Vicianin, Prunasin, and β-Cyanoalanine in Common Vetch Seed as Sources of Urinary Thiocyanate in the Rat

Charlotte Ressler* and Jayant G. Tatake

Department of Pharmacology, University of Connecticut Health Center, Farmington, Connecticut 06030-6125

When young rats were fed a diet containing common vetch seed for 1 month, they excreted in the urine ~7 times more thiocyanate than they had ingested. Vicianin, prunasin, and β-cyanoalanine were identified as principal dietary sources of the excreted thiocyanate. Vicianin was isolated by chromatography and crystallization. Its structure was confirmed by mass spectrometry and by identification of its monosaccharides and aglycon. Prunasin was identified chromatographically by HPLC. The combined seed content of vicianin (0.68 μmol/g) and prunasin (0.16 μmol/g) corresponded to the cyanogen content of the seed (0.91 ± 0.14 μmol/g; $n = 7$), determined as cyanide after autolysis. When vicianin was fed, the urinary thiocyanate output was 21% of the ingested amount of vicianin, whereas β-cyanoalanine yielded a urinary thiocyanate output of <0.2%. Calculations show that 73% of the thiocyanate can be derived from vicianin and prunasin, with 27% derived from β-cyanoalanine. High urinary output of thiocyanate has been associated with endocrine and neurological disorders.

Keywords: *Cyanide; thiocyanate; sulfate; common vetch; Vicia sativa L.; vicianin; prunasin; legume cyanogen; β-cyanoalanine*

INTRODUCTION

The seed of the common vetch (*Vicia sativa* L.) is marketed presumably as a lower priced substitute for the lentil (*1*, *2*). The seed contains toxins, including β-cyanoalanine (**1**), cyanogen, and vicine (*3*, *4*). Although both **1** and vetch seed are highly neurotoxic for chicks (*5*), no information is available as to whether they are also toxic for humans. Chronic cyanide toxicity of dietary origin has been associated with a variety of neurological disorders, such as tropical ataxic neuropathy, spastic paraparesis, ankle clonus, and konzo (*6*−*9*). In a study of the safety of common vetch seed when consumed as a food, we have examined the formation of cyanide by determining the excretion of thiocyanate, the major metabolite of cyanide, which is less volatile and can serve as a good marker of in vivo cyanide formation. For this purpose rats were fed for 1 month on a diet of 85% common vetch seed, which contains **1** largely in the form of γ-glutamyl-β-cyanoalanine (γ-glu-**1**). **1** was also fed alone in a semipurified diet. Inasmuch as cyanogen found in common vetch seed may be another source of cyanide and thiocyanate, we isolated the major cyanogen by a five-step procedure followed by crystallization and identified it by spectrometry as well as by its component sugars and aglycon after hydrolysis. A minor cyanogen was also identified chromatographically. HPLC systems were developed to facilitate the identification and quantitation of these legume cyanogens. Figure 1 shows the structures of the major toxins in common vetch seed. Conversion of the isolated, purified vicianin (**2**) to urinary thiocyanate has been evaluated in the rat and compared with **1**.

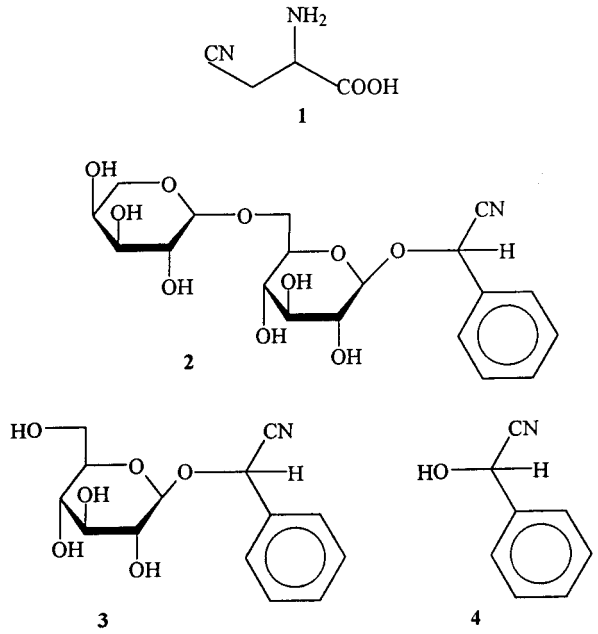

Figure 1. Structures of **1**, β-cyanoalanine; cyanogenic glycosides **2**, vicianin, and **3**, prunasin, in common vetch seed; and **4**, the related cyanohydrin, mandelonitrile.

MATERIALS AND METHODS

Amberlite XAD-2 resin was from Supelco, Bellefonte, PA; Bio-Sil A was from Bio-Rad, Richmond, CA; Dowex 50W-X8, *Helix pomatia* β-glucuronidase, and prunasin were from Sigma, St. Louis, MO. Amygdalin was from Aldrich, Milwaukee, WI. β-Cyanoalanine was synthetic material (*10*).

Common vetch seed, thought to be an improved Willamette variety, was purchased from Seedway, Inc. (York, PA) in 1993 and 1995. The specimen used for isolation contained 0.036% (0.91 μmol/g) cyanogen, 0.49% (20.2 μmol/g) γ-glu-**1**, and 0.09%

* Author to whom correspondence should be addressed [telephone (860) 679-2510; fax (860) 679-3693; e-mail ressler@nso2.uchc.edu].

10.1021/jf010343w CCC: $20.00 © 2001 American Chemical Society
Published on Web 09/13/2001

(7.89 μmol/g) **1**. The specimen fed contained 23.2% protein, 0.027% (0.67 μmol/g) cyanogen, 0.128 μmol/g thiocyanate, 0.74% (30.45 μmol/g) γ-glu-**1**, and 0.02% (1.75 μmol/g) **1** (*4*). The control diet (Teklad), obtained from Harlan Teklad, Madison, WI, was a semipurified 15% protein diet based on casein and was well balanced with the exception of the protein content, 3.76 kcal/g; 0.44% total sulfur amino acid; 0.043% cys; 0.036 and 0.018 μmol/g thiocyanate in two batches. The vetch diet consisted of 85% vetch seed plus 15% Teklad. It contained 22.0% protein, 3.88 kcal/g; 0.32% total sulfur amino acid; 0.12% cys; and 0.114 μmol/g thiocyanate. The rat chow, Teklad LM-485 from Harlan Teklad, contained 19.92% protein, 4.05 kcal/g; 0.7% total sulfur amino acid; and 0.033 μmol/g thiocyanate.

Male Sprague−Dawley weanling rats were from Charles River Laboratories, Wilmington, MA, and were maintained on food and water ad libitum. Animals were acclimated to a 12-h light−dark cycle prior to use. Treated rats were housed individually in raised floor cages. Urine was collected in plexiglass metabolism cages as 24-h samples in the presence of a few crystals of thymol.

Thiocyanate in urine was determined as described previously (*11*). Recoveries ranged from 89 to 100%. The thiocyanate content of common vetch seed and the control diets was determined after three extractions with water (5 mL/g) and washing of the extract with ether. Cyanogen in urine was determined by incubation of 0.25 mL with 0.25 mL of dialyzed common vetch seed cyanogen hydrolase containing 1.75 mg of protein for 24 h, followed by distillation and determination of cyanide as previously described (*4*). Inorganic sulfate in urine was determined turbidimetrically (*12*). Benzaldehyde was identified chromatographically (*4*). Arabinose and glucose as constituent sugars were identified by hydrolysis of common vetch cyanogen in dilute trifluoroacetic acid as described for fern vicianin (*13*), modified by use of the aniline−diphenylamine−phosphoric acid spray for detection (*14*). Low-resolution field desorption mass spectrum (FDMS) analysis was performed on a Micromass 70-VSE spectrometer. The reversed-phase HPLC system has been previously described (*15*).

Administration of Common Vetch Seed, β-Cyanoalanine, and Vicianin. A total of 10 weanling rats received 85% common vetch seed/15% Teklad casein (15% protein) diet, and 10 weanling rats received the casein diet as controls. Two trials, 3 years apart, lasting 30 and 21 days, were run, and the data combined. Urine was collected intermittently from the treated rats between days 9 and 29 (19 samples) and on the following day from the controls (15 samples) (see Table 2).

Three weanling rats, maintained on the control diet for 10 or 13 days, were placed in metabolism cages and daily urine collection was started. Two days later, when they weighed 109−131 g, 0.35% of **1** was added to the control diet for 2 days followed by the control diet (without **1**) for another 2 days.

Four groups of two weanling rats each were placed in metabolism cages. One group on the control diet received per os 23.5 mg/100 g of **2**. The second group on laboratory chow received 2.7 mg/100 g, a lower dose that approximates the daily intake of **2** on the 85% vetch diet. The other groups served as respective controls. Urine was collected daily for 6 days from all animals.

Body weight, food consumption, and urine volume were measured daily, and thiocyanate, sulfate, and cyanogen were determined in the urine samples.

Isolation and Identification of Common Vetch Cyanogens. Common vetch seed, 2 × 250 g, was finely pulverized in the cold in 2 × 750 mL of 10% trichloroacetic acid in a Waring blender. The suspension was centrifuged at 18000*g*, and the residue was re-extracted with 500 mL of trichloroacetic acid. The combined supernatant was shaken four times with ether. The extract was freed of ether and then adjusted to pH 6.5. After storage overnight in the cold, the white precipitate was removed by centrifugation. The extract was concentrated to 250 mL and passed in two portions over a column (2.6 × 18 cm) of Amberlite XAD-2, washed with 200 mL of water, and eluted with 200 mL of MeOH (*16*). Cyanogen-containing fractions were identified initially by a spot test (*17*) and subsequently by the HPLC method we developed (see below). The MeOH eluate was taken to dryness. The residue was dissolved in water, adjusted to pH 2 with 1 N HCl, and applied to a column (0.9 × 4 cm) of Dowex 50W-X8, 200 mesh. The flow-through and water wash were collected, adjusted to pH 6.5 with dilute NH_4OH, and taken to dryness. The residue was taken up in 5 mL of MeOH, and the solution was applied to silica (3 g) and then chromatographed on a column of silica (2.5 × 15 cm) equilibrated with MeOH/EtOAc (5:95) (*18*). The material was eluted with 10% MeOH/EtOAc in fractions of 3−3.5 mL that were analyzed by HPLC (Figure 2). Fractions containing the cyanogen were pooled, concentrated, and then rechromatographed on a silica column (1.5 × 20 cm), with fractions of 2.5−3 mL. Fractions 24−60 on concentration yielded a white residue that was crystallized from MeOH/benzene (wt = 42 mg of **2** of 95% purity).

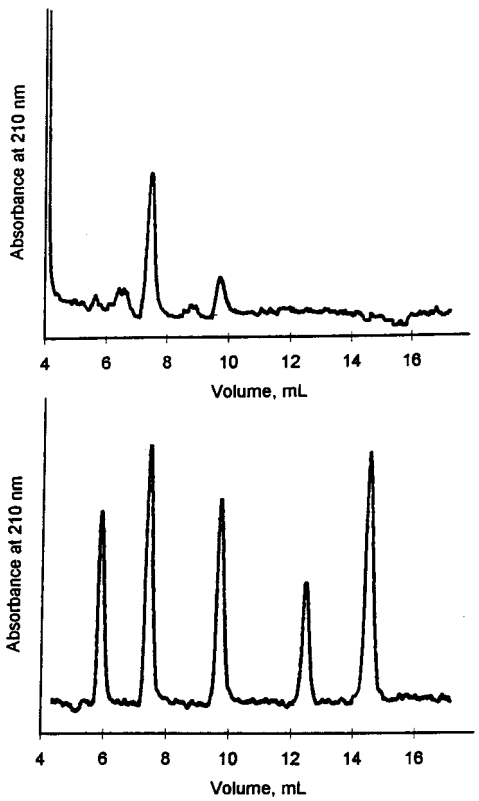

Figure 2. High-performance liquid chromatograms of aromatic cyanogenic glycosides and reference alcohols; (lower) reference, in sequence, amygdalin, vicianin, prunasin, benzyl alcohol, and anisyl alcohol (see Table 1), all on a Rainin Microsorb-MV C_{18} (5 μm, 100 A) column (4.6 mm × 25 cm) with 15% acetonitrile, flow rate = 1 mL/min (standard calibration equation for vicianin is $y = 1.144x - 0.719$, where y is peak area in mm^2 and x is concentration in μg/mL); (upper) an 80% methanol extract of 1.33 mg of common vetch seed showing vicianin and prunasin.

The content of **2** in the seed (0.03%) was obtained by autolysis (*4*) with correction for the content of **3** that was obtained by HPLC of 80% MeOH extract of the seed. In the other samples **2** was determined directly by HPLC as in Figure 2. The yield of **2** in each step was 80−85% except for 69% on crystallization. Purification was >3100-fold.

Prominent FDMS peaks were *m*/*z* 116.1 (cyanobenzyl), 133.0 (mandelonitrile−H)$^+$ and (terminal arabinose cleaved at glycosidic O), 428 (vicianin−H)$^+$, and 450 (vicianin−Na)$^+$, in agreement with that reported by Dreifuss et al. (*19*). On HPLC the purified material showed a single peak (R_v 7.2−7.8 mL, 210 nm) in 15% acetonitrile and at R_v 12 mL in the gradient 0−40% acetonitrile/water. Incubation of 31 μg of the material at pH 6.4 for 20 h with a dialyzed extract of the vetch seed containing 0.47 mg of protein resulted in complete loss of the HPLC peak, with a cyanide yield of 93.5%. The

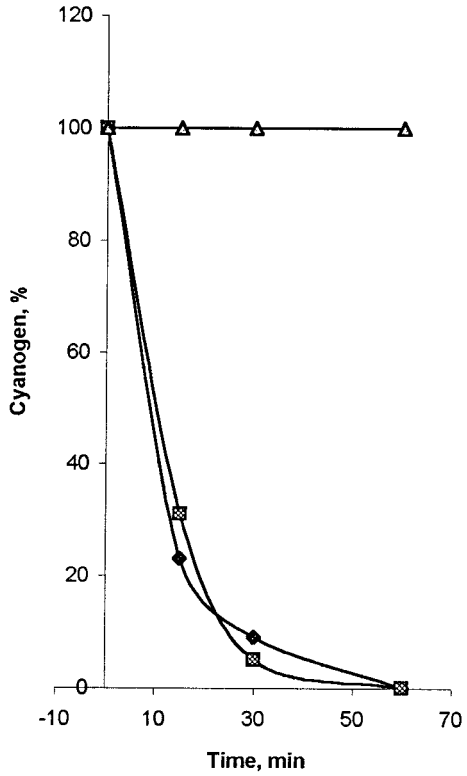

Figure 3. Relative reactivity of cyanogens to *H. pomatia* glucuronidase. Solutions of 10 mM amygdalin (◆), prunasin (■), and vicianin (△) were incubated in 130−169 μL of 0.05 M sodium acetate buffer with 60 units of *H. pomatia* glucuronidase at 30 °C. Samples were taken at intervals, diluted with an equal volume of acetonitrile, and centrifuged. Cyanogen was determined by HPLC as in Figure 2.

concentration of vicianin, determined chromatographically, in 10% trichloroacetic acid extracts of the seed was 0.029% (0.68 μmol/g).

Prunasin (**3**) was detected chromatographically, at a seed concentration of 0.16 μmol/g, in the 10% trichloroacetic acid extract, as well as in an 80% MeOH extract of the seed pulverized in liquid nitrogen. It was removed during subsequent chromatography of the product on silica. When a solution of the trichloroacetic acid extract was treated with *H. pomatia* β-glucuronidase (*20*) for 3 h, the HPLC peak corresponding to **3** was lost quantitatively, 97% of **2** was retained, and benzaldehyde was formed qualitatively. By limiting the incubation to 1 h, it was possible to detect the less stable mandelonitrile chromatographically. The relative reactivity of authentic prunasin, amygdalin, and the isolated crystalline vicianin toward *H. pomatia* glucuronidase is shown in Figure 3. Although stable as compared to prunasin and amygdalin, vicianin nevertheless decomposed slowly by 59, 86, and 96% on further incubation on days 1, 2, and 3, respectively. Table 1 lists the retention volumes on HPLC of the three cyanogens as well as of reference benzyl alcohol (BA) and anisyl alcohol (AA) in two systems.

RESULTS

Thiocyanate Formation. Table 2 lists the urinary excretion of thiocyanate and the recovery of cyanogen under the various conditions studied. Urinary output is on a daily basis. For **1** and **2** as precursors, thiocyanate and cyanogen values represent the average daily accumulation in the 4 or 6 days, respectively, following administration. The rats fed the 85% common vetch seed diet excreted 6.3 μmol/24 h of thiocyanate, an amount ∼7 times greater than that ingested. Administration of **2** as a single dose of 23.5 mg/100 g led in the first 24 h to a peak excretion of 3 mM thiocyanate, which was 18 times the control value. A lower dose, 2.7 mg/100 g, approximating the daily consumption of cyanogen in the 85% vetch seed diet, led to 0.2−0.8 mM thiocyanate (data not shown). Recovery of cyanogen in urine from **2** at the two dosages was <10%. The diet incorporating 0.35% of **1** resulted in a low (0.52 μmol/24 h) but elevated excretion of thiocyanate as compared to controls ($P < 0.001$). Cyanogen excretion was extremely low (0.083 μmol) but was likewise elevated as compared to controls ($P = 0.001$).

Table 1. HPLC Chromatographic Constants of Common Vetch Cyanogenic Glycosides, Amygdalin, and Two Reference Alcohols

	system[a]					
	A			B		
compound	V_r	$V_r/V_{r,BA}$	$V_r/V_{r,AA}$	V_r	$V_r/V_{r,BA}$	$V_r/V_{r,AA}$
amygdalin	6.0	0.48	0.41	5.3	0.48	0.42
vicianin	7.6	0.61	0.52	6.7	0.60	0.53
prunasin	9.8	0.78	0.68	7.4	0.67	0.59
BA	12.5	1.00	0.86	11.1	1.00	0.88
AA	14.5	1.16	1.00	12.6	1.14	1.00

[a] System A, 15% aqueous acetonitrile; system B, 30% aqueous methanol on a Rainin Microsorb MV 5 μm reversed phase 4.6 mm × 25 cm column; flow rate = 1 mL/min; λ 210 nm.

Figure 4A shows the marked increases in the thiosulfate/sulfate ratio in urine resulting from a single oral dose of **2** in two rats. The lack of increase in the control not adminstered **2** is also seen. The response to fed β-cyanoalanine is shown in Figure 4B. The effect of **2** was ∼5 times that of **1** at 11% the dosage of **1**. There was no clear effect of **1** on the excretion of sulfate.

DISCUSSION

Certain tropical populations that consume the important food staple cassava, which after improper preparation still contains the cyanogenic glycoside linamarin or its derivatives, excrete large amounts of thiocyanate in their urine (*9*, *21*). By analogy with the cassava effect, it occurred to us that cyanogen in vetch might also cause increased urinary output of thiocyanate. Although recognized, the significance of cyanogen in legumes has not always been well appreciated. In common vetch seed concentrations have been reported that vary between 0.1 mg/100 g and the potentially lethal 52 mg/100 g, expressed as cyanide potential (*22*−*25*). The specimens on hand contained 0.9−2.5 mg/100 g (0.33−0.91 μmol/g) (*4*).

In the present study the major cyanogen in *V. sativa* has been isolated by pulverization and extraction of the seed in trichloroacetic acid to inactivate hydrolases, followed by chromatography and group fractionation on aromatic and cation exchange resins. This was followed by chromatography and rechromatography on silica and crystallization, the latter procedures used for the isolation of vicianin from the fern *Davallia bullata* (*18*). The major cyanogen has been confirmed to be vicianin (mandelonitrile vicianoside, **2**) on the basis of its hydrolysis in acid to the expected arabinose and glucose, our previous identification of the aglycon as mandelonitrile (*4*), and comparison of its mass spectrum with that reported on material isolated from *V. sativa* without information (*19*).

Vicianin was first isolated and identified from *Vicia angustifolia* seeds in 1906 (*26*). It was later found in a

Table 2. Urinary Excretion of Thiocyanate and Cyanogen by Rats Fed Common Vetch Seed, β-Cyanoalanine, and Vicianin[a]

		thiocyanate[c]		cyanogen	
diet	body wt[b] (g)	μmol/24 h	yield (%)	μmol/24 h	recovery (%)
control[d]	128.1 ± 3.2 (n = 15)	0.15 ± 0.04* (n = 15)		0.043 ± 0.004** (n = 9)	
+0.35% β-cyanoalanine	129.9 ± 3.3 (n = 8)	0.52 ± 0.08[e]* (n = 12)	0.16	0.083± 0.008** (n = 7)	0.01
+85% common vetch seed[f–h]	100.1 ± 6.6 (n = 11)	5.33 ± 0.36[i,j] (n =12)	63.3[k]	0.19 ± 0.06 (n = 5)	2.22
	100.9 ± 15.9 (n = 8)	2.49 ± 0.2[l] (n = 7)	33.7[k]	0.33 ± 0.03 (n = 8)	3.32
+vicianin, 18.8 mg	97.6 ± 4.6 (n = 10)	1.56 ± 0.69[e] (n = 12)	21.2	0.53 ± 0.24 (n = 12)	7.14

[a] Values expressed as means ± SEM. Values within parentheses indicate the number of samples. *P < 0.001; **P = 0.001. [b] On days urine samples were taken for analysis. [c] Corrected for dietary intake of thiocyanate. [d] Teklad semipurified, 15% protein. [e] Average daily excretion within 4 days of administration of β-cyanoalanine; 6 days for vicianin. [f] Common vetch seed specimen contained 0.67 μmol/g of cyanogen and 0.74% γ-glutamyl-β-cyanoalanine (*4*). [g] Upper figures, 1997 data; lower, 2000 data. [h] Corresponding controls weighed 143.1 ± 13.5 (n = 6) and 184.2 ± 14.2 g (n = 6). [i] Data from four rats in a 20-day period selected among days 9–29. [j] Uncorrected for intake, value is 6.26 ± 1.24 (n = 12). [k] Thiocyanate excreted/cyanogen in seed fed × 100. [l] Data from four rats on days 13 and 19.

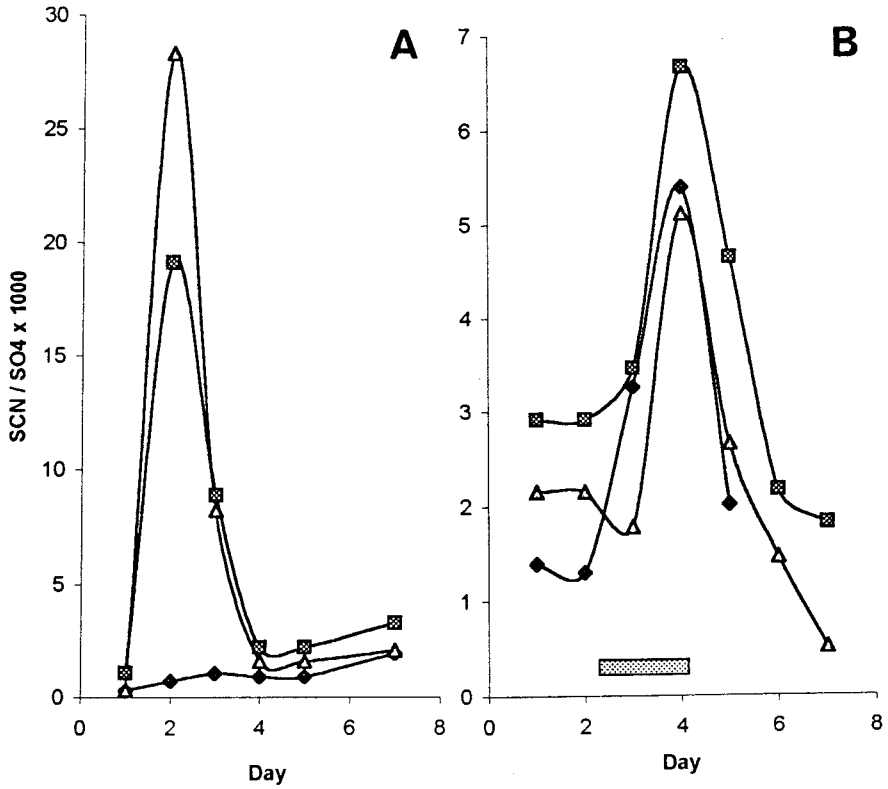

Figure 4. Daily thiocyanate/sulfate ratios in urine normalized to consumption of 10 g of food: (A) before and after administration of vicianin, 44 μmol per os, to two male rats, 80 g (■, △), on day 2 [the control received the vehicle (◆)]; (B) before and after a diet of 0.35% β-cyanoalanine was given to three male rats, 116–131.5 g, for the 2 days shown by the shaded rectangle. The average sulfate excretions were 142.6 ± 8.5 (A) and 129 ± 4.9 μmol/24 h/10 g of food (B), respectively.

number of fern species (*13*, *18*, *27*). Until now, the lack of reference material made it necessary to extract and purify **2** and to subject it to degradation, MS, and ^{1}H and ^{13}C NMR for identification. Other cyanogenic glycosides have been determined by HPLC with detection by refractometry or postcolumn enzymatic cleavage followed by electrochemical detection of cyanide (*20*, *28*). In this investigation we show that common vetch vicianin and prunasin, and commercially available amygdalin, can be characterized by HPLC on a C_{18} reversed phase column in two simple isocratic systems and detected by UV absorption. Retention volumes of the three cyanogens have been expressed individually and also relative to benzyl and anisyl alcohols. When applicable, our techniques can identify and determine vicianin while avoiding the need for repeated isolation and extensive spectroscopy.

Prunasin constituted 19–25% of the cyanogen found in common vetch seed. Its identity was based on chromatographic comparison with authentic material and was consistent with its susceptibility to rapid degradation by *H. pomatia* β-glucuronidase (*20*), during which benzaldehyde and mandelonitrile were detectable. Under the same conditions vicianin was stable, although subject to slow degradation over a period of 3 days. Because two different methods of seed extraction yielded comparable concentrations, it seems unlikely that the prunasin arose hydrolytically from vicianin as an artifact of extraction.

Source of Thiocyanate Following Consumption of Vetch. The vetch diet containing **1**, largely as 0.63% γ-glu-**1**, and 0.023% cyanogen (av mol wt = 395) caused a significant excretion of thiocyanate. Even when given as a single dose, purified **2** caused thiocyanate excretion to increase markedly. By contrast, feeding **1** at a level comparable to that found in the seed resulted in a low but statistically significant rise in thiocyanate excretion. In the case of **1**, thiocyanate output in the urine

constitutes a more sensitive probe of cyanogenesis than cyanide generation in vitro (*29*).

In conclusion, there is little question that the cyanogens that occur in common vetch seed, that is, vicianin and prunasin, are a source of the increased urinary output of thiocyanate in the rat. Moreover, β-cyanoalanine is not an efficient precursor of cyanide and thiocyanate. However, because the quantity of total **1** is much greater than that of cyanogen (32.2 and 0.67 μmol/g, respectively), a minor fraction of the ingested β-cyanoalanine might contribute to the thiocyanate output. When the thiocyanate content of the diet is taken into account, the amount of thiocyanate excreted could represent either up to 63% conversion of the cyanogen or 1−2% conversion of the **1**. In a semipurified diet the yield of thiocyanate was 0.16% from **1** and 21% from **2**. In other words, 27%, $[(0.0016 \times 32.2)/(0.21 \times 0.67 + 0.0016 \times 32.2)] \times 100$, of the thiocyanate could have originated from the β-cyanoalanines and 73% from the cyanogens in the seed. Although in vetch the exact contribution of each precursor cannot be defined by the method used here, it seems likely that both cyanogens and β-cyanoalanines contribute to the biosynthesis of thiocyanate. This inference is reflected also in the increases in the urinary thiocyanate/sulfate ratios shown when each was administered separately in the same basal diet (Figure 4). That other precursors of thiocyanate, such as glucosinolates (*30*), occur in common vetch seed has not been ruled out. The recovery of **2** as cyanogen in urine at two dosages was 7−9%, somewhat lower than the 19% observed for linamarin in rats (*31*). Recovery of cyanogen from vetch was even less (2−3%). It is uncertain whether this represents extensive metabolism of **2** or excretion through an uninvestigated route.

Thiocyanate excretion is confirmed in this study to be a reasonable reflection of cyanide exposure in the rat. However, it is not quantitative. A variety of other pathways are recognized to contribute to the disposition of cyanide, including expiration through the lungs and conversion to cyanomethemoglobin (*32*), further metabolism of thiocyanate, oxidation to cyanate, and incorporation into 2-aminothiazolidine-4-carboxylic acid (*33*). The latter two metabolites, usually minor, have been suggested to be mediators of aspects of chronic cyanide neurotoxicity in the presence of sulfur amino acid deficiency, when they increase in amount (*34*−*36*).

On the assumption that the dietary supply of cystine for humans is sufficient to allow detoxification of the cyanide to thiocyanate derived from the cyanogens and β-cyanoalanines in vetch, the question arises as to whether the thiocyanate in turn could be harmful. A cassava-eating population from Zaire had goiter rates of 60−70% at the same time excreting 0.33 mM thiocyanate in the urine (*21*). However, a low iodine intake is likely to have contributed to the goiter incidence (*37*).

Thiocyanate has been considered to be a candidate etiologic agent for the upper motor neuron degeneration in konzo (*38*). Children and adults consuming cassava and affected by konzo had urine concentrations of 0.76 and 0.9 mM thiocyanate, respectively (*9*).

Thiocyanate is an allosteric regulator of the AMPA-selective (quisqualate subtype) Glu receptor, stabilizing AMPA binding to this receptor (*39*) and thereby enhancing desensitization (*40*). Seizures, learning, and memory are all considered to be adversely affected by AMPA receptor desensitization (*41*). Whether the generation of thiocyanate following excessive vetch consumption also plays a role in AMPA-related conditions should be considered.

If a diet does not contain sufficient cystine to detoxify cyanide and has a high content of common vetch seed, it may be capable of eliciting chronic cyanide toxicity. A simple procedure to avoid potential toxic effects is to modify the cooking procedure for vetch by decanting off the broth containing the cyanogens, β-cyanoalanine, and vicine (*4*, *42*, *43*).

ABBREVIATIONS USED

AA, anisyl alcohol; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; BA, benzyl alcohol; FDMS, field desorption mass spectrum; HPLC, high-performance liquid chromatography.

ACKNOWLEDGMENT

Low-resolution field desorption mass spectrum was kindly obtained by S. L. Mullen in the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois. The 70-VSE mass spectrometer was purchased in part with a grant from the Division of Research Resources, National Institutes of Health (RR 04648).

LITERATURE CITED

(1) Tate, M. E.; Enneking, D. A mess of red pottage. *Nature* **1992**, *359*, 357−358.
(2) Tate, M. E.; Rathjen, J.; Delaere, I.; Enneking, D. Covert trade in toxic vetch continues. *Nature* **1999**, *400*, 207.
(3) Ressler, C. Isolation and identification from common vetch of the neurotoxin β-cyano-L-alanine, a possible factor in neurolathyrism. *J. Biol. Chem.* **1962**, *237*, 733−735.
(4) Tatake, J. G.; Ressler, C. Cyanogen, vicine, and β-cyanoalanine toxins in a common vetch (*Vicia sativa* L.) food, and their removal. In *Recent Research Developments in Agricultural and Food Chemistry*; Pandalai, S. G., Ed.; Research Signpost: Trivandrum, India, 1999; Vol. 3, pp 435−444.
(5) Ressler, C.; Nigam, S. N.; Giza, Y.-H. Toxic principle in vetch. Isolation and identification of γ-L-glutamyl-L-β-cyanoalanine from common vetch seeds. Distribution in some legumes. *J. Am. Chem. Soc.* **1969**, *91*, 2758−2765.
(6) Osuntokun, B. O. Cassava diet, chronic cyanide intoxication and neuropathy in the Nigerian Africans. *World Rev. Nutr. Diet.* **1981**, *36*, 141−173.
(7) Cliff, J.; Essers, S.; Rosling, H. Ankle clonus correlating with cyanide intake from cassava in rural children from Mozambique. *J. Trop. Pediatr.* **1986**, *32*, 186−189.
(8) Howlett, W. P.; Brubaker, G. R.; Mlingi, N.; Rosling, H. Konzo, an epidemic upper motor neuron disease studied in Tanzania. *Brain* **1990**, *113*, 223−235.
(9) Tylleskar, T.; Banea, M.; Bikangi, N.; Cooke, R. D.; Poulter, N. H.; Rosling, H. Cassava cyanogens and konzo, an upper motorneuron disease found in Africa. *Lancet* **1992**, *339*, 208−211.
(10) Ressler, C.; Ratzkin, H. Synthesis of β-cyano-L-alanine and γ-cyano-α-L-aminobutyric acid, dehydration products of L-asparagine and L-glutamine, a new synthesis of amino acid nitriles. *J. Org. Chem.* **1961**, *26*, 3356−3360.
(11) Lundquist, P.; Kagedal, B.; Nilsson, L. An improved method for determination of thiocyanate in plasma and urine. *Eur. J. Clin. Chem. Clin. Biochem.* **1995**, *33*, 343−349.
(12) Lundquist, P.; Martensson, J.; Sorbo, B.; Ohman, S. Turbidimetry of inorganic sulfate, ester sulfate, and total sulfate in urine. *Clin. Chem.* **1980**, *26*, 1178−1181.

(13) Lizotte, P. A.; Poulton, J. E. Identification of (*R*)-vicianin in *Davallia trichomanoides* Blume. *Z. Naturforsch.* **1986**, *41C*, 5−8.
(14) Smith, I. *Chromatographic and Electrophoretic Techniques*; Interscience: New York, 1960; Vol. 1, p 251.
(15) Ressler, C.; Knapp, M. M. Liquid chromatography of oxidized sulfanilamide derivatives. *J. Liq. Chromatogr.* **1985**, *8*, 1445−1453.
(16) Chassagne, D.; Crouzet, J. C.; Bayonove, C. L.; Baumes, R. L. Identification and quantification of passion fruit cyanogenic glycosides. *J. Agric. Food Chem.* **1996**, *44*, 3817−3820.
(17) Feigl, F.; Anger, V. Replacement of benzidine by copper ethylacetoacetate and tetra base as spot-test reagent for hydrogen cyanide and cyanogen. *Analyst* **1966**, *91*, 282−284.
(18) Kofod, H.; Eyjolfsson, R. Cyanogenesis in species of the fern genera *Cystopteris* and *Davallia*. *Phytochemistry* **1969**, *8*, 1509−1511.
(19) Dreifuss, P. A.; Wood, G. E.; Roach, J. A. G.; Brumley, W. C.; Andrzejewski, D.; Sphon, J. A. Field desorption mass spectrometry of cyanogenic glycosides. *Biomed. Mass Spectrom.* **1980**, *7*, 201−204.
(20) Brimer, L.; Dalgaard, L. Cyanogenic glycosides and cyanogens in plant tissues. Qualitative and quantitative determination by enzymatic post-column cleavage and electrochemical detection, after separation by high-performance liquid chromatography. *J. Chromatogr.* **1984**, *303*, 77−88.
(21) Bourdoux, P.; Delange, F.; Gerard, M.; Mafusa, M.; Hanson, A.; Ermans, A. M. Evidence that cassava ingestion increases thiocyanate formation: a possible etiological factor in endemic goitre. *J. Clin. Endocrinol. Metab.* **1978**, *46*, 613−621.
(22) Porley, C. M. F.; Echenique, L. *Vicia sativa*. *Chem. Abstr.* **1943**, *37*, 1739.
(23) Wokes, F.; Willimott, S. G. The determination of cyanide in seeds. *J. Pharm. Pharmacol.* **1951**, *3*, 905−917.
(24) Kingsbury. J. M. *Poisonous Plants of the United States and Canada*; Prentice-Hall: Englewood Cliffs, NJ, 1964.
(25) Montgomery, R. D. Observations on the cyanide content and toxicity of tropical pulses. *West Indian Med. J.* **1964**, *13*, 1−11.
(26) Bertrand, G. Vicianin, new cyanogenic glucoside in vetch seed. *Compt. Rend. Acad. Sci. (Paris)* **1906**, *143*, 832−834.
(27) Wajant, H.; Forster, S; Selman, D.; Effenberger, F.; Pfizenmaier, K. Purification and characterization of a novel (*R*)-mandelonitrile lyase from the fern *Phlebodium aureum*. *Plant Physiol.* **1995**, *109*, 1231−1238.
(28) Stochmal, A.; Oleszek, W. Determination of cyanogenic glucosides in white clover (*Trifolium repens* L.) by high performance liquid chromatography. *Phytochem. Anal.* **1994**, *5*, 271−272.
(29) Pfeffer, M.; Ressler, C. β-Cyanoalanine, an inhibitor of rat liver cystathionase. *Biochem. Pharmacol.* **1967**, *16*, 2299−2308.
(30) Van Etten, C. H.; Wolff, I. A. Natural sulfur compounds In *Toxicants Occurring Naturally in Foods*, 2nd ed.; National Academy of Science: Washington, DC, 1973; pp 210−223.
(31) Barratt, M. D.; Hill, D. C.; Alexander, J. C.; Zitnak, A. Fate of orally dosed linamarin in the rat. *Can. J. Physiol. Pharmacol.* **1977**, *55*, 134−136.
(32) Schultz, V. Clinical pharmacokinetics of nitroprusside, cyanide, thiosulfate and thiocyanate. *Clin. Pharmacokin.* **1984**, *9*, 239−251.
(33) Wood, J. L.; Cooley, S. L. Detoxication of cyanide by cystine. *J. Biol. Chem.* **1956**, *218*, 449−557.
(34) Swenne, I.; Ericksson, U. J.; Christoffersson, R.; Kagedal, B.; Lundquist, P.; Nilsson, L.; Tyleskar, T.; Rosling, H. Cyanide detoxification in rats exposed to acetonitrile and fed a low protein diet. *Fundam. Appl. Toxicol.* **1996**, *31*, 66−71.
(35) Tor-Agbidye, J.; Palmer, V. S.; Lasarev, M. R.; Craig, A. M.; Blythe, L. L.; Sabri, M. I.; Spencer, P. S. Bioactivation of cyanide to cyanate in sulfur amino acid deficiency: Relevance to neurological disease in humans subsisting on cassava. *Toxicol. Sci.* **1999**, *50*, *228−235*.
(36) Tor-Agbidye, J.; Palmer, V. S.; Spencer, P. S.; Craig, A. M.; Blythe, L. L.; Sabri, M. I. Sodium cyanate alters glutathione homeostasis in rodent brain: relationship to neurodegenerative diseases in protein-deficient malnourished populations in Africa. *Brain Res.* **1999**, *820*, 12−19.
(37) Cliff, J.; Lundquist, P.; Rosling, H.; Sorbo, B.; Wide, L. Thyroid function in a cassava-eating population affected by epidemic spastic paraparesis. *Acta Endocrinol. (Copenhagen)* **1986**, *113*, 523−528.
(38) Spencer, P. S. Food Toxins, AMPA Receptors, and Motor Neuron Diseases. *Drug Metab. Rev.* **1999**, *31*, 561−587.
(39) Nielsen, E. O.; Cha, J.-H. J.; Honore, T.; Penney, J. B.; Young, A. B. Thiocyanate stabilizes AMPA binding to the quisqualate receptor. *Eur. J. Pharmacol.* **1988**, *157*, 197−203.
(40) Donevan, S. D.; Rogawski, M. A. Allosteric regulation of α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate receptors by thiocyanate and cyclothiazide at a common modulatory site distinct from that of 2,3-benzodiazepines. *Neuroscience* **1998**, *87*, 615−629.
(41) Yamada, K. A. Modulating excitatory synaptic neurotransmission: Potential treatment of neurological disease? *Neurobiol. Dis.* **1998**, *5*, 67−80.
(42) Ressler, C.; Tatake, J. G.; Kaizer, E.; Putnam, D. H. Neurotoxins in a vetch food: stability to cooking and removal of γ-L-glutamyl-L-β-cyanoalanine and β-cyanoalanine and acute toxicity from common vetch (*Vicia sativa* L.) legumes. *J. Agric. Food Chem.* **1997**, *45*, 189−194.
(43) Darre, M. J.; Minior, D. N.; Tatake, J. G.; Ressler, C. Nutritional evaluation of detoxified and raw common vetch seed (*Vicia sativa* L.) using diets of broilers. *J. Agric. Food Chem.* **1998**, *46*, 4675−4679.

Received for review March 13, 2001. Revised manuscript received July 24, 2001. Accepted July 25, 2001.

JF010343W