ketone. Other compounds inactive against CP-22 were the terpene hydrocarbons (−)-β-pinene, (+)-α-pinene, *dl*-α-pinene, and the terpene ethers, α-pinene oxide, β-pinene oxide, and cineole. In contrast, α- and β-pinene and cineole have inhibitory activity against higher plants (Muller et al., 1964). The bioassays of these compounds demonstrated that chemical structure is important for activity and that the fungus is not generally sensitive to volatile compounds in the C-4 to C-10 range of molecular weights.

The most active compounds were *trans*-2-hexenal, 2,4-hexadienal, furfural, β-ionone, and 1-nonanol which occur naturally in the corn ear (Flath et al., 1978; Jones et al., 1980; Lueck and Cejka, 1958). β-Ionone also reduced sporulation of *A. flavus* CP-22 but allowed growth with delayed morphological development (Wilson et al., 1981).

The inhibitory action of these volatile, naturally occurring compounds perhaps serves as a barrier to pollination to protect the embryos during the silking stage from intrusion of pollen or spores of other species and thus may enhance the survival rate of the embryos. For instance mycelial growth of *A. flavus* has been found to proceed along the silks (Payne, 1983; Jones et al., 1980). Thus, an additional effect of the volatile inhibitory compounds may be that the young kernels are protected from mycelial growth of invading fungi.

This implies that resistance to *A. flavus* may be bred into corn by selecting for strains that produce higher levels of volatiles. Further, if 2,4-hexadienal could be induced to oxidze to the well-known fungal inhibitor, sorbic acid (2,4-hexadienoic acid), a longer lasting effect of fungal inhibition might be obtained by decreasing the loss of the inhibitors by volatilization. The possibility of further manipulation of the corn plant metabolites with plant growth regulators also exists.

Registry No. *trans*-2-hexenal, 6728-26-3; 2,4-hexadienal, 80466-34-8; furfural, 98-01-1; *trans*-cinnamaldehyde, 14371-10-9; β-ionone, 79-77-6; 3-penten-2-one, 625-33-2; 4-methyl-3-penten-2-one, 141-79-7; 4-(2-furyl)-3-buten-2-one, 623-15-4; *trans*-2-hexen-1-ol, 928-95-0; 2,4-hexadien-1-ol, 111-28-4; β-ionol, 22029-76-1; 3-penten-2-ol, 1569-50-2; 4-(2-furyl)-3-buten-2-ol, 4229-85-0; 1-nonanol, 143-08-8.

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Canavanine to Arginine Ratio in Alfalfa (*Medicago sativa*), Clover (*Trifolium*), and the Jack Bean (*Canavalia ensiformis*)

Samuel Natelson

The canavanine to arginine ratio (C/A) was determined in six alfalfa varieities (*Medicago sativa*), three clover varieties (*Trifolium*), and the jack bean (*Canavalia ensiformis*). A low-pressure chromatographic procedure was used with a Dowex 50-x-8 column (0.5 × 30 cm) for the separation of the guanidino compounds. Fluoroscence was measured (excitation 307 nm, emission 447 nm) after heating the column eluate with phenanthrenequinone in 2 M NaOH, cooling, and acidifying with 4 M acetic acid. The C/A g kg^{-1} ratios found were for the jack bean 30.9, for the alfalfas Team 11.6, Classic 10.7, Weevlchek 9.78, Saranac A.R. 9.65, Arc 9.55, and Buffalo 8.65. For the clovers the C/A ratios were red 0.824, ladino 0.779, and white 0.600. The canavanine content (g kg^{-1}) of the alfalfas ranged from 8.33 for the least weevil resistant Buffalo to 13.6 for the highly weevil resistant Weevlchek. The other weevil resistant varieties, Saranac A.R. 10.9, Arc 10.8, Team 10.7, and Classic 9.90, were not significantly different from each other.

INTRODUCTION

The distribution of canavanine in the seeds of the leguminosae has been used to classify the various species. (Bell, 1950; Birdsong, et al., 1960; Bell and Tirimann, 1965; Turner and Harborne, 1967; Tschiersch, 1961; Mannhalter and Michl, 1974). Canavanine is toxic to animals, insects, and other plant pests (Tschiersch, 1962; Isogai et al., 1973). Its production appears to be a defense mechanism (Rosenthal, 1977). Alfalfas (*Medicago sativa*) contain substantial quantities of canavanine in their seeds, and it has been suggested that the difference in the resistance of various alfalfa cultivars to the alfalfa weevil (*Hypera postica*) is due, at least partly, to their difference in canavanine content (Natelson and Bratton, 1984; Natelson, 1985).

The toxicity of canavanine is due, largely, to its similarity in structure to that of arginine (Hegdekar, 1970; Vanderzant and Chremos, 1971; Crine and Lemieux, 1982). Canavanine contains an oxygen linked to the guanidino group instead of a carbon. In Figure 1 the structures of canavanine and arginine are compared.

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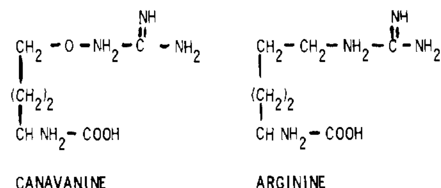

Figure 1. The structures of canavanine and arginine compared.

Thus canavanine binds to many enzymes which react with arginine, interfering in their action. It can also be incorporated into proteins, in place of arginine, which cannot then perform the function for which they were designed. (Crine et al., 1982). Arginine reduces the toxic effect of canavanine in in vitro experiments (Wright and Srb, 1950).

For these reasons, it was felt that the arginine content of the alfalfas should be determined along with their canavanine levels. This would permit comparison of the canavanine/arginine ratio. A cultivar with a high C/A ratio should be more toxic than another with the same canavanine content but a lower ratio. This type of study, along with field studies, may serve to design better weevil resistant varieties.

Some doubt has been raised as to which plants contain canavanine (Rosenthal and Davis, 1975; Rosenthal, 1978; Rosenthal and Dahlman, 1982). For this reason the technique chosen for assay had to include some validation process for the identification of the canavanine. For this purpose, an automated fluorometric system (Natelson, 1984) was improved and used for simultaneous canavanine, arginine, and guanidine assay. In the procedure reported earlier, acidification was with 4 M HCl. Excitation was at 305 nm and emission at 395 nm. In the present procedure, 4 M acetic acid was used and 2 M NaOH was substituted for 1 M NaOH so that the solution entering the cuvet had pH 4.6–5.2. This shifted the excitation and emission wavelengths to 307 and 447 nm, respectively. Sensitivity was about the same with these modifications, but the patterns were smoother and proportionality between peak heights and concentration was improved.

Canavanine is reduced readily to yield homoserine and guanidine (Kalyankos et al., 1958; Takahara et al., 1978; Natelson, 1985). In the reaction between phenanthrenequinone and the guanidino compound the fluorescence with guanidine is about seven times greater than that for canavanine on a molar basis. Advantage was taken of that fact by preparing a chromatogram before and after reduction of the sample. If canavanine is present, its peak disappears from the tracing after reduction, and a marked increase in the height of the guanidine peak is observed. This confirms the presence of canavanine. The system is especially effective for the clovers since they contain very little guanidine initially. This technique was applied to six common cultivars of alfalfa, three of clover (*Trifolium*), and the jack bean which has been analyzed repeatedly as a reference.

EXPERIMENTAL SECTION

Reagents. Phenanthrenequinone (0.5 mM). Phenanthrenequinone (208 mg) was dissolved in 20 mL of dimethylformamide and diluted to 500 mL with isopropyl alcohol. Aliquots of this 2 mM solution were diluted 4-fold with 50% isopropyl alcohol in water.

Buffers. A stock buffer was made 0.1 M with respect to citric acid, 0.25 M with respect to NaOH, and 0.1 M with respect to boric acid. Aliquots of this buffer were brought to pH 4.0, 5.0, 6.0, 8.1, and 10.2, respectively, by adding 3 M HCl to the aliquot slowly.

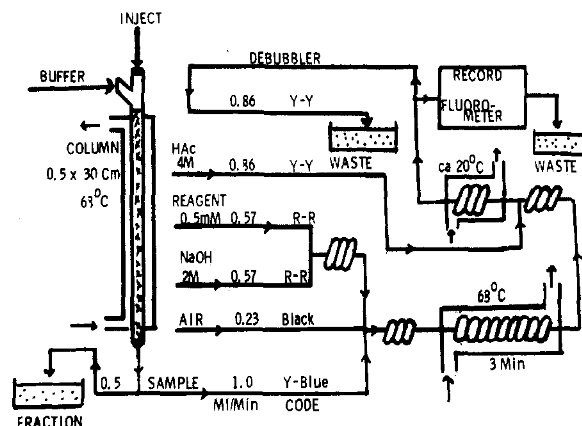

Figure 2. Schematic representation of the continuous flow assembly for analyzing the effluent from the column. The buffer is pumped onto the column by a peristaltic pump and the effluent (1.5 mL min^{-1}) is divided between a fraction collector and the analyzer whose flow is also maintained with the peristaltic pump.

Standards. The standard mixture contained 2 mM L^{-1} guanidinosuccinic acid, 1 mM L^{-1} each of guanidinobutyric acid, guanidine, and arginine, and 5 mM L^{-1} of canavanine.

$BaSO_4$–Pd Catalyst. To 177 mg of $PdCl_2$ (1 mmol) was added 100 mL of 0.08 M H_2SO_4. This solution was added slowly to a slurry of 3 g of $Ba(OH)_2 \cdot 8H_2O$ in 50 mL of H_2O stirring vigorously. The mixture was stirred for 30 min, 1 M NaOH was added to pH 10.5, and stirring was continued for an additional hour. The solution was filtered on a Buchner funnel and washed well with H_2O. The residue was then dried in a vacuum oven at 70 °C for 3 h. This contained 6% Pd (as the oxide) on $BaSO_4$.

Extraction Procedures. (a) The finely ground seeds (1 g) were shaken for 2 h with 10 mL of a 5% trichloroacetic acid solution in 50% isopropyl alcohol containing 1 mM L^{-1} of guanidinobutyric acid as the internal standard. The mixture was allowed to stand overnight, shaken again for 15 min, and centrifuged in a plastic tube at 5000*g*. The clear supernatant (50 μL) was injected onto the column (Figure 2). For the jack bean, 500 mg was extracted instad of 1 g and treated as described.

(b) The finely ground seeds (1 g) were shaken with 20 mL of 0.2 M H_2SO_4 and centrifuged at 5000*g*. The supernatant was decanted and shaken with 10 mL of chloroform and again centrifuged as before. Protein collects at the water–chloroform interface. The aqueous layer was transferred to a polypropylene test tube and stirred with finely ground $Ba(OH)_2$ to pH 6.0–6.9. The solution was centrifuged as before and a 10-mL aliquot was taken. An equal volume of isopropyl alcohol was added, and the solution was centrifuged to remove the polysaccharides. A 10-mL aliquot was lyophilized to dryness in a 25-mL beaker. The residue was dissolved in 2.5 mL of H_2O containing 1 mM L^{-1} guanidinobutyric acid and 50 μL was injected for assay as described below.

(c) The ground seed (1 g) was stirred with 30 mL of 20% acetic acid in water for 3 h. The mixture was allowed to stand at room temperature overnight and shaken for 15 min. The mixture was centrifuged at 5000*g*, and 15 mL of isopropyl alcohol was added to a 15-mL aliquot of the supernatant. The polysaccharides were removed by centrifugation, and 15 mL of the supernatant was lyophilized after removing about half under vacuum at 40 °C. The residue was dissolved in 2.5 mL of H_2O containing 1 mM L^{-1} guanidinobutyric acid, and 50 μL was assayed as described below.

Reduction of Canavanine. To 2 mL of extract from b or c above (not a) was added 100 mg of sodium borate

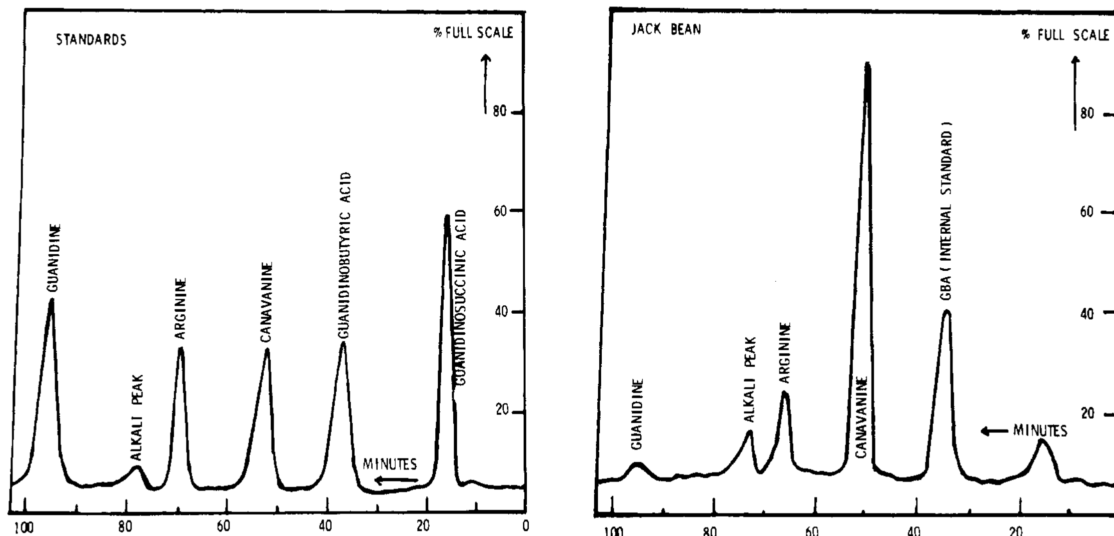

Figure 3. (A, left) Elution pattern obtained with the standards. The peak heights represent 50 nmol of arginine, guanidine, and guanidinobutyric acid (GBA), 100 of nmol guanidinosuccinic acid, and 250 nmol canavanine. (B, right). Tracing obtained with an extract of 2.5 mg of the jack bean. The pH changes were 5, 6, and 8.1 (0.2 M buffer 15 min each) and 10.2 (20 min, 0.25 M NaOH 10 min, and 1.5 M NaOH 25 min).

($Na_2B_4O_7{\cdot}10H_2O$) plus 1 mL of H_2O. The extract with trichloroacetic acid (a) poisons the catalyst. The solution was adjusted to pH 8.5 with HCl or NaOH and 50 mg of the $BaSO_4$–Pd catalyst was added. While mixing, hydrogen gas was bubbled into the solution for 2 h. The catalyst turned black. The solution was diluted to 5 mL and centrifuged, and 125 μL was injected onto the column.

Assay Procedure. The apparatus shown in Figure 2 was assembled and the resin (Dowex 50-x-8, 200–400 mesh) was rinsed successively with H_2O (15 min), 3 M HCl (15 min), H_2O (15 min), 1.5 M NaOH (15 min), water (15 min), and pH 4.0 buffer (20 min). The outlet of the column was now attached to the analyzer. The sample was injected, and the pH 5.0 buffer was pumped through the column for 15 min. The other buffers followed in sequence: pH 6.0, 15 min; pH 8.1, 15 min; pH 10.2, 20 min; 0.25 M NaOH, 10 min; 1.5 M NaOH, 25 min. The fluorometer was set at about 307 nm excitation and 447 nm emission. Prior to attaching the column to the analyzer, it was necessary to sample a guanidinosuccinic acid standard of 25 nmol/mL to maximize the excitation and emission response. The Farrand spectrofluorometer was employed and set at 1/100th its maximum sensitivity.

The heating coil was kept at 68 °C by circulating water from a Lauda water bath which also heated, in series, the column jacket. The cooling coil was cooled with tap water. To regenerate the column, after a run, it was only necessary to rinse the column for 20 min with water and 20 min with the pH 4.0 buffer. After about 20 runs the column was rinsed with H_2O then 3 M HCl (15 min), H_2O (20 min), 2 M NaOH (15 min), H_2O (15 min), and pH 4.0 buffer (20 min).

Recovery Studies. To 1-g aliquots of ground alfalfa (Buffalo) was added 5 mg of canavanine. Extraction was then carried out by each of the procedures (a, b, and c) on the 1-g aliquots with eight 1-g samples in each set. The assay procedure was then carried out as described above.

The mean recovery and standard deviation from 5 mg of canavanine added, with 8 replicates in each set, were for method 4.78 mg ± 0.21 b 4.48 ± 3.3, and c 4.61 ± 2.9. Thus by method a the mean recovery was 95.6%, b 89.6%, and c 92.2%.

Calculations. Figure 3 compares the chromatogram obtained with the standards to that from the jack bean. When 50 μL of this standard was injected, 50 nmol were added for arginine, guanidine, and GBA, 100 nmol for GSA, and 250 nmols for canavanine. Dividing these values by the peak heights, the nmol/division could be calculated as 1.79 for arginine, 1.37 for guanidine, 1.72 for GBA, 1.72 for GSA, and 9.26 for canavanine.

This was practicable since it was shown by adding increments of guanidinobutyric acid that a straight line, going through the origin, was obtained when peak height was plotted against concentration from 5 to 90% of the recorder scale; a regression line with a correlation coefficient (r) of 0.968 was obtained.

Chromatograms prepared in the absence of GBA showed that there was little interference at about 34–39 min for the clovers, jack bean, and alfalfas, and this substance was chosen as an internal standard. The concentrations of canavanine, arginine, and guanidine were calculated with the factors for nmol/division listed above. These were then corrected for variance in the height of the GBA peak.

Other diketones were explored as substitutes for phenanthrenequinone. These included 1,2-naphthoquinone-4-sulfonic acid and benzil. Excitation, when these compounds reacted with GSA, was at 307 and emission at about 435 nm. Sensitivity was about half that observed with phenanthrenequinone.

Benzoin, which has also been recommended (Kai et al., 1983), resembled benzil in its behavior and is probably oxidized to benzil in the strongly alkaline solution.

RESULTS

From Figure 3B, for the jack bean, a canavanine content of about 24–27 g kg^{-1}, with a mean at 26.5 g kg^{-1}, was found (Table I). This is in agreement with the values observed in the earlier report (Natelson et al., 1984). For the jack bean the canavanine to arginine ratio was about 31:1. The value is about the same whether mol kg^{-1} or g kg^{-1} is calculated, since the formula weights for canavanine (176) and arginine (174.2) are similar.

Figure 4 parts A and B compares the chromatograms obtained before and after reduction of the canavanine in red clover (*Trifolium pratense*). In Figure 4A, the canavanine peak is less than 10% of full scale, indicating that it is present in low concentration. After reduction (Figure 4B) the guanidine formed plus the small amount of endogenous guanidine result in a peak of about 50% full

Table I. Canavanine, Arginine, and Guanidine Content of Alfalfas, Clovers, and the Jack Bean as Determined by Column Chromatography

seed	canavanine,[a] g kg^{-1} (% coeff var)	arginine,[a] g kg^{-1} (% coeff var)	guanidine,[a] g kg^{-1} (% coeff var)	canavanine to arginine ratio
		Alfalfas (*M. sativa*)		
Weevlchek	13.6 (4.9)	1.39 (8.7)	0.0903 (10.2)	9.78
Saranac	10.9 (5.8)	1.13 (9.1)	0.0620 (11.1)	9.65
Team	10.7 (6.1)	0.920 (10.2)	0.0673 (10.0)	11.6
Arc	10.8 (6.7)	1.13 (8.0)	0.0903 (9.7)	9.55
Classic	9.90 (5.3)	0.929 (10.2)	0.0626 (9.8)	10.7
Buffalo	8.33 (5.3)	0.963 (8.1)	0.0655 (9.9)	8.65
		Clovers (*Trifolium*)		
ladino (*repens*)	1.94 (10.1)	2.49 (7.3)	0.0556 (10.1)	0.779
white (*repens*)	1.83 (9.8)	3.05 (7.9)	0.0425 (11.1)	0.600
red (*pratense*)	1.73 (9.7)	2.10 (8.5)	0.0528 (11.0)	0.824
jack bean (*C. ensiformis*)	26.5 (4.3)	0.857 (8.6)	0.203 (9.1)	30.9

[a] Mean values of 5 determinations.

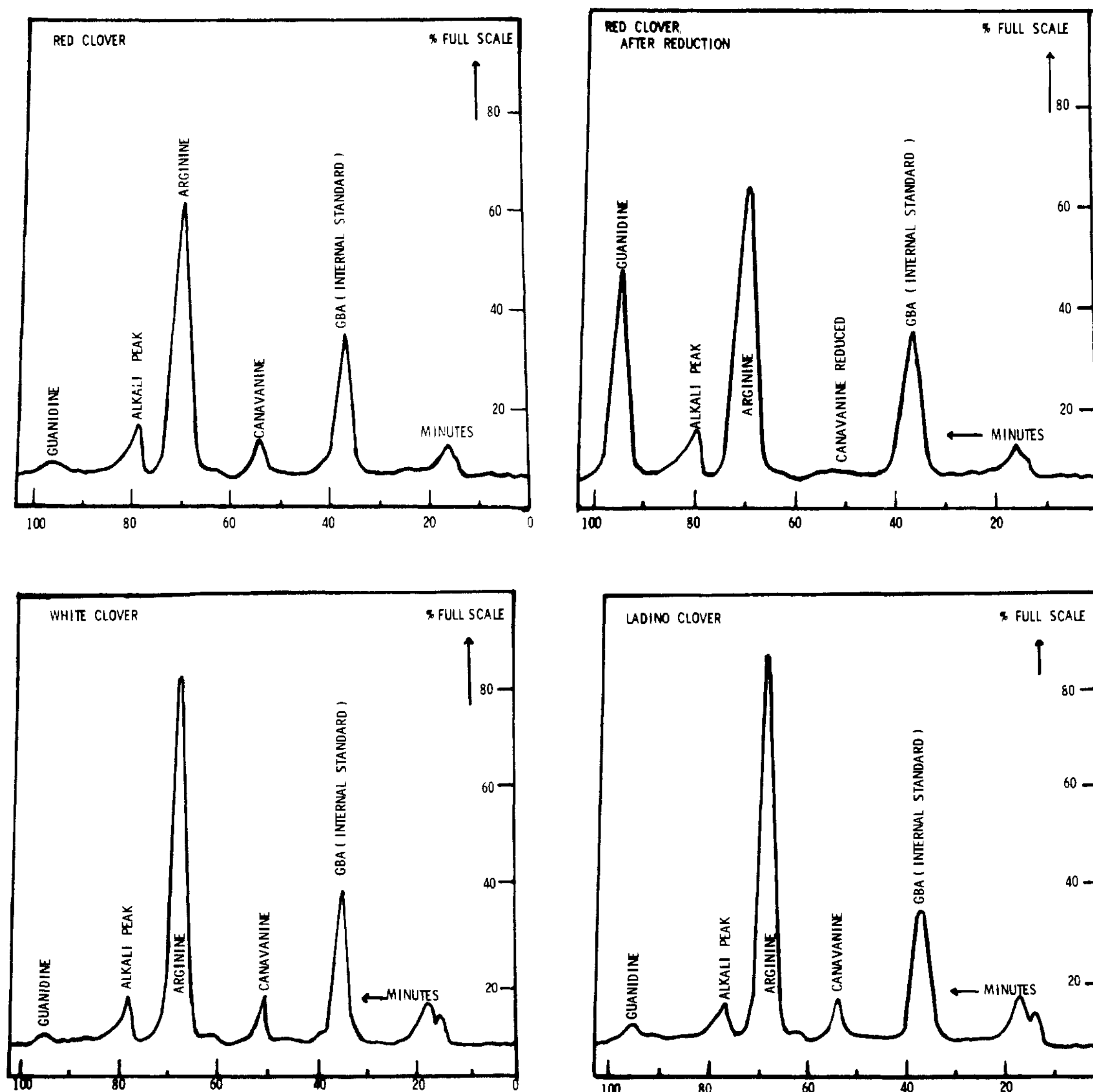

Figure 4. (A, upper left) Chromatogram obtained with the extract of 5 mg of red clover. (B, upper right) Tracing after the reduction of the extract showing the disappearance of canavanine and an increase in the guanidine peak. (C and D, lower left and right). The tracings obtained with white and ladino clover, respectively, before reduction.

scale. The guanidine formed by reduction is a measure of the amount of canavanine present.

This procedure has advantages other than the increase in sensitivity by a factor of $9.26/1.37 = 6.76$. After reduction, the fluorescing interfering substances which are not reduced can be seen in Figure 4B as a small rise above the base line. Repeated reduction did not eliminate this interference. It is partly due to the presence of histidine. A red or amber color could also be seen in the solution collected in the fraction collector at the canavanine peak. This is bleached on reduction. For this reason, in the present study, the mean canavanine content of the clovers

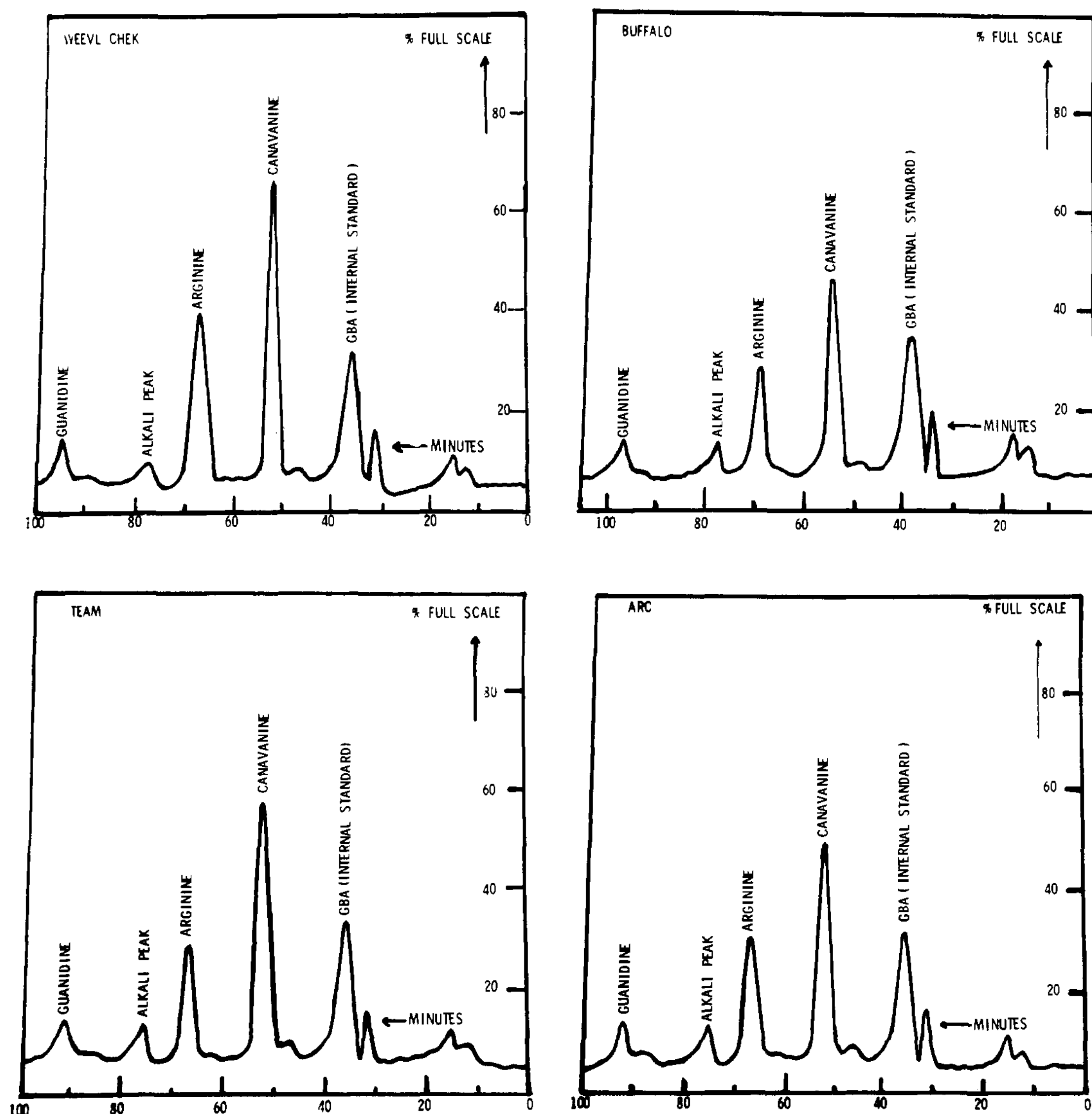

Figure 5. A (upper left) and B (upper right) compare the chromatograms obtained with 5-mg extracts of the alfalfa seeds containing canavanine in the highest (Weevlchek) and lowest (Buffalo) concentration of those tested. The lower part of the figure (C and D) compares the tracings of Team and Arc, two closely related alfalfa varieties.

(Table I) was significantly lower than those reported before by analyzing for canavanine in the clover directly without reduction. (Natelson and Bratton, 1984).

From Figure 4 parts A, C, and D it can be seen that red clover had the lowest canavanine and arginine content. Because of its high arginine content, white clover had the lowest C/A ratio, which was significant at the 0.01 probability level when the t-test for significance of mean differences was applied.

The guanidine content of the clovers is very low and is of the order of only about 50 mg kg^{-1} of seed (Table I). The peaks of guanidine and methylguanidine were not resolved under the chromatographic conditions used. However, colorimetric tests with the Sakaguchi reagent and the diacetyl reagent on the effluent in the fraction collector showed that the peak at 93–96 min was over 90% guanidine. In the jack bean, the canavanine concentration was high and the arginine concentration was only about $^1/_3$ that found in the clovers. This resulted in a very high C/A ratio. The guanidine level was about four times that found in the clovers (Table I).

Figure 5 parts A and B compares the chromatograms from a highly weevil resistant strain of alfalfa, Weevlchek (Farmers Forage Res. Coop., Lafayette, IN 47906, 1971), with that of a less resistant older variety, Buffalo (Kansas Ag. Exp. St., 1943). From Table I it can be shown that the concentration of canavanine in Weevlchek is significantly higher than that found in Buffalo, $P = <0.01$, confirming the observations in a previous study (Natelson and Bratton, 1984). In Weevlchek, the arginine concentration is significantly higher than that for Buffalo, $P = <0.05$. Thus the canavanine to arginine ratio (C/A) is not as high for Weevlchek as would be expected from the canavanine levels. The highest C/A ratio (11.5) was observed with Team (Figure 5C). The canavanine level in Team was intermediate between Weevlchek and Buffalo but the arginine level was low. This may explain partially the high weevil resistance of this alfalfa variety (Barnes et al., 1970). Arc, which was developed subsequently from Team (USDA Tech. Bull. No. 1571, 1977), has about the same canavanine content as Team. However the arginine content is significantly higher than that of Team and thus the C/A ratio is lower (Table I).

An alfalfa variety developed at Cornell University in 1965 is Saranac. There is also a later variety, Saranac A.R., introduced in 1976, which was analyzed here (Figure 6B). This contained about the same concentration of canavanine as Team or Arc and about the same amount of arginine. Thus, with regard to C/A ratio, Saranac and Arc are similar.

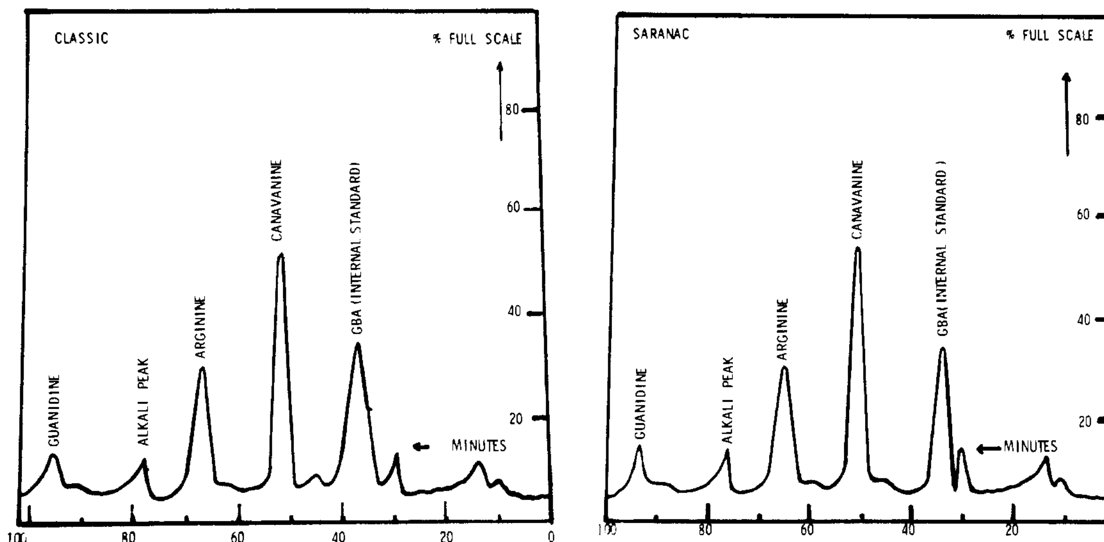

Figure 6. The alfalfa variety Classic (A, left), a widely used alfalfa variety in Eastern Tennessee, is compared with another commonly used variety, Saranac A.R. (B, right). The tracings represent extracts of 5 mg of the seeds.

Classic is an alfalfa variety used widely in east Tennessee. It was developed and marketed in about 1979 by the Farmers Forage Res. Coop. This is the same group that developed Weevlchek. Like Team, its arginine level is low. Its canavanine level is somewhat lower than Team but its C/A ratio approaches that of Team, being 10.7—second highest only to Team.

The arginine concentrations in the seeds of the alfalfas (mean = 1.08 g kg^{-1}) are less than half those found in the clovers (mean 2.55 g Kg^{-1}).

DISCUSSION

The alfalfa weevil (*Hypera postica*, Gyllenhal) was first found in the United State (Utah) in 1904. It did not appear in Tennessee until 1959. By 1968 the depredation of the weevil had reduced the alfalfa acreage under cultivation in Tennessee from a high of over 250 000 to about 50 000 acres (Bennett, 1968). The use of insecticides and the introduction of weevil resistant varieties caused a resugence of interest in alfalfa as a forage crop, and by the end of 1983 about 140 000 acres of alfalfa were under cultivation (Tenn. Farm Facts, 1984). While chemical sprays are effective in controlling the insect pests, their toxicity limits the types which have been permitted to be used. For example, Hexachlor has been banned in the United States. Further, as insecticides are used repeatedly, resistant strains of insects emerge.

Canavanine has been shown to be toxic to all species including neurospora (Horowitz and Srb, 1948), bacteria (Volcani and Snell, 1948), insects (Rehr et al., 1973; Dahlman and Rosenthal, 1976; Isogai et al., 1973; Vanderzant and Chremos, 1971), and mammalian normal and tumor cells (Doyle et al., 1983; Green et al., 1980; Naha, 1980). This is due mainly to competition with arginine, required for protein synthesis, for binding sites (Neurath et al., 1979). Thus, an insect whose arginine *t*-RNA synthetase is highly specific for arginine binding over canavanine can tolerate substantial amounts of canavanine (Rosenthal et al., 1972, 1977). In addition, canavanine resistant bacteria as well as all mammals have reductases which detoxify canavanine by cleavage to homoserine and guanidine (Kalyankos et al., 1958; Takahara et al., 1978; Natelson, 1984). Alternatively the canavanine can be hydrolyzed by arginase to yield canaline and urea. In certain insects urease is present in high concentration and can hydrolyze the urea to reutilize the nitrogen (Dahlman and Rosenthal, 1976; Rosenthal et al., 1972; Rosenthal, 1977). The canaline can then be reduced to ammonia and homoserine. Where canavanine is present in excess it usually does not kill the larva of insects but after they pupate the insect does not emerge.

Certain intestinal bacteria can detoxify canavanine by deamination (Kihara and Snell, 1957; Kalyankos et al., 1958). This explains why ruminants can tolerate large amounts of canavanine.

The jack bean has been known as the chickasaw lima bean since it was cultivated and used as food and fodder, after grinding and washing with water whereby substantial amounts of canavanine were removed. Excess canavanine is also excreted in the urine (Natelson, 1984).

The common alfalfa from Spanish sources (Chilean) such as Buffalo is a lesser weevil resistant variety. The other five varieties, Weevilchek (mainly Indian) and Team, Arc, and Saranac, which are derived from Flemish sources, are considered weevil resistant strains (USDA Tech Bull., 1977).

All of the work in developing weevil resistant varieties has ignored the canavanine content of the alfalfa as a significant factor. Table I suggests a set of criteria which may also be considered in developing a highly insect resistant variety. This suggests that a high canavanine, low arginine variety be developed, which approaches the jack bean in the canavanine/arginine ratio, and has desirable field characteristics. The significant difference exemplified between Weevlchek and Buffalo suggests that this could be achieved practically.

The procedure of comparing the chromatograms before and after reduction has the advantages of an almost 7-fold increase in sensitivity and the assurance that canavanine is being assayed. For example, in the case of the clovers, substantially higher canavanine concentrations were reported because of the presence of interfering substances which could not be removed by columm chromatography (Natelson and Bratton, 1984). This suggests a solution to an analytical problem which has been stressed by others (Rosenthal and Davis, 1975; Rosenthal and Dahlman, 1982).

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Registry No. Canavanine, 543-38-4; arginine, 74-79-3.

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Direct Detection of Solanesol in Tobacco by ^{1}H and ^{13}C Magic Angle Spinning NMR

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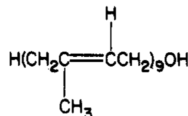
^{1}H and ^{13}C NMR have been used to detect solanesol directly in tobacco without destroying or modifying the sample. Magic angle sample spinning was employed to remove the resonance line broadening due to variations of magnetic susceptibility within the sample. ^{13}C line widths of ca. 10 Hz were obtained. The ^{1}H MAS spectrum of tobacco allows the solanesol signals to be resolved from the broad signal of exchangeable protons. ^{13}C spin-lattice relaxation times (T_1) and nuclear Overhauser enhancements (NOE) of solanesol in chloroform solution, in intact tobacco, and as neat oil indicate that the polyisoprene chain motion in tobacco is restricted relative to the motion in solution but still sufficient to average out the dipolar couplings between protons and carbons.

INTRODUCTION

Solanesol in Tobacco. Polyisoprenoid alcohols are found in diverse life forms including higher plants, mammalian tissues, amd microorganisms. These natural products are composed of isoprene units linked head-to-tail to form linear chains containing between 6 and 24 units depending on the source. Most of the polyprenols consist of both cis and trans units but solanesol is recognized to be composed of all trans isoprene units. Solanesol is a



common leaf constituent in plants which was first isolated from tobacco by Rowland et al. in 1956. In tobacco, solanesol occurs at levels as high as 4% of the dry leaf lamina weight, making solanesol the most abundant tobacco terpenoid. In other plant species solanesol occurs at significantly lower levels. In bacteria and mammalian tissues, phosphodiesters of polyprenols act as chemical carriers of saccharide units in the synthesis of complex polysaccharides (McCloskey and Troy, 1980; Morton, 1972). The physiological function of solanesol in green plants, however, remains undefined, and the significance of the greater solanesol content in tobacco relative to other plant species is not understood (Sheen et al., 1978).

Despite the high levels of solanesol in tobacco, there does not appear to be a direct relationship between solanesol and leaf quality (Davis, 1976). Solanesol is likely to influence cigarette smoke aroma, however, since pyrolysis at temperatures up to 550 °C yields mono- and diterpenes as well as isoprene (Grossman et al., 1962; Grossman et al., 1963). At temperatures above 650 °C, solanesol pyrolysis

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