Incorporation of L-Canavanine into Proteins and the Expression of Its Antimetabolic Effects

Gerald A. Rosenthal*,†,‡ and Douglas L. Dahlman‡,§

T. H. Morgan School of Biological Sciences, Graduate Center for Toxicology, and Department of Entomology, University of Kentucky, Lexington, Kentucky 40506

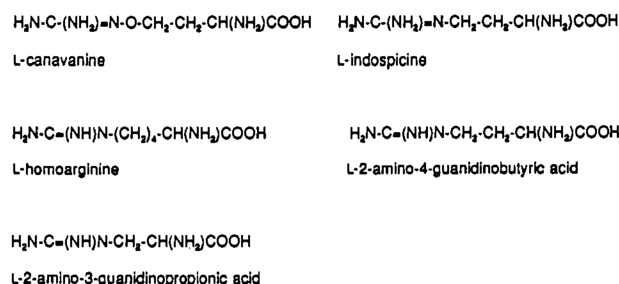
L-Canavanine [2-amino-4-(guanidinooxy)butyric acid], a potent arginine antimetabolite, is incorporated readily into the newly synthesized proteins of larvae of the tobacco hornworm, *Manduca sexta*. Canavanine causes massive developmental aberrations in *M. sexta*. The relationship between the production of aberrant, canavanine-containing proteins and their adverse developmental effects was assessed by studies of such arginine analogues as L-canavanine, L-indospicine [L-2-amino-6-(amidino)hexanoic acid], L-homoarginine [L-2-amino-6-guanidinohexanoic acid], L-2-amino-4-guanidinobutyric acid, and L-2-amino-3-guanidinopropionic acid. These studies required development of novel methods for the radiochemical synthesis of all of these analogues except canavanine. Of the tested arginine analogues only canavanine was incoporated into larval proteins, and only canavanine-treated larvae exhibited significant developmental aberrations. Our study strengthens the contention that anomalous, canavanyl protein formation is responsible for the growth-inhibiting and adverse developmental effects of canavanine.

INTRODUCTION

The higher plant nonprotein amino acid L-canavanine [L-2-amino-4-(guanidinooxy)butyric acid] can be a potent toxicant; for example, its deleterious and insecticidal properties are manifested readily in canavanine-sensitive insects (Rosenthal, 1977a; Rosenthal and Bell, 1979). Because of its structural similarity to arginine, canavanine is a substrate for arginyl-tRNA synthetase which activates and aminoacylates canavanine, attaching it to arginyl-transfer RNA (Allende and Allende, 1964; Mitra and Mehler, 1967). Replacement of arginine by canavanine places a much less basic amino acid into the protein (Boyar and Marsh, 1982; Greenstein and Winitz, 1961). This decrease in basicity has the potential to affect amino acid residue interactions, disrupt tertiary and/or quaternary structure, and influence adversely protein conformation.

Canavanine consumption by larvae of the tobacco hornworm, *Manduca sexta*, an insect sensitive to the antimetabolic effects of canavanine, reduces growth severely and produces pupae and adults that exhibit massive developmental aberrations. Larvae often perish in a futile attempt at larval-pupal metamorphosis (Dahlman and Rosenthal, 1975). Incorporation of canavanine into the vitellogenin of the migratory locust, *Locusta migratoria migratorioides*, produces a canavanine-containing vitellogenin whose conformation is altered significantly relative to the native macromolecule (Rosenthal et al., 1989a). Other studies have established that canavanine incorporation into the diptericins of larvae of the fly *Phormia terranovae* curtails sharply the protective efficacy of these antibacterial proteins (Rosenthal et al., 1989b). Thus, production of canavanine-containing proteins that are conformationally altered and functionally impaired contributes significantly to the expression of canavanine's antimetabolic properties.

To evaluate the relationship between the production of aberrant, canavanyl proteins and canavanine's deleterious effect on insect growth and development, methods were devised for the synthesis of a group of arginine analogues. The studied arginine analogues include L-canavanine [L-2-amino-4-(guanidinooxy)butyric acid], L-indospicine [L-2-amino-6-amidinohexanoic acid], L-homoarginine [L-2-amino-6-guanidinohexanoic acid], L-2-amino-4-guanidinobutyric acid, and L-2-amino-3-guanidinopropionic acid. These compounds were tested for their effect on the



growth and development of larval *M. sexta*. Methods for the radiochemical synthesis of these arginine analogues were also developed. The availability of these labeled substances permitted an evaluation of the relationship between a compound's deleterious growth effects and its assimilation into newly synthesized proteins.

L-Indospicine is produced by leguminous plants of the *Indigofera* (Hegarty and Pound, 1970). Provided at 2 g kg^{-1}, this nonprotein amino acid is hepatotoxic to sheep, rabbits, cows (Nordfeldt et al., 1972), and the Sprague-Dawley rat (Christie et al., 1969). Indospicine also causes somatic dwarfism in the fetal rat (Pearn, 1967). The effect of this arginine analogue on invertebrates has not been determined. L-Homoarginine occurs in many members of *Lathyrus* (Bell, 1962). This natural product, administered at 10 g kg^{-1}, produces convulsions and death in the rat (O'Neal et al., 1968). Homoarginine affects adversely the growth of a number of microorganisms; for example, it is a potent growth inhibitor of *Chlorella vulgaris* and is toxic to *Escherichia coli* (Walker, 1955a). Its deleterious effects on insects have been established (Janzen et al., 1977). L-2-Amino-3-guanidinopropionic acid and L-2-amino-4-guanidinobutyric acid have not been evaluated in insects.

* Author to whom correspondence should be addressed.
† T. H. Morgan School of Biological Sciences.
‡ Graduate Center for Toxicology.
§ Department of Entomology.

0021-8561/91/1439-0987$02.50/0 © 1991 American Chemical Society

MATERIALS AND METHODS

Chemicals and Biochemicals. Unless otherwise indicated, chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI) and biochemicals were obtained from Sigma Chemical Co. (St. Louis, MO). Canavanine was prepared from acetone-defatted jack bean seeds (*Canavalia ensiformis*) and purified by repetitive recrystallization (Rosenthal, 1977b). L-[*Guanidinooxy*-^{14}C]canavanine was synthesized by reacting L-canaline with [^{14}C]-cyanamide–Zn^{2+} after the method of Ozinskas and Rosenthal (1986). L-2-Amino-4-guanidinobutyric acid and L-2-amino-3-guanidinopropionic acid were obtained from Calbiochem (San Diego, CA).

Synthesis of D,L-Indospicine. The synthesis of D,L-indospicine drew upon the experimental efforts of Wieczorkowska and Hegarty (1986) and Gaudry (1948).

2,6-Dihydroxyhexanenitrile. 3,4-Dihydropyran (100 g, 1.19 mol) was mixed with 400 mL of 0.02 N HCl and heated, under refluxing conditions, at no more than 75 °C until the two immiscible liquids dissolved. Heating was continued for an additional 20 min. The reaction mixture was adjusted to pH 7.0–7.2 with 2 N NaOH, cooled in an ice–water bath, and treated with 125 g (1.2 mol) of sodium bisulfite. Stirring was continued until the sodium bisulfite was dissolved completely. At that time, KCN (78 g, 1.2 mol) in 100 mL of deionized water was added slowly to the reaction mixture. The reaction was allowed to continue for 5 h at 22 °C. Afterward, the reaction mixture was extracted twice with anhydrous ether and the ethereal layer concentrated by rotary evaporation in vacuo. The yield of crude 2,6-dihydroxyhexanenitrile was 95 ± 2%.

5-(4-Hydroxybutyl)hydantoin. The nitrile was reacted with ammonium carbonate (230 g, 2.4 mol), dissolved in 400 mL of deionized water, at 55 °C for 4 h. At that time, the reaction mixture was evaporated in vacuo; water was added and the evaporation in vacuo repeated. Finally, the residue was dissolved in a minimum amount of hot, deionized water and placed at 3 °C overnight. The resulting crystals were washed with cold deionized water, ethanol, and finally anhydrous ether. The typical yield of the hydroxybutylhydantoin was 20%: mp 151 °C. Anal. Calcd for $C_7H_{12}O_3N_2$: C, 48.8; H, 7.0; N, 16.3. Found: C, 48.6; H, 7.1; N, 16.5.

5-(4-Bromobutyl)hydantoin. After the hydroxybutylhydantoin (10 g) was refluxed in 100 mL of concentrated HBr at 90 °C for 3 h, the reaction mixture was evaporated in vacuo at no more than 45 °C. The residue was dissolved in 40 mL of deionized water, treated with 1.5 g of decolorizing charcoal, and refluxed for 15 min. The hot reaction mixture was filtered over two sheets of Whatman No. 50 paper. The hot filtrate was placed at 3 °C overnight; the crystalline product was collected by filtration in vacuo. Typical yield of the bromobutylhydantoin was 85 ± 3%: mp 125 °C. Anal. Calcd for $C_7H_{11}O_2N_2Br$: C, 35.8; H, 4.7; N, 11.9. Found: C, 36.0; H, 4.7; N, 12.0.

5-(4-Cyanobutyl)hydantoin. The bromobutylhydantoin (10 g) was mixed with 35 mL of absolute ethanol and treated with 3.4 g of KCN in 13 mL of deionized water at 23 °C for 4 h. The reaction mixture was taken to pH 4–5 with 6 N NCl in a *well-ventilated* hood. After the solvent was removed under a gentle stream of air, the residue was treated with 40 mL of anhydrous methanol and filtered. The filtrate solvent was removed by rotary evaporation in vacuo. Finally, the residue was extracted with 100 mL of ethyl acetate overnight at 23 °C. After the ethyl acetate was concentrated by rotary evaporation in vacuo, the remaining solvent was washed twice with deionized water and once with brine. The ethyl acetate was dried over anhydrous Na_2SO_4 and filtered and the solvent removed by rotary evaporation in vacuo.

The residue was dissolved in a minimum amount of hot ethyl acetate and precipitated by cooling in an ice–water bath. The crude crystaline product was dried overnight at no more than 40 °C. The cyanobutylhydantoin was purified by dissolving in 50% aqueous ethanol and treating with decolorizing charcoal. The solvent was removed by rotary evaporation in vacuo and dissolved in a minimum amount of hot absolute ethanol. Cyanobutylhydantoin was precipitated from the mother liquor with cold, deionized water. The yield of 5-(4-cyanobutyl)hydantoin was 77%: mp 118 ± 1 °C. Anal. Calcd for $C_8H_{11}O_2N_3$: C, 53.0; H, 6.1; N, 23.2. Found: C, 52.8; H, 6.2; N, 23.0.

5-(4-Amidinobutyl)hydantoin. Cyanobutylhydantoin (1 g) was treated with 40 mL of dry chloroform and 2 mL of absolute ethanol. The reaction mixture was saturated with dry HCl and allowed to react overnight at 23 °C. After the solvent was removed by rotary evaporation in vacuo, methanol was added to the residue and the evaporation process was repeated twice. The residue, containing ethyl 5-(5-hydantoinyl)pentanimidate, was dried in vacuo for 30 min at 23 °C prior to dissolving in 40 mL of absolute methanol. The reaction mixture was saturated with NH_3 and allowed to react overnight at 23 °C. The evaporation process was repeated as above. The resulting 5-(4-amidinobutyl)hydantoin was dissolved in a minimum of hot deionized water, decolorized with charcoal, and filtered and the filtrate concentrated by rotary evaporation in vacuo. The residue was dissolved in a minimum of hot, deionized water and placed at 3 °C overnight. The yield of 5-(4-amidinobutyl)hydantoin (indospicine hydantoin) was 52%: mp 118 ± 1 °C. Anal. Calcd for $C_8H_{14}O_2N_4$: C, 48.5; H, 7.1; N, 28.3. Found: C, 48.7; H, 7.2; N, 28.0. Indospicine hydantoin was dissolved in ^2H_2O and analyzed for its ^1H NMR spectrum (Varian XL-300). The assignments for the α, β, γ, δ, and ϵ indospicine hydantoin protons were 4.31, 1.87, 1.79, 1.87, and 3.52 ppm, respectively.

D,L-2-Amino-6-amidinohexanoic Acid (D,L-Indospicine). Indospicine hydantoin was refluxed with 6 N HCl at 115–120 °C for 48 h. The evaporation process was repeated as above, and the reaction mixture, containing D,L-indospicine, was taken to pH 3.5 and applied to a 20 × 60 mm column of Dowex 50 (NH_4^+). The column was washed exhaustively with deionized water and developed with 0.2 N ammonia. The effluent was concentrated by rotary evaporation in vacuo. The yield of D,L-indospicine was 32 ± 3%. Anal. Calcd for $C_7H_{15}O_2N_3$: C, 48.5; H, 8.7; N, 24.3. Found: C, 48.7, H, 8.8; N, 24.3. L-Indospicine: The assignments for the α, β, γ, and ϵ indospicine protons were 3.61, 1.75, 1.30, 1.62, and 2.38 ppm, respectively.

Resolution of D,L-Indospicine. The D,L-indospicine racemate was resolved by chemical conversion to their corresponding N-acylated derivatives and then treatment with acylase I to create racemically pure L-indospicine (Greenstein and Winitz, 1961). L-Indospicine was isolated by ion-exchange chromatography with Dowex 50 (NH_4^+) as described above and purified by repetitive crystallization from water with ethanol.

Synthesis of D,L-[*Amidino*-^{14}C]-2-amino-6-amidinohexanoic Acid (D,L-[^{14}C]Indospicine). D,L-[^{14}C]Indospicine was synthesized by reacting 7.4 MBq of Na[^{14}C]CN (185 Bq/mmol) with 2 mmol of 5-(4-bromobutyl)hydantoin in a *well-ventilated* hood. The radiochemical synthesis and purification of D,L-[^{14}C]-indospicine was conducted as described for D,L-indospicine (see Figure 1).

Synthesis of the Other Arginine Analogues. L-[*Guanidino*-^{14}C]-2-amino-6-guanidinohexanoic acid, L-[*guanidino*-^{14}C]-2-amino-4-guanidinobutyric acid, or L-[*guanidino*-^{14}C]-2-amino-3-guanidinopropionic acid were synthesized by reacting the copper salt of L-lysine, L-2,4-diaminobutyric acid, or L-2,3-diaminopropionic acid, respectively, with [^{14}C]-*O*-methylisourea after the method of Ozinskas and Rosenthal (1986). The radiolabeled amino acid was treated with H_2S, CuS was removed by filtration, and the filtrate was taken to pH 3.5. The filtrate was placed on a 20 × 45 mm column of Dowex 50 (H^+), washed with deionized water, and developed with 1 N NH_3. After the column effluent was concentrated by rotary evaporation in vacuo, the radiolabeled amino acid was stored at –60 °C.

Insects. All experiments were conducted with newly ecdysed (<12 h old) fifth-instar larvae of *M. sexta* obtained from a continuous colony maintained at the University of Kentucky; rearing procedures were described elsewhere (Yamamoto, 1969). All insects were anesthetized with carbon dioxide prior to parental injection or hemolypmh collection. Treated animals received their amino acid in sterile, deionized water. Control animals received only water.

Growth Inhibition Studies. Nine larvae, constituting a single treatment group, received 10 or 15 μmol of amino acid delivered in 50 μl of deionized water gram of fresh body weight. These treated larvae received L-indospicine, L-homoarginine, L-2-amino-4-guanidinobutyric acid, or L-amino-3-guanidinopropionic acid. L-Canavanine was provided at 5.0 or 10.0 μmol/g of fresh body weight. The initial mean larval weight was 1.20 ± 0.05 g.

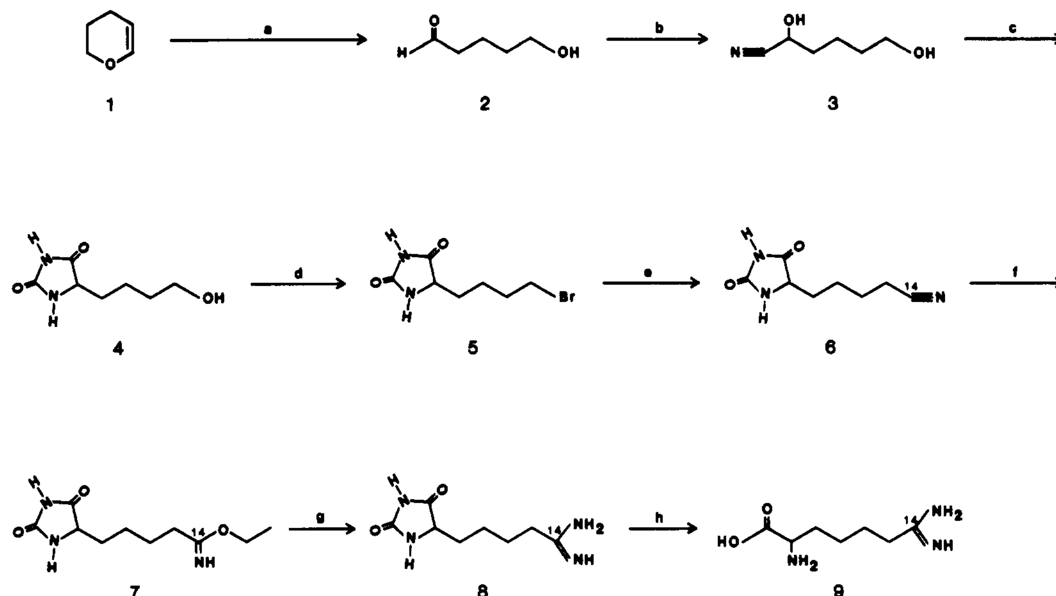


Figure 1. Synthesis of D,L-indospicine. Reaction conditions: (a) 0.02 N HCl 75 °C/35 m; (b) pH 7.0–7.2/2 N NaOH/$NaHSO_3$–$Na_2S_2O_5$/KCN/H_2O/5 h/22 °C; (c) $(NH_4)_2CO_3$/H_2O/55 °C/4 h; (d) HBr/90 °C/3 h/; (e) KCN/EtOH–H_2O/23 °C/4 h/; (f) HCl–EtOH/23 °C/24 h; (g) NH_3/23 °C/24 h; (h) HCl/115–120 °C/48 h.

Fresh larval weight was determined daily. The larvae were allowed to ecdyse to pupae and adults to assess adverse effects on metamorphosis and adult development.

^{14}C Incorporation Studies. Each of the nine larvae received 5 μCi of racemically pure carbon-14-labeled amino acid with 10 μmol of carrier amino acid/g of fresh weight. In contrast, the indospicine-treated larvae received 10 μCi of D,L-[^{14}C]indospicine. One day after drug administration, hemolymph was obtained by cutting a proleg and permitting the hemolymph to drain from the body cavity. Hemolymph (100 μL) was taken from each of three larvae, pooled into a single sample, and treated with an equal volume of 25% (w/v) trichloroacetic acid (TCA). Three such pooled samples were processed as described below. After the hemolymph was drained, the gut and gut contents were discarded; any residual hemolymph was removed by blotting with absorbent paper. The remaining tissues, designated "body wall" and consisting primarily of musculature and fat body, were processed as described.

The TCA-treated hemolymph proteins were collected by centrifugation with a Beckman Model B microfuge. After careful removal of the supernatant solution, the pellet was extracted three times with 10% (w/v) TCA, twice with anhydrous ether–ethanol (1:1 v/v), and finally with anhydrous ether. The pellet was air-dried and treated with 0.3 mL of TS-1 tissue solubilizer (Research Products International) at 50 °C overnight. The dissolved pellet was transferred to a liquid scintillation vial by using three 1-mL portions of scintillation medium and counted by liquid scintillation spectroscopy.

Radiolabeled proteins, obtained from the whole body samples, were also evaluated. Body wall samples from three insects were combined into a single sample and ground with a Sorvall Omnimixer at full power for 30 s. A sample of the resulting homogenate was processed as described for the radiolabeled hemolymph proteins. The data represent the mean ± SEM for three samples each consisting of three larvae.

L-[*Guanidinooxy*-^{14}C]canavanine Incorporation into Proteins. Incorporation of [^{14}C]canavanine into newly synthesized, radiolabeled, insectan proteins was determined by an enzyme-based, radiometric assay (Rosenthal and Thomas, 1985). The TCA-precipitated hemolymph or whole body proteins were hydrolyzed with 6 N HCl under N_2 overnight at 100 °C. The protein hydrolysate was subjected to ion-exchange chromatography to isolate L-[*guanidinooxy*-^{14}C]canavanine. The radiolabeled canavanine content of the hydrolysate was determined by a radiometric assay of the column eluent that employed arginase (EC 3.5.3.1) and urease (EC 3.5.1.5) to release the ^{14}C of L-[*guanidinooxy*-^{14}C]canavanine as $^{14}CO_2$ (Rosenthal and Thomas, 1985).

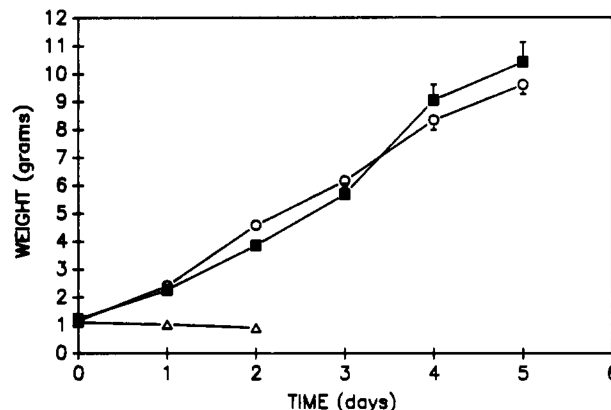


Figure 2. Effect of various arginine analogues on the growth of *M. sexta*. Larvae were injected with 10 μmol/g of fresh body weight canavanine (△) or 15 μmol of indospicine (■); control larvae received deionized water (○). The growth of larvae given 15 μmol/g of fresh body weight homoarginine, 2-amino-4-guanidinobutyric acid, or 2-amino-3-guanidinopropionic acid did not differ significantly from that of the control larvae (data not presented).

RESULTS AND DISCUSSION

Novel methods for the chemical and radiochemical synthesis of L-indospicine and other potentially useful L-arginine analogue have been developed. Administration of 10.0 μmol of canavanine/g of fresh larval weight by parenteral injection terminated the growth of *M. sexta* larvae. All of the treated larvae expired within 48 h (Figure 2). These dramatic effects attest to canavanine's marked insecticidal quality. In sharp contrast, providing a 2- or 3-fold greater dose of any of the other tested arginine analogues failed to affect growth adversely. Moreover, all of the treated larvae ecdysed successfully to pupae and ultimately adults without manifesting adverse developmental effects.

The newly synthesized proteins of the body wall musculature and hemolymph were analyzed for the incorporation of the administered radiolabeled arginine analogues. As revealed in Table I, only significant canavanine was present in the isolated proteins. The amount of [^{14}C]canavanine-labeled protein present after 24 h was nearly 40% of the [^{14}C]arginine-labeled protein level

Table I. Incorporation of [^{14}C]Arginine and Certain of Its Analogues into Protein by *M. sexta* Larvae

amino acid	amino acid incorporation, % administered dose	
	hemolymph	body wall
L-arginine	8.4 ± 0.3	0.87 ± 0.04
L-canavanine	3.2 ± 0.11	0.45 ± 0.02
L-homoarginine	0.4 ± 0.12	0.07 ± 0.01
L-2-amino-3-guanidinopropionic acid	ND[a]	ND
L-2-amino-4-guanidinobutyric acid	tr	ND
L-indospicine	0.1 ± 0.06	0.03 ± 0.02

[a] ND, not detected.

(Table I). Prior double-label, protein-turnover experiments with *M. sexta* larvae injected with [^{14}C]canavanine- and [^{3}H]arginine-containing hemolymph proteins established that canavanine-containing proteins were degraded preferentially relative to their native counterparts (Rosenthal and Dahlman, 1986). Thus, the production of canavanyl proteins relative to native proteins exceeded the level indicated by the data of Table I. There was no significant incorporation of homoarginine, indospicine, L-2-amino-3-guanidinopropionic acid, or L-2-amino-4-guanidinobutyric acid into newly synthesized larval proteins (Table I).

Of the tested arginine analogues only canavanine was incorporated into the proteins of actively growing *M. sexta* larvae. At the same time, only canavanine elicited discernible developmental aberrations. Our experimental findings strengthen our contention that aberrant, canavanyl protein formation is responsible for the growth-inhibiting and adverse developmental effects of canavanine in this insect.

ACKNOWLEDGMENT

We gratefully acknowledge the support of the National Science Foundation (DCB-8901749), Biomedical Research Grant (2-S07-RR07114-21), and the Graduate School of the University of Kentucky for the research described in this paper. This is Paper No. 90-7-141 of the Kentucky Agricultural Experiment Station, Lexington, KY.

LITERATURE CITED

Allende, C. C.; Allende, J. E. Purification and substrate specificity of arginyl-ribonucleic acid synthetase from rat liver. *J. Biol. Chem.* **1964**, *239*, 1102–1106.

Bell, E. A. The Isolation of L-Homoarginine from Seeds of *Lathyrus cicera*. *Biochem. J.* **1962**, *83*, 225–231.

Boyar, A.; Marsh, R. E. L-Canavanine, a Paradigm for the Structures of Substituted Guanidines. *J. Am. Chem. Soc.* **1982**, *104*, 1995–1998.

Christie, G. S.; Madsen, N. P.; Hegarty, M. P. Acute Biochemical Changes in Rat Liver Induced by the Naturally Occurring Amino Acid Indospicine. *Biochem. Pharmacol.* **1969**, *18*, 693–701.

Dahlman, D. L.; Rosenthal, G. A. Non-Protein Amino Acid-Insect Interactions. I. Growth Effects and Symptomology of L-Canavanine Consumption by the Tobacco Hornworm, *Manduca sexta* (L.). *Comp. Biochem. Physiol.* **1975**, *51A*, 33–36.

Gaudry, R. The Synthesis of D,L-α-Amino-ϵ-Hydroxycaproic acid and a New Synthesis of D,L-Lysine. *Can. J. Res. (B)* **1948**, *26*, 387–397.

Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids*; Wiley: New York, 1961; Vol. 1–3.

Hegarty, M. P.; Pound, A. W. Indospicine, a Hepatotoxic Amino Acid from *Indigofera spicata*: Isolation, Structure, and Biological Studies. *Aust. J. Biol. Sci.* **1970**, *23*, 831–842.

Janzen, D. H.; Juster, H. B.; Bell, E. A. Toxicity of Secondary Compounds to the Seed-Eating Beetle, *Callosobruchus maculatus*. *Phytochemistry* **1977**, *16*, 223–227.

Mitra, S. K.; Mehler, A. H. The Arginyl Transfer Ribonucleic Acid Synthetase of *Escherichia coli*. *J. Biol. Chem.* **1967**, *242*, 5490–5494.

Nordfeldt, S.; Henke, L. A.; Marita, H.; Matsumoto, H.; Takahashi, H.; Young, O. R.; Willers, E. H.; Cross, R. F. *Tech. Bull.—Hawaii Agric. Exp. Sta.* **1972**, *15*.

O'Neal, R. M.; Chen, C.-H.; Reynolds, C. S.; Meghal, S. K.; Koeppe, R. E. The Neurotoxicity of L-2,4-Diaminobutyric Acid. *Biochem. J.* **1968**, *106*, 699–704.

Ozinskas, A. J.; Rosenthal, G. A. L-Canavanine Synthesis by Zinc-Mediated Guanidination of L-Canaline with Cyanamide. *Bioorg. Chem.* **1986**, *14*, 157–162.

Pearn, J. H. Effects on the Liver in the Rat of Ingestion of *Indigofera spicata*, a Legume Containing an Inhibitor of Arginine Metabolism. *Br. J. Exp. Pathol.* **1967**, *48*, 620–627.

Rosenthal, G. A. The Biological Effects and Mode of Action of L-Canavanine, a Structural Analogue of L-Arginine. *Q. Rev. Biol.* **1977a**, *52*, 155–178.

Rosenthal, G. A. Preparation and Colorimetric Analysis of L-Canavanine. *Anal. Biochem.* **1977b**, *77*, 147–151.

Rosenthal, G. A.; Bell, E. A. Naturally Occurring, Toxic Nonprotein Amino Acids. In *Herbivores—Their Interaction with Plant Metabolites*; Rosenthal, G. A., Janzen, D. H., Eds.; Academic: New York, 1979.

Rosenthal, G. A.; Dahlman, D. L. L-Canavanine, Insect Protein Synthesis, and Higher Plant Chemical Defense. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 14–18.

Rosenthal, G. A.; Thomas, D. A. A Radiometric Assay for Determining the Incorporation of L-Canavanine and L-Arginine into Protein. *Anal. Biochem.* **1985**, *147*, 428–431.

Rosenthal, G. A.; Reichhart, J.-M.; Hoffmann, J. A. L-Canavanine Incorporation into Vitellogenin and Macromolecular Conformation. *J. Biol. Chem.* **1989a**, *264*, 13693–13696.

Rosenthal, G. A.; Lambert, J.; Hoffmann, D. Canavanine Incorporation into the Antibacterial Protein of the Fly, *Phormia terranovae* (Diptera) and its Effect on Biological Activity. *J. Biol. Chem.* **1989b**, *264*, 9768–9771.

Walker, J. B. Canavanine and Homoarginine as Antimetabolites of Arginine and Lysine in Yeast and Algae. *J. Biol. Chem.* **1955a**, *212*, 207–212.

Walker, J. B. Homoarginine Inhibition of *Escherichia coli* B. *J. Biol. Chem.* **1955b**, *212*, 617–622.

Wieczorkowsha, E.; Hegarty, M. P. Synthesis of D,L-[7-^{14}C]Indospicine and D,L-2-Amino-[7-^{14}C]Pimelic Acid. *J. Lab. Comp. Radiopharm.* **1986**, *24*, 1273–1280.

Yamamoto, R. T. Mass Rearing of the Tobacco Hornworm. II. Larval Rearing and Pupation. *J. Econ. Entom.* **1969**, *62*, 1427–1431.

Received for review August 14, 1990. Revised manuscript received November 15, 1990. Accepted December 2, 1990.

Registry No. 1, 110-87-2; **3**, 133447-70-8; **4**, 68469-36-3; **5**, 87052-63-9; **6**, 115375-10-5; **7**, 133447-72-0; **8**, 133447-71-9; L-**9**, 16377-00-7; D,L-**9**, 24185-98-6; D,L-[^{14}C]-**9**, 133447-73-1; $H_2NC(=NH)NHOCH_2CH_2CH(NH_2)CO_2H$, 543-38-4; $H_2NC(=NH)NH(CH_2)_4CH(NH_2)CO_2H$, 156-86-5; $H_2NC(=NH)NHCH_2CH_2CH(NH_2)CO_2H$, 14191-90-3; $H_2NC(=NH)NHCH_2CH(NH_2)CO_2H$, 14191-91-4; KCN, 151-50-8; HBr, 10035-10-6; Na[^{14}C]CN, 133447-74-2; L-Lys·Cu, 21512-99-2; L-$HO_2CCH(NH_2)CH_2CH_2NH_2$, 1758-80-1; L-$HO_2CCH(NH_2)CH_2NH_2$, 4033-39-0; L-[*guanidinooxy*-^{14}C]canavanine, 87578-85-6; L-canaline, 496-93-5; L-[*guanidine*-^{14}C]-2-amino-6-guanidinohexanoic acid, 133447-75-3; L-[*guanidino*-^{14}C]-2-amino-4-guanidinobutyric acid, 133447-76-4; L-[*guanidino*-^{14}C]-2-amino-3-guanidinopropionic acid, 133447-77-5; [^{14}C]*O*-methylisourea, 133447-78-6.