THE OCCURRENCE OF PUTRESCINE AND A NEW GUANIDINO AMINO ACID IN SEEDS OF LATHYRUS TINGITANUS

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The natural occurrence of L-homoarginine in seeds of Lathyrus sativus (Rao, Ramachandran and Adiga, 1963), of L. cicera, L. cymenum and others (Bell, 1962a) has recently been noted. Further, seeds of L. tingitanus contain the unusual amino acid lathyrine — β-(2-amino-pyrimidine-4-yl) alanine (Bell, 1962b). The possible role of homoarginine as a precursor of lathyrine has been suggested (Rao et al., 1963; Bell, 1962c), and it has also been speculated that γ-hydroxyhomoarginine or its lactone — which may spontaneously undergo ring closure to yield tetrahydrolathyrine — could be expected to occur in L. tingitanus (Rao et al., 1963). We present herein some evidence for the presence of a new guanidino amino acid in the seeds. Some of the properties noted for this amino acid are akin to those anticipated for the lactone of γ-hydroxyhomoarginine. The seeds also contain a substantial amount of putrescine.

EXPERIMENTAL AND RESULTS

Materials. L. tingitanus seeds were kindly made available by E. A. Lewis and R. L. Brown of the Soil Conservation Service, U. S. Department of Agriculture.

Extraction of Seeds. Seeds (3 kg) were blended with 95% ethanol in a steel Waring blender, and the slurry transferred to a Buchner funnel, and the alcohol sucked off. The cake was washed repeatedly with 95% ethanol until the filtrate showed little color. The filter cake was dried in air, and then blended again with sufficient water to make a thin slurry. The latter was

filtered through cheesecloth, and the extract was clarified by centrifugation. The clear supernatant was adjusted to pH 3.6 with 4 N HCl and the protein coagulum removed by filtration. The clear filtrate was heated to 70°, kept for 10 minutes at that temperature, and then cooled. The slight flocculum was filtered and discarded. The volume of the clear yellow extract at this stage was 2700 ml.

The Isolation and Some Properties of a New Amino Acid. The aqueous extract equivalent to 100 g of seeds was concentrated to a volume of 15 ml by evaporation in vacuo. An aliquot of the extract was applied as a spot to a Whatman 3 MM sheet, 9 x 18 inch, and electrophoresis was conducted for 1 hr at 750 volts, using a pyridine-acetate buffer of pH 6.5 (pyridine, 100; acetic acid, 4; water, 100). The sheet was dried in air and sprayed with ninhydrin. The presence of various ninhydrin-reacting compounds is shown in Fig. 1. Spot C was unique in staining yellow, unlike the others. The color of this spot changed gradually to a greyish purple on long storage of the paper, or on prolonged heating. The rest of the concentrated extract was now streaked across the center of several sheets of Whatman 3 MM paper, 9" from either end, and paper electrophoresis conducted at 1000 V for 1 hr and 10 min. The sheets were dried, and tracer strips treated with ninhydrin, revealing good separation of band C from B and D. All the sheets were cut, and the bands corresponding to C removed and eluted with water. The eluate was concentrated to dryness in vacuo, yielding 9 mg of glassy material.

On reelectrophoresis at pH 6.5 and 3.5, the above material moved as a single homogeneous spot. The material reacted in the Sakaguchi test like a guanidino compound. The infrared spectrum of the material showed a moderate intensity band at 1765 cm^{-1}, indicating the presence of a lactone = CO, besides others. Two milligrams of the material was hydrolyzed with baryta under conditions used earlier on homoarginine (Rao et al., 1963), and the Ba^{++} removed as the sulphate. A portion of the new amino acid present in solution was treated with alkaline periodate, but no ammonia or formaldehyde was detected. This would indicate that the carbons adjacent to those carrying an amino

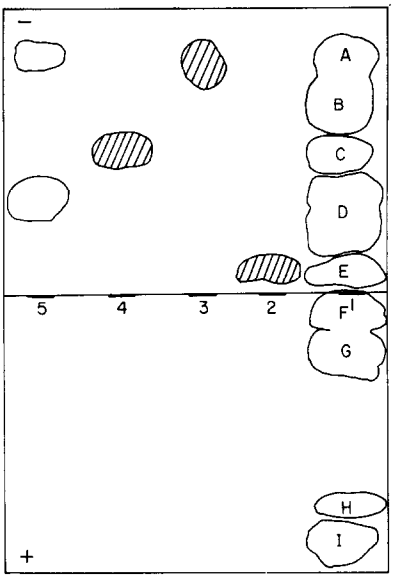

Fig. 1

Sketch of paper electropherogram of aqueous extract of L. tingitanus seeds and compounds isolated. 750 volts, 45 min.; pH 6.5. 1- Aqueous extract of seeds, spot C stains yellow with ninhydrin and spot E orange-red; 2- isolated lathyrine; 3- putrescine isolated from seeds; 4- γ-hydroxy-homoarginine lactone isolated from seeds; 5- authentic homoarginine and putrescine.

group were not substituted by a hydroxyl group. Also, on paper chromatograms the amino acid found in alkaline hydrolyzates had a R_f different from that of δ-hydroxylysine isolated from gelatin. The rest of the baryta hydrolyzate was heated in a sealed tube with 10 mg. red phosphorous and 0.2 ml. of hydriodic acid. The reaction mixture after purification, by adsorption on Dowex 50 x 4 in the H^+ form and elution with 1.7 N NH_4OH, revealed on paper chromatography in the three solvent systems used earlier (Rao et al., 1963) the presence of a single amino acid having the same R_f as lysine. The same behaviour would be expected of the lactone of γ-hydroxyhomoarginine whose natural occurrence has been anticipated. Methods for the large-scale isolation, and conclusive characterization, of this compound as well as methods for its synthesis are being worked out.

The Identification and Isolation of Putrescine. The aqueous extract from 3 kg. of seeds was passed in four separate batches, after adjustment to pH 2-2.2, through a column of Dowex 50 x 8 (50-100 mesh, Na form, 130 ml). The column after passing the extract through was washed with 150 ml water, and then elution started with a sodium acetate-acetic acid buffer (0.2 M in Na) of pH 4.65. Fifteen ml fractions were collected up to tube 120, and then the column washed successively with 120 ml water, 150 ml 1.5 N NH_4OH, and 150 ml water. A method for the isolation of lathyrine from fractions 30-110 in yields of 1 g/100 g of seeds will be reported elsewhere. The columns at this stage were washed successively with 150 ml 4 N HCl and 100 ml water. The combined acid effluent, containing the lactone of γ-hydroxy-homoarginine and several other compounds, was diluted 20-fold with water and passed through a 150 ml column of Dowex 50 x 8 in the H^+ form, and elution started with a gradient of HCl — 4 N HCl running into a mixing chamber containing 125 ml 1 N HCl. Fractions of 2 ml were collected, and by analysis of aliquots by the ninhydrin reaction, three peaks were located. The peaks were found between tube numbers 20-110, 130-200, and 210-290. The contents of tubes 130-200 was pooled and taken to dryness in vacuo. The slightly hygroscopic residue was recrystallized from aqueous ethanol. Yield 432 mg. No unsaturation could be detected in the compound. Analysis: Found; C 29.94, H 8.90, N 17.50, Cl 43.76 corresponding to $C_4H_8 \cdot 2NH_2 \cdot 2HCl$; calculated; C 29.84, H 8.77, N 17.39, Cl 44.04. Comparison of the above material with authentic putrescine (1,4-diamino-butane) dihydrochloride revealed the complete identity of the two on paper electrophoresis, paper chromatography and in infrared absorption. Both the isolated and authentic materials on reaction with ninhydrin in solution showed a peak of absorption at 501 mμ, unlike reaction products with amino acids which have the main peak at 569-70 mμ, and a hump at 410-412 mμ. It is estimated that the seeds contain about 0.03-0.04% of putrescine. The peak between tubes 210-290 was found to contain at least three guanidino amino acids whose identity remains to be established.

Discussion. The role of putrescine in the nitrogen metabolism of the plant is not clear. It is conceivable that this material is formed in the plant by degradation of agmatine, α,δ-diamino-adipic acid, or ornithine. The recognition of a new guanidino amino acid, with a behaviour similar to that expected for the lactone of γ-hydroxyhomoarginine, indicates that the L. tingitanus plants can perhaps hydroxylate homoarginine, and the final characterization of this new guanidino amino acid may provide a clue to the details of biosynthesis of lathyrine. The results of studies on the biosynthesis of lathyrine using C^{14}-labeled lysine and homoarginine will be reported elsewhere.

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NOTE: Since the communication of the above results, a report in the literature has firmly identified the new amino acid in the seed extracts, staining yellow with ninhydrin, to be γ-hydroxyhomoarginine lactone (Bell, 1963).

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