Finally, the bioacetylation of alcohols showed no dependence on the light:dark cycle to which the insects were subjected. Even the glands sampled during the 'non-calling' time were able to carry out the alcohol-acetate transformation.

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Separation of canavanine and canaline by high performance liquid chromatography

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Summary. The plant amino acid canavanine and its hydrolytic product canaline were successfully separated and identified by Reverse Phase High Performance Liquid Chromatograph (RP-HPLC). This procedure was used to demonstrate the arginase-mediated cleavage of canavanine to yield canaline and urea, and the subsequent formation of a Schiff's base complex between canaline and pyridoxal phosphate. Both aforementioned reactions were demonstrated by RP-HPLC.
Key words. Canavanine; canaline; alfalfa; fava; high performance liquid chromatography; arginase.

The pharmacological and physiological properties of the plant amino acid canavanine and its hydrolytic product canaline have been the subject of intense research[1-5], since both have toxic effects in a great range of organisms. Canavanine interferes with arginine metabolism[6], inhibits protein synthesis[7,8], blocks RNA and DNA synthesis[9] and affects the immune system[10]. While the toxic effect of canaline appears to be mainly the inhibition of pyridoxal phosphate-dependent enzyme reactions[11,12], these biochemical events are also of great interest nutritionally since canavanine is found in several edible legumes[13,14]. The isolation and identification of the aforementioned amino acids have usually been performed using a cation exchange resin, such as Dowex-50, followed by thin layer chromatography or electrophoresis[15]. Quantitation of canavanine has routinely been performed with a colorimetric method, utilizing pentacyanoammonium ferrate[16] or fluorometrically with phenanthrenequinone[17], while canaline has been estimated by the rather unspecific Jaffe reaction[18], after extensive sample clean-up or indirectly by conversion to 2-amino-4-ureidooxybutyric acid[19]. RP-HPLC offers a simple and direct solution to the detection, identification and quantitation of canavanine and canaline.

Materials and methods. L-Canavanine, L-canaline, L-arginine, amino acids for standards and tripotassium citrate were purchased from Sigma Chemical Co. (St. Louis, MO 63178 USA). Solvents for HPLC were obtained from Alltech Associates (Los Altos, CA 94022, USA).

Pre-o-phthaldialdehyde (OPT) derivatization followed by RP-HPLC has been used extensively for the detection and quantitation of α-amino acids[20,21]. The method employed in this investigation is basically that of Burbach et al.[21] with the following modifications: Potassium citrate (0.05 M) was substituted for 0.1 M sodium citrate and the gradient (mobile phase) scheme based on A: 90% 0.05 M potassium citrate-5% dioxane-5% iso-propanol (v/v) and B: 85% methanol. The gradient was changed to 100% A, 0% B (initial) then concave 10 min to 50% A-50% B followed by a 4-min isocratic run of 50% A-50% B, then concave 12 min to 15% A-85% B, followed by 2 min isocratic at 15% A-85% B. An Alltech Econosphere C-18 (250 × 4.6 mm) reverse phase column (Alltech Associates, Los Altos, Ca 94022, USA) in conjunction with a Waters HPLC system with dual pumps, a data module (Waters Associates, Milford, MA 01757, USA) and a Schoeffel Model 970FS (Schoeffel Instruments, Westwood, N.J. 07675, USA) were used for chromatography and analysis. Samples containing protein were deproteinized with 6% perchloric acid and neutralized with 5 M potassium carbonate.

Experimental design. Hydrolysis of canavanine was demonstrated by incubating 20 mM L-canavanine (Sigma No. C9758) in 10 mM Tris buffer, pH 9.3, with a dilute Mn^{++}-activated rat liver homogenate (particle free) for 0 (A), 15 (B) and 30 (C) min at 37 °C (fig. 1). A pH of 9.3 was chosen, because it is the optimum pH for mammalian liver arginase. This pH also facilitates the detection of minor canavanine hydrolysis; for instance, at pH 9.3 it was found that homogenates of rat heart, brain, and intestine hydrolyzed easily detectable amounts of L-canavanine; these three homogenates had activities of respectively 1/70th, 1/25th, and 1/4th of that of liver. In contrast, at pH 7.2, the hydrolysis of L-canavaline was not discernible in the homogenates of rat heart, brain, and intestine.

When L-canavanine or L-canaline was added to fava bean homogenate approximately 98% was recovered by HPLC analysis. Addition of the same amino acids to pooled mouse serum (Sigma Chemical Co.) resulted in a recovery of 95% for canavanine and only 90% for canaline (mean of five assays).

Formation of a canaline pyridoxal phosphate complex is illustrated in figure 2. When a mixture of α-amino acids plus L-canavanine and L-canaline were preincubated with an aldehyde prior to OPT derivatization the quantity of canaline (peak 8) was reduced.

To satisfy the need for detection of canavanine and canaline in plant material, two different leguminous seeds, fava (*Vicia faba*) and alfalfa (*Medicago sativa*) were obtained from a

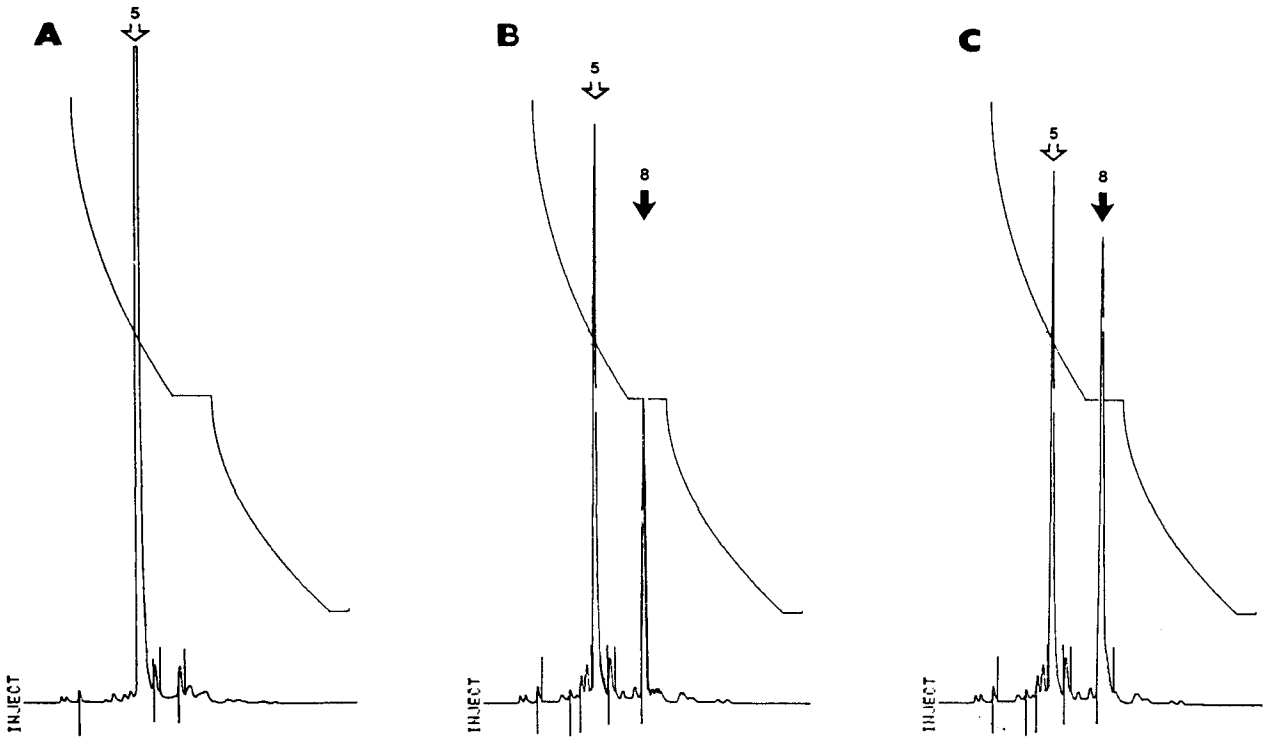

Figure 1. Demonstration of ureohydrolysis of canavanine by rat liver homogenate. Chromatograms of the substrate (canavanine) mixture at 0, 15, and 30 min incubation are shown in *A*, *B* and *C*, respectively.
A dilute supernatant of Mn^{++}-activated rat liver homogenate was incubated with 20 mM canavanine in 10 mM Tris at pH 9.3 at 37 °C. Samples were deproteinized with 6% perchloric acid and neutralized with 5 M potassium carbonate prior to OPT derivatization and chromatography. Peak 5 = canavanine; peak 8 = canaline. For identification of other peaks, see fig. 2.

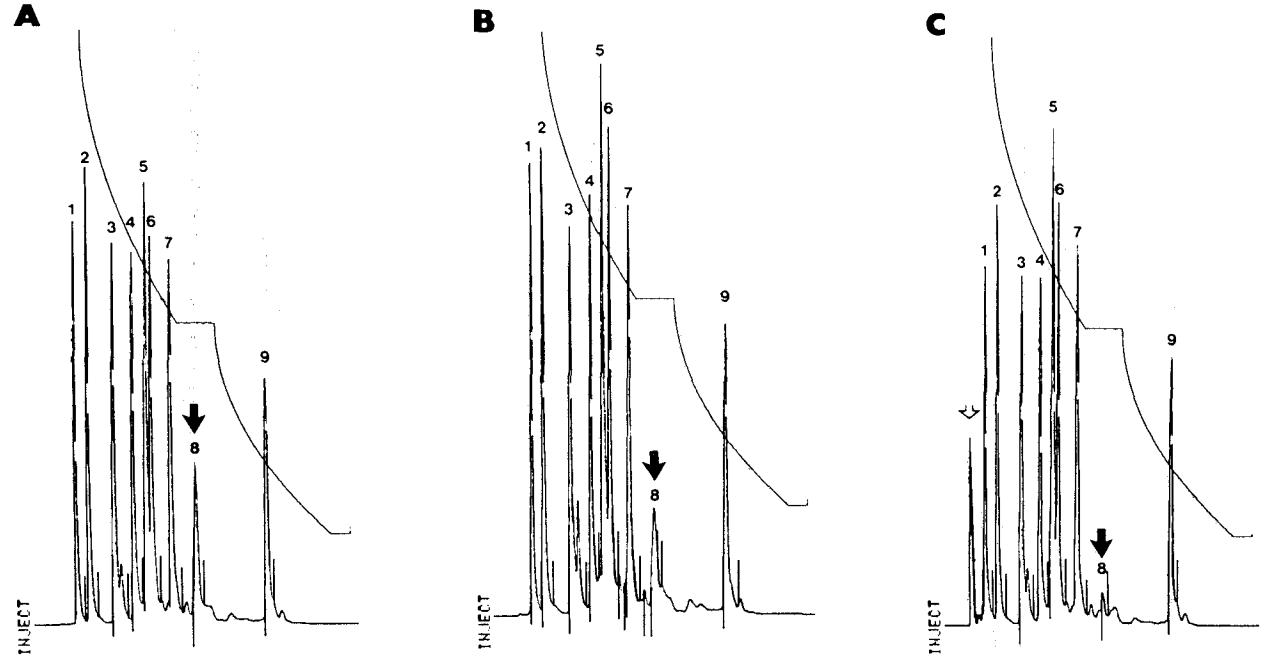

Figure 2. The formation of a canaline aldehyde complex. A mixture of α-amino acids plus canavanine and canaline was chromatographed by RP-HPLC without and with incubation with chlorobenzaldehyde or pyridoxal phosphate prior to OPT derivatization. *A* Elution profile of OPT derivatized amino acid: 1, ASP; 2, GLU; 3, ASN; 4, HSD; 5, L-canavanine; 6 ARG; 7, GABA; 8, L-canaline; and 9, ORN. All peaks (monitored an excitation wavelength of 310 nm and emission wavelength of 417 nm at 0.2 μA *AUFS*) represent 1 nmole amino acid except canaline which is 2 nmoles. *B* Same amino acid mixture as *A* preincubated with 10 nmoles of chlorobenzaldehyde for 30 min at room temperature prior to OPT derivatization. *C* Same amino acids as in *A* except preincubated at room temperature with 10 nmoles, pyridoxal phosphate for 30 min prior to OPT derivatization. Note the appearance of a very acidic compound (open arrow) and almost complete elimination of canaline, peak 8 (solid arrow).

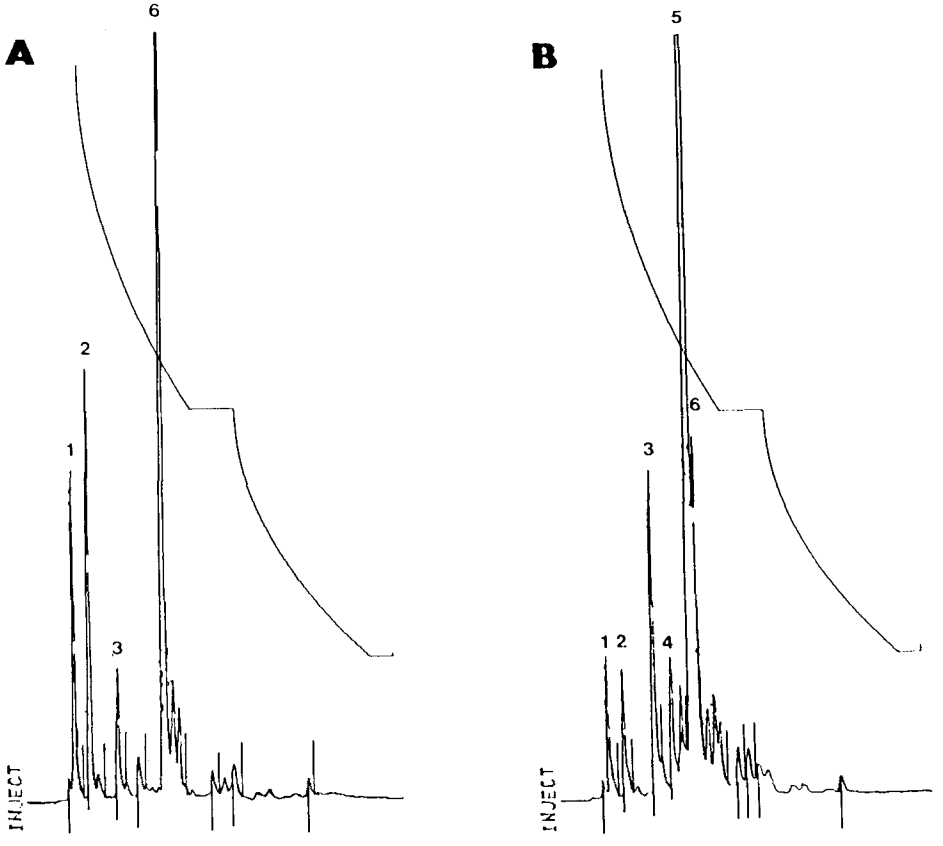

Figure 3. RP-HPLC of two different leguminous seeds, Acid extracts were prepared as described in text. *A* OPT derivatized amino acids of fava bean *(Vicia faba)*. B OPT derivatized alfalfa *(Medicago sativa)* seed extract. Note the absence of canavanine (peak 5) in *A* and the relatively high canavanine concentration in *B*, almost overshadowing ARG. Each chromatogram is equivalent to 0.4 mg dry seed. For peak identification see fig. 2.

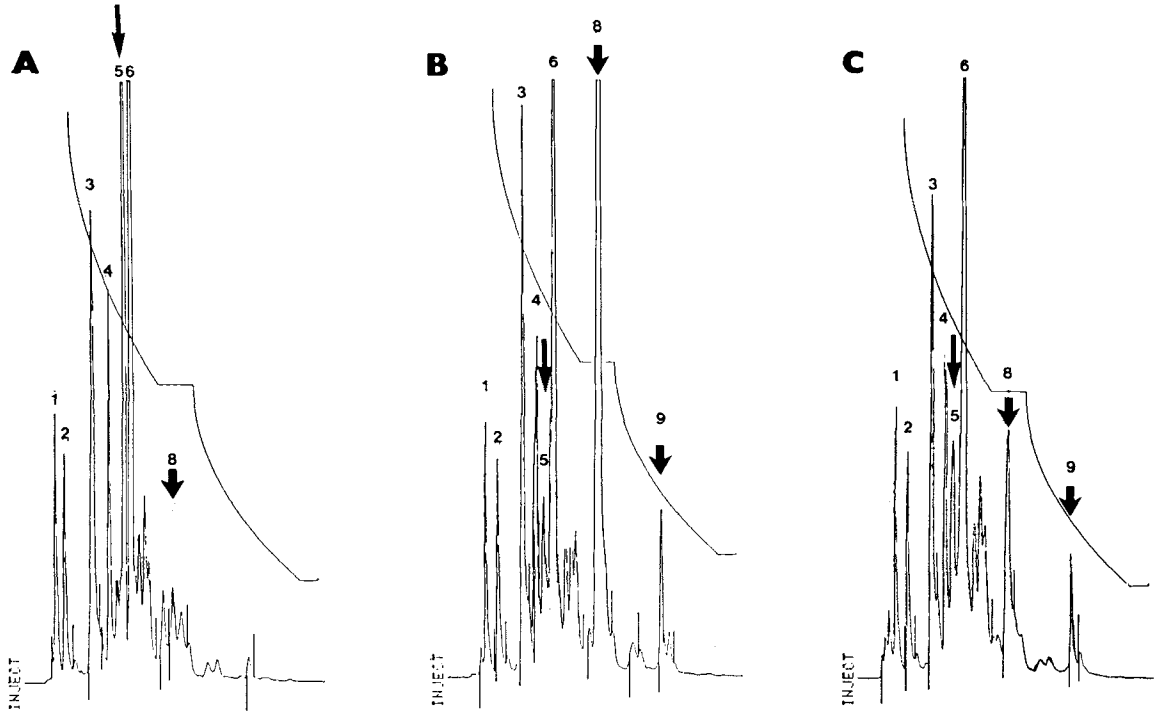

Figure 4. Confirmation of the presence of canavanine in an alfalfa seed extract by RP-HPLC after hydrolysis with calf liver arginase and OPT derivatization. *A* Alfalfa extract representing approximately 1.2 mg of dried seed mixed with 0.7 U arginase (Boehringer Mannheim No 102881) in 10 mM Tris buffer, pH 9.3, immediately acidified (zero time). *B* Same reaction mixture as *A* incubated 30 min at 37 °C. Note the appearance of canaline (peak 8) and ornithine (peak 9). *C* Similar conditions as in *B* except that the mixture was incubated with 10 nmoles pyridoxal phosphate for 15 min at room temperature prior to OPT derivatization. In all three cases an equivalent of 0.4 mg dried seed was chromatographed. Note the reduction of the canaline peak and the unchanged ornithine peak.

local store and homogenized in 6% perchloric acid with a polytron tissue grinder (Kinematica GmbH, Luzerne, Switzerland). After centrifugation (20,000 × g, 15 min) the clear supernatant was neutralized with 5 M potassium carbonate and recentrifuged to remove the potassium perchlorate. A volume equivalent to 0.4 mg of dry seed was derivatized with OPT and subjected to HPLC. Figure 3A shows the chromatogram of fava bean and 3B that of alfalfa seed. The latter contained approximately 0.4% canavanine (peak 5). The aforementioned observations led to the following analytical design.

When alfalfa seed extract (see fig. 4A) was incubated in 10 mM Tris, pH 9.3, containing 0.7 U Mn^{++} activated calf liver arginase (Boehringer Mannheim No. 102881) the appearance of canaline (peak 8) and ornithine (peak 9) were clearly demonstrated (fig. 4B). Preincubation with pyridoxal phosphate prior to OPT derivatization caused a reduction of canaline but not that of ornithine (fig. 4c). A similar experiment conducted with fava bean extract showed only the formation of ornithine (data not shown).

By utilizing a combination of arginase treatment, OPT derivatization and RP-HPLC it is possible to detect, identify and quantitate canavanine and/or canaline. The procedure is relatively rapid compared to previous methods[15, 18, 19] and the two amino acids can be assayed simultaneously in the picomole range.

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How to grow a tropical national park: basic philosophy for Guanacaste National Park, northwestern Costa Rica

1 All tropical regions should have national parks, rather than have national parks occur as historical afterthoughts, or as emotional reactions to the last fragments of a dwindling majesty. Today, for many very large areas of the tropics, the possession of a national park requires a philosophy and technology of park reconstruction, of restoration ecology. That is to say, a national park is up there along with schools, public health, electrical services, etc.; it can be put in place, and for the same reasons.

2 Given that a major habitat is to be restored into a National Park as part of its preservation process, to which vegetation types shall we restore it?

a) There are many possibilities, and if they have an endpoint in common, it is tens of centuries down the road (e.g., wind- vs vertebrate-generated forests invade abandoned pastures)

b) What kinds of habitats will give the highest yields of biodiversity preservation? What kinds of habitats will make the park the most user-friendly (community involvement value, recreational value, interest-generating value, example value, seed and gene stock value, tourist income value, watershed value)?

c)What are the degrees of irreversibility of the various habitat possibilities (can introduced species be eliminated, what life forms are hard to remove or control)?

d) The existence of restoration alternatives calls for a whole new level of action and decision-making presently absent from the planning and realization of tropical national parks.

3 Why are we restoring a tropical dry forest national park?

a) Because there is no pristine vegetation left of this major forest type (it once occupied more than half of the tropics), and the vegetation remnants are being rapidly obliterated. Less than 2% of the western Mesoamerican dry forest (it once occupied an area the size of France) is even approximately intact, and 0.09% has conservation status. In a few areas, such as the one occupied by Guanacaste National Park (GNP), there are sufficient population and habitat fragments to fully restore the 700 km^2 dry forest block and its rain forest refuges.

b) Because this is the only way to conserve the approximately 30,000 species of insects, 500 birds, 3,000 plants, 160 mammals and 200 herps that occupy the GNP area.

c) Because by returning introduced and artificial grassland pastures to forest, we eliminate the risk that the forest will be roasted off the map by human-generated fires; virtually all of the fire problem originates in the pastures and then moves into the forest.

d) Because the active process itself allows

1. explicit construction of a user-friendly biological system and
2. community participation in the planning of the structure itself, and in the mechanics of its growth, thereby engendering a desire to retain it aside from its innate or taught attractiveness.

4 The tropical dry forest is 40–90% as species-rich as is the rainforest (e.g., 2800 species of moths at one light over the year). However, the dry forest once covered more of the tropics than did rainforest. It species are not replicated in the rainforest, and its habitats and life forms are not either.

5 The restoration process can proceed at minimal cost and management, through nothing more than stopping the hunting and the fires. Or, it can be speeded up in proportion to the amount of money that someone wishes to invest in labor or minimal technology.