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The Isolation of L-Homoarginine from Seeds of *Lathyrus cicera*

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The occurrence of appreciable concentrations (1% or more as judged by the intensity of the ninhydrin spots) of free ninhydrin-reacting compounds in the seeds of 45 species of *Lathyrus* has been reported by Bell (1962a). The genus was divided into groups of species characterized by peculiar associations of these compounds in their seeds. Most of the compounds are probably non-protein amino acids and the associated groups may represent final storage products together with their respective precursors.

The distribution and relative concentrations of two of the compounds suggested that they may be related. These substances have now been isolated and identified. Both are amino acids that have not been found previously in natural sources. The first, lathyrine, was isolated from seeds of *Lathyrus tingitanus* by Bell (1961) and shown to be 2-amino-pyrimidin-4-ylalanine (Bell & Foster, 1962). The second was identified by Bell (1962a, b) as homoarginine (α -amino- ϵ -guanidinocaproic acid), and

the present paper describes its isolation from seeds of *Lathyrus cicera*. The isolated amino acid was shown to be L-homoarginine by direct comparison with the synthetic compound and by the identification of homocitrulline (α -amino- ϵ -ureidocaproic acid) and lysine as the principal products of alkaline hydrolysis.

The possible role of L-homoarginine (I) as the natural precursor of lathyrine (II), and the relationship between the groups of species in which these two amino acids occur, are discussed.

EXPERIMENTAL

Chromatography. One-dimensional chromatograms were prepared by the descending technique on Whatman no. 1 paper. The chromatograms were developed with butan-1-ol-acetic acid-water (12:3:5, by vol.) and lutidine-water (11:5, v/v) for 40 hr. and with phenol-water (4:1, w/v), in the presence of the vapour from aq. ammonia (sp.gr. 0.88) added to the tank, for 17 hr.

Ionophoresis. Ionophoresis was conducted on Whatman no. 1 paper in buffer solutions of pH 1.9 [formic acid (98–100%)-acetic acid-water (33:148:1819, by vol.)], pH 3.6 [acetic acid-pyridine-water (10:1:190, by vol.)], and pH 6.5 [acetic acid-pyridine-water (1:25:475, by vol.)]. A horizontal method essentially that of Gross (1961) was used, a potential difference of 75 v/cm. being applied for 30 min.

Development of colours. Chromatography and ionophoresis papers were dipped in ninhydrin reagent [0.2% (w/v) ninhydrin in 95% (v/v) acetone] and the modified Ehrlich's reagent [1.0% (w/v) *p*-dimethylaminobenzaldehyde in acetone-10N-hydrochloric acid (9:1, v/v)] of Smith (1953) and the modified Sakaguchi's reagent [0.1% (w/v) 8-hydroxyquinoline in acetone, followed by 0.2% (v/v) bromine in 0.5N-sodium hydroxide] of Jepson & Smith (1953). Ninhydrin colours were developed at 80°.

Infrared spectra. Spectra were determined with a double-beam spectrophotometer by using the KBr-pressed-disk technique, and a rock-salt prism.

Detection of homoarginine. Homoarginine was detected as a ninhydrin-reacting Sakaguchi-positive compound which moved slightly faster than arginine in the chromatographic solvents described and a little more slowly than arginine during ionophoresis at pH 1.9, 3.6 and 6.5.

Isolation of L-homoarginine

Finely-ground seed of *Lathyrus cicera* (500 g.) was extracted for 7 hr. with acetone and for 14 hr. with ethanol in a modified Soxhlet apparatus. The defatted seed was shaken for 2 hr. with 1.5 l. of ethanol-N-hydrochloric acid (2:1, v/v) and the suspension filtered after 17 hr. at room temperature. Two further extractions of the seed were made, 1.5 l. of ethanol-water (2:1, v/v) being used for each. The acid-ethanol and water-ethanol extracts were combined and the ethanol was removed under reduced pressure; after being filtered, the concentrated extract was passed through a column (3 cm. × 80 cm.) of De-Acidite FF resin which was washed with water until a colourless, neutral and ninhydrin-negative effluent was obtained.

The alkaline effluent and the combined washings were passed through a column (3 cm. × 50 cm.) of Zeo-Karb 226 resin which retained the Sakaguchi-positive fraction. The column was washed with water until the effluent was free of ninhydrin-reacting compounds, and the guanidino amino acid was then eluted with N-ammonia. Fractions giving a positive Sakaguchi reaction were combined and concentrated *in vacuo* at room temperature over CaCl₂ and H₂SO₄. The viscous residue was made acid to litmus by the dropwise addition of 5N-hydrochloric acid, and aniline (1 ml.) was added to ensure the formation of the monohydrochloride (Cox, 1928).

The crude hydrochloride was stirred several times with 20–30 ml. of ethanol to remove aniline hydrochloride and excess of aniline. After the ethanol had been decanted off,

the residual oil crystallized on standing. The decanted ethanol was evaporated to an oil from which further hydrochloride was precipitated by stirring with 5–10 ml. of fresh ethanol.

The combined hydrochlorides were stirred in warm ethanol and water was added dropwise until solution was complete. After the solution had been stirred with activated charcoal and filtered, the hydrochloride separated in colourless prisms on standing at 4°; it was recrystallized twice from aqueous ethanol in the same manner. The purified material (1.0 g.) had m.p. 206° (uncorr.), unchanged by admixture with authentic L-homoarginine hydrochloride, kindly supplied by Professor C. E. Dent (Found: C, 37.5; H, 7.6; Cl, 16.2; N, 25.1. Calc. for C₇H₁₄N₄O₂·HCl: C, 37.4; H, 7.6; Cl, 15.8; N, 25.0%). Observed for monohydrochloride: $[\alpha]_D^{25} + 13 \pm 1^\circ$ (c 0.8 in N-HCl); calculated for free base: $[\alpha]_D^{25} + 16 \pm 1^\circ$. Stevens & Bush (1950) found $[\alpha]_D^{25} + 18^\circ$ for synthetic L-homoarginine (c 1.2 in N-HCl).

The infrared spectra of the hydrochlorides of synthetic L-homoarginine and of the isolated amino acid are given in Fig. 1. All absorption peaks appear in both spectra.

Alkaline hydrolysis of isolated amino acid

Hydrolysis with sodium hydroxide. The amino acid hydrochloride (3.8 mg.) was refluxed with 0.1N-NaOH (5 ml.) for 3 hr., the solution evaporated to dryness at room temperature under reduced pressure and the residue redissolved in 1 ml. of 10% (v/v) propan-2-ol and brought to pH 7 with N-hydrochloric acid. Spots (10 μ l.) of the solution were chromatographed and subjected to ionophoresis on paper with the solvent systems and buffer solutions described. Each spot was run with markers of L-homoarginine, homocitrulline, lysine, urea, arginine, citrulline and ornithine. Papers run in each solvent system and buffer were developed with the ninhydrin, Ehrlich and Sakaguchi reagents.

The hydrolysate gave a strong ninhydrin-reacting spot, which corresponded on all papers to lysine, and two weaker spots. One of these gave a ninhydrin reaction and also a yellow spot with Ehrlich's reagent, and corresponded to homocitrulline; the other gave only a yellow spot with Ehrlich's reagent and was identified as urea. The approximate R_F values of homocitrulline in butan-1-ol-acetic acid-water, lutidine-water and phenol-water were 0.21, 0.09 and 0.67 respectively.

Fig. 1. Infrared spectra of L-homoarginine hydrochloride. A, Synthetic material; B, natural material. The KBr-disk method was used for the determination of both spectra.

Hydrolysis with silver oxide. The amino acid hydrochloride (7.7 mg.) was refluxed with silver oxide (23.8 mg.) in 5 ml. of water for 3 hr. The hydrolysate was filtered, evaporated to dryness at room temperature under reduced pressure and the residue redissolved in 1 ml. of 10% (v/v) propan-2-ol. Chromatography and ionophoresis as above gave a strong spot of unchanged homoarginine and two weaker spots corresponding to lysine and homocitrulline.

Identification of homocitrulline on paper. Ureides normally give a yellow colour on paper with Ehrlich's reagent, whether applied directly or after development with ninhydrin or isatin in a multiple dipping sequence (Jepson & Smith, 1953). Homocitrulline gives the expected yellow when treated directly, but when the Ehrlich's reagent is applied after ninhydrin or isatin the colours given with these reagents rapidly fade and are replaced by an intense rose red. This reaction is more sensitive than the simple Ehrlich reaction. Hydroxyproline (Jepson & Smith, 1953) and 4-methyleneproline (Gray & Fowden, 1962) are reported to show colour changes with these reagents, but the development of a stable rose red appears to be specific for homocitrulline.

DISCUSSION

L-Homoarginine was detected in the seeds of those 17 species of *Lathyrus* known to contain lathyrine and also in those of 19 other species of this genus (Bell, 1962a). In the 17 species containing lathyrine the concentrations of L-homoarginine and lathyrine appear complementary, a high concentration of one being associated with a low concentration of the other. This relationship and the structural similarity of the two compounds suggest that lathyrine may be formed by the cyclization and dehydrogenation of L-homoarginine and that the fundamental difference between the species containing lathyrine and those containing

only homoarginine is the presence or absence of an enzyme system capable of bringing about this transformation.

The high nitrogen content and extremely low solubility of lathyrine at normal biological pH values agree with its supposed role in nitrogen storage. L-Homoarginine, with a similar nitrogen content, is more soluble.

SUMMARY

1. The natural occurrence of L-homoarginine has been confirmed by isolation from seeds of *Lathyrus cicera*.

2. The possible role of L-homoarginine as the precursor of lathyrine is discussed.

3. A colour reaction for the detection of homocitrulline is described.

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Univalent Cations and the Respiration of Brain-Cortex Slices

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The peculiar ability of brain slices to respond metabolically to certain stimuli was first observed with potassium as the stimulating agent (Ashford & Dixon, 1935; Dickens & Greville, 1935). Studies on the metabolic response to electrical pulses have been numerous (McIlwain, 1951, 1956), and the potassium effect, although frequently confirmed in a qualitative way, has not been examined quantitatively to a comparable extent.

The metabolic response to stimulation comprises increased respiratory rate and aerobic glycolysis, breakdown of creatine phosphate and ATP, and an increase in inorganic phosphate; it is found in brain tissue, but is not shown by slices from kidney, liver, testis, and tumour tissue. L. Hertz & T. Clausen (unpublished work) showed that even within the central nervous system there is considerable variation from one area to another