1 g of Raney nickel. On cooling the mixture was filtered, and the catalyst was washed twice with 25 ml of water. The combined filtrate and wash was lyophilized to give 20 mg of a white powder. Paper chromatography indicated the presence of only one amino acid, indistinguishable from α-aminobutyric acid.

Action of L- *and* D-*Amino Acid Oxidases.*—L-Amino acid oxidase was a lyophilized snake venom preparation (*Crotalus adamanteus*) from the Ross Allen Reptile Institute, Silver Springs, Fla. D-Amino acid oxidase was a hog kidney preparation from Worthington Biochemical Corporation, Freehold, N. J. Quantitative amino acid analyses were carried out by paper chromatography.

Results on the oxidation of the isolated amino acid and of the α-aminobutyric acid from the nickel desulfurization by these enzymes are summarized in Table I. It is clear that the $C_8H_{16}O_4N_2S$ acid is completely oxidized by the L-oxidase and inert to the D-oxidase. The α-aminobutyric acid is about 70% L-isomer and 30% D-isomer.

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The Isolation and Characterization of L-Homoarginine from Seeds of *Lathyrus sativus**

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A method of isolation of the new plant amino acid, L-homoarginine, from seeds of *Lathyrus sativus*, in a yield of 0.96 g per kg, is presented. The amino acid has been characterized on the basis of the properties of the free base, the monoflavianate, and the monopicrate. The properties of L-homoarginine monopicrate and the infrared spectrum of the monoflavianate are reported. It is suggested that homoarginine may act as a precursor to lathyrine in *L. tingitanus*.

The study of the chemical constituents of seeds of *Lathyrus sativus* is of considerable interest for two reasons. The seed meal is widely used by people in certain parts of India as a principal dietary constituent. Such people, in course of time, develop a nervous disorder termed "lathyrism" and are afflicted by varying degrees of physical disability, particularly of the lower limbs (Ganapathy and Dwivedi, 1961). During an investigation of the toxic constituents of the above seed, the presence of L-homoarginine in high concentration was discovered, and we report below the method of isolation of this constituent and its characterization. On completion of this work a report appeared in the literature (Bell, 1962a,b) on the recognition of homoarginine in three species of *Lathyrus* (*sativus*, *cicera*, and *cymenum*) and on the isolation of the amino acid from the species *L. cicera*. The method of isolation and identification used by Bell (1962b) differs substantially from techniques used by us. Our independent work confirms the presence of the new naturally occurring amino acid, L-homoarginine, in the plant kingdom.

Experimental and Results

L. sativus seed meal (3 kg) was extracted for 90 minutes, under refluxing conditions, with 4.5 liters of 75% EtOH. The extraction was repeated thrice, with fresh portions of solvent. The pooled extracts were filtered and concentrated to an eighth of the volume *in vacuo* (40–45°). Paper chromatographic examination [solvent–*n*-butanol (4), pyridine (1), H_2O (1), HOAc (2)] of the concentrate revealed the presence of two ninhydrin-reacting constituents with R_F values 0.09 and 0.24, which according to tests described later turned out to be different from other known constituents of plants. The concentrate was extracted with 1.5 liters of $CHCl_3$, and the $CHCl_3$ layer containing most of the color and lipids was rejected. The aqueous layer (1.5 liters, *p*H 5–6) was applied to a 180-ml column of Dowex 50-X8 (200–400 mesh) in the H form. After the passage of the sample the column was washed with 2 liters of water, securing the complete elution of the constituent with R_F 0.09 whose nature has been dealt with in a separate communication (Adiga *et al.*, 1962). Then 2.5 liters of 1.0 N HCl was passed through the column and the eluate rejected. After this stage, 1.5 N HCl was used as eluant and fractions of 10-ml volume were collected. All the arginine in the extract emerged in fractions 11–28, and the compound with R_F 0.24 emerged in fractions 30–216. The contents of tubes 30–216 were pooled and diluted four-fold with water and applied to a 99-ml column of Dowex 50-X8 in the H form. The column was washed with water till the effluents were Cl^- free, and the adsorbed amino acid was eluted with 1 N NH_4OH, completion of elution being checked by paper chromatography. The eluate was lyophilized. The yield was 2.8 g, and the material, which was slightly hygroscopic, was stored dry in a desiccator.

The substance in solution in water gave an alkaline reaction and in the test for elements was found to contain nitrogen but neither sulfur nor phosphorus. The ninhydrin reaction for amino groups (Rosen, 1957) and the Sakaguchi test for guanidino groups (MacPherson, 1946) were answered by the compound, while

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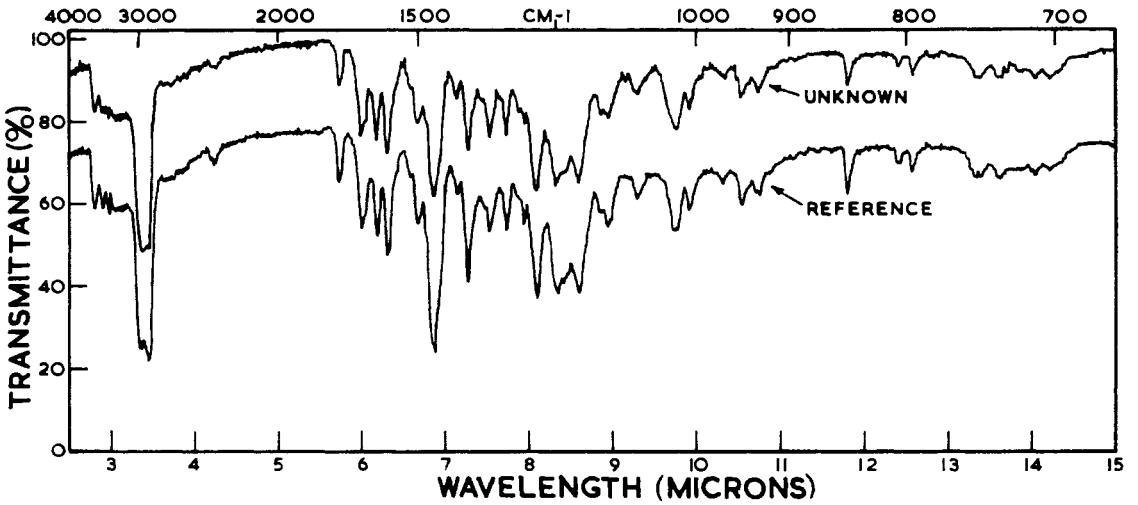

FIG. 1.—The infrared spectra of authentic and isolated homoarginine flavianate.

other tests showed the absence of phenolic hydroxyl and imidazole groups. In the platinic chloride reaction, on paper, the compound showed reducing property, but essentially no carbohydrate was detectable by the anthrone reaction. While at acid reaction (HOAc present) no decolorization of Br_2 occurred, indicating absence of unsaturation, decolorization did ensue at neutral *p*H as with guanidines. On acid hydrolysis (24 hours or longer) no breakdown was detectable, indicating the stability of the compound. On paper chromatography, with 80% EtOH as solvent, the hydrochloride of the untreated compound moved with an R_F 0.16, while the sample esterified with MeOH-HCl showed an R_F of 0.7 (reference homoarginine methyl ester HCl had the same R_F, 0.7), indicating the presence of an esterifiable group. A direct comparison of R_F values of the isolated unknown and homoarginine revealed identity of the one with the other (Table I). A mixed chromatogram of the two also yielded a single spot with the same R_F as homoarginine.

TABLE I

R_F VALUES OF ARGININE, HOMOARGININE, AND THE UNKNOWN COMPOUND

Solvent System	Argi-nine	Un-known	Homo-arginine
Isoamyl alcohol (4)–pyridine (8)–HOAc (1)–H_2O (4)	0.25	0.30	0.30
n-BuOH (30)–pyridine (20)–HOAc (6)–H_2O (24)	0.27	0.31	0.31
Phenol saturated with H_2O	0.64	0.72	0.72

Formation of the Monoflavianate.—About 0.5 g of the base in 2 ml of water was treated with a solution of 1.5 g flavianic acid in 12 ml of water, and the flask was warmed in the water bath for 30 minutes and then left at room temperature overnight. The deposit of solid was filtered and washed with 0.5% flavianic acid solution and then with EtOH. Yield, 1.43 g. The material was recrystallized by the procedure used by Vickery (1940) for arginine monoflavianate. The sample had a m.p. (decomp., with darkening and shrinking) 245–50° when the capillary tube was heated from 170°. Authentic L-homoarginine monoflavianate showed the same decomposition point under the same conditions. Analysis of the isolated monoflavianate: calcd. for $C_7H_{16}O_2N_4 \cdot C_{10}H_6O_8N_2S$: C, 40.7; H, 4.43; N, 16.76; S, 6.39; Found: C, 40.69; H, 4.56; N, 16.68; S, 6.99. The infrared spectra of the above monoflavianate and of authentic material were identical (Fig. 1), with prominent bands associated with the

C=NH, carboxyl, guanidino, and sulfonic group frequencies.

Formation of the Monopicrate.—Three hundred milligrams of the base in 2 ml of water was treated with an excess of picric acid in suspension in 25 ml of water. The flask was heated on the water bath till solution occurred, and thereafter cooled. Excess picric acid was removed by extraction with Et_2O, and the aqueous phase was concentrated to a small volume when deposition of crystals started. The material was filtered, washed with EtOH, and dried. Yield, 520 mg, m.p. (decomp.) 191–2°. Recrystallization from EtOH-H_2O yielded a sample with m.p. (decomp.) 202–3°. Analysis of the isolated picrate: calcd. for $C_7H_{16}O_2N_4 \cdot C_6H_3N_3O_7$, N, 23.50; found: N, 24.07.

Other Properties.—Some of the picrate was decomposed with HCl, and the liberated amino acid hydrochloride showed a ratio for presence of guanidino to amino groups of 0.8.

Also, the amino acid, regenerated from the picrate as homoarginine base, showed a $[M]_D = +42.4°$, $c = 0.452$ (1.02 N HCl, 24°). Reported $[M]_D$ for homoarginine in 1 N HCl at 23° is +40° (Greenstein and Winitz, 1961).

The dinitrophenyl derivative of the isolated compound in solution in 1% $NaHCO_3$ showed two peaks typical of DNP-α-amino acids, one at 267–9 mμ and the other at 362–6 mμ. The ratio of extinction at 390 mμ to that at 360 mμ was 0.64, in the same range as those for other DNP-α-amino acids.

Hydrolysis of the free base in 40% $Ba(OH)_2 \cdot 4H_2O$ at 107° for 72 hours led to complete loss of the guanidino function and the formation of another amino acid, identified as lysine by paper chromatography.

Biological Activity.—In preliminary test, L-homoarginine is found to cause 50% growth inhibition of *S. aureus* and *C. albicans* at levels of 8 μg/ml and complete inhibition at levels of 16 μg/ml. That this amino acid is nontoxic to *N. crassa* and inhibitory to *E. coli* has been reported by an earlier worker (Walker, 1955).

DISCUSSION

All the properties of the isolated free base, the analyses on the monoflavianate and the picrate, the identity of the infrared spectrum of the monoflavianate

with that of authentic L-homoarginine monoflavianate, the decomposition of the base to lysine on alkaline hydrolysis, and the observed $[M]_D$ value of +42.4° would be consistent with the formulation of the compound as L-homoarginine.

In this connection it is interesting to point out that, while homoarginine is present in several species of *Lathyrus* (Bell, 1962a), *L. tingitanus* is distinguished in that it contains lathyrine (Bell and Foster, 1962). It is tempting to speculate that the biosynthesis of lathyrine in the above species is from homoarginine and that it proceeds via γ-hydroxy homoarginine. Should this be so, the presence of γ-hydroxy homoarginine and its lactone in *L. tingitanus* may be anticipated. The natural occurrence of the lower homolog, γ-hydroxyarginine, has recently been recognized (Makisuma, 1961). Since the present report was communicated, Bell (1962c) has published details on the isolation of L-homoarginine from *L. cicera*, and he has also suggested that L-homoarginine may be the natural precursor to lathyrine, which is formed by cyclization and dehydration, and that the difference between plants which contain lathyrine and those containing only homoarginine is the presence or absence of an enzyme system capable of bringing about this transformation.

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Preparation, Metabolism, and Toxicity of Certain Acyl Derivatives of β-Aminopropionitrile*

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Several N-acyl derivatives of β-aminopropionitrile were synthesized and tested in rats for their ability to produce osteolathyrism. Only those derivatives which were cleaved *in vivo*, as evidenced by the appearance of cyanoacetic acid and free β-aminopropionitrile in the urine, were effective as lathyrogens. The γ-glutamyl (whether L-, D-, or DL-), glycyl, L-leucyl, and DL-phenylalanyl derivatives were active, whereas the acetyl, succinyl, glutaryl, β-alanyl, DL-pantoyl, and 4-hydroxybutyryl derivatives were not. The results point to an absolute requirement for a free amino group for osteolathyrism production and are consistent with the hypothesis that β-aminopropionitrile acts by direct binding through its amino group to some structural feature of soluble collagen (tropocollagen) which must be free to allow the formation of highly polymerized, mature collagen fibers.

The connective tissue damage and metabolic disturbances produced by β-aminopropionitrile have been widely studied since 1955, when it was identified as the active toxic principle of *Lathyrus odoratus* (Strong, 1956; Schilling and Strong, 1955). In *L. odoratus* seeds the compound occurs combined with glutamic acid as the N-(γ-L-glutamyl) derivative, which elicits the same symptoms as β-aminopropionitrile when fed to rats. However, it was found that the N-acetyl derivative of β-aminopropionitrile is nontoxic (Bachhuber *et al.*, 1955). In the present study, a number of other N-acyl derivatives of β-aminopropionitrile have been synthesized and administered to rats, both by feeding and by intraperitoneal injection, in order to determine whether they would produce the characteristic symptoms of β-aminopropionitrile toxicity and whether they would give rise to urinary excretion of free β-aminopropionitrile and cyanoacetic acid (Lipton *et al.*, 1958) as does the N-(γ-L-glutamyl) compound.

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EXPERIMENTAL

Preparation of N-Acyl β-Aminopropionitrile Derivatives ($RCONHCH_2CH_2CN$).—Data on the compounds synthesized are summarized in Table I. Synthetic methods employed were derived from previously described procedures, with modifications as given in footnotes to the table. The L and DL forms of N-γ-glutamyl-β-aminopropionitrile were prepared as previously described (Schilling and Strong, 1955).

Method A (Anhydride).—The reaction of an acid anhydride with an equimolar amount of free β-aminopropionitrile (Buc, 1947) was run in dioxane solution at 5° as previously described (Schilling and Strong, 1955) unless otherwise noted. The products of these very exothermic reactions separated either by direct crystallization or after the addition of ether.

Method B (Lactone).—The reaction of equimolar amounts of a lactone and β-aminopropionitrile was carried out by heating the reactants together with or without a solvent.

Method C (Acyl Chloride).—The reaction of an excess of free β-aminopropionitrile with phthalimidoacyl chlorides corresponding to the amino acids glycine, β-