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THE ISOLATION OF α- AND γ-OXALYL DERIVATIVES OF α,γ-DIAMINOBUTYRIC ACID FROM SEEDS OF *LATHYRUS LATIFOLIUS*, AND THE DETECTION OF THE α-OXALYL ISOMER OF THE NEUROTOXIN α-AMINO-β-OXALYL-AMINOPROPIONIC ACID WHICH OCCURS TOGETHER WITH THE NEUROTOXIN IN THIS AND OTHER SPECIES

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Abstract—Two oxalyl derivatives of α,γ-diaminobutyric acid have been isolated from the seeds of *Lathyrus latifolius*. The reversible transfer of the oxalyl group between the α- and γ-amino groups of the amino acid with the establishment of an equilibrium has been demonstrated. α-Oxalylamino-β-aminopropionic acid, an isomer of the neurotoxin α-amino-β-oxalylaminopropionic acid, has been found together with the neurotoxin in seed extracts of this same species and also in seed extracts of *L. sativus*, *L. clymenum* and *L. cicera*, species implicated in classical "lathyrism", a neurolathyrism, which affects man and domestic animals. An analogous transfer of the oxalyl group between the α- and β-amino groups of α,β-diaminopropionic acid has also been shown. The phylogenetic and toxicological significance of these findings is discussed.

INTRODUCTION

THREE compounds toxic to higher animals have been isolated from various species of *Lathyrus*. These are β(*N*-γ-glutamyl)-aminopropionitrile from *L. odoratus*,[1] L-α,γ-diaminobutyric acid from *L. latifolius*[2] and L-α-amino-β-oxalylaminopropionic acid from *L. sativus*.[3,4] The last-mentioned of these substances has been found in twenty-one other species of the genus[5] (including *L. cicera* and *L. clymenum*, species implicated with *L. sativus* in classical "lathyrism", a neurological disease affecting man and domestic animals). In ten of these it is accompanied by two acidic derivatives of α,γ-diaminobutyric acid one of which has been identified as α-amino-γ-oxalylaminobutyric acid.[6] A study of these derivatives of α,γ-diaminobutyric acid was undertaken not only because they were of interest in themselves but also because it was thought that the establishment of their identity might provide data for the biochemical evaluation of phylogenetic relationships between species of this genus. The possibility that they might be toxic to higher animals was also borne in mind.

The present paper describes the isolation and characterization of these compounds, their relationship as readily interchangeable isomers, and the discovery of a similar relationship between the corresponding derivatives of α,β-diaminopropionic acid. Of these α,β-diaminopropionic acid compounds, the α-oxalyl derivative has been found in relatively low concentrations in species which synthesize the toxic β-derivative, but due to the ease with which transformation occurs as much as 40–50 per cent (judged by colour yield) of the β-derivative may

[1] E. D. SCHILLING and F. M. STRONG, *J. Am. Chem. Soc.* 77, 2843 (1955).
[2] C. RESSLER, P. A. REDSTONE and R. H. ERENBERG, *Science* **134**, 188 (1961).
[3] S. L. N. RAO, P. R. ADIGA and P. S. SARMA, *Biochemistry* 3, 432 (1964).
[4] V. V. S. MURTI, T. R. SESHADRI and T. A. VENKITASUBRAMANIAN, *Phytochem.* 3, 73 (1964).
[5] E. A. BELL, *Nature* **203**, 378 (1964).
[6] E. A. BELL, *Fed. Europ. Biochem. Socs. Abst.* 1, 53 (1964).

be converted to the α-oxalyl isomer during isolation procedures unless suitable precautions are taken; a factor which cannot be overlooked when studying the toxicity of either.

RESULTS AND DISCUSSION

When freshly prepared extracts of *L. latifolius* seed are subjected to high-voltage ionophoresis on paper at pH 3·6 the fastest-moving acidic compound readily detectable with ninhydrin is the β-oxalyl derivative of α,β-diaminopropionic acid. Moving slightly slower and in comparable concentration (about 1 per cent dry wt of seed) is the compound which has been identified as α-amino-γ-oxalylaminobutyric acid and this in turn is followed by a second derivative of α,γ-diaminobutyric acid which occurs in lower concentration.

After elution from the ionophoresis papers and acid hydrolysis, the only products of the slower-moving compound which could be detected by ionophoresis were the same as those found after hydrolysis of the α-amino-γ-oxalylaminobutyric acid, namely oxalic acid and α,γ-diaminobutyric acid.

After standing at 4° for several weeks it was observed that the concentration of the slower-moving derivative of α,γ-diaminobutyric acid in seed extracts (50% ethanol) had increased and that of the faster-moving α-amino-γ-oxalylaminobutyric acid had decreased. Complete disappearance of the faster compound did not occur however, suggesting that an equilibrium between the two might exist. Support for this suggestion came from the work of Rao[7] who observed a second acidic compound in solutions of synthetic α-amino-γ-oxalylaminobutyric acid which he prepared by the action of ethoxalyl chloride upon the copper salt of α,γ-diaminobutyric acid. The two components of the synthetic mixture (kindly given by Dr. S. L. N. Rao) when subjected to high-voltage ionophoresis at different values of pH behaved in all respects as the two derivatives of α,γ-diaminobutyric acid found in the seed extracts.

The isolation of the two compounds from a concentrated extract of *L. latifolius* seed was attempted by passing the extract successively through columns of cation and anion exchange resins and then displacing the acidic compounds from the anion exchange resin with 0·4 N acetic acid. In this way a series of fractions containing varying proportions of the two α,γ-diaminobutyric acid derivatives and α-amino-β-oxalylaminopropionic acid (later shown to be a mixture of isomers) was obtained.

When isolated from the first fractions the slowest moving of these compounds analysed as the monohydrate of a monooxalyl derivative of α,γ-diaminobutyric acid and on hydrolysis with 4 N-hydrochloric acid gave oxalic acid and L-α,γ-diaminobutyric acid.

The unhydrolysed compound gave with ninhydrin an atypical grey–purple which developed slowly. The colour was not affected by pre-treatment with cupric nitrate however, indicating that the compound did not contain a free amino group in the α-position.[8]

As the compound moved towards the cathode on ionophoresis at pH 1·9 it contained at least one free basic group, and the presence of ionizable groups with apparent pK values of 2·0, 3·0 and 10·2 (cf. 10·2 for the γ-amino group of α,γ-diaminobutyric acid), were found on titration. Its i.r. absorption spectrum showed absorption maxima at 3380 (.CO.NH.), 3110 (NH_3^+ stretch), 1715 (COOH), 1620–1660 (.CO.NH. + NH_3^+ deformation) and 1520 cm^{-1} (amide II: secondary noncyclic amides only[9]) which were consistent with the chemical evidence that this slow-moving derivative was L-α-oxalylamino-γ-aminobutyric acid.

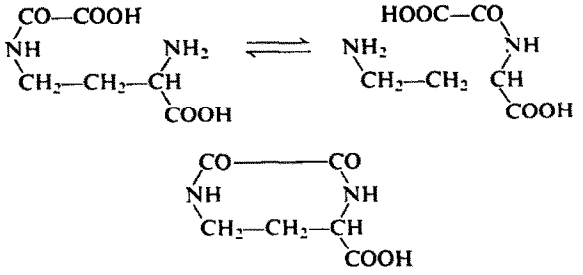
[7] S. L. N. RAO, Private communication.
[8] P. O. LARSEN and A. KJAER, *Biochem. Biophys. Acta* **38**, 148 (1960).
[9] L. J. BELLAMY, *The Infra-red Spectra of Complex Molecules* (2nd Ed.), p. 205. Methuen, London (1960).

The fractions which contained the faster-moving derivative, previously identified as α-amino-γ-oxalylaminobutyric acid, together with traces of the α-oxalyl isomer were bulked, freeze-dried, and the residue, after redissolving in a small volume of water, precipitated with acetone. The product obtained still contained traces of α-oxalylamino-γ-aminobutyric acid however and attempts were made to purify it by recrystallization from hot aqueous acetone and other solvents. These failed, the recrystallized material containing a higher proportion of the α-oxalyl isomer than the original mixture. On recrystallization from hot water with slow cooling large crystals of two species, elongated prisms and regular flat hexagons were obtained. Most of the material was in the form of aggregates containing both crystal types, but individual crystals did exist and examples of each were separated by hand-picking. On dissolving the crystals in water and subjecting them to ionophoresis it was found that the elongated prisms belonged to α-oxalylamino-γ-aminobutyric acid and the hexagons to α-amino-γ-oxalylaminobutyric acid. Elementary analysis of the hexagonal crystals agreed with the previous identification of the fast-moving compound. The i.r. absorption spectrum of these crystals showed maxima at 3325 (.CO.NH.), 1630–1660 (.CO.NH.), and 1590 cm^{-1} (CO_2^-), but no maxima corresponding to NH_3^+ stretch or COOH. The absence of absorption at these wavelengths and the presence of a strong C:O absorption at 1775 cm^{-1} (comparable with the .C:O absorption found in strained ring systems) suggests that this isomer exists as a hydrated lactam in the solid state. In solution however the compound is positively charged at pH 1·3 and forms a chelate with cupric ions confirming the presence of a free α-amino group and the open structure originally assigned to it.

Insufficient of this compound was isolated for a direct determination of its configuration to be made, but knowing that the slower-moving derivative was L-α-oxalylamino-γ-aminobutyric acid an aggregate of mixed crystals containing approximately 50 per cent of each compound was hydrolysed and the α,γ-diaminobutyric acid derived from the mixture shown to be of the L-isomer, thereby confirming that both compounds were derivatives of L-α,γ-diaminobutyric acid the faster-moving being L-α-amino-γ-oxalylaminobutyric acid.

Demonstration of an Equilibrium between the Two Oxalyl Derivatives of α,γ-Diaminobutyric Acid

Changes in the relative concentrations of the two oxalyl derivatives in seed extracts and the difficulty of obtaining the γ-oxalyl compound pure from hot solvents led to the conclusion that we were dealing with an equilibrium mixture. This was confirmed by dissolving pure crystals of each compound in 0·4 N acetic acid (the acid previously used for displacement). On standing at room temperature a slow reversion to an isomeric mixture occurred in each solution, the same change also took place in water, the rate of change increasing rapidly with temperature.

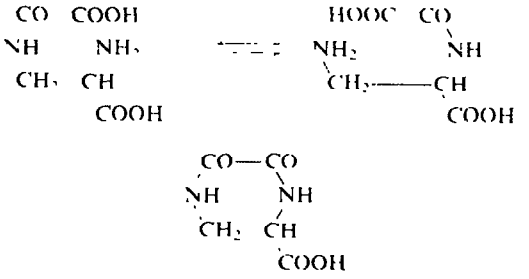


(Possible intermediate)

The mechanism of this change has not been examined but it may possibly involve the formation and hydrolysis of an unstable cyclic intermediate.

Detection of α-Oxalylamino-β-aminopropionic Acid in Seeds of L. latifolius, L. sativus, L. clymenum *and* L. cicera *and the Existence of an Equilibrium between it and its β-Oxalyl Isomer*

The finding of an equilibrium between the two α,γ-diaminobutyric acid derivatives led us to examine the possibility that the α-oxalyl derivative of α,β-diaminopropionic acid might exist in species of *Lathyrus* and undergo reversible transformation to the neurotoxic β-derivative. By applying seed extracts to ionophoresis papers in fine stripes and continuing ionophoresis for longer periods than before the presence of a compound occurring in lower concentration than the other acidic derivatives, giving a grey–purple with ninhydrin, and moving immediately behind α-amino-β-oxalylaminopropionic acid was detected. A much higher proportion of this compound was found in the recrystallized material, nominally α-amino-β-oxalylaminopropionic acid, which had been isolated from the large-scale extract of *L. latifolius* seed. Samples of the two components were obtained by elution from ionophoresis papers and on hydrolysis both gave oxalic acid and α,β-diaminopropionic acid. On heating in aqueous solution the individual components reverted to a mixture in the same manner as the α,γ-diaminobutyric acid derivatives. The slower-moving of the two α,β-diaminopropionic acid derivatives reacted with cupric nitrate and ninhydrin as if it were an amino acid containing a substituted α-amino group[8] indicating that it bears the same relationship to the neurotoxin as does α-oxalylamino-γ-aminobutyric acid to its isomer, and that an analogous equilibrium between the lower homologues exists.



(Possible intermediate)

The relative concentrations of the ω-oxalyl to the α-oxalyl derivatives in the plants suggests that enzymic oxylation occurs preferentially on the amino group furthest from the carboxylic acid group. Indeed the possibility that the α-oxalyl derivatives in the plant may arise by isomeric change *in vivo* rather than by enzymic oxylation cannot be excluded at present. That the α-oxalyl derivatives are not artifacts was confirmed by the analysis of extracts immediately after their preparation in the cold.

Phylogenetic Significance

The genus *Lathyrus* has been sub-divided on the basis of ninhydrin-reacting compounds which occur in high concentrations in the seeds of its species.[10,11] In Table 1, the distribution

[10] E. A. BELL, *Biochem. J.* **83**, 225 (1962).
[11] E. A. BELL, In *Comparative Phytochemistry* (Edited by T. SWAIN), p. 195. Academic Press, New York (1966).

of these compounds in the species of groups I and II are re-stated, two of the original "unknowns" being now identified as the oxalyl derivatives of α,γ-diaminobutyric acid.

TABLE 1. THE DISTRIBUTION OF NINHYDRIN-REACTING COMPOUNDS OCCURRING IN HIGH CONCENTRATION IN SEEDS OF TWO GROUPS OF *Lathyrus* SPECIES

Group	No.	Name	B_1	B_2	N_2	A_1	A_2	A_3	Arg.
I	1	*L. aurantius*		++					T
	2	*L. luteus*		++					T
	3	*L. laevigatus* sp. *aureus*		++					T
	4	*L. sylvestris*		++	T	+	+	T	+++
	5	*L. latifolius*		++		+	+	T	++
	6	*L. heterophyllus*		++		+	+	T	++
	7	*L. gorgoni*		++		+	+	T	T
	8	*L. grandiflorus*		++		+	+	T	T
	9	*L. cirrhosus*		++		+	+	T	+
	10	*L. rotundifolius*		++	T	+	+	T	+
	11	*L. tuberosus*		++		+	+	T	+
	12	*L. multiflora*		++	T	T	+	T	+
	13	*L. undulatus*		++	+	T	++	T	+
II	14	*L. alatus*	++			++			T
	15	*L. articulatus*	++			++			T
	16	*L. arvense*	++			++			+
	17	*L. setifolius*	++			+++			T
	18	*L. pannonicus*	++			++			T
	19	*L. ochrus*	++			++			T
	20	*L. clymenum*	++			++			T
	21	*L. sativus*	++			T			T
	22	*L. megallanicus*	++			T			T
	23	*L. quadrimarginatus*	++			T			T
	24	*L. cicera*	++			T			T

T=trace; B_1=L-homoarginine; B_2=L-α,γ-diaminobutyric acid; N_2="unknown"; A_1=L-α-amino-β-oxalylaminopropionic acid; A_2=L-α-amino-γ-oxalylaminobutyric acid; A_3=L-α-oxalylamino-γ-aminobutyric acid; Arg.=arginine.

On re-examining the relationships (with respect to the biosynthesis of amino acids and derivatives) which exist between the species of these groups in the light of these new identifications several points of interest emerge. Firstly if the ninhydrin "patterns" given by the seed extracts of the last ten species in group I are compared with those given by the species of group II it will be seen that they resemble each other in one respect (the presence of α-amino-β-oxalylaminopropionic acid) and differ in three respects, namely the replacement of α,γ-diaminobutyric acid in group I by homoarginine in group II and the occurrence of two additional acidic compounds in group I. Knowing the identity of the additional acidic spots and that the same enzyme system is responsible for the oxylation of both diamino acids (as preliminary experiments in this laboratory indicate) then it is apparent that the presence of the two oxalyl derivatives of α,γ-diaminobutyric acid can be discounted when considering biochemical similarities and differences between these two groups of species, as the information they represent (that these species can synthesize α,γ-diaminobutyric acid and contain an oxylating system) is already available to us in the presence of the identified "spots" of free α,γ-diaminobutyric acid and α-amino-β-oxalylaminopropionic acid. Paradoxically however, the absence of the two acidic derivatives of α,γ-diaminobutyric acid from the seeds of the

first three species of group I cannot be ignored in studying the relationship which exists between these three and the remaining species of the same group or between these three and the species of group II, for their absence tells us that they differ from all the other species of the two groups not only in failing to synthesize α,β-diaminopropionic acid but also in lacking an oxylating system. The superficially complex "pattern" difference seen between the last ten species of group I and the species of group II resolves itself therefore into a single alternative between homoarginine and α,γ-diaminobutyric acid synthesis, while the first three species of group I appear to be correctly placed, furthest from group II, in that they differ in two other respects, their inability to synthesize α,β-diaminopropionic acid or to oxylate the diamino acid which they do synthesize. The oxalyl derivatives of α,γ-diaminobutyric acid provide here interesting examples of characters which are of no significance when comparing one group of species (No. 4–13) with a second group (No. 14–24) but are of significance when comparing either of these groups to a third (No. 1–3).

The Toxicity of Oxalyl-Amino Acids in Animals

The ingestion of seeds of *L. sativus*, *L. cicera* and *L. clymenum*, three species of group II, have been reported to induce classical lathyrism (a neurolathyrism) in man and higher animals.[12] The isolation from *L. sativus* of a compound identified as α-amino-β-oxalylaminopropionic acid which produced neurotoxic symptoms in chicks provided a likely explanation of this toxicity. The results reported in the present paper however leave some degree of doubt as to whether the toxic effects are due entirely to α-amino-β-oxalylaminopropionic acid, to its α-oxalyl isomer or to both compounds. The toxicity of these two derivatives and also the toxicity of the two oxalyl derivatives of α,γ-diaminobutyric acid are being investigated.

EXPERIMENTAL

Ionophoresis

Ionophoresis was conducted on Whatman 3 MM paper using a horizontal method essentially that of Gross.[13] Buffer solutions of pH 1·9, 3·6 and 6·5 (prepared as previously described[14]), and of pH 1·3 formic acid (98–100%)-acetic acid–water (200:147:2000, by vol.) and pH 3·0 (acetic acid–pyridine–water (170:10:1820, by vol.) were used, a potential difference of 60 V/cm being applied for 30–40 min. At pH 3·0 the four oxalyl derivatives were completely resolved in 40 min.

Infra-red Absorption Spectra

Infra-red absorption spectra were determined with a Perkin–Elmer spectrophotometer (No. 235) using the KCl disc technique.

Extraction of L. latifolius *Seed*

Finely ground seed of *L. latifolius* (2 kg), obtained from Sutton and Sons Ltd., Reading, was extracted with acetone in a modified Soxhlet apparatus to remove fat and pigments. The defatted seed was shaken with cold 50% ethanol (4 × 5 l., 8 hr each time). The combined extracts were reduced to 9 l. by distillation under reduced pressure and passed successively through a column (120 cm × 7 cm) of weakly acidic cation exchange resin (Zeo-Karb 226) in

[12] H. SELYE, *Rev. Can. Biol.* **16**, 3 (1957).
[13] D. GROSS, *J. Chromatog.* **5**, 194 (1961).
[14] F. A. BELL and A. S. I. TIRIMANNA *Biochem. J.* **97**, 104 (1965).

the H form and a column (250 cm × 4 cm) of strongly basic anion exchange resin (De-Acidite FF) in the OH form. The De-Acidite column was washed with 20 l. of water and the basic, neutral and weakly acidic ninhydrin-reacting compounds (glutamic acid and aspartic acid) displaced with 0·1 N acetic acid (5 l.). After an equal volume of 0·2 N acetic acid had been passed through the column without displacing the strongly acidic compounds the concentration of the acid was raised to 0·4 N. After adding 600 ml of this acid the least acidic of the α,γ-diaminobutyric acid derivatives was detected in the effluent. The next 5 l. of effluent contained this compound together with small amounts of α-amino-γ-oxalylaminobutyric acid and yielded 2·3 g of solid when freeze-dried. The following 5 l. yielded 3·5 g of solid which was mainly α-amino-γ-oxalylaminobutyric acid but contained traces both of the less acidic α,γ-diaminobutyric acid derivative and of α-amino-β-oxalylaminopropionic acid. All remaining ninhydrin-reacting material was displaced in the next 8 l. of effluent which on freeze-drying yielded 2·6 g of solid which was mainly the "α-amino-β-oxalylaminporopionic acid" (later shown to be an isomeric mixture) with traces of α-amino-γ-oxalylaminobutyric acid.

Isolation of α-Oxalylamino-γ-Aminobutyric Acid

The solid obtained from the first 5 l. of ninhydrin-positive effluent was dissolved in water (50 ml), the solution shaken in the cold with charcoal, filtered and acetone added until it became cloudy. After 3 days at 4° the needles which separated were filtered off, redissolved in water and reprecipitated with acetone. Yield 1 g, m.p. softens at 140, dec. 178° (Found: C, 34·72; H, 5·84; N, 13·91. $C_6H_{10}O_5N_2$, H_2O requires: C, 34·61; H, 5·77; N, 13·46%) $[\alpha]_D^{20} - 15{\cdot}65$ (*c* 1·3, H_2O). A solution of the crystals in water was found on ionophoresis to contain only the slower-moving derivative of α,γ-diaminobutyric acid. On titration (60 mg with 0·5 N HCl and 0·5 N NaOH) the compound was found to contain three ionizable groups (apparent values of p*K*, 2·0, 3·0 and 10·2). The isolated compound (80 mg) was hydrolysed with 4 N HCl (3 ml) in a sealed tube at 80° for 1·5 hr and the solution taken to dryness at room temperature under reduced pressure. The crystalline residue was triturated several times with boiling ether, the ether extracts were combined and evaporated, leaving a white powdery residue. This residue was dissolved in water (0·5 ml) and the solution allowed to evaporate at room temperature. Large colourless crystals were obtained, m.p. 102°, no depression of m.p. was observed when these were admixed with the dihydrate of oxalic acid (Found: C, 19·56; H, 5·16. Calc. for $C_2H_2O_4.2H_2O$: C, 19·6; H, 4·8%). The crystals gave the characteristic reactions of oxalic acid with diphenylamine, indole and sulphuric acid and they decolorized acid potassium permanganate.

The second hydrolysis product which did not dissolve in ether was warmed with methanol, and water added drop by drop until solution was complete. On standing at 4° long colourless needles separated, m.p. 210°, $[\alpha]_D^{20} + 11{\cdot}71$ (*c* 1·3, H_2O) (Found: C, 27·93; H, 6·63; N, 15·98. Calc. for $C_4H_{10}O_2N_2$ $1\frac{1}{2}$ HCl: C, 27·8; H, 6·65; N, 16·2%). No depression of m.p. was observed when this compound was admixed with the sesquichloride of L-α,γ-diaminobutyric acid prepared by recrystallizing the synthetic dihydrochloride from aqueous methanol in the same way (cf. Wilkinson[15]). The identity of the compound was confirmed by subjecting it to ionophoresis at pH values of 1·9, 3·6 and 6·5 using authentic L-α,γ-diaminobutyric acid as a marker and by showing its i.r. spectrum to be identical with that of the authentic compound.

[15] S. WILKINSON, *J. Chem. Soc.* 104 (1951).

Isolation of α-Amino-γ-Oxalylaminobutyric Acid

The freeze-dried material (3·5 g) which was predominantly α-amino-γ-oxalylaminobutyric acid was dissolved in hot water (10 ml), boiled briefly with charcoal, filtered, cooled slowly and kept at 4° for 17 hr. After this period a crop of large crystals (1·1 g) had separated and was removed by filtration. Under magnification (×8) it was seen that two distinct crystalline species were present (flat hexagons and elongated prisms). Examples of each type were separated by hand-picking and the flat hexagons were found on ionophoresis to be the crystals of α-amino-γ-oxalylaminobutyric acid. Sufficient of these were separated for elementary and i.r. analysis; m.p. softens 180°, dec. 204° (Found: C, 37·46; H, 5·76; N, 14·12. $C_6H_{10}O_5N$ requires: C, 37·89; H, 5·26; N, 14·74%). On dissolving the hexagonal crystals in water and adding ethanol fine needles were obtained. (Found: C, 37·94; H, 5·53; N, 14·11%.) Both hexagonal crystals and needles gave the same i.r. spectrum which is described above. An aggregate of crystals (0·2 g) containing approximately equal amounts of L-α-oxalylamino-γ-aminobutyric acid and the faster-moving L-amino-γ-oxalylaminobutyric acid (probably a lactam in the solid state) was hydrolysed with 4 N HCl (3 ml) in a sealed tube for 17 hr at 80°. The hydrolysate was taken to dryness at reduced pressure, extracted with boiling ether and the ether-soluble material shown to be oxalic acid by mixed m.p. and the colour reactions previously described. The residue which did not dissolve in ether was recrystallized from aq. methanol. (Found: C, 27·92; H, 6·85; N, 15·7; Cl, 31·0. Calc. for $C_4H_{10}O_2N_2$ $1\frac{1}{2}$HCl: C, 27·8; H, 6·65; N, 16·2; Cl, 31·5%.) $[\alpha]_D^{20}+18{\cdot}47$ (*c* 2·7, 5 N HCl) cf. synthetic sesquihydrochloride of L-α,γ-diaminobutyric acid $[\alpha]_D^{20}+19{\cdot}5$ (*c* 2·7, 5 N HCl).

The Interconversion of the α- and γ-Oxalyl Derivatives of α,γ-Diaminobutyric Acid

α-Amino-γ-oxalylaminobutyric acid (1·5 mg of hexagonal crystals recrystallized from water) and α-oxalylamino-γ-aminobutyric acid (1·5 mg precipitated with acetone) were dissolved in 0·4 N acetic acid (1 ml each). After 4 days at room temperature each solution was found to contain two ninhydrin-reacting components; a high concentration of the original derivative being accompanied in each solution by a low concentration (2–3 per cent) of its isomer. On standing at 50° for 17 hr the proportions of the minor constituent in each solution had increased, the ratio of major to minor constituent now being in the order of 2:1. An equally marked transformation was observed when solutions of the same concentration in water were heated at 80° for 17 hr.

The Isolation of "α-Amino-β-Oxalylaminopropionic Acid" (Subsequently Shown to be an Isomeric Mixture)

The freeze-dried material (2·6 g) obtained from the last fractions displaced from the De-Acidite column was recrystallized twice from hot 50% ethanol to give a colourless crystalline solid (0·8 g), m.p. 164–167° dec. (Found: C, 31·85; H, 5·58; N, 15·30. Calc. for $C_5H_8O_5N_2$ $\frac{1}{2}H_2O$ (cf. Ref. 4): C, 32·4; H, 4·9; N, 15·13%.) When subjected to ionophoresis at pH 3·6 (60 V/cm for 15 min) this material appeared to contain only one component.

The Identification of α-Oxalylamino-β-Aminopropionic Acid in Seeds of L. sativus, L. cicera *and* L. clymenum *and in the "α-Amino-β-Oxalylaminopropionic Acid" from* L. latifolius

Seed of these species (100 mg) was ground and shaken with 1 ml 50% ethanol in the cold for 4 hr, filtered and the filtrate applied as a thin stripe to paper and subjected to ionophoresis at pH 3·6 for 40 min. Immediately behind the concentrated band of α-amino-β-oxalylaminopropionic acid was a compound which gave with ninhydrin a slowly developing grey–purple

which was not affected by pre-treatment with cupric nitrate. The concentration of this compound was about 5 per cent of that of the β-oxalyl derivative.

On ionophoresis under the same conditions it was found that the "α-amino-β-oxalyl-aminopropionic acid", obtained by recrystallizing the last freeze-dried fraction of the large-scale extraction of *L. latifolius* seeds from 50% ethanol, contained approximately 50 per cent of this slower-moving compound. These two components were separated by ionophoresis and eluted from the paper with water. On hydrolysis in N HCl for 7 hr at 80° each component gave the same two derivatives, α,β-diaminopropionic acid (identified by ionophoresis at pH 1·9, 3·6 and 6·5 using authentic material for a marker, and by the typical blue–green given by this amino acid when treated successively on paper with ninhydrin and Ehrlich's reagent), and oxalic acid (identified by chromatography[16] and the colour reactions already described).

The Interconversion of the α- and β-Oxalyl Derivatives of α,β-Diaminopropionic Acid

Solutions (approximately 0·01 M) of the two oxalyl derivatives of α,β-diaminopropionic acid, prepared by eluting the compounds from ionophoresis papers with water, were heated at 80° for 17 hr in sealed tubes. On ionophoresis each of the heated solutions was found to contain a mixture of isomers; in each the concentrations of the two components were approximately the same (as judged by colour intensity with ninhydrin).

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[16] H. A. W. BLUNDSTONE, *Nature* **197**, 377 (1963).