Davis, M. A. F.; Gidley, M. J.; Morris, E. R.; Powell, D. A.; Rees, D. A. Intermolecular Association in Pectin Solutions. *Int. J. Biol. Macromol.* **1980**, *2*, 330–332.
Deckers, H. A.; Olieman, C.; Rombouts, F. M.; Pilnik, W. Calibration and Application of High Performance Size Exclusion Columns for Molecular Weight Distributions of Pectins. *Carbohydr. Polym.* **1986**, *6*, 361–378.
Fishman, M. L.; Pepper, L. A. Reconciliation of the Differences in Number Average Molecular Weights ($\bar{M}_n$) By End Group Titration and Osmometry for How Methoxy Pectins. In *New Developments in Industrial Polysaccharides*; Dea, I. M., Crescenzi, V., Stivala, T., Eds.; Gordon and Breach: New York, 1985; pp 159–166.
Fishman, M. L.; Pfeffer, P. E.; Barford, R. A.; Doner, L. W. Studies of Pectin Solution Properties by High Performance Size Exclusion Chromatography. *J. Agric. Food Chem.* **1984**, *32*, 372–378.
Fishman, M. L.; Pepper, L. A.; Pfeffer, P. E. Dilute Solution Properties of Pectin. In *Water Soluble Polymers*; Glass, E. D., Ed.; Advances in Chemistry Series 213; American Chemical Society: Washington, DC, 1986a.
Fishman, M. L.; Pepper, L. A.; Damert, W. C.; Phillips, J. G.; Barford, R. A. A Critical Re-Examination of Molecular Weight and Dimensions for Citrus Pectins. In *Chemistry and Function of Pectins*; Fishman, M. L., Jen, J. J., Eds.; ACS Symposium Series 310; Washington, DC, 1986b; Chapter 3.
Fishman, M. L.; Damert, W. C.; Phillips, J. G.; Barford, R. A. Evaluation of Root Mean Square Radius of Gyration as a parameter for Universal Calibration of Polysaccharides. *Carbohydr. Res.* **1987**, *160*, 215–225.
Flory, P. J. Determination of Molecular Weights. In *Principles of Polymer Chemistry*; Cornell University Press: Ithaca, NY, 1953; p 312.
Lecacheaux, D.; Lesec, J. Measurement of the Dead Volume Between Concurrent Detectors in Gel Permeation Chromatography. *J. Liq. Chromatogr.* **1982**, *5*, 2227–2239.
Malihi, F. B.; Kuo, C.; Koehler, M. E.; Provder, T.; Kah, A. F. Development of a Continuous Gel Permeation Chromatography Viscosity Detector. In *Size Exclusion Chromatography*; Provder, T., Ed.; ACS Symposium Series 245: American Chemical Society: Washington, DC, 1984.
Michel, F.; Thibault, J. F.; Doublier, J. L. Viscometric and Potentiometric Study of High-Methoxyl Pectins in the Presence of Sucrose. *Carbohydr. Polym.* **1984**, *4*, 283–297.
Pals, D. T. F.; Hermans, J. Sodium Salts of Pectin and of Carboxyl Methyl Cellulose in Aqueous Sodium Chlorides I. Viscosities. *Recl. Trav. Chim. Pays-Bas* **1952**, *71*, 433–457.
Tanford, C. Transport Processes, Viscosity. In *Physical Chemistry of Macromolecules*; Wiley: New York, 1961.
Yau, W. W.; Kirkland, J. J.; Bly, D. D. Retention. In *Modern Size Exclusion Chromatography*; Wiley: New York, 1979.

Received for review May 6, 1988. Accepted October 24, 1988.

Catabolism of L-Canavanine and L-Canaline in the Jack Bean, *Canavalia ensiformis* (L.) DC. (Leguminosae)

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The metabolism of L-canavanine and its primary metabolite L-canaline was investigated in the jack bean, *Canavalia ensiformis* (L.) DC. (Leguminosae). L-[1,2,3,4-^{14}C]Canavanine and L-[U-^{14}C]canaline were synthesized from L-[U-^{14}C]homoserine. After 1.5 h, 35% of the administered radiolabeled canaline was converted to acetone-soluble products; this amount decreased with time to 5.3% at 12 h. This fraction was primarily responsible for respiratory loss of ^{14}C as $^{14}CO_2$, which reached 52% of the administered canaline after 12 h. The water-soluble, neutral fraction accounted for no more than 10% of the injected canaline. The water-soluble, charged materials contained 35–40% of the ^{14}C at each time period; five compounds were identified within this fraction. They are homoserine, phosphohomoserine, lysine, canavanine, and canaline glyoxylate oxime. Canavanine-administered plants provided a degradation pattern remarkably similar to that of canaline-administered plants, indicating the importance of arginase-mediated hydrolysis of canavanine to canaline in the process of canavanine catabolism.

L-Canavanine, the 2-amino-4-(guanidinooxy)butyric acid antimetabolite of L-arginine, has been isolated from 1200 legumes (Bell et al., 1978). It is the principal nonprotein amino acid of the jack bean, *Canavalia ensiformis* (L.) DC. (Bell, 1958). This potentially toxic secondary plant metabolite can play an important role in legume chemical defense against herbivores, particularly insects (Rosenthal, 1986, 1988). It also functions as a nitrogen-storing metabolite, able to support de novo amino acid synthesis (Rosenthal and Rhodes, 1984; Rosenthal et al., 1988).

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Previous studies of canavanine catabolism in the jack bean reveal that this arginine analogue is cleaved hydrolytically by arginase (EC 3.5.1.5) to L-canaline and urea. The latter compound is degraded by urease (EC 3.5.3.1) to CO_2 and NH_3. Working in consort, these enzymes are responsible for mobilizing two of the three nitrogens of canavanine's guanidinooxy group (Rosenthal, 1971). But what is the metabolic fate of L-canaline? In 1970, Töpfer et al. reported that [^{3}H]canaline gave rise to tritiated homoserine in germinating seeds of *Caragana spinosa*, but they did not provide data on how significant this reaction was in overall canaline degradation. Thus, an objective of this study is to employ L-[U-^{14}C]canaline and L-[1,2,3,4-^{14}C]canavanine to determine to what extent homoserine functions in the catabolism of these nonprotein amino acids.

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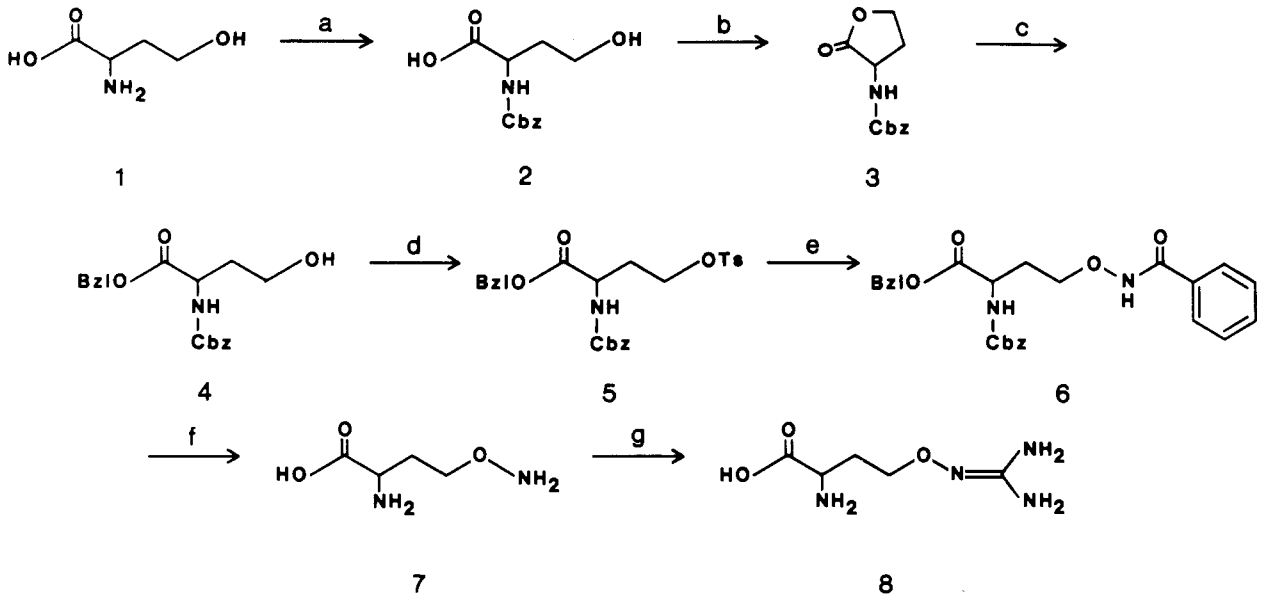

Figure 1. Reaction conditions for synthesis of L-[U-^{14}C]canaline and L-[1,2,3,4-^{14}C]canavanine: (a) ClCOO*Ph*/$NaHCO_3$/H_2O; (b) H_2O/citric acid/Δ; (c) NaOH/ETOH/H_2O, evaporate, benzyl bromide/DMF; (d) tosyl chloride/ET_3N/THF; (e) benzohydroxamic acid/NaH/DMF; (f) HCl/ETOH/Δ, HCl/H_2O/Δ; (g) NH_4OH/pH 6.2, CuO, H_2NCN/$ZnCl_2$, H_2S.

Homoserine is important in the nitrogen metabolism of canavanine-producing legumes, for it supports transsulfuration and direct sulfhydration reactions leading to cystathionine, cysteine, and methionine formation (Giovanelli et al., 1979). Previous study of L-[U-^{14}C]homoserine metabolism in jack bean (Rosenthal, 1982) disclosed that homoserine served as a precursor for lysine production. Thus, if canavanine is converted to homoserine, it would also support primary metabolic reactions via lysine biosynthesis. We also sought to determine if and how reactions other than those involving canaline contribute to the metabolic fate of the aliphatic chain of canavanine.

EXPERIMENTAL SECTION

Preparation of L-[U-^{14}C]Canaline and L-[1,2,3,4-^{14}C]Canavanine. Synthesis of L-[U-^{14}C]canaline and L-[1,2,3,4-^{14}C]-canavanine (Figure 1) were achieved by modification of the method of Ozinskas and Rosenthal (1986a). Analytical data on all of the reaction products were provided elsewhere (Ozinskas and Rosenthal, 1986a).

L-2-[(Carbobenzyloxy)amino]-4-hydroxybutyric Acid (**2**). Two millimoles of L-homoserine (1) and 3.7 MBq of L-[U-^{14}C]homoserine (Amersham; 1.48 GBq/mmol) were dissolved in 7 mL of 1 N $NaHCO_3$, and the resultant mixture was cooled to 0 °C and reacted with 1.2 equiv of benzyl chloroformate as four aliquots over a 1-h period. After 2 h on ice, the reaction mixture was stirred at 23 °C for 24 h and extracted with ether (2 × 10 mL). After the aqueous phase was acidified to pH 2–3 with 3 N HCl, it was extracted with ethyl acetate (3 × 10 mL). The extract was dried over Na_2SO_4, filtered, and evaporated in vacuo. Final yield of **2** was 90 ± 3%.

L-2-[(Carbobenzyloxy)amino]-4-butyrolactone (**3**). Compound **2** was refluxed with 10 mL of 150 mM citric acid for 6 h at 115 °C; the reaction mixture was extracted with ethyl acetate (3 × 10 mL). After the ethyl acetate was concentrated, it was treated with Na_2SO_4, filtered, and evaporated in vacuo. Final yield of **3** was 97 ± 2%.

Benzyl L-2-[(Carbobenzyloxy)amino]-4-hydroxybutyrate (**4**). Compound **3** was dissolved in 5 mL of absolute ethanol and treated with 1.05 equiv of NaOH in 0.5 mL of deionized water. Formation of the sodium salt of **3** was allowed to proceed at 23 °C for 60 min. The reaction mixture was dried, twice resuspended in absolute ethanol, and finally dried exhaustively in vacuo. The residue was dissolved in 3 mL of dry DMF and reacted in the dark with 2 equiv of benzyl bromide for 24 h at 50 °C. The reaction mixture was transferred to ethyl acetate, washed with deionized water, twice with saturated $NaHCO_3$, and with saturated NaCl, dried over Na_2SO_4, filtered, and concentrated. The ethyl acetate fraction was purified by silica gel flash chromatography with ethyl acetate/hexane (1:1, v/v). Final yield of **4** was 96 ± 2%.

Benzyl L-2-[(Carbobenzyloxy)amino]-4-[(p-tolylsulfonyl)-oxy]butyrate (**5**). Compound **4**, dissolved in 1.15 mL of THF (filtered over alumina) and 1.05 mL of triethylamine, was cooled to −10 °C and reacted with 2 equiv of *p*-toluenesulfonyl chloride in 1.25 mL of alumina-filtered THF. The reaction mixture was stirred mechanically at 4 °C for 48 h and terminated by removing the solvent under a stream of N_2. The residue was taken up in 10 mL of ethyl acetate, extracted twice with 0.5 N HCl, saturated $NaHCO_3$, and saturated NaCl, dried over Na_2SO_4, and filtered. After the ethyl acetate was concentrated, it was purified by silica gel flash chromatography with ethyl acetate/hexane (1:3, v/v). Final yield of **5** was 90 ± 1%.

Benzyl L-2-[(Carbobenzyloxy)amino]-4-(benzamidooxy)-butyrate (**6**). A solution of 3.1 mmol of sodium benzohydroxamate and 75 mg (3.0 mM) of NaH in 4 mL of DMF was allowed to react at 23 °C for 15–20 min or until H_2 evolution ceased. To this reaction mixture was added 1.6 mmol of **5** in 4 mL of DMF and the resultant solution reacted at 55 °C for 24 h. After the DMF was concentrated by rotary evaporation in vacuo, 10 mL of ethyl acetate was added and the mixture extracted with 0.5 N HCl, H_2O, saturated $NaHCO_3$, and saturated NaCl. After being dried over Na_2SO_4, the extract was filtered, concentrated, and purified by silica gel flash chromatography with ethyl acetate/hexane (4:5, v/v). Final yield of **6** was 80 ± 2%.

L-2-Amino-4-(aminooxy)butyric Acid (L-*Canaline*) (**7**). Compound **6** was refluxed with 7 mL of ethanolic HCl for 4 h at 95 °C. After the solvent was removed by rotary evaporation in vacuo, the residue was dissolved in 7 mL of 3 N HCl and refluxed for 3 h at 110 °C. Ether (2 × 10 mL) was used to extract the reaction mixture prior to removing HCl by rotary evaporation in vacuo.

If L-[1,2,3,4-^{14}C]canavanine is desired, the residue is taken to pH 6.2 with 1 N NH_4OH and the solvent removed in vacuo, resuspended in deionized water, and then reacted with CuO to generate L-canaline–Cu^{2+} (Ozinskas and Rosenthal, 1986b). The latter substance is guanidinated with zinc cyanamide to yield L-1,2,3,4-^{14}C]canavanine after the method of Ozinskas and Rosenthal (1986b). If L-[U-^{14}C]canaline is sought, the canaline hydrochloride salt is treated with triethylamine and the free base isolated as described (Ozinskas and Rosenthal, 1986a). The overall yield of L-[1,2,3,4-^{14}C]canavanine from L-[U-^{14}C]homoserine is 45 ± 5%.

Preparation of L-[G-^{14}C]Canaline and L-[G-^{14}C]Canavanine from L-[G-^{14}C]Homoserine. L-[G-^{14}C]Homoserine is prepared by feeding 10-day-old pea plants, *Pisum sativum*, with $Na_2{}^{14}CO_3$. In our procedure, 92.5 MBq of $Ba^{14}CO_3$ (Amersham; 2.0–3.6

MBq/μmol) was dissolved in 4 mL of deionized water, treated with a 2-fold excess of Na_2SO_4, and reacted at 30 °C overnight. The solution, clarified by filtration, was diluted to 925 kBq/mL.

Paired 10-day-old pea plants were carefully removed from a loamy potting soil and rinsed free of soil and the roots of both plants placed in a 10-mL, narrow-mouthed, serum bottle that contained 5 mL of ^{14}C-labeled sodium carbonate (4.63 MBq) in vigorously aerated, half-strength Hoagland's solution. The plants were allowed to take up the radiolabeled materials for 24 h on sunny day in which the temperature did not exceed 25 °C, harvested, and stored at −60 °C.

The frozen pea plants (n = 40) were ground in excess acetone and the acetone-defatted plants extracted with 50% aqueous ethanol containing 0.1 N H_2SO_4 overnight at 4 °C. After the extract was centrifuged at 12000g for 12 min, the supernatant solution was filtered over Whatman 541 paper and concentrated in vacuo. The pH was taken to 2–3 with a saturated solution of $Ba(OH)_2$. After 15 min at 4 °C, $BaSO_4$ was removed by centrifugation as above. The supernatant solution was filtered over paper as above, adjusted to pH 3.5 with 1 N HCl, and placed on a 20 × 75 mm column of Dowex 50 (H^+). The column was washed with 0.7 L of deionized water and developed with a 0.5 L of 200 mM ammonia and the column effluent concentrated by rotary evaporation in vacuo. After treatment with decolorizing charcoal, the amino acid laden effluent was taken to complete dryness by rotary evaporation in vacuo.

Homoserine lactone was prepared by refluxing the homoserine-containing residue in ethanolic hydrochloric acid for 90 min at 105 °C and drying in vacuo at 55 °C for an additional 30 min after solvent removal. Deionized water was added to the residue and the drying process repeated twice. The residue was then dissolved in 3 N HCl and refluxed for 90 min at 115 °C. Afterward, the HCl was removed *exhaustively* by rotary evaporation in vacuo and the residue dissolved in deionized water, taken to pH 3.0 with 1 N ammonia, and placed on a 20 × 75 mm column of Dowex 50 (NH_4^+). The column was washed with 0.7 L of deionized water and homoserine plus the basic amino acids eluted with 0.5 L of 200 mM ammonia. Homoserine lactone was converted to homoserine in situ when the column was treated with 200 mM ammonia.

The effluent was dried by rotary evaporation in vacuo and the residue dissolved in deionized water, taken to pH 3.0–3.5 with 2 N ammonia, and applied to a second Dowex 50 (NH_4^+) as described above. Basic amino acids were retained on the column while homoserine was eluted with 0.7 L of deionized water. The effluent was concentrated in vacuo and treated with decolorizing charcoal. Automated amino acid analysis of the final product revealed it was free of ninhydrin-positive substances other than L-homoserine. L-Homoserine yield was about 6% of the pea on a dry weight basis; the specific activity was 1.32 kBq/μmol.

Growth of the Plants and ^{14}C-Labeled Compound Administration. The ^{14}C-labeled compounds were administered to 9-day-old jack bean plants by direct injection into the fleshy, green cotyledons (the storage tissues of the seed). Each of the two segments constituting the cotyledons received half of the administered dose: radiolabeled amino acid (29.6 KBq) and carrier compound (15 μmol) when applicable, in sterile water.

The plants were fed in the morning of a bright fall day in which the temperature reached a maximum of 24 °C. All treatments were replicated three times, and the above-ground parts were collected at the indicated time intervals and stored at −60 °C. Conditions for the growth of the plants prior to ^{14}C administration were described previously (Rosenthal, 1982).

Preparation of the Plant Extract. Frozen plant materials were ground in a large excess of acetone at full power with a Sorvall Omni-mixer. The resulting homogenate was filtered and the acetone-defatted plant material allowed to dry at 22 °C.

The acetone-defatted plant material was extracted with 150 mL of 50% aqueous ethanol containing 0.2 N sulfuric acid by mechanically stirring overnight at 4 °C. After centrifugation at 12000g for 15 min, the supernatant solution was concentrated by rotary evaporation in vacuo and adjusted to approximately pH 4 with $Ba(OH)_2$; $BaSO_4$ was eliminated by centrifugation as above. The supernatant solution was rotary evaporated in vacuo to dryness, dissolved in deionized water, and evaporated as above. After the residue was taken up in a minimum volume of deionized water, it was adjusted to pH 3.5 with 1 N HCl, filtered, and stored at −20 °C.

Purification of the Plant Extract. The plant extract was purified on a 20 × 120 mm column of Dowex 50 (H^+). After the column was washed with 1 L of deionized water to secure the water-soluble, neutral fraction, the water-soluble, charged fraction was obtained by elution with 0.5 L of 200 mM NH_3.

The water-soluble, charged fraction was treated as described to convert homoserine to its lactone. After conversion, the final residue was dissolved in a minimum of deionized water, taken to pH 3–3.5 with 1 N NH_4OH, and placed on a 20 × 120 mm column of Dowex 50 (NH_4^+). The column was washed with 0.5 L of deionized water; the effluent contained phosphohomoserine and a canaline oxime (Figure 1). Homoserine, lysine, and canavanine were obtained by treating the column with 0.5 L of 200 mM ammonia. The column effluent was concentrated by evaporation in vacuo, taken to pH 3.5 with 1 N HCl, and rechromatographed on Dowex 50 (NH_4^+) as described above. Radiolabeled homoserine eluted with the water wash, while lysine and canavanine were obtained by developing the column with 200 mM ammonia.

The ^{14}C content of lysine and canavanine was determined by automated amino acid analysis in which the column effluent was collected without reacting with ninhydrin. The column effluent was then assayed by liquid scintillation spectroscopy. Radiolabeled homoserine accounted for all of the ^{14}C found in the appropriate column effluent and was not analyzed further by automated amino acid analysis.

Phosphohomoserine and the canaline oxime possessed a very similar elution volume, but phosphohomoserine eluted first. These two compounds were reacted with an equal volume of 6 N HCl at 100 °C overnight in vacuo. This procedure dephosphorylated and lactonized the homoserine derivative. The two compounds were then separated by ion-exchange chromatography with Dowex 50 (NH_4^+). The canaline oxime was eluted with deionized water and homoserine with 200 mM ammonia.

The canaline oxime was identified as canaline glyoxylate by treating the canaline oxime with hydrogen at 22 °C for 3–4 h in the presence of palladium black (Rosenthal, 1979). This procedure reductively cleaved canaline glyoxylate to form homoserine and glycine in stoichiometric yield. The reaction products were identified by automated amino acid analysis.

Processing the L-[*guanidinooxy*-^{14}C]Canavanine-Treated Plants. Nine-day-old jack bean plants treated with 185 KBq of L-[*guanidinooxy*-^{14}C]canavanine were processed as described above to prepare the standard plant extract. The plant extract was placed on a 20 × 120 mm column of Dowex 50 (H^+), washed with 1 L of deionized water, and developed with 0.5 L of 200mM ammonia to remove amino acids and any hydroxyguanidine that might have formed. The column was then treated with 2 N NaOH to elute [^{14}C]guanidine that might have been produced from L-[*guanidinooxy*-^{14}C]canavanine; the effluent was taken to neutrality with 2 N HCl and assayed by liquid scintillation spectroscopy. The amino acid containing effluent was applied to Dowex 50 (NH_4^+), washed with 0.5 L of deionized water, and developed with 200 mM ammonia. L-[*guanidinooxy*-^{14}C]Canavanine and any [^{14}C]hydroxyguanidine would be present in the final effluent.

The final effluent was evaporated in vacuo and the residue dissolved in 3 mL of 50 mM sodium tricine buffer (pH 7.6) and treated with a sufficient excess of jack bean leaf arginase and urease to insure complete hydrolysis of the L-[*guanidinooxy*-^{14}C]canavanine (Rosenthal and Thomas, 1985). The reaction mixture was deproteinized with 2 mL of 50% (w/v) trichloroacetic acid and centrifuged at 12000g for 12 min and the supernatant solution extracted three times with anhydrous ether. The reaction mixture was concentrated in vacuo and taken to pH 3.5 with N NH_4OH, placed on a 20 × 75 mm column of Dowex 50 (NH_4^+), and washed with 0.5 L of deionized water. [^{14}C]Hydroxyguanidine, generated from L-[*guanidinooxy*-^{14}C]canavanine, would be eluted with 200 mM ammonia.

RESULTS AND DISCUSSION

L-[U-^{14}C]Canaline is rapidly metabolized by 9-day-old jack bean plants (Table I). Within 1.5 h, 35% of the administered radiolabeled canaline is converted to ace-

Table I. Catabolism of L-[U-^{14}C]Canaline by 9-Day-Old Jack Bean, *C. ensiformis*

fraction or substance	% administered time, h 1.5	3.0	6.0	^{14}C: 12.0
I. acetone-soluble	35.1	22.8	14.6	5.3
II. water-soluble				
a. neutral	3.9	10.4	8.9	2.9
b. charged (total)	37.6	39.9	38.9	35.1
homoserine	21.5	18.9	15.4	13.4
phosphohomoserine	3.5	4.8	4.6	2.9
canaline glyoxylate	10.6	12.5	15.5	14.4
lysine	1.2	2.1	1.6	2.3
canavanine	0.8	1.6	1.8	2.1
max respiratory loss	18.9	22.4	33.1	52.1

tone-soluble components. These ^{14}C-bearing metabolites are rapidly degraded; at 12 h postadministration, only 5.3% of the administered ^{14}C remains in this fraction. Degradation of the acetone-soluble components is primarily responsible for the respiratory loss of ^{14}C. Analysis of the 12-h-treated plants reveals that more than half of the initial ^{14}C is absent from the tested fractions (Table I). Metabolites such as triglycerides and fatty acids would be acetone soluble and readily respired to CO_2 and subsequently lost. This finding indicates that most the aliphatic carbon skeleton of L-[U-^{14}C]canaline is converted to acetone-soluble components that are subsequently lost, most probably through degradation and conversion to carbon dioxide. If so, canaline actively supports the energy requirements of the developing plant.

The radiolabeled water-soluble neutral fraction, containing such metabolites as monosaccharides, disaccharides, and polysaccharides, increases to about 10% of the administered ^{14}C after 3 h and declines thereafter (Table I). Since the total water-soluble charged fraction accounted for about 38 ± 2% of the administered L-[U-^{14}C]canaline throughout the experiment, the water-soluble neutral components has to be produced from the acetone-soluble materials or their degradation products. Recovery of the ^{14}C from the acetone powder for the samples used in this study was 96 ± 1%; thus, virtually all of the administered ^{14}C was accounted for by the data of Table I. Overall, the acetone-soluble constituents increase rapidly and then decline sharply; water-soluble neutral compounds build more slowly, but they too are efficiently utilized.

A substantial part of the L-[U-^{14}C]canaline is converted to L-homoserine and L-phosphohomoserine. If canaline is converted directly to homoserine, this reaction would require a novel enzyme able to mediate a reductive deamination of the aminooxy group of canaline. No enzyme is known presently that can cleave an O–N linkage. Moreover, isolation of radiolabeled phosphohomoserine suggests that homoserine formed from canaline is itself phosphorylated. Phosphohomoserine functions in the biosynthesis of cystathionine, cysteine, and methionine (Giovanelli et al., 1979). These three sulfur-bearing amino acids do not accumulate sufficiently to evaluate the storage in these metabolites of ^{14}C derived from radiolabeled canaline catabolism. Nevertheless, the evidence is strong that via the reactions shown in Figure 2 canavanine and canaline support the primary nitrogen metabolism of this plant.

Canaline also supports the production of lysine (Table I), but this undoubtedly results from the ability of L-[U-^{14}C]homoserine to produce radiolabeled lysine (Rosenthal, 1982). In addition, canaline provides the carbon skeleton for an unusual higher plant conjugate, an oxime originating from canaline and glyoxylic acid.

A particularly interesting observation is the finding the L-[U-^{14}C]canaline gives rise to L-[U-^{14}C]canavanine. At

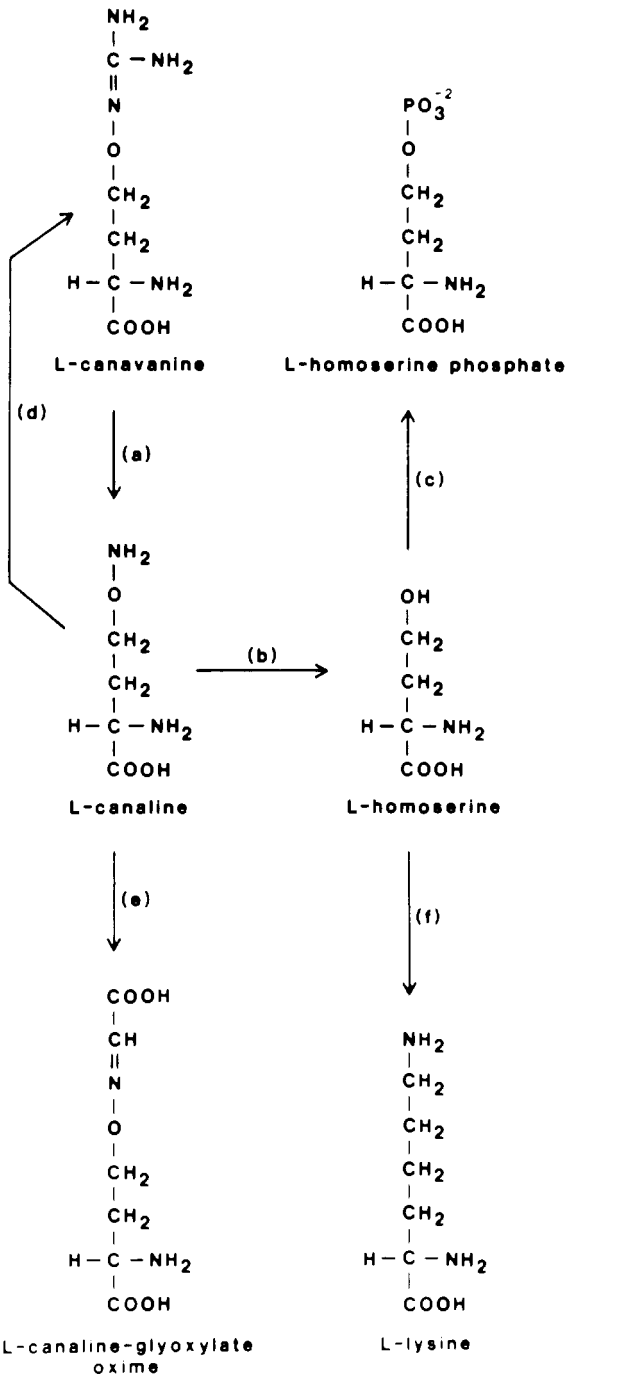

Figure 2. Metabolic reactions of L-canavanine in *C. ensiformis*: (a) hydrolytic cleavage employing arginase (EC 3.5.3.1); (b) reductive deamination, enzyme unknown; (c) phosphorylation employing homoserine kinase (EC 2.7.1.37); (d) biosynthesis employing ornithine carbamoyltransferase (EC 2.1.3.3), argininosuccinic acid synthetase (EC 6.3.4.1), and argininosuccinic acid lyase (EC 4.3.2.1); (e) oxime formation between canaline and glyoxylate; (f) lysine formation via 2,3-dihydropicolinic acid or *meso*-2,6-diaminopimelic acid.

present, there are only two ways in which this can occur; transamidination involving a nitrogen donor such as arginine or via the anabolic portion of the reactions of the Krebs–Henseleit ornithine–urea cycle (Rosenthal, 1982). To distinguish between these possibilities, L-[*guanidino*-^{14}C]arginine was fed to comparable 9-day-old jack bean plants along with cold canaline. No significant production of L-[*guanidinooxy*-^{14}C]canavanine was noted. Thus, our earlier demonstration that L-[*guanidino*-^{3}N-^{15}N]arginine serves in the production of L-[*guanidinooxy*-^{3}N-^{15}N]canavanine (Rosenthal et al., 1988) means that this reaction

Table II. Water-Soluble, Charged Radiolabeled Products 12 h after Administration of L-[U-^{14}C]Canaline or L-[1,2,3,4-^{14}C]Canavanine to 9-Day-Old Jack Bean, *C. ensiformis*

admin radiolabeled compd	radiolabeled product, % admin ^{14}C			
	homoserine	phosphohomoserine	canaline glyoxylate	lysine
canavanine	12.2	2.1	13.8	3.1
canaline	12.4	2.4	13.2	2.9

must occur by a route other than by direct transamidination of canaline to form canavanine.

As mentioned previously, it is not possible to prove that canavanine is made from canaline, via the formation of *O*-ureido-L-homoserine and L-canavaninosuccinic acid, since these compounds do not accumulate in the jack bean plant. However, canaline does produce canavanine. Can this occur via the partial reactions of the ornithine–urea cycle, i.e. those fostering canavanine production from canaline via *O*-ureido-L-homoserine? This biosynthetic route was evaluated by providing 15 μmol of carrier carbamoyl phosphate at the time of [^{14}C]canaline administration. The addition of exogenous carbamoyl phosphate doubled radiolabeled canavanine formation after 12 h. This can only occur if carbamoyl phosphate fosters *O*-ureido-L-homoserine formation as a prerequisite to canavanine synthesis. On the other hand, the amount of radiolabeled canavanine found in jack bean plants administered L-[U-^{14}C]canaline is small and the present evidence fails to support a role for canaline biosynthesis from homoserine. Other than by arginase-mediated hydrolysis of canavanine, there is presently no evidence that canaline can be biosynthesized by a higher plant and thereby produce sufficient canaline to sustain canavanine biosynthesis. Moreover, nearly all of the catabolized canaline is diverted into homoserine and its derivative or oxime formation. This is inconsistent with a major role for canaline in canavanine biosynthesis. In conclusion, while canaline can serve as a canavanine precursor, metabolism of this ornithine analogue does not appear to be sufficiently active to account for the in vivo canavanine production that occurs in young jack bean plants.

Analysis of 9-day-old jack bean plants provided L-[1,2,3,4-^{14}C]canavanine for 12 h reveals a degradation pattern that agrees with that obtained for canaline (Table II). This establishes the importance of arginase-directed catabolism of canavanine to canaline in the degradation of this arginine analogue. This conclusion is supported by another line of inquiry. Namely, that administration of L-[*guanidinooxy*-^{14}C]canavanine to comparable jack bean plants failed to provide evidence for the production of guanidine or hydroxyguanidine. Thus, canavanine is not degraded directly to yield homoserine and guanidine or hydroxyguanidine. These degradative pathways operate in canavanine processing by procaryotes (Kihara et al., 1957). Rather, canavanine is first converted to canaline and homoserine produced via the catabolism of canaline. It appears that the eventual production of ammonia from urea is of such importance to the developing jack bean plant that canavanine hydrolysis to canaline and urea is the overwhelming pathway of canavanine catabolism.

ACKNOWLEDGMENT

The financial support of the National Science Foundation (Grant PCM-82-19721) is gratefully acknowledged.

Registry No. 1, 672-15-1; 2, 119769-67-4; 3, 119769-68-5; 4, 119769-69-6; 5, 119769-70-9; 6, 119769-71-0; 7, 119769-72-1; 8, 119769-73-2; L-[U-^{14}C]homoserine, 119736-88-8; benzyl chloroformate, 501-53-1; *p*-toluenesulfonyl chloride, 98-59-9; sodium benzohydroxamate, 22513-32-2; phosphohomoserine, 4210-66-6; canaline glyoxalate oxime, 92759-10-9; L-lysine, 56-87-1; canavanine, 543-38-4; canaline, 496-93-5.

LITERATURE CITED

Bell, E. A. Canavanine and Related Compounds in Leguminosae. *Biochem. J.* **1958**, *70*, 617–619.

Bell, E. A.; Lakey, J. A.; Polhill, R. M. Systemic Significance of Canavanine in the Papilionoideae (Faboideae). *Biochem. System. Ecol.* **1978**, *6*, 201–212.

Giovanelli, J.; Mudd, S. H.; Datko, A. H. Homocysteine Biosynthesis in Plants. In *Third International Meeting of Low Molecular Weight Sulfur-Containing Natural Products*; Cavallini, D., et al., Eds.; Plenum Press: New York, 1979.

Kihara, H.; Prescott, J. M.; Snell, E. E. The Bacterial Cleavage of Canavanine to Homoserine and Guanidine. *J. Biol. Chem.* **1957**, *226*, 497.

Ozinskas, A. J.; Rosenthal, G. A. Synthesis of L-Canaline and γ-Functional 2-Aminobutyric Acid Derivatives. *J. Org. Chem.* **1986a**, *51*, 5047–5050.

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

Rosenthal, G. A. An Ontogenic Study of Canavanine Formation in the Fruit of Jack Bean, *Canavalia ensiformis* (L.) DC. *Plant Physiol.* **1971**, *47*, 209–211.

Rosenthal, G. A. L-Canavanine Metabolism in the Jack Bean, *Canavalia ensiformis* (L.) DC. [Leguminosae]. *Plant Physiol.* **1982**, *69*, 1066–1069.

Rosenthal, G. A. Biochemical Insight into Insecticide Properties of L-Canavanine, A Higher Plant Protective Allelochemical. *J. Chem. Ecol.* **1986**, *12*, 1145–1156.

Rosenthal, G. A. The Protective Action of a Higher Plant Toxic Product. *Bioscience* **1988**, *38*, 104–109.

Rosenthal, G. A.; Rhodes, D. L-Canavanine Transport and Utilization in Developing Jack Bean, *Canavalia ensiformis* (L.) DC. *Plant Physiol.* **1984**, *76*, 541–544.

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.; Berge, M. A.; Ozinskas, A. J.; Hughes, C. G. The Ability of L-Canavanine to Support Nitrogen Metabolism in the Jack Bean, *Canavalia ensiformis* (L.) DC. *J. Agric. Food Chem.* **1988**, *36*, 1159–1163.

Töpfer, V. R.; Miersch, J.; Reinbothe, H. Studies on Canavanine Degradation in Fabaceae. *Biochem. Physiol. Pflanzen.* **1970**, *161*, 231–242.

Received for review July 11, 1988. Accepted October 24, 1988.