil, but, on dilution of the oil with water and addition of acetone, crystals were produced, 1·09 g. from the main fraction and 0·07 g. from the late peak. After one recrystallization, examination of the late peak fraction showed it to be pure LL-diaminopimelic acid monohydrochloride (Table 1). The main fraction, with the same analysis, had a specific rotation of +19·0. It is evident that the unusually high rotation of this main fraction was due to the fact that it was crystallized throughout as the hydrochloride; the LL isomer was therefore not removed by recrystallization as was usually the case in routine preparations where crystallization was carried out as the free amino acid.

Table 1. *Analysis and optical rotation in* 5N-HCl *of naturally occurring isomers of diaminopimelic acid monohydrochloride*

Isomer	Source	N (%)	Cl (%)	$[\alpha]_D$
meso	'Diphtheria'	12·5	15·6	0
	'Mutant'†	12·0	15·6	0
LL	'Mutant'*	11·9	15·6	+39·4
	'Mutant'†	12·0	15·6	+37·8
	Synthetic	12·3 (theor.)	15·7 (theor.)	+38·5

* Resolved on Zeocarb 225.
† Resolved by crystallization.

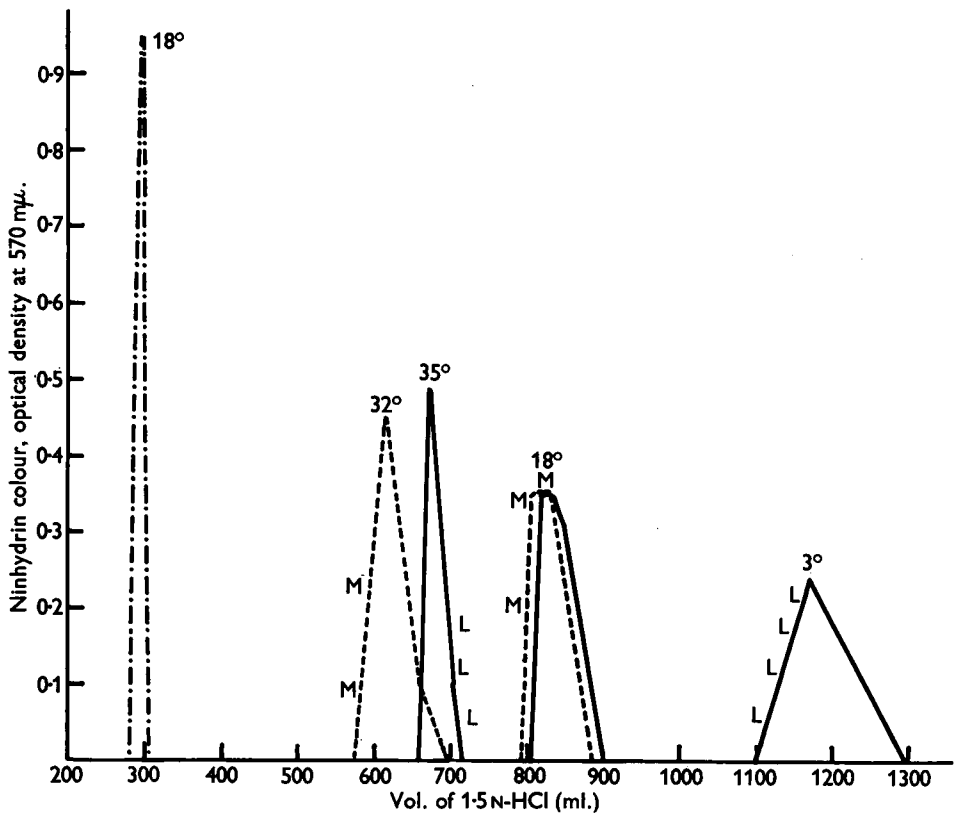

Fig. 2. Chromatography of a mixture of *meso*- and LL-diaminopimelic acid (12 mg.) on Zeocarb 225 column (0·9 cm. × 50 cm.) with 1·5N-HCl as eluent, at the temperatures shown. - - - -, 12%-Cross-linked resin; ——, 8%-cross-linked resin; ·—·—·, 4%-cross-linked resin. Unless indicated against the curve, both isomers were present: L indicates presence of only LL isomer; M indicates presence of only *meso* isomer.

Attempted separation of LL *and* meso *isomers by ion exchange*

Subsequent attempts to repeat the separation of LL isomer during routine preparations of 'mutant' diaminopimelic acid with the large column were not always successful. Experiments were therefore carried out on a small scale under carefully controlled conditions. When 100 cm. or 50 cm. columns of Zeocarb 225, 8% cross-linked, 100–200 mesh were used, no separation was achieved with 2·5N-HCl as eluting agent, the diaminopimelic acid peak being completely symmetrical. However, when 1·5N-HCl was used on a 50 cm. column, the peak was not symmetrical (see Fig. 2), the shape being dependent on the temperature. Chromatographic examination of various fractions at different stages of the elution showed that each isomer was affected differently by changes in temperature. At room temperature the *meso* isomer emerged slightly ahead of the LL isomer; at +2° the LL isomer was the first to appear. On raising the temperature to 35°, the slowest-moving fraction contained only the LL isomer. It was concluded from these results that, as the original separation of pure LL isomer from the later fraction of the large (unjacketed) column occurred in July 1953, a slightly raised room temperature was possibly responsible for the effect, the actual separation occurring during the passage of the 1·5N-HCl through the column. Reducing the degree of cross-linkage of the resin to 4% produced a symmetrical peak with 1·5N-HCl at 18°, whereas 12% cross-linked resin produced similar results to the 8% at 18°.

In all cases where a separation of isomers was achieved, it was so slight as to be useless for preparative work.

Enzyme studies: action of diaminopimelic acid decarboxylase on isomers

Samples of diaminopimelic acid, prepared as described from the mutant culture fluid, were incompletely decarboxylated by purified decarboxylase preparations, the extent of decarboxylation varying from 75 to 90% in different preparations. In contrast, diaminopimelic acid isolated from *C. diphtheriae* was completely decarboxylated. Crude enzyme preparations completely decarboxylated diaminopimelic acid from both sources. The residual diaminopimelic acid from decarboxylation of the 'mutant' samples by the purified enzyme was separated by paper electrophoresis and found to be completely decarboxylated to cadaverine by crude enzyme, but to undergo no appreciable decarboxylation in the presence of the purified enzyme. These results indicated the presence of two components in the mutant material, the proportions varying from batch to batch. The exact identity of these components was not established until pure isomers of diaminopimelic acid had been prepared and tested with the different decarboxylase preparations.

The effect of crude and purified decarboxylase preparations on the isomers of diaminopimelic acid is shown in Figs. 1 and 3 and Table 2. The

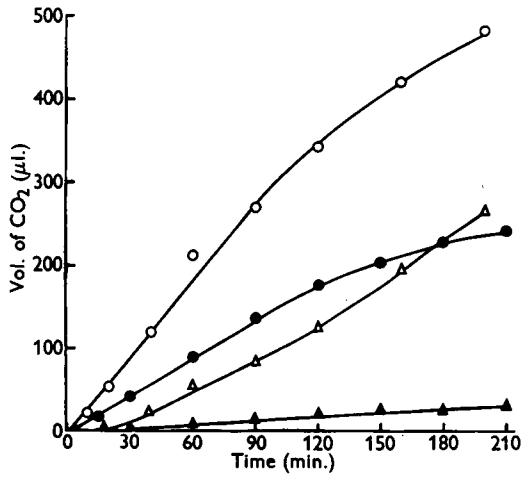

Fig. 3. Decarboxylation of LL and *meso* isomers by crude and purified enzyme preparations. Diaminopimelic acid isomer (5×10^{-3}M) in 0·1M phosphate pH 6·8, $1·6 \times 10^{-5}$M pyridoxal phosphate, to a final volume of 2·5 ml. with a suspension of crude enzyme (40 mg.) or a solution of purified enzyme (3 mg.); reaction in N_2, 37°. ○, Crude enzyme + *meso*; △, crude enzyme + LL; ●, purified enzyme + *meso*; ▲, purified enzyme + LL. (The gas evolution with crude enzyme was greater than that with the purified enzyme, since the crude enzyme contained lysine decarboxylase.)

Table 2. *Decarboxylation of diaminopimelic acid isomers by crude and purified decarboxylase preparations*

2 ml. of phosphate buffer (0·1M, pH 6·8) containing enzyme (40 mg. of crude, or 10 mg. of purified) + 10 μg. of pyridoxal phosphate, incubated in N_2 at 37° in a Warburg apparatus with 0·5 ml. of diaminopimelic acid ($2·5 \times 10^{-3}$M in buffer).

Diaminopimelic acid		Decarboxylase: Crude	Decarboxylase: Purified	
Isomer	Source	Q_{CO_2}	Q_{CO_2}	Total gas evolution (% of theoretical)
meso	Resolved	3·4	57	100
	'Diphtheria'	3·1	57	100
	'Mutant'	3·1	57	98
LL	Resolved	1·0*	—	21
	'Mutant'	1·0*	2·5	19
DD	Resolved	0·0	0·0	0

* 15 min. lag period.

DD isomer was not decarboxylated by either preparation, and did not inhibit the decarboxylation of the *meso* isomer when the isomers were present in equimolar concentration. The LL isomer was slowly and completely attacked, after a lag of 10–20 min., by the crude cells. No two purified enzyme preparations had the same effect on the LL isomer; some had practically no effect, while others produced a slow, incomplete decarboxylation. The ratio, Q_{CO_2} *meso*/LL, varied between 30 and 12 and appeared to drop on prolonged storage of the enzyme preparations. The behaviour of each of the pure isomers obtained from mutant culture fluid was identical with that of the respective resolved isomers (Table 2). Chromatographic examination of the reaction products of the natural diaminopimelic acid samples treated with the crude enzyme showed that all the diaminopimelic acid present had been decarboxylated to cadaverine, proving the absence of the DD isomer.

Diaminopimelic acid from hydrolysates of Corynebacterium diphtheriae

This optically inactive material was identical with *meso*diaminopimelic acid. It was completely decarboxylated to lysine by purified decarboxylase, and to cadaverine by the crude enzyme preparation: in both cases it was decarboxylated at rates identical with the rates of decarboxylation of authentic *meso*diaminopimelic acid (Table 2). Chromatographic evidence showed that it was completely free from the LL isomer (Fig. 1, spot 10).

Diaminopimelic acid in unfractionated bacterial culture filtrates, cell extracts and hydrolysates

The culture filtrate from *Esch. coli* mutant 26–26 was examined by paper chromatography in methanol–HCl–pyridine, without preliminary concentration or desalting (Fig. 1, spot 11). It contained approximately equal amounts of LL- and *meso*-diaminopimelic acid and no other detectable amino acids at the level examined. The desalted culture filtrate was treated with crude decarboxylase; all the diaminopimelic acid present was converted into cadaverine, indicating the absence of the DD isomer. This mutant accumulated diaminopimelic acid only when the original lysine content of the growth medium was 10–15 mg./l. (Dr B. D. Davis, private communication). At lysine levels of 30 mg./l. or over, no diaminopimelic acid was detected even when large amounts of desalted medium were examined.

Alcoholic extracts of the *Esch. coli* cells which had accumulated diaminopimelic acid in the culture filtrate also contained large amounts of both isomers in approximately equal proportions (spot 12). Mutant cultures grown on 30 mg. of lysine/l. and wild-type *Esch. coli* ATCC 9637 had no diaminopimelic acid in their alcoholic extracts. The hydrolysed ethanol-extracted cell residues of mutant or wild-type *Esch. coli* cultures contained only *meso*diaminopimelic acid.

Crude or electrodialysed hydrolysates of various bacteria were examined by paper chromatography (see Fig. 4 for examples). In most cases,

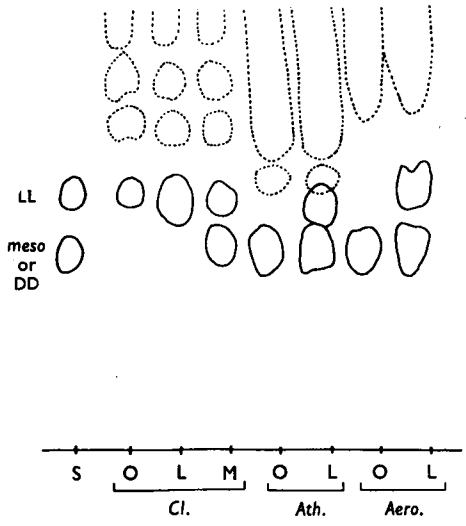

Fig. 4. Portion of chromatograms of bacterial hydrolysates treated with H_2O_2. Technique as in Fig. 1. Spots with continuous outline represent diaminopimelic acid; when freshly developed they were olive green and changed to permanent yellow. Spots with dotted outline represent other amino acids; they were purple or blue and faded rapidly. Each spot represents the following organisms: *Cl.*, *Cl. welchii* equivalent to 1·5 mg. (dry wt.); *Ath.*, *Athiorhodaceae* sp. equivalent to 2·5 mg. (dry wt.); *Aero.*, neutral amino acid fraction from 5 mg. (dry wt.), of *Aero. aerogenes*; O, organism alone; L, added LL-diaminopimelic acid (10 μg.); M, added *meso*diaminopimelic acid (10 μg.); S, synthetic diaminopimelic acid marker (20 μg.).

the isomeric form was checked by chromatography in the presence of added isomers and by decarboxylation of the neutral amino acid fraction (to prove the absence of the DD isomer). In the following hydrolysed organisms only the *meso* isomer of diaminopimelic acid was found: *Vibrio comma*, *Azotobacter chroococcum*, *Rhizobium* sp., *Esch. coli*, *Aero. aerogenes*, *Brucella abortus*, *Haemophilus pertussis*, *Lactobacillus plantarum* (*arabinosus*), *Bacillus megatherium*, *B. subtilis*, *Clostridium acetobutyricum*, *Rhodopseudomonas spheroides*, *Cytophaga globulosa*, *Anaboena cylindrica*, *Myco. tuberculosis*, *C. diphtheriae*. LL-Diaminopimelic acid was found in *Cl. perfringens* (*welchii*), *Propionibacter jensenii* and *Pr. thoenii*.

Growth-promoting activity of isomers of diaminopimelic acid for Escherichia coli 173–25

The diaminopimelic acid auxotroph, *Esch. coli* 173–25 (Davis, 1952), was sown on agar plates containing minimal medium (Davis & Mingioli, 1950) supplemented with one of the three isomers of diaminopimelic acid (0·1 μM), each with or without additional L-lysine (0·1 μM). After 24–48 hr. at 37° the organism had grown equally well on *meso*- and LL-diaminopimelic acid, growth being in both cases much greater when lysine was also present. There was no growth on the DD isomer with or without lysine.

DISCUSSION

At the time when the *meso* structure was originally suggested for the optically inactive diaminopimelic acid from *C. diphtheriae*, no other naturally occurring *meso* amino acid was known. Since then, the lanthionine of antibiotics has been shown by chemical degradation to be the *meso* isomer (Alderton & Fevold, 1951). Diaminopimelic acid, unlike lanthionine, cannot be degraded to optically active amino acids, so rigorous proof for the *meso* form could not be provided without reference to the pure authentic isomers, which were not then available. Following the resolution of synthetic diaminopimelic acid, the required evidence for the *meso* form was obtained. The only alternative to the *meso* form is a racemic mixture; this was discounted by paper chromatography and enzymic decarboxylation. Confirmation for the *meso* structure was also obtained with the L-amino acid oxidase of *Neurospora*, which oxidized only one amino group of both 'diphtheria' and synthetic *meso*diaminopimelic acid, but oxidized the two amino groups of the LL isomer (Work, 1955).

The *meso* isomer differs so much from the other forms in water solubility and crystallizability from aqueous ethanol that it is possible to crystallize pure *meso* isomer from mixtures of two or three isomers such as occur in diaminopimelic acid obtained either from *Esch. coli* 26–26 mutant culture filtrate or by chemical synthesis. The *meso* isomer exhibits another characteristic property: aqueous solutions on prolonged standing (even at −10°) slowly yield a white insoluble precipitate. The differential solubility of the isomers is reversed in dilute hydrochloric acid–acetone mixtures, and thus pure LL-diaminopimelic acid monohydrochloride can be crystallized from a mixture of *meso* and LL isomers, provided that the LL isomer predominates.

Hirs, Moore & Stein (1954) reported a good separation of *meso*cystine from the other isomers by chromatography on sulphonated-polystyrene resin; under their conditions, we obtained no separation of diaminopimelic acid isomers. The occasional slight separation of the LL isomer achieved on routine large-scale chromatography of the mutant culture filtrate, although insufficient for preparative purposes, did produce a small amount of dextrorotatory material. At this time Wright & Cresson (1953) had already suggested the presence of LL-diaminopimelic acid in 'mutant' diaminopimelic acid, but our dextrorotatory material behaved so unexpectedly towards crude and purified decarboxylase preparations that it was not then identified as LL-diaminopimelic acid. Subsequently the optical rotation and enzymic behaviour of the resolved LL isomer showed that this natural material was indeed LL-diaminopimelic acid. The apparent decarboxylation of the LL isomer by crude preparations is due to a preliminary racemization to the *meso* isomer (Hoare & Work, 1955*b*), which was subsequently decarboxylated (Hoare, 1955).

'Mutant' diaminopimelic acid was originally prepared for use as a substrate for the decarboxylase. Only one or two crystallizations of the free amino acid were carried out then, since recrystallization did not influence the susceptibility of the product to the *crude* decarboxylase, although it did reduce optical rotation. The confusing results on the variable incomplete decarboxylation of 'mutant' diaminopimelic acid by *purified* decarboxylase were not explained until it was shown that recrystallization removed variable amounts of the less susceptible LL isomer, and that different purified decarboxylase preparations varied in their capacity to attack this isomer.

It has not yet been possible to decide whether diaminopimelic decarboxylase has an absolute specificity towards *meso*diaminopimelic acid or whether it also slowly attacks the LL form. In certain cases the *meso* isomer was decarboxylated at least 30 times faster than the LL isomer. Since the relative activities towards the two isomers were so variable, both with storage time and with different preparations, the decarboxylase may be contaminated with a racemase. The product of decarboxylation of *meso*diaminopimelic acid is L-lysine (Dewey *et al.* 1954); it follows that the carboxyl group which is removed is attached to the carbon atom in the D configuration. As the DD isomer is neither a substrate for nor an inhibitor of the decarboxylase, one carbon atom in the L configuration is apparently necessary for interaction with the decarboxylase.

Wright & Cresson (1953) reported that synthetic diaminopimelic acid and their 'mutant' amino acid had identical growth-promoting activities for the diaminopimelic acid auxotroph *Esch. coli* 173–25. This does not agree with our findings that only the LL and *meso* isomers support growth of this mutant. Racemization of these isomers might

account for the similarity of their growth-promoting activities.

Paper chromatography of the untreated mutant culture filtrate showed that diaminopimelic acid was present as an approximately equimolar mixture of LL and *meso* isomers; this proved that the isomer mixture isolated by column chromatography was not an artifact produced during the purification. The soluble intracellular diaminopimelic acid of this mutant resembled closely that found in the culture filtrate. The intracellular diaminopimelic acid racemase, acting in the absence of the decarboxylase, is probably responsible for the accumulation and subsequent excretion by the cells of this isomer mixture. The order of synthesis of the isomers is not yet known. It is interesting to note that the bound diaminopimelic acid of this mutant, like that of normal *Esch. coli*, is the *meso* form. With a few exceptions, the *meso* form was found in hydrolysates of bacteria, including both *C. diphtheriae* and *Myco. tuberculosis*. This proves that the *meso* isomer isolated from these two organisms was the only form present and was not separated from an isomer mixture by fractional crystallization, as was the *meso* isomer isolated from the *Esch. coli* 'mutant' culture filtrate. So far, LL-diaminopimelic acid has been found only in *Cl. welchii* and the two strains of *Propionibacter* examined; the significance of this distribution is not yet known.

SUMMARY

1. Approximately equal amounts of LL- and *meso*-diaminopimelic acid are produced and excreted by a lysine-requiring mutant of *Esch. coli* 26–26.

2. The isolation of each isomer from the culture filtrate is described.

3. Diaminopimelic acid isolated from hydrolysed *C. diphtheriae* is the *meso* isomer.

4. Proof of isomeric form was obtained by optical rotation, paper chromatography and enzymic decarboxylation.

5. *meso*Diaminopimelic acid is decarboxylated by a partially purified preparation of diaminopimelic acid decarboxylase; the LL isomer is only slightly attacked. Both these isomers are completely decarboxylated by crude acetone-dried bacterial cells, the *meso* isomer being the more susceptible. The DD isomer is not decarboxylated.

6. The diaminopimelic-acid-requiring mutant *Esch. coli* 173–25 grows equally well on the *meso* and LL isomers, but not on the DD isomer.

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REFERENCES

Alderton, G. & Fevold, H. (1951). *J. Amer. chem. Soc.* **73**, 463.
Cocking, E. C. & Yemm, E. W. (1954). *Biochem. J.* **58**, xii.
Davis, B. D. (1952). *Nature, Lond.*, **169**, 534.
Davis, B. D. & Mingioli, E. S. (1950). *J. Bact.* **60**, 17.
Dewey, D. L., Hoare, D. S. & Work, E. (1954). *Biochem. J.* **58**, 523.
Hirs, C. H. W., Moore, S. & Stein, W. H. (1954). *J. Amer. chem. Soc.* **76**, 6063.
Hoare, D. S. (1955). *Biochem. J.* **59**, xxii.
Hoare, D. S. & Work, E. (1955*a*). *Biochem. J.* **60**, ii.
Hoare, D. S. & Work, E. (1955*b*). *Proc. 3rd Int. Congr. Biochem., Brussels*, p. 37.
Lindan, O. & Work, E. (1951). *Biochem. J.* **48**, 337.
Milner, H. W., Lawrence, N. S. & French, C. S. (1950). *Science*, **111**, 633.
Rhuland, L. E., Work, E., Denman, R. F. & Hoare, D. S. (1955). *J. Amer. chem. Soc.* **77**, 4844.
Salton, M. R. J. (1953). *Biochim. biophys. Acta*, **10**, 512.
Work, E. (1950). *Biochim. biophys. Acta*, **5**, 204.
Work, E. (1951). *Biochem. J.* **49**, 17.
Work, E. (1953). *Proc. 6th Congr. int. Microbiol., Rome*, **1**, 161.
Work, E. (1955). *Biochim. biophys. Acta*, **17**, 410.
Work, E., Birnbaum, S. M., Winitz, M. & Greenstein, J. P. (1955). *J. Amer. chem. Soc.* **77**, 1916.
Work, E. & Denman, R. F. (1953). *Biochim. biophys. Acta*, **10**, 183.
Work, E. & Dewey, D. L. (1953). *J. gen. Microbiol.* **9**, 394.
Wright, L. D. & Cresson, E. L. (1953). *Proc. Soc. exp. Biol., N.Y.*, **82**, 354.