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THE AMINO ACIDS OF THE GENUS *ACACIA*

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Abstract—A survey of the amino acids present in seeds of about forty species of the genus *Acacia* has revealed that members of the series Gummiferae can be distinguished readily from other species by their distinctive composition: among other differences, they alone contain *N*-acetyldjenkolic acid, together with small amounts of a related oxidized derivative, but they lack *S*-carboxyethyl- and *S*-carboxyisopropyl-cysteines, β-acetyl-α,β-diaminopropionic acid and albizziine, which are distributed widely among other *Acacia* species. L-β-*N*-Acetyl-α,β-diaminopropionic acid has been characterized as a plant constituent for the first time, and evidence is presented that sulphoxide derivatives of *S*-carboxyethylcysteine, djenkolic acid and *N*-acetyldjenkolic acid coexist with the corresponding thioethers in many seeds of the *Acacia* genus.

INTRODUCTION

THE SEEDS of many species from the sub-family Mimosoideae are particularly rich sources of a number of amino acids whose distribution elsewhere within the plant kingdom is very restricted. Many of the compounds are encountered in species forming part of the large and complex *Acacia* genus. For example, pipecolic acid and its related 4- and 5-hydroxy derivatives occur together in the seeds of some *Acacia* species.[1-3] Another group of structurally-related compounds found either in the genera *Acacia* or *Mimosa* include α,β-diaminopropionic acid,[4] albizziine (α-amino-β-ureidopropionic acid),[5] willardine [β-(uracil-1-yl)-α-aminopropionic acid],[6] and mimosine [β-*N*-(3-hydroxy-4-pyridone)-α-aminopropionic acid].[7] *S*-Substituted cysteine derivatives encountered in *Acacia* species include *S*-carboxyethylcysteine[5] and *S*-carboxyisopropylcysteine;[8] djenkolic acid and *N*-acetyldjenkolic acid, isolated from *A. farnesiana*,[9,10] form examples of more complex thioethers, in which two sulphur atoms are linked by a methylene group.

During a study of diamino acid metabolism in plants (see Seneviratne and Fowden[11]), *Acacia* species were employed for some of the experiments and our attention was drawn to the presence of additional amino acids in a number of the species used. Among these was L-β-acetamido-α-aminopropionic acid (L-β-acetyl-α,β-diaminopropionic acid), which now has been isolated from seed of *A. armata* and characterized by comparison with synthetic material. Other new ninhydrin-positive substances have been partially characterized. The distribution of these newly- and previously-identified amino acids of *Acacia* has been surveyed

[1] A. I. VIRTANEN and S. KARI, *Acta Chem. Scand.* **8**, 1290 (1954).
[2] A. I. VIRTANEN and S. KARI, *Acta Chem. Scand.* **9**, 170 (1955).
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[5] R. GMELIN, G. STRAUSS and G. HASENMAIER, *Z. Naturforsch.* **13b**, 252 (1958).
[6] R. GMELIN, *Hoppe-Seylers Z. Physiol. Chem.* **316**, 164 (1959).
[7] J. RENZ, *Hoppe-Seylers Z. Physiol. Chem.* **244**, 153 (1936).
[8] R. GMELIN and P. K. HIETALA, *Hoppe-Seylers Z. Physiol. Chem.* **322**, 278 (1960).
[9] R. GMELIN, G. HASENMAIER and G. STRAUSS, *Z. Naturforsch.* **12b**, 687 (1957).
[10] R. GMELIN, A. KJAER and P. O. LARSEN, *Phytochem.* **1**, 233 (1962).
[11] A. S. SENEVIRATNE and L. FOWDEN, *Phytochem.* **7**, 1047 (1968).

in about forty species of the genus, and the collated data used to ascertain what value these non-protein[12] amino acids may have as chemical characters for classifying the Acacias.

RESULTS

The Identity of the Amino Acids from Acacia

The diagram in Fig. 1 illustrates the positions on two-dimensional paper chromatograms of the non-protein amino acids encountered most frequently in extracts of *Acacia* seeds. Often additional ninhydrin-positive spots were present on chromatograms but these substances are not recorded because they represented minor constituents, restricted to relatively few species of seed. Previous investigations describing the amino acids of *Acacia* species did not record substances in positions B, D, H, I, K and L (Fig. 1), and so the nature of these compounds has been investigated.

FIG. 1. DIAGRAMMATIC REPRESENTATION OF THE POSITIONS OF THE MAIN NON-PROTEIN AMINO ACIDS PRESENT IN *Acacia* SPECIES (SOLID SPOTS) IN RELATION TO THE CHROMATOGRAPHIC PATTERN OF COMMONLY OCCURRING AMINO ACIDS (OPEN SPOTS).
Key to spots: 1, aspartic acid; 2, glutamic acid; 3, serine; 4, glycine; 5, threonine; 6, alanine; 7, glutamine; 8, lysine; 9, arginine; 10, proline; 11, ethanolamine; 12, tyrosine; 13, γ-aminobutyric acid; 14, valine; 15, phenylalamine; 16, leucines; A, *S*-carboxyethylcysteine; B, *S*-carboxyethylcysteine sulphoxide; C, *S*-carboxyisopropylcysteine; D, β-acetyl-α,β-diaminopropionic acid; E, albizziine (overlays asparagine); F, willardiine; G, djenkolic acid; H and I, oxidized forms of djenkolic acid; J, *N*-acetyldjenkolic acid; K, oxidized form of *N*-acetyldjenkolic acid; L, γ-glutamyldjenkolic acid; M, pipecolic acid; N, 5-hydroxypipecolic acid; and O, 4-hydroxypipecolic acid.

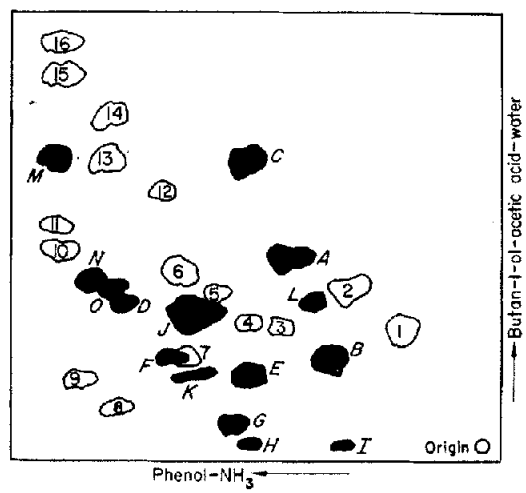

FIG. 1.

Compound B was invariably detected in seed extracts containing high concentrations of *S*-carboxyethylcysteine. It was isolated in small quantity by elution from chromatograms prepared from an extract of *A. aneura* seed. *B* was acidic, being retained by a Dowex-1 anion-exchange resin; it moved slower than aspartic acid and faster than glutamic acid when subjected to electrophoresis at pH 3·45.

Treatment with 6 N-HCl at 100° for 16 hr converted *B* mainly into *S*-carboxyethylcysteine. This behaviour is reminiscent of the conversion of methionine sulphoxide into methionine under similar conditions.[13] When *S*-carboxyethylcysteine was treated with 15 per cent (w/v) hydrogen peroxide at room temperature for 2 hr, the single oxidation product was shown to be inseparable from *B* in solvents 1–5 listed in the experimental section. Similar treatment of methionine oxidizes the thioether largely to its sulphoxide. These observations suggest that *B* is *S*-carboxyethylcysteine sulphoxide.

Compound D is found as a predominant amino acid component of the seeds of *A. armata*

[12] L. FOWDEN, *Endeavour* **21**, 35 (1962).
[13] L. R. NJAA, *Acta Chem. Scand.* **16**, 1359 (1962).

and *A. dealbata*, and is also a major constituent of several other seed species. The compound was isolated (1·04 g) from seed of *A. armata* (2 kg), together with pure samples of *S*-carboxyethylcysteine (4·7 g), albizziine (7·5 g) and djenkolic acid (0·73 g).

D was identified as L-β-acetyl-α,β-diaminopropionic acid. Like other *N*-acetyl derivatives, it was hydrolyzed to the parent amino acid by treatment with N-HCl at 100° for a few hours. The presence of α,β-diaminopropionic acid in the hydrolysate was indicated by comparison with authentic material by paper chromatography and electrophoresis, and by a specific colour reaction (see Experimental section). The identity of *D* was confirmed by synthesizing L-β-acetyl-α,β-diaminopropionic acid and comparing the isolated and synthetic compounds using chromatographic, polarimetric and nuclear magnetic resonance spectroscopic techniques. *D* could be separated from α-acetyl-α,β-diaminopropionic acid on paper chromatograms developed in solvents 2–6.

Compounds H and I were present on chromatograms when djenkolic acid formed a major constituent of a seed extract, i.e. in *A. georginae*. Two substances possessing chromatographic properties identical with those of *H* and *I* were obtained when a sample of authentic djenkolic acid was oxidized with 15 per cent (w/v) hydrogen peroxide at normal temperature. Substances *H* and *I* then may represent oxidized forms of djenkolic acid in which one or both, respectively, of the sulphur atoms have been converted into the corresponding sulphoxide form.

Compound K was observed on chromatograms prepared from those *Acacia* species assigned to the sub-genus Gummiferae; it was associated always with much larger quantities of *N*-acetyldjenkolic acid. Oxidation of *N*-acetyldjenkolic acid with hydrogen peroxide gave a single product which behaved identically with *K* on paper chromatograms developed in solvents 1–3. Therefore *K* probably represents a sulphoxide derivative of *N*-acetyldjenkolic acid.

Compound L was eluted from paper chromatograms prepared from a seed extract of *A. georginae*. *L* was destroyed by treatment with N-HCl at 100° for 3 hr: glutamic and djenkolic acids were identified in the hydrolysate by paper chromatographic and electrophoretic techniques. The lability of *L* to dilute acid strongly suggests that it was the γ-glutamyl peptide of djenkolic acid. Many previous instances are recorded of the co-occurrence in seeds of a non-protein amino acid and its related γ-glutamyl peptide,[14] especially when the amino acid forms a major constituent of the soluble-nitrogen fraction (see Table 1 for components of *A. georginae* seed).

The ease with which the sulphoxides of *S*-carboxyethylcysteine, djenkolic and *N*-acetyldjenkolic acids are formed by hydrogen peroxide oxidation of the corresponding thioethers makes it uncertain whether compounds *B*, *H*, *I* and *K* should be regarded as artefacts arising by oxidation during extraction and chromatography. Certainly, it is usual to find that methionine is converted partially into its sulphoxide when plant extracts are similarly processed. However, since the sulphoxides of other *S*-substituted cysteines (e.g. the methyl, allyl and propenyl derivatives) are established as non-artefact components of various *Brassica* and *Allium* species,[15] the normal existence of further sulphoxides of the type now described would appear probable. The distribution data contained in Table 1 further indicate that fairly large, interspecific variation occurs in the relative content of thioethers and the corresponding sulphoxides. For example, *S*-carboxyethylcysteine was usually present in

[14] J. F. THOMPSON, C. J. MORRIS, W. N. ARNOLD and D. H. TURNER, In *Amino Acid Pools*, pp. 54–64 (edited by J. T. HOLDEN), Elsevier, Amsterdam (1962).
[15] A. I. VIRTANEN, *Phytochem.* **4**, 207 (1965).

TABLE 1. THE DISTRIBUTION OF NON-PROTEIN AMINO ACIDS IN SEEDS OF *Acacia* SPECIES†

	S-Carboxyethylcysteine (*A*)	*S*-Carboxyethylcysteine sulphoxide (*B*)	*S*-Carboxyisopropyl-cysteine (*C*)	β-Acetyl-α,β-diamino-propionic acid (*D*)	Albizziine (*E*)	Willardiine (*F*)	Djenkolic acid (*G*)	Djenkolic acid sulphoxide (*H*)	Djenkolic acid disulphoxide (*I*)	*N*-Acetyldjenkolic acid (*J*)	*N*-Acetyldjenkolic acid sulphoxide (*K*)	γ-Glutamyldjenkolic acid (*L*)	Pipecolic acid (*M*)	5-Hydroxypipecolic acid (*N*)	4-Hydroxypipecolic acid (*O*)
Phyllodineae															
iv Calamiforma															
Acacia calamifolia	S	M	M	W	S*	O	T	T	O	O	O	T	W	M	W
A. rigens	O	O	O	M	S*	O	W	W	O	O	O	T	W	M	O
vi Uninerves															
A. armata	S*	M	M	S*	S*	O	M	T	O	O	O	W	W	W	W
A. brachybotrya	S*	M	W	W	S*	O	W	T	O	O	O	T	W	W	W
A. cultriformis	S*	M	M	M	S	O	W	T	O	O	O	W	M	W	W
A. gladiiformis	S	M	M	M	S*	O	W	W	O	O	O	W	W	S	O
A. hakeoides	S	M	W	M	S*	O	W	W	O	O	O	W	W	M	M
A. lineata	M	M	W	W	S	O	W	T	O	O	O	W	O	M	T
A. montana	S	M	M	W	S*	O	W	W	O	O	O	W	M	M	W
A. podalyriæfolia	S*	M	M	S	M	M	M	T	O	O	O	O	W	M	M
A. pycnantha	M	M	W	M	S*	O	W	W	O	O	O	T	W	W	W
A. rhetinoides	S*	W	W	M	S*	O	W	T	O	O	O	T	W	W	W
A. salicina	T	T	T	W	S*	O	W	W	W	O	O	O	T	W	W
A. suaveolens	S*	M	M	T	O	O	W	T	O	O	O	T	M	T	W
A. wattsiana	M	M	W	W	S*	O	W	T	O	O	O	W	W	M	M
vii Plurinerves															
A. cyclopsis	W	M	T	M	S*	O	W	W	O	O	O	W	M	M	M
A. georginae	O	O	O	T	S*	O	M	M	O	O	O	S	W	M	M
A. longifolia	M	W	M	W	S*	O	M	W	O	O	O	T	M	W	W
A. melanoxylon	S	M	M	S	S*	O	W	T	O	O	O	W	M	M	W
A. oswaldi	O	W	T	T	O	O	W	W	O	O	O	W	M	M	M
A. stenophylla	O	T	O	M	S*	O	W	M	O	O	O	T	M	T	W
viii Juliflorae															
A. aneura	S*	M	M	W	O	O	M	W	O	O	O	W	M	W	M
Bipinnatae															
ix Botryocephalae															
A. baileyana	S*	M	M	W	S	O	W	T	O	O	O	O	W	M	M
A. dealbata	S*	M	S	S*	S*	M	M	W	O	O	O	O	M	M	M
A. decurrens	M	M	W	M	S*	O	W	T	O	O	O	T	W	M	M
xi Gummiferae															
A. bidwillii	O	O	O	O	O	O	M	T	O	S*	M	O	T	O	O
A. farnesiana	O	O	O	O	O	O	M	O	O	S*	M	T	W	O	M
A. grandicornuta	O	O	O	O	O	O	W	O	O	S*	M	M	O	O	M
A. karoo	O	O	O	O	O	O	W	O	O	S*	M	M	W	O	M
A. nilotica	O	O	O	O	O	O	W	T	O	S*	M	T	T	O	W
A. robusta	O	O	O	O	O	O	W	O	O	S*	M	M	O	O	O
A. suberosa	O	O	O	O	O	O	S	O	O	S*	M	T	W	O	M
A. tortilis	O	O	O	O	O	O	W	O	O	S*	M	T	T	O	M
A. woodii	O	O	O	O	O	O	M	O	O	S*	M	O	W	O	M

* Denotes that the compound forms the predominant spot on the chromatogram.

† Symbols denote relative strengths of chromatographic spots: S, strong; M, medium; W, weak; T, trace; O, not detected.

amounts considerably in excess of those of the sulphoxide, but the latter compound predominated in *A. cyclopsis*, *A. oswaldi* and *A. stenophylla*: similarly, although compound *H* was normally observed in species containing djenkolic acid, the sulphoxide form could not be detected in many members of the Gummiferae containing considerable quantities of djenkolic acid. Such marked variation in the relative content of non-oxidized and oxidized forms of these sulphur-containing amino acids in different *Acacia* extracts prepared under comparable conditions cannot be rationalized with an entirely artefact character of the sulphoxides.

The unequivocable characterization of compound *D* as β-acetyl-α,β-diaminopropionic acid adds a further representative to a related group of plant amino acids based upon the diaminopropionic acid structure. In addition to albizziine, willardiine and mimosine, two other β-substituted derivatives have been described recently: these are the toxic lathyrogen, β-oxalyl-α,β-diaminopropionic acid, from *Lathyrus* species,[16,17] and β-*N*-methyl-α,β-diaminopropionic acid, another toxic compound from *Cycas circinalis*.[18]

Substitution at the ω-amino-N atom of diamino acids appears to be more common in plants than similar replacement at the α-amino-N. For instance, δ-acetylornithine is quite widespread in plants,[19] whilst γ-acetyl-α,γ-diaminobutyric acid has been isolated from *Euphorbia pulcherrima*,[20,21] In addition, γ-oxalyl-α,γ-diaminobutyric acid forms another *Lathyrus* constituent.[22] Lysine occurs in the form of both N^{α}- and N^{ϵ}-substituted derivatives: α-substitution is seen in lysopine (N^{α}-D-carboxyethyl-L-lysine), occurring in crown gall tissue of several higher plants,[23] while ε-*N*-methyl-lysine is a component of the flagellar protein of *Salmonella typhimurium*[24] and ε-acetyl-lysine has been implicated as an intermediate in lysine degradative processes.[25]

The Distribution of Amino Acids in Acacia *Seeds*

Samples of pulverized seed were extracted with 75 per cent (v/v) ethanol under comparable conditions and finally an aliquot (representing 0·2 g seed material) of the cationic fraction obtained from each seed was applied to a two-dimensional paper chromatogram. Normally, amino acids were detected using ninhydrin as the chromogenic reagent but occasionally duplicate chromatograms were sprayed with the palladium reagent of Toennies and Kolb[26] to confirm the identity of sulphur-containing amino acids. The patterns of distribution of the non-protein amino acids observed in different species are recorded in Table 1, where the species are grouped following the classification of Bentham.[27]

Many species of *Acacia* contained albizziine as the predominant seed amino acid but in most species the sulphur-containing amino acids represented a substantial proportion of the total α-amino-N. Some seeds had *S*-carboxyethylcysteine as the major free amino acid, while massive amounts of *N*-acetyldjenkolic acid characterized all members of the Gummiferae. β-Acetyl-α,β-diaminopropionic acid, pipecolic acid and its hydroxy derivatives were widely distributed throughout the *Acacia* genus.

[16] S. L. N. Rao, P. R. Adiga and P. S. Sarma, *Biochemistry* **3**, 432 (1964).
[17] E. A. Bell, *Fed. Europ. Biochem. Soc. Abstr.* **1**, 53 (1964).
[18] A. Vega and E. A. Bell, *Phytochem.* **6**, 759 (1967).
[19] G. Reuter, *Flora* **145**, 326 (1957).
[20] I. Liss, *Phytochem.* **1**, 87 (1962).
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[22] E. A. Bell and J. P. O'Donovan, *Phytochem.* **5**, 1211 (1966).
[23] K. Biemann, C. Lioret, J. Asselineau, E. Lederer and J. Polonsky, *Bull. Soc. Chim. Biol.* **42**, 979 (1960).
[24] R. P. Ambler and M. W. Rees, *Nature* **184**, 56 (1959).
[25] A. Kjaer and P. O. Larsen, *Acta Chem. Scand* **15**, 750 (1961).
[26] G. Toennies and J. J. Kolb, *Anal. Chem.* **23**, 823 (1951).
[27] G. Bentham, in *Flora Australiensis*, Vol. 2, pp. 301–421, Lovell Reeve, London (1864).

A very sharp distinction exists between the amino acid composition of seeds of members of the Gummiferae and those of other sub-generic groups. The Gummiferae alone contained *N*-acetyldjenkolic acid and the associated sulphoxide (compound *K*): the species forming this group also completely lack *S*-carboxyethyl- and *S*-carboxyisopropyl-cysteines, and albizziine and β-acetyl-α,β-diaminopropionic acid, substances almost invariably present in the seeds of other *Acacia* species. These characteristics are so clearly defined and constant for those members of the Gummiferae examined that one would feel justified in assigning a species to this sub-genus if it possessed this type of amino acid composition. The differences of composition between members of other sub-generic groups listed in Table 1 are relatively slight, and no constant pattern of amino acid distribution emerges for all the members constituting any single group.

A ninhydrin-positive spot in a position associated with dichrostachinic acid was present on chromatograms prepared from most of the *Acacia* species. This sulphur-containing amino acid was first characterized from seed of *Dichrostachys glomerata*,[28] but it has been identified subsequently in a number of other genera forming the tribes Mimoseae and Adenanthereae and isolated from seed of *Leucaena leucocephala*.[29] However, crucial proof of its presence in the genus *Acacia* will necessitate isolation, since other plant amino acids move to similar positions on chromatograms developed in our routine combination of solvent systems.

EXPERIMENTAL

Chromatographic and Electrophoretic Methods

Descending paper chromatography was performed on Whatman no. 3 MM filter paper. The following solvents were used: 1, 75 per cent (w/w) phenol in the presence of ammonia vapour; 2, butan-1-ol-acetic acid-water (90:10:29, by vol.); 3, *tert*-amyl alcohol–acetic acid–water (20:1:20, by vol., upper phase); 4, ethylmethylketone-butan-1-ol–10 N-NH_3 (3:5:2, by vol.); 5, ethyl acetate–pyridine–water (2:1:2, by vol., upper phase); 6, butan-1-ol saturated with 3 N-NH_3; and 7, 75 per cent (v/v) isopropanol. Solvents 1 and 2 were used in that order to develop two-dimensional chromatograms.

High voltage paper electrophoresis was performed on Whatman 3 MM filter paper using a Locarte Co. (London) apparatus having plates 1 m long. The following buffers were used: 1, pH 2·0, formic acid (61 ml), acetic acid (97 ml) and water to 2 l.; 2, pH 3·45, acetic acid–pyridine–water (10:1:190, by vol.); 3, pH 6·5, acetic acid–pyridine–water (4:100:1900, by vol.).

Routine Preparation of Seed Extracts

Finely ground seed material of the different *Acacia* species was shaken with 75 per cent (v/v) ethanol (25 ml/g dry seed) for about 24 hr and, after centrifuging, the residue was re-extracted. The two supernatants were combined and the amino acid fraction present in each extract was separated by absorption upon and elution from small cation-exchange resin columns.[30] The eluates were evaporated to dryness and stored until required. Aliquots equivalent to 0·2 g of original seed material were applied to two-dimensional chromatograms to resolve individual amino acid constituents present in each extract.

Compounds *B*, *H*, *I*, *K* and *L* were obtained by combining appropriate areas of paper cut from several replicate chromatograms and subsequently eluting the amino acids with hot water.

Isolation of Amino Acids from A. armata *Seed*

Finely ground seed (2 kg) was mixed with 75 per cent (v/v) ethanol (20 l.) and occasionally stirred over a 2-week period. The supernatant was decanted and the residue again extracted with a further 70 l. of aqueous ethanol. The combined alcoholic extracts were reduced to 1·5 l. at 35–40° in a Vortex evaporator, adjusted to pH 4·5, and decolourized with charcoal. The clarified extract was extracted finally with ether to remove residual seed oil.

An initial fractionation of the amino acids in the extract was obtained using a Dowex-50W (× 8) resin column (H^+ form, length 90 cm, dia. 3·7 cm). After absorbing the amino acids and thoroughly washing the column to remove non-cationic materials present in the extract, 0·2 N-NH_3 was used to elute the amino

[28] R. GMELIN, *Hoppe-Seylers Z. Physiol. Chem.* **327**, 186 (1962).
[29] I. K. SMITH, Ph.D. Thesis, London Univ. (1967).
[30] P. M. DUNNILL and L. FOWDEN, *Phytochem.* **4**, 933 (1965).

acids. Fractions (260 × 10 ml) were collected and analysed by paper chromatography. Compound *D* (β-acetyldiaminopropionic acid) was present in fractions 16–64 together with aspartic and glutamic acids, *S*-carboxyethylcysteine and *S*-carboxyisopropylcysteine, and some serine, threonine and 4-hydroxypipecolic acid. Fractions 90–122 contained almost pure albizziine and, after evaporation to about 50 ml, 7·5 g of pure L-β-ureido-α-aminopropionic acid crystallized; $[\alpha]_D^{20} - 64°$ (C = 2, in H_2O), Gmelin *et al.*[5] report $[\alpha]_D^{20} - 66·9°$ (C = 3·6, in H_2O). The concentration of amino acids in fractions 160–260 was low but L-djenkolic acid (0·73 g) separated when the combined volume was reduced to about 70 ml; isolated material had $[\alpha]_D^{20} - 62°$ (C = 1, in N-HCl), literature[10] value $[\alpha]_D^{20} - 60·2°$ (C = 1, in N-HCl).

Fractions 20–60 were combined, evaporated to about 50 ml and cooled at 4°. *S*-β-Carboxyethyl-L-cysteine (4·7 g) separated and was removed: the sample had $[\alpha]_D^{20} - 11°$ (C = 1, in N-HCl); authentic[5] rotation $[\alpha]_D^{20} - 9·3°$ (C = 1, in N-HCl). The remaining solution of amino acids was adjusted to 100 ml and pH 7·0 by addition of dilute ammonia, and then applied to a Dowex-1 (× 10) column (acetate form, length 28 cm, dia. 5 cm). The non-acidic amino acids (serine, threonine, 4-hydroxypipecolic acid and β-acetyldiaminopropionic acid) were not held by the resin and were collected in the eluate (200 ml) at this stage. The pH of the eluate was re-adjusted to 4·5, and the solution applied to a second Dowex-50W (× 8) column (H^+ form, length 80 cm, dia. 1·5 cm); after washing, the amino acids were eluted with 0·15 N-NH_3, fractions (50 × 5 ml) being collected after the initial breakthrough of amino acids. Fractions 1–17, containing mainly β-acetyldiaminopropionic acid together with a trace of threonine, were combined, decolourized, and evaporated to yield a residue (1·04 g), which gave pure L-β-acetyl-α,β-diaminopropionic acid (0·28 g) after recrystallizing from 75 per cent (v/v) ethanol.

Properties of the Isolated β-acetyl-α,β-diaminopropionic Acid

The isolated material had the following analysis: (Found: C, 41·5; H, 6·7; N, 18·8. Calc. for $C_5H_{10}N_2O_3$: C, 41·1; H, 6·8; N, 19·2 per cent). Optical rotation measurements on the isolate gave $[\alpha]_D^{20} - 87°$ (C = 8, in H_2O) and $-35°$ (C = 4, in 6 N-HCl); values determined for synthetic L-β-acetyl-α,β-diaminopropionic acid were $[\alpha]_D^{20} - 81°$ (C = 8, in H_2O) and $-33°$ (C = 4, in 6 N-HCl), while Greenstein and Winitz[31] (p. 2466) quote $[\alpha]_D^{25} - 42·5$ (in 5 N-HCl). The isolated and chemically-synthesized materials were inseparable when run on chromatograms in all seven solvent systems, and on electrophoretograms developed at pH 2·0 and 3·45, but the isolate could be separated chromatographically from α-acetyl-α,β-diaminopropionic acid using solvents 2–6.

The NMR spectra obtained from 4 per cent (w/v) solutions of the isolate and the synthetic β-acetyl derivative in D_2O were identical in every respect. In contrast, the i.r. spectra of nujol mulls of the two materials differed slightly, presumably due to differences in crystalline form.

Like other N-acetylamino acids, the isolated material was readily hydrolysed by dilute mineral acid at 100° to give a basic amino acid characterized as α,β-diaminopropionic acid by chromatography and electrophoresis in the solvents listed above. Spots of diaminopropionic acid present on paper chromatograms gave a novel sequence of colour changes when treated with the following reagents: the ninhydrin chromaphore is a normal purple colour, but this changed quickly to a yellow-orange colour when oversprayed with the modified Ehrlich's reagent (containing *p*-*N*-dimethylaminocinnamaldehyde in place of *p*-dimethylaminobenzealdehyde); on moistening with water, an intense blue–purple colour was produced. α,γ-Diaminobutyric acid, ornithine, lysine and albizziine gave pink-red colours when treated with ninhydrin followed by the modified Ehrlich's reagent, but these remained unchanged when moistened with water.

Synthesis of N-acetyl Derivatives of L-α,β-diaminopropionic Acid

Acetylation under the alkaline conditions of the Schotten–Baumann reaction lead to substitution on the β-amino group of α,β-diaminopropionic aicd (Greenstein and Winitz,[31] p. 2467). L-α,β-Diaminopropionic acid (30 mmoles) and $Ba(OH)_2 . 8H_2O$ (13 g) were dissolved in water (190 ml) and cooled to $-5°$. Precooled acetic anhydride (30 mmoles) was added dropwise over 30–40 min together with a further quantity (13 g) of $Ba(OH)_2 . 8H_2O$. After standing at 30° for 1 hr, Ba was precipitated as $BaSO_4$, and then the pH of the supernatant was adjusted to 4·5. β-Acetyldiaminopropionic acid was separated from any unchanged material by fractionation on a Dowex-50W (× 8) column (H^+ form, length 80 cm, dia. 1·5 cm). 0·2 N-Ammonia was used to elute the amino acids and fractions containing only the β-acetyl derivative were evaporated to yield 0·87 g. This residue was recrystallized from aqueous ethanol to give pure L-β-acetyl-α,β-diaminopropionic acid (0·47 g) (Found: C, 41·1; H, 6·9; N, 18·8. Calc. for $C_5H_{10}N_2O_3$: C, 41·1; H, 6·8; N, 19·2 per cent).

α-Acetyl-α,β-diaminopropionic acid was prepared on a small scale from β-acetamidoacrylic acid following Greenstein and Winitz[31] (p. 2465).

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[31] J. P. GREENSTEIN and M. WINITZ, in *Chemistry of the Amino Acids*, Vol. 3, Wiley, New York (1961).