# Purification and Some Properties of Vicine and Convicine Isolated from Faba Bean (*Vicia faba* L.) Protein Concentrate

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A procedure was developed for the simultaneous isolation of pure vicine [2,6-diamino-4,5-dihydroxypyrimidine 5-(β-D-glucopyranoside)] and convicine [2,4,5-trihydroxy-6-aminopyrimidine 5-(β-D-glucopyranoside)] from faba beans (*Vicia faba* L.). The procedure involves the extraction of the glycosides from faba bean protein concentrate by a neutral or basic acetone–water solution and concentration of the extract to a minimal volume, followed by lowering of the pH to neutrality when required. After standing approximately 3 days the vicine and convicine crystals are harvested and washed by centrifugation. Vicine can be readily obtained from the mixed crystals by extraction at a low pH since vicine is highly soluble while convicine is highly insoluble at this pH. Vicine can be recrystallized by adjustment of the pH of the acidic or basic solutions to neutrality. The relatively insoluble convicine is readily solubilized in a basic solution and can be recrystallized by lowering the pH to neutrality. Initial studies on the solubility and stability of vicine and convicine at different pH levels provided the basis for the development of the isolation procedure.

Faba beans (*Vicia faba* L.) contain both thermolabile (Marquardt and Ward, 1979; Cansfield et al., 1980) and thermostable antinutritional compounds (Olaboro et al., 1981a,b). Recent studies by Olaboro et al. (1981a–c) and Muduuli et al. (1981, 1982) have demonstrated that the thermostable egg-size-depressing factors in faba beans were vicine and convicine. These compounds when fed to laying hens reduced egg size, egg production rate, yolk size, and the fertility and hatchability of egg, increased yolk fragility and incidence of yolk blood spots, elevated the levels of plasma lipid and lipid peroxides, liver peroxides, glutathione, and erythrocyte hemolysis in vitro, and depressed plasma vitamin E levels. Vitamin E supplementation of the diet alleviated the vicine-induced depression in fertility and hatchability of eggs.

Vicine and convicine and their respective aglycons have also been implicated as the causative agents of favism (Mager et al., 1965, 1969). Favism is a metabolic disease that causes hemolytic anemia in glucose-6-phosphate dehydrogenase deficient individuals that have consumed faba beans. This disease constitutes a major health problem in countries throughout the Middle East, North Africa, Italy, and Greece (Belsey, 1973; Bottini, 1973).

More detailed studies on the metabolic effects of vicine and convicine have been hampered by the absence of an isolation procedure that yields large quantities of vicine and convicine from faba beans, the failure to identify a suitable animal model, and the unavailability of a simple and rapid chemical assay. Recent studies in our laboratory, as indicated above, have identified the laying hen as being an excellent model animal for studying the metabolic effects of vicine and convicine and have also lead to the development of a rapid reverse-phase liquid chromatographic method for the quantitation of these compounds (Marquardt and Frohlich, 1981). Previously reported procedures for the isolation of vicine and convicine, however, are rather involved or provide relatively low yields. Also, these procedures yield only vicine or convicine (Levene, 1914; Lin and Ling, 1962; Bein et al., 1968). A procedure, however, has been developed for the isolation of relatively large quantities of vicine from faba bean protein concentrate (Olaboro et al., 1981c). This method is rather time consuming and involves several fractionation steps and does not yield convicine.

The objective of the current study was to modify the latter procedure so as to reduce the number of fractionation steps and to maximize the recovery of vicine. An additional objective was to simultaneously isolate convicine from the mixture. Information on the stability and solubilities of these compounds at different pH levels were also established in order to facilitate the development of this procedure.

## MATERIALS AND METHODS

**Source of Faba Bean Protein Concentrate and Chemicals.** Faba beans (cultivar Diana) were dehulled in a plate type grinder followed by mechanical removal of the hulls in a commercial seed cleaner (No. 245 Carter Seed Cleaner, Simon Day, Ltd., Winnipeg, Manitoba, Canada). The dehulled beans which contained 32% protein ($N \times 6.25$) were pin milled (Type A250 CW, Alpine Augsburg, Natick, MA) and separated by using air classification (Type 132 MP, Alpine Augsburg) into a protein concentrate (FBPC) containing approximately 66% protein and a starch-rich fraction.

**Stability of Vicine and Convicine.** Vicine (0.50 mM, 162 μg/mL) and convicine (0.50 mM, 161 μg/mL) were dissolved in duplicate in 1 N NaOH, 0.1 or 1.0 N HCl, or $H_2O$ and maintained at 2 or 30 °C for 0, 24, or 168 h. The samples were diluted at the appropriate times with 4 volumes of 6% perchloric acid and were assayed for vicine and convicine (Marquardt and Frohlich, 1981).

**Solubility of Vicine and Convicine As Influenced by pH and Temperature.** An excess quantity of pure vicine or convicine were mixed with 3 mL of water and the pH was adjusted with 3 N HCl or NaOH to the desired level. The suspensions were stirred for 1 h at 25 °C, centrifuged at 20000*g* for 20 min and diluted 50–30000-fold with cold 0.1 N HCl. Absorbances were measured at 274 nm and the concentration of vicine or convicine was determined from standard vicine or convicine curves prepared in 0.1 N HCl. The solubilities of vicine and convicine in water at 2, 25, and 60 °C were determined as described above.

**Extractability and Purity of Vicine and Convicine As Influenced by pH and Proportion of Water and Acetone.** FBPC (4 g) and different volumes of water or

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0021-8561/83/1431-0839$01.50/0 

Table I. Quantity and Purity of Vicine and Convicine Extracted from FBPC in the Presence of Different Volumes of Water and Acetone[a]

| | treatments | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| extraction volumes, mL[b] | 45 (67) | 35 (57) | 30 (50) | 42 (71) | 32 (62) | 27 (56) |
| vicine | | | | | | |
| % solubilized[c] | 74 ± 2 | 76 ± 1 | 73 ± 2 | 66 ± 2 | 68 ± 3 | 71 ± 0 |
| % in supernatant[d] | 60 ± 2 | 60 ± 2 | 58 ± 2 | 55 ± 1 | 56 ± 2 | 56 ± 1 |
| mg/g of dry matter[e] | 107 ± 2 | 85 ± 0 | 65 ± 4 | 114 ± 2 | 99 ± 4 | 84 ± 4 |
| convicine | | | | | | |
| % solubilized[c] | 66 ± 0 | 65 ± 2 | 66 ± 0 | 60 ± 2 | 58 ± 4 | 61 ± 2 |
| % in supernatant[d] | 53 ± 1 | 53 ± 2 | 53 ± 0 | 46 ± 1 | 47 ± 3 | 48 ± 1 |
| mg/g of dry matter[e] | 44 ± 1 | 35 ± 1 | 27 ± 1 | 44 ± 1 | 39 ± 3 | 33 ± 1 |

[a] Values represent the average of duplicate sample ± SE. [b] Values in parentheses represent the percent acetone in the total volume (v/v). [c] Values represent the percent of total glycoside in FBPC that was dissolved. [d] Values represent the percent glycoside that was recovered in the supernatant after centrifugation of the sample. [e] Values represent the amount of glycoside per unit of dried extract.

Table II. Quantity and Purity of Vicine and Convicine Extracted from FBPC by Different Volumes and Proportions of Water, NaOH, and Acetone[a]

| | treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| extraction volume, mL[b] | 30 (67) | 36 (67) | 45 (67) | 52 (77) | 30 (67) | 36 (67) | 45 (67) | 52 (77) |
| vicine | | | | | | | | |
| % solubilized[c] | 64 ± 3 | 66 ± 1 | 72 ± 0 | 58 ± 0 | 62 ± 1 | 70 ± 5 | 74 ± 0 | 55 ± 0 |
| % in supernatant[c] | 51 ± 4 | 52 ± 1 | 55 ± 0 | 48 ± 2 | 47 ± 0 | 52 ± 2 | 56 ± 1 | 45 ± 1 |
| mg/g of dry matter[c] | 118 ± 7 | 116 ± 4 | 109 ± 1 | 122 ± 3 | 105 ± 5 | 113 ± 6 | 106 ± 0 | 118 ± 0 |
| convicine | | | | | | | | |
| % solubilized[c] | 51 ± 0 | 54 ± 0 | 61 ± 0 | 48 ± 0 | 60 ± 0 | 67 ± 5 | 72 ± 0 | 45 ± 3 |
| % in supernatant[c] | 41 ± 1 | 43 ± 0 | 47 ± 0 | 39 ± 2 | 45 ± 1 | 50 ± 2 | 54 ± 2 | 36 ± 3 |
| mg/g of dry matter[c] | 44 ± 1 | 43 ± 1 | 43 ± 1 | 46 ± 1 | 47 ± 2 | 50 ± 3 | 48 ± 0 | 51 ± 3 |

[a] Values represent average of duplicate samples ± SE. [b] Values in parentheses represent the percent acetone in the total volume (v/v). In addition to water, treatments 5–8 contained 0.7 mL of 3 N NaOH. [c] See Table I for a definition of the terms.

water plus NaOH were mixed with the aid of a homogenizer (Polytron PT-10, Brinkman, Westbury, NY) in the proportions outlined in Tables I and II. Acetone in the proportions given in Tables I and II was immediately added to the suspensions and the mixtures were rehomogenized. After being allowed to stand for 15 min the samples were centrifuged at 13000*g* for 15 min. A 1-mL aliquot of each supernatant was evaporated to dryness at 37 °C under nitrogen followed by the addition of 2 mL of water. These and all subsequent samples were analyzed for vicine and convicine by the method of Marquardt and Frohlich (1981). The supernatant dry matter contents were determined on separate 5-mL aliquots. After evaporation to dryness at 37 °C under nitrogen the samples were further dried at 65 °C for 14 h, placed in a desiccator for 6 h, and weighed.

**Isolation of Vicine and Convicine.** Water (6.2 L) or water (5.75 L) and 3 N NaOH (0.45 L) were added to FBPC (2.5 kg) in the ratio of 1:2.5 (w/v). The mixture was homogenized for 5 min (Tekmar, SD45N homogenizer, Cincinnati, OH), an additional 5 volumes (12.5 L) of acetone was added, and the suspension was mixed for an additional 15 min with a propellar-type mixer (Ohmite Manufacturing Co., Skokie, IL). After the mixture was allowed to stand 30–60 min at room temperature, the clear supernatant was siphoned off, and the remaining slurry was transferred into cotton bags and squeezed in a 9-L mechanical press (F. Dick, Sausage-extruder). Antifoam B (6 mL, Fisher Scientific Co.) was added to the filtered extract, and the extract was concentrated in a cyclone (steam) evaporator at reduced pressure to approximately 0.08–0.12 volume (200–300 mL). The pH of the concentrated extract was lowered to approximately 7.2 with 8 N HCl and the preparation was allowed to stand 24–96 h at 1–4 °C until crystallization was complete. The crystalline suspension was harvested by centrifugation (13000*g* for 30 min) and was washed successively with 150 and 100 mL of distilled water and 200 mL of acetone with centrifugation for 20, 20, and 30 min, respectively, at 13000*g* after each wash. The mixed vicine and convicine crystals were dried overnight at 60 °C.

The mixed crystals were resuspended in water (10 mL/g) and the pH was adjusted to 1.0 by using 8 N HCl. The suspension was mixed for 15 min at 24 °C and was centrifuged at 13000*g* for 30 min. The pellet was washed successively with 2.5 mL of 0.2 N HCl and 10 mL of water/g of wet pellet. The supernatant and the first washings were pooled and the pH was immediately raised to 7.2 with 8 N NaOH. The crystalline suspension was allowed to stand overnight at 1–4 °C. Vicine crystals were harvested by centrifugation at 13000*g* for 30 min and were washed twice with water (2.5 mL/g wet weight) and finally acetone (4 mL/g wet weight). This fraction (vicine, first crystallization) was dried overnight at 50–60 °C.

The first vicine crystals were recrystallized. They were suspended in water (10 mL/g), and the pH was adjusted to approximately 10.5–11 with 8 N NaOH until all of the sample was dissolved. The mixture was centrifuged at 13000*g* for 20 min, the pH of the supernatant was lowered with 8 N HCl over a 30 min period to 9.0, and the suspension was allowed to stand overnight at 1–4 °C during which time crystallization was completed. The pure vicine (second crystallization) was harvested, washed, and dried as described above except that the volumes of water, water, and acetone wash solutions were 5, 5, and 10 mL/g of dry vicine (first crystallization), respectively.

The washed convicine rich pellet from the mixed crystals was suspended in water (4 mL/g wet weight), the pH was adjusted to 11 with 8 N NaOH, and the mixture was centrifuged at 13000*g* for 20 min. The pH of the super-

natant was lowered to 7.2 with 4 N HCl and the preparation was allowed to crystallize overnight at 4 °C. Convicine (first crystallization) was harvested, washed, and dried in a manner similar to that described for vicine (first crystallization). The convince was recrystallized by using the same procedures as used in the first crystallization except the volumes of water, water, and acetone wash solutions were 10, 10, and 20 mL/g of dry convicine (first crystallization).

**Influence of Time, Temperature, and Volume on the Yield of Crystalline Vicine and Convicine.** Vicine and convicine in the concentrated extract (4 mL) which was prepared as described in the previous section were recrystallized as follows: A, 0 °C for 3 days; B, 0 °C for 7 days; C, 20 °C for 3 days; D, 20 °C for 7 days; E, 0 °C for 3 days in a solution that contained 4 mL of extract plus 2 mL of water; F, same as E except the time was 7 days; G, 0 °C for 3 days in a solution that contained 4 mL of extract plus 4 mL of water; H, same as G except the time was 7 days. The fractions were centrifuged at 20000*g* for 20 min at the indicated times and the supernatant and precipitate were assayed for amounts of vicine and convicine.

**Other Procedures.** Microscopic observations of the crystalline compounds were carried out by using a Cambridge Stero Scan Mark 2A electron microscope at 10 kV. The crystals were suspended in acetone, transferred by Pasteur pipet to an aluminum stub, allowed to air-dry, and subsequently coated with 1000 nm of gold in a Balzer Union Spatter Coater. Photographs of representative samples were taken.

Nitrogen analysis was by the Kjeldahl procedure (Association of Official Agricultural Chemists, 1970) and the ninhydrin test as outlined by Rosen (1957). Vicine and convicine were hydrolyzed in the presence of acid and glucosidase to divicine and isouramil, and the reaction of hydrolyzed products with a reducing reagent (2 M phenol reagent, Fisher Scientific Co.) was followed (Bendich and Clements, 1953; Mager et al., 1965; Bein et al., 1968; Lin and Ling, 1962). The melting points of the two compounds were determined according to the procedure of Vogel (1956). The absorbance spectra of vicine and convicine in 0.1 M HCl and 0.1 M NaOH were determined as reported previously (Olaboro et al., 1981c). The refractive index was monitored by using a Waters (Milford, MA) R401 differential refractometer. All analyses were carried out in duplicate unless otherwise stated. Statistical analyses were as described by Snedecor and Cochran (1967).

## RESULTS AND DISCUSSION

**Stability and Solubility of Vicine and Convicine As Influenced by pH and Temperature.** The results from an initial experiment demonstrated that vicine and convicine were stable in 1 N NaOH at 2 or 30 °C and in water at 2 °C during a 168-h storage period. These compounds were also relatively stable in water at 30 °C; the average percent decrease ± SE in vicine and convicine concentrations when stored for 168 h were only 5 ± 3 and 10 ± 5, respectively. Vicine was stable in 0.1 or 1.0 N HCl at 2 °C but decreased in concentration by 17 ± 1 and 83 ± 1%, respectively, when stored at 30 °C for 168 h. Convicine was more readily hydrolyzed in acid than vicine. The average percent decrease in the concentration of convicine ± SE when stored for 168 h was 22 ± 4 at 2 °C and 96 ± 0 at 30 °C in 0.1 N HCl and 79 ± 5 at 2 °C and 100 ± 0 at 30 °C in 1 N HCl. The percent decrease ± SE in concentration of vicine and convicine in acid medium when stored for 24 h was 1 ± 2 and 2 ± 1 at 2 °C in 0.1 N HCl, −2 ± 3 and 33 ± 2 at 30 °C in 0.1 N HCl, −1 ± 4 and 14 ± 7 at 2 °C in 1 N HCl, and 16 ± 1 and 93 ± 2 at 30 °C in 1 N HCl, respectively. Overall these results demonstrate that vicine and convicine can be stored in a neutral or basic solution for long periods of time but that they should not be stored at a low pH during a similar time period. These compounds, however, are relatively stable in the presence of concentrated acids and low temperatures for short periods of time. Other researchers (Bendich and Clements, 1953; Bien et al., 1968; Lin and Ling, 1962; Marquardt and Frohlich, 1981) have also reported that these compounds are unstable in an acid media and that they are rapidly hydrolyzed to their aglycons at high temperatures in the presence of concentrated acids.

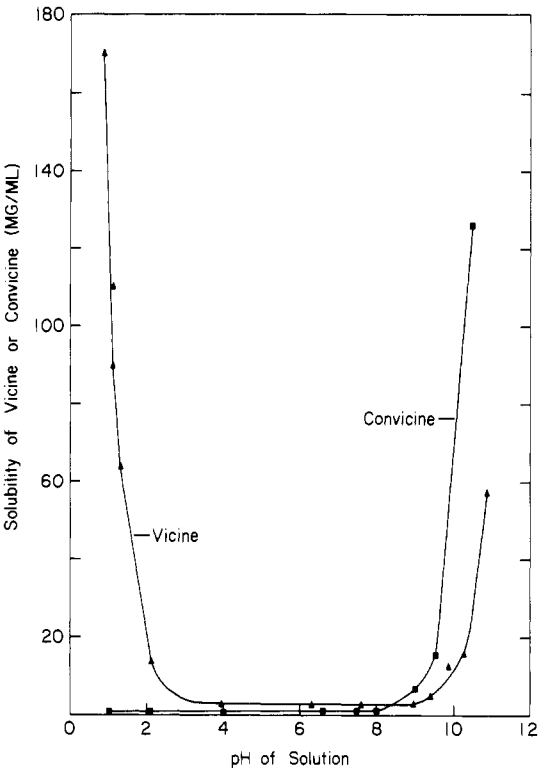


**Figure 1.** Influence of pH on the solubility of vicine and convicine. Values represent averages of duplicate or triplicate samples. SE values are not shown as they were less than 5% of mean values.

The results of the second experiment demonstrated that the solubility of vicine and convicine was markedly influenced by pH (Figure 1). Convicine was minimally soluble at pH levels between 1.0 and 8.0, the values ± SE being 0.4 ± 0.1 and 1.2 ± 0.1 mg/mL, respectively. Vicine was minimally soluble at pH levels of between 4.0 and 9.0, the corresponding values ± SE being 3.0 ± 0.2 and 3.6 ± 0.2 mg/mL, respectively. Vicine, however, was highly soluble at pH values of less than 1 or greater than 10.5. Convicine was also highly soluble in high-pH solutions but in contrast to vicine had a low solubility at a low pH. Vicine and convicine therefore can be readily resolved by extraction at a pH level where the solubilities of the two compounds differ.

The results from a third experiment demonstrate the vicine and convicine solubilities are markedly influenced by temperature. The solubility of vicine in mg/mL ± SE at 2, 25, and 60 °C was 1.5 ± 0.1, 3.3 ± 0.1, and 6.5 ± 0.2, respectively. The corresponding values for convicine were 0.11 ± 0.01, 0.26 ± 0.1, and 0.72 ± 0.02. The final pH of all solutions ranged from 7.0 to 7.1. Lin and Ling (1962) reported that the solubility of vicine in an aqueous solution was 10 mg/mL. These authors, however, did not indicate

Table III. Purification of Vicine and Convicine from FBPC[a]

| fraction[b] | % recovery[c] | | x-fold purification[c] | |
|---|---|---|---|---|
| | vicine | convicine | vicine | convicine |
| FBPC | 100 ± 2 (100) | 100 ± 3 (100) | 1.0 ± 0.02 (1.0) | 1.0 ± 0.03 (1.0) |
| extract | 64 ± 0.3 (62) | 50 ± 3 (63) | 8.3 ± 0.3 (8.1) | 7.0 ± 1.1 (7.9) |
| crude mixed crystals[d] | 44 ± 1 (43) | 42 ± 1 (50) | 49.1 ± 0.1 (45.9) | 50.8 ± 0.2 (57) |
| vicine, 1st crystallization | 32 ± 1 (31) | 0.6 ± 0.0 (0.6) | 71.8 ± 0.1 (71.8) | |
| vicine, 2nd crystallization | 24 ± 0.6 (23) | 0 (0) | 71.9 ± 0.0 (71.9) | |
| convicine, 1st crystallization | 0.6 ± 0.0 (0.6) | 31 ± 0.7 (36) | | 157 ± 0.2 (157) |
| convicine, 2nd crystallization | 0 (0) | 23 ± 0.7 (27) | | 163 ± 0.2 (163) |

[a] All fractionation studies were carried out in duplicate. [b] The initial weight of FBPC was 2.5 kg. The average recovery of extract was 13.0 or 14.4 L when the respective initial extracting solvent was water-acetone (nonparenthesized values) or NaOH-acetone (parenthesized values). The initial concentration of vicine and convicine ± SE in FBPC were 13.9 ± 0.3 and 6.1 ± 0.2 mg/g, respectively. [c] Values ± SE represent the percent recovery or x-fold purification of glycoside relative to that of FBPC. SE for parenthesized values were similar to nonparenthesized value. [d] The crude mixed crystals contained an average of 64.9% vicine, 31.8% convicine, and 3.3% other compounds.

tmeperature or pH level at which these measurements were made. Little or no other information has been reported in the solubilities of these compounds.

**Yield and Purity of Vicine and Convicine When Extracted under Different Conditions.** Previous studies have demonstrated that an essential step in the purification of vicine or convicine was the initial extraction of FBPC with an aqueous–organic solvent mixture (Olaboro et al., 1981b). The role of water in the extracting solution was to dissolve the glycosides whereas the organic solvent which is not able to solubilize either vicine or convicine markedly increased the purity of these compounds as demonstrated subsequently, compared to that obtained when water alone was the extracting solution. Acetone was selected as the organic solvent in the current study as it is relatively inexpensive, has a low boiling point, and is therefore readily evaporated and is a very effective protein denaturing agent. Other organic solvents could probably be substituted for acetone. FBPC was used in the current study as it was not possible to isolate vicine and convicine from whole faba beans or dehulled faba beans by using the procedure outline in this paper.

The results presented in Table I established the effects of different levels of acetone and water on the yield and purity of vicine and convicine in an extract prepared from FBPC. The amount of vicine or convicine that was recovered in the supernatant decreased by an average of only 6 or 11% when the total volume of water in the extracting solution was decreased by 25% (15–12 mL; treatments 1, 2, and 3 vs. 4, 5, and 6). The percent vicine and convicine solubilized also followed a similar pattern. The proportion acetone in the total solution did not markedly influence the solubility or extractability of vicine or convicine at a fixed volume of water but it has a pronounced influence on the concentration of glycoside per unit dry matter extracted. The concentration of glycoside per unit of dry matter increased dramatically with increasing acetone concentrations with the highest values being obtained in the of a 71:29% acetone–water mixture.

The results presented in Table II further established the influence of different extracting solutions on the yield and purity of vicine and convicine. A 33% reduction of solvent volume (treatments 3 and 7 vs. 1 and 5) resulted in a 11.7 and 14.7% average decrease in amount of vicine and convicine recovered in the supernatant. The corresponding increases in glycosides per unit volume of solvent were 23 and 24%. These results demonstrate that extraction of FBPC with the lower amount of solvent, which is the minimal volume that can be used, results in a proportionately greater reduction in solvent volume than in total recovery of glycosides. Subsequent isolations were therefore designed to minimize solvent volumes at the expense of a reduced yield of glycosides as the costs for solvents and time required to evaporate the solvents were greater than those for FBPC.

Increasing the pH of the 67:33% acetone–water extracting solution from 6.9 (treatments 1, 2, and 3) to 8.9 (treatments 5, 6, and 7) did not markedly influence the purity or the percent vicine recovered from FBPC but increased the relative recovery and purity of convicine by 14 and 12%, respectively. The greater recovery of convicine may be attributed to its greater solubility at the high pH. Vicine presumably was maximally solubilized under these conditions.

Increasing the percent acetone in the extracting solution from 67 to 77 at a fixed volume of water (treatments 2 vs. 4 and 6 vs. 8) resulted in a considerable reduction in the percent vicine and convicine either solubilized or recovered in the supernatant. This reduction was more pronounced in the basic extracting solution. The purity of the extract (mg of glycoside/g of dry matter), however, was only slightly improved. These results together with results of the previous study would suggest that the optimal concentration of acetone in the extracting solution is approximately 67%. A lower proportion of acetone results in a greatly reduced purity of the preparation, whereas yields are reduced but purity is not greatly increased when the acetone concentration is increased above 67% acetone.

The results of another study demonstrated that the yields of vicine and convicine were reduced by approximately 25% when 0.7 N HCl was substituted for water in the extracting solution. These results together with the previous data on increased rate of hydrolysis would suggest that the initial extraction of the two glycosides should not be carried out at a low pH. A final study in this series of experiments demonstrated that the order of the addition of extracting solvents, duration of extraction (1 min vs. 2 h), or ionic concentration of the extracting solution (0 vs. 1.0 M NaCl) only had a slight effect on the concentration of vicine and convicine in the extraction solution. Overall it may be concluded that the most satisfactory procedure for extracting vicine and convicine from FBPC would be to initially add 2.5 volumes of water or water plus NaOH to FBPC so as to facilitate the formation of a homogeneous paste followed by the addition of the required amount of acetone (5 volumes).

**Isolation of Vicine and Convicine.** A summary of the isolation procedure is outlined in Table III. The overall procedure involves the extraction of vicine and convicine from FBPC by a neutral or basic acetone–water solution and concentration of the extract to a minimal volume, followed by lowering of the pH to neutrality. After the mixture was allowed to stand for several hours to days, vicine and convicine crystals form and are harvested by

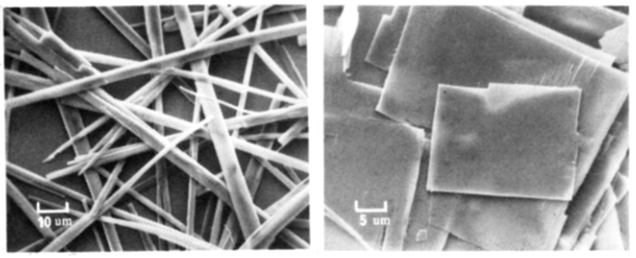


**Figure 2.** Vicine (left) and convicine (right) crystals observed by using a Cambridge Stero Scan Mark II at 875- and 2187.5-fold magnification, respectively.

centrifugation. Vicine and convicine are separated from the mixed crystals by the selective extraction of vicine at a low pH. The two glycosides can be readily solubilized by adjusting the pH of the solution. The final yields ● SE of vicine (second crystallization) and convicine (second crystallization) were 7.8 ± 0.2 and 3.5 ± 0.1 g, respectively, when the initial extracting solution was water–acetone. The corresponding yields when the solvent was NaOH–acetone were 7.6 ● 0.6 and 4.2 ± 0.1 g.

As indicated previously, this procedure cannot be used to purify vicine or convicine from whole or dehulled beans. Persumably the high starch content of these beans interferes with the isolation. This procedure may nevertheless be adapted to the isolation of these compounds from seed in the early development stage. These seeds tend to have greatly elevated vicine and convicine and low carbohydrate concentrations (Pitz et al., 1981).

Extraction in a basic as compared to a neutral solution in addition to increasing the yield of convicine (Table III) also greatly increased the degree of frothing that occurred during evaporation and the viscosity of the concentrated extract. Frothing of the extract was eliminated by the use of an antifoaming agent and the highly viscous containing material was readily removed from the concentrated extract following the subsequent purification step.

Approximately 30% of the vicine and 20% of the convicine from the extract was lost relative to that present in the original extract following centrifugation of the mixed crystals. Part of this loss may be attributed to a loss or destruction of vicine and convicine during the evaporation step and part to the incomplete crystallization of these compounds. In a separate study, the influence of volume of the concentrated extract and time and temperature on the yields of crystalline compounds was established. Doubling the volume of the concentrated extract reduced the average yield of the two glycosides by 10 and 3% when they were allowed to stand for 3 and 7 days, respectively, at 0 °C. The yield of crystalline material seemed to be independent of temperature as similar yields were obtained at 0 and 20 °C during a 3- or 7-day trial. Overall these results would suggest that maximum yields of vicine and convicine are obtained over a 7-day period particularly when the volume was increased and that near maximum values are obtained during a 3-day period. Pure glycosides, in contrast, rapidly crystallize from solution particularly when solutions of these compounds are highly concentrated and the pH is in the neutral range.

**Purity and Properties of Vicine and Convicine.** UV and refractive index monitoring following reverse-phase liquid chromatography were similar to those reported previously (Marquardt and Frohlich, 1981). They did not reveal any other compounds in the recrystallized vicine or convicine. Convicine (second crystallization), however, contained 0.2 ± 0.1% vicine whereas vicine (second crystallization) did not contain any detectable convicine (i.e., less than 0.1% convicine). The addition of β-glycosidase to either vicine or convicine and incubation as described by Mager et al. (1965) resulted in the complete disappearance from the reverse-phase chromatogram of both vicine and convicine and the appearance of new peaks corresponding to their hydrolytic products, divicine and isouramil. Acid hydrolysis of the two glycosides yielded similar results. The nitrogen content, melting point values, reactivity with ninhydrin reagent or phenol reagent (Folin-Ciocalteau reagent) before and after acid hydrolysis, and ultraviolet absorption spectra in both acid and basic solutions for both vicine and convicine were the same as reported by previous authors (Bendich and Clements, 1953; Lin and Ling, 1962; Mager et al., 1965, 1969; Being et al., 1968; Olaboro et al., 1981b; Marquardt and Frohlich, 1981). The crystalline structure of vicine is needlelike while that of convicine appears to be platelike with one plate or needle readily forming from the other (Figure 2).

**Conclusion.** A procedure has been developed for the simultaneous preparation of pure vicine and convicine from FBPC. This procedure is simple to carry out and yields relatively large quantities of pure vicine and convicine. In addition, the crystalline form of convicine and the solubility and stability of vicine and convicine which are important factors in the development of the isolation procedure were established. This procedure should facilitate future animal research on metabolic effects of these compounds.

ACKNOWLEDGMENT

We thank Aniko Bernatsky, Sheryl Stern, and Wayne Hayward for skilled technical assistance and Dr. B. Dronzek and B. Luit for photographing the crystals.

**Registry No.** Vicine, 152-93-2; convicine, 19286-37-4.

LITERATURE CITED

Association of Official Agricultural Chemists "Official Methods of Analysis", 11th ed.; AOAC: Washington, DC, 1970.
Belsey, M. A. *Bull. W. H. O.* **1973**, *48*, 1.
Bendich, A.; Clements, G. C. *Biochim. Biophys. Acta* **1953**, *12*, 462.
Bien, S.; Salemnik, G.; Zamir, L.; Rosenblum, M. *J. Chem. Soc. C* **1968**, 496.
Bottini, E. *J. Med. Genet.* **1973**, *10*, 154.
Cansfield, P. E.; Marquardt, R. R.; Campbell, L. D. *J. Sci. Food Agric.* **1980**, *31*, 802.
Levene, P. A. *J. Biol. Chem.* **1914**, *18*, 305.
Lin, J.-Y.; Ling, K.-H. *J. Formosan Med. Assoc.* **1962**, *61*, 484.
Mager, J.; Glasser, G.; Razin, A.; Izak, G.; Bien, S.; Noam, M. *Biochim. Biophys. Acta* **1965**, *20*, 235.
Mager, J.; Razin, A.; Hershko, A. "Toxic Constituents of Plant Foodstuffs"; Liener, E. D., Ed.; Academic Press: New York, 1969; p 293.
Marquardt, R. R.; Frohlich, A. A. *J. Chromatogr.* **1981**, *208*, 373.
Marquardt, R. R.; Ward, A. T. *Can. J. Anim. Sci.* **1979**, *59*, 781.
Muduuli, D. S.; Marquardt, R. R.; Guenter, W. *Can. J. Anim. Sci.* **1981**, *61*, 757.
Muduuli, D. S.; Marquardt, R. R.; Guenter, W. *Br. J. Nutr.* **1982**, *47*, 53.
Olaboro, G.; Campbell, L. D.; Marquardt, R. R. *Can. J. Anim. Sci.* **1981a**, *61*, 751.
Olaboro, G.; Marquardt, R. R.; Campbell, L. D. *J. Sci. Food Agric.* **1981b**, *32*, 1074.
Olaboro, G.; Marquardt, R. R.; Campbell, L. D.; Frohlich, A. A. *J. Sci. Food Agric.* **1981c**, *32*, 1163.
Pitz, W. J.; Sosulski, F. W.; Rowland, C. G. *J. Sci. Food Agric.* **1981**, *32*, 1.
Rosen, M. A. *Arch. Biochem. Biophys.* **1957**, *67*, 10.
Snedecor, G. W.; Cochran, W. G. "Statistical Methods", 6th ed.; Iowa State University Press: Ames, IA, 1967.
Vogel, A. I. "Practical Organic Chemistry Including Qualitative Organic Analysis", 3rd ed.; Longmans, Green and Co: New York, 1956; p 72.

Received for review August 2, 1982. Revised manuscript received February 7, 1983. Accepted February 18, 1983. This investigation was supported by the New Crop Development Fund, Food Production and Marketing Branch, Agriculture Canada, the National Science and Engineering Council of Canada, a Manitoba Department of Agriculture grant to the Faculty of Agriculture, and the University of Manitoba. A CIDA graduate student assistantship was held by D.S.M.

# Characteristics of Pindak Bean Starch

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Pindak bean (a cross between the pinto and Japanese bush bean) starch was characterized and compared to pinto bean and HRW wheat starches. Pindak bean flour yielded 43.5% starch compared to 40.0% for pinto bean flour. The pindak and pinto bean starches were irregular and somewhat elliptical with a few small, round granules. The amylose content of the pindak bean was 27.2% for the colorimetric method and 33.6% for the potentiometric method. The solubilities at 85 °C for all starches were similar, and the swelling power at 85 °C was more variable, with pindak starch having the highest value. The pindak and pinto bean starches had a type C viscosity pattern with viscosities higher than that of wheat starch. The initial pasting temperature for pindak starch was 77.5 °C. Further studies need to be done on the food uses of dry edible bean starches.

The pinto bean is one of the major dry edible beans grown in the United States. The pindak bean, a cross between the pinto bean and the Japanese bush bean, has been recently released by North Dakota State University (Schneiter et al., 1981). Pindak beans appear to have a more uniform size distribution and a greater degree of disease resistance than pinto beans. Consequently, pindak beans have potential for large-scale commercial production.

Starch has been shown to be a major component of legumes (Schoch and Maywald, 1968). Starch is used extensively throughout the food industry as a filler, extender, thickener, stabilizer, and texture modifier (Wurzburg, 1968). A nontraditional method for utilizing beans may involve its fractionation into protein and starch component by air classification (Satterlee, 1981). This process could be especially important for developing food uses for culled beans. According to Satterlee (1981), approximately 100 million pounds of low-cost culled beans are available each year in the United States. The objective of this study is to evaluate the characteristics of pindak bean starch and compare them with those of pinto and wheat starch.

## EXPERIMENTAL PROCEDURES

The pindak beans were obtained from North Dakota State University (1981 crop). The pinto beans were obtained commercially (1981 crop). Bean starches were isolated by using method A of Schoch and Maywald (1968). The starch was air-dried (3 days) and passed through a 70-mesh sieve. The standard starch source was commercially isolated hard red winter (HRW) wheat.

Standard AOAC (1980) methods were used to determine starch moisture, nitrogen, and ash. Acid detergent fiber (ADF) was determined by the method of Goering and Van Soest (1970). Bean starch content was determined by a modification of the Osborne and Voogt (1978) method using amyloglucosidase. Starch digestibility of pindak starch with $\alpha$-amylase (Type VII-A, Sigma Chemical Co.) is determined by a modification of methods from Rao (1969) and Kayisu and Hood (1979). The digestion products glucose and maltose were determined by using HPLC analysis with a carbohydrate analysis column (Waters Associates), mobile phase 70:30 (acetonitrile–water), flow rate 1.8 mL/min, and detection using a 401 refractometer. The amylose content (blue value) was determined by the method of Williams et al. (1970). Iodine affinity for the pindak starch was determined by potentiometric titration (Schoch, 1964). Swelling power and solubility of the starches were determined at 85 °C according to the method of Schoch (1964). Water binding capacity of the starches was determined by the procedure of Medcalf and Gilles (1965). Pasting curves at 12% starch in distilled water were determined with a Brabender VISCO/amylo/GRAPH by using a standard cycle of heating from 25 to 95 °C, holding at 95 °C for 15 min and cooling to 50 °C (Naivikul and D'Appolonia, 1979). The size and shape of each starch were studied with a SO Spencer light microscope equipped with a 35-mm Kodak camera. Photomicrographs were taken at a magnification of 100 by using normal light for the measurement of granule size.

## RESULTS AND DISCUSSION

The starch yield and chemical data on the starch are shown in Table I. The yield of starch (dry weight basis) from pindak bean flour was 43.5%, which was similar to the starch yield from pinto bean flour (40.0%). The starch yields in this study were comparable with the legume values obtained by Naivikul and D'Appolonia (1979). The dry bean starch levels were lower than the 70–80% levels obtained for wheat flour (Pomeranz, 1971). The nitrogen content of the starches was low (0.01–0.07%) with values similar to those reported by Naivikul and D'Appolonia (1979). The oil content was extremely low and not reported due to the high degree of error. The ash content was low (0.01–0.02%) for the laboratory-isolated bean starches with the commercially available starch having a higher ash content (0.61%). The higher ash content of the wheat starch is probably due to the variation in industrial vs. laboratory isolation methods. The acid detergent fiber content of the starches ranged from 0.03 to 0.08%, which was slightly lower that the values reported by Naivikul and D'Appolonia (1979). Part of the difference in compositional data from previous work may be due to the methods of analysis used.

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0021-8561/83/1431-0844$01.50/0 