This work was supported financially by the North Dakota Agricultural Experiment Station, Fargo, ND, by the Red River Valley Potato Growers Association, East Grand Forks, MN, and by a NSF-URP summer fellowship to M.T.K. Presented, in part, at the 168th National Meeting of the American Chemical Society, Atlantic City, NJ, Sept 1974, PEST 44. Authorized for publication as Paper No. 1118 in the Journal Series of the North Dakota Agricultural Experiment Station.

A Simple and Rapid Colorimetric Method for Determination of Vicine and Convicine

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A simple and rapid colorimetric procedure for determination of vicine and convicine is described based on complex formation between their corresponding aglycons and titanium reagent. The inability of other compounds such as nucleosides and nucleotides to interfere with the titanium complex is a distinct advantage over the UV procedure for measurement of vicine and convicine in fababean products.

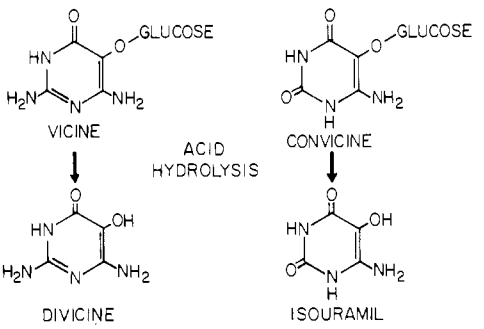
The aglycons of vicine and convicine have been implicated as the causative factors for favism in fababeans (Jamalian et al., 1977; Lin and Ling, 1962a,b; Mager et al., 1965). Two spectrophotometric methods for measuring vicine and convicine have been reported, including one based on the reduction of Folin–Ciocalteau phenol reagent (Higazi and Read, 1974) while the other method involved direct UV spectrophotometric scanning of a protein-free fababean extract (Collier, 1976). This paper reports a sensitive colorimetric procedure for measuring vicine and convicine based on complex formation of the corresponding aglycons with $TiCl_4$ (20% in concentrated HCl). This reagent has been developed in our laboratory to determine hydrogen peroxide (Gupta et al., 1977), lipid hydroperoxides (Eskin and Frenkel, 1976), phenolic compounds (Eskin et al., 1978), and sinapine (Ismail and Eskin, 1979) and as a chromogenic reagent for phenolic compounds (Eskin and Frenkel, 1978) and vicine and convicine (Hoehn et al., 1980) on thin-layer plates. While the reaction mechanism remains to be delineated, possible chelation via the oxygen or an oxidation–reduction type of reaction may be involved.

EXPERIMENTAL SECTION

Materials. Pure samples of vicine and convicine were provided by W. J. Pitz, Department of Crop Science, University of Saskatchewan, Canada, and Dr. R. R. Marquardt, Department of Animal Science, University of Manitoba, Canada. Titanium tetrachloride was purchased from British Drug Houses (Toronto, Canada). Fababeans (*Vicia faba* minor var. Diana) were supplied by the Department of Plant Science, University of Manitoba, Winnipeg, Canada. The beans were dehulled and then ground in a pinmill. The flour (30% protein) was air classified into a protein concentrate (70% protein) and starch fraction (7% protein) according to the method described by Vose et al. (1976).

Formation of Aglycons. Vicine and convicine do not react with the titanium reagent, but their corresponding aglycons, divicine and isouramil, form colored complexes due to the availability of the C-5 hydroxyl for interaction with the titanium salt. To establish the formation of aglycons, we hydrolyzed the glucosides in strong acid (Scheme I). So that the optimum conditions for aglycon formation were established, vicine (20 mg) was dissolved in 1.0 mL of concentrated HCl and heated at 60, 70, 80, and 90 °C over a time period of 0.5–5 min. Following each treatment the hydrolyzed solution was diluted to a final volume of 5 mL with concentrated HCl to give a concentration range of 0–1.0 mg/mL. To each solution was added 0.2 mL of $TiCl_4$ (20% in concentrated HCl) and mixed thoroughly on a vortex for a few seconds. The colored complexes were scanned between 400 and 700 nm in a Unicam SP800 spectrophotometer while individual absorbances were read at 480 nm by using a Unicam SP600 spectrophotometer against an equivalent blank.

Scheme I. Acidic Hydrolysis of Vicine and Convicine to Their Respective Aglycons, Divicine and Isouramil



Titanium Reagent Level and Absorbance of the Divicine–Titanium Complex. The relationship between titanium reagent levels and absorbance of the divicine–titanium complex was examined. Vicine (20 mg) was dissolved in 1 mL of concentrated HCl and hydrolyzed at 80 °C for 1.5 min. The hydrolyzed solution was further diluted with concentrated HCl to give solutions ranging from 0 to 0.5 mg/mL. Increasing amounts of titanium tetrachloride (0.05–3.2 mL) were added to the divicine solutions (5 mL), and absorbance at 480 nm was measured against an equivalent blank by using a Unicam SP600 spectrophotometer.

Preparation of Divicine and Isouramil Standards. Ten milligrams of pure vicine and convicine were each

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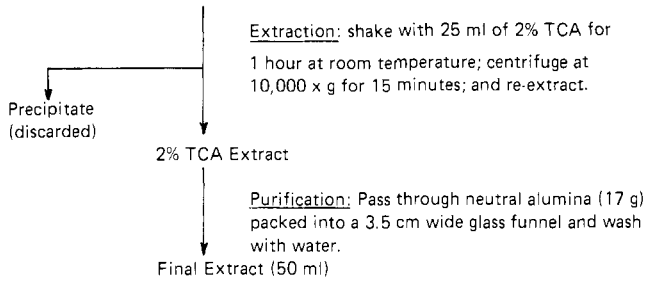
0021-8561/82/1430-0144$01.25/0

Scheme II. Procedure for Extraction of Vicine and Convicine from Fababeans



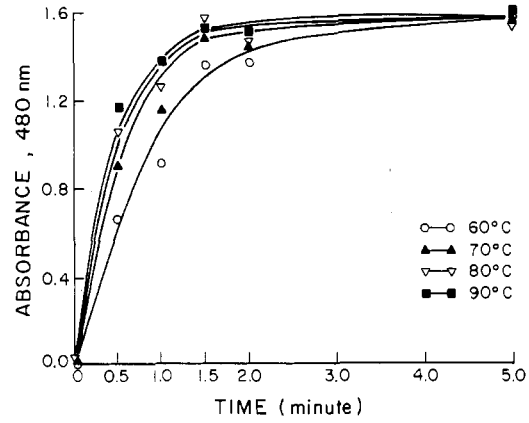


Figure 1. Effect of temperature and time on the hydrolysis of vicine with concentrated HCl.

dissolved in 10 mL of 2% trichloroacetic acid (Cl_3AcOH) and 10 mL of 0.1 N NaOH, respectively. This was necessary for convicine due to its poor solubility in 2% Cl_3AcOH. Aliquots of 0–0.5 mL were removed and made up to 5 mL with concentrated HCl representing 0–100 μg/mL vicine and convicine. Each glycoside was then hydrolyzed at 80 °C for 1.5 min, followed by the addition of 0.8 mL of $TiCl_4$ (20% in concentrated HCl). Individual absorbances were recorded at 480 nm as described previously.

Extraction of Vicine and Convicine from Fababeans. Extraction of these pyrimidines was performed initially on fababean flour using four different extractants: 2% Cl_3AcOH (Higazi and Read, 1974); 0.1 N NaOH (Pitz and Sosulski, 1979); ethanol–water, 3:1 (Olsen and Anderson, 1978); ethanol:water 3:2 (Olaboro, 1979). Subsequent extraction and purification of vicine and convicine were based on that described by Higazi and Read (1974) using 2% Cl_3AcOH (Scheme II). Extraction with 2% Cl_3AcOH produces a solution free from protein, nucleic acids, and other high molecular weight materials. Potentially interfering substances, such as phenols and aromatic amino acids, were then removed from Cl_3AcOH extracts by treatment with neutral alumina. Samples of the final extract were subjected to hydrolysis to produce the aglycons which were then reacted with the titanium reagent. For comparison the vicine and convicine content was also determined by the UV method of Collier (1976) and the Folin method of Higazi and Read (1974). Data obtained were individually subjected to the analysis of variance, and differences among treatments were determined by using the Student's t test (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The rate of hydrolysis of vicine to the aglycon divicine, as measured at 480 nm, increased as the temperature rose from 60 to 90 °C, reaching a plateau after 1.5 min (Figure 1). Complete hydrolysis was evident at 70, 80, and 90 °C after 1.5 min as no further hydrolysis was apparent on prolonged heat treatment (2–5 min). Thus, hydrolysis at 80 °C for 1.5 min was employed as the standard technique for aglycon formation in subsequent assays.

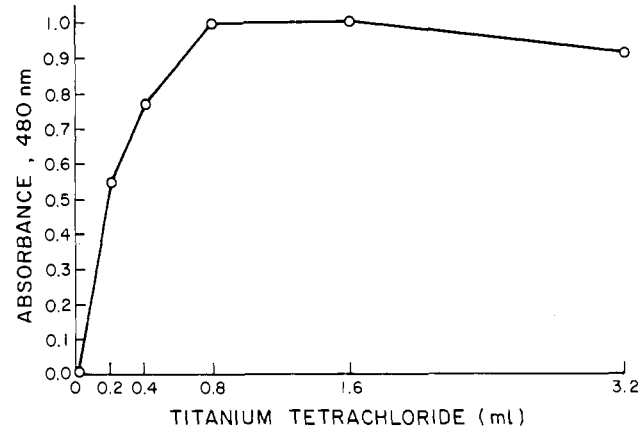


Figure 2. Effect of titanium reagent level on the absorbance of the divicine–titanium complex.

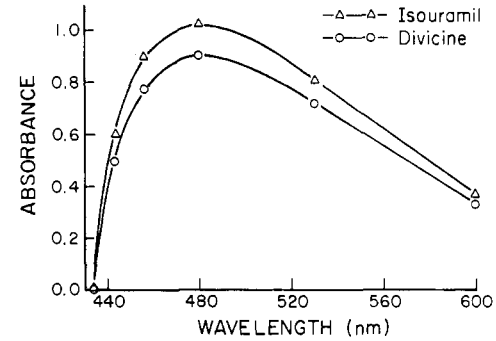


Figure 3. Absorption spectra of the divicine–titanium complex and the isouramil–titanium complex.

Table I. Spectrophotometric Data for the Aglycons-Titanium Complex and Their Corresponding Glycosides

	aglycon–titanium complex		glycoside[b]		glycoside[c]	
compd	max, nm	$\epsilon^a \times 10^{-3}$	max, nm	$\epsilon^a \times 10^{-3}$	max, nm	$\epsilon^a \times 10^{-3}$
vicine	480	1.50	273.5	16.30	650	7.50
convicine	480	1.66	271	17.30	650	5.70

[a] The molar absorption coefficient, ϵ, was calculated on the molecular weight basis of 304 for vicine (Bendich and Clements, 1953) and 305 for convicine (Bien et al., 1968). [b] Collier (1976). [c] Determined according to the method of Higazi and Read (1974).

The effect of increasing the amount of titanium reagent on the absorbance of the divicine–titanium complex is shown in Figure 2. The highest absorbance readings were obtained following the addition of 0.8 mL of titanium reagent. Further increase in the level of the reagent did not increase absorbance ($P < 0.01$) so that 0.8 mL of $TiCl_4$ (20% in concentrated HCl) was used in all assays. The absorbance spectra for divicine–titanium and isouramil–titanium complexes are shown in Figure 3. Both complexes exhibited similar spectra with absorbance maxima at 480 nm.

This indicated that the titanium reagent cannot differentiate between the two aglycons. It was apparent that the divicine–titanium complex showed a lower absorbance compared to the isouramil–titanium complex when measured at equivalent concentrations (0.6 mM). A linear Beer's law plot was obtained for both divicine and isouramil over a range of 0–100 μg/mL of the corresponding

Table II. Influence of Extraction Procedures on Estimation of Total Vicine Contents by the Titanium Reagent Method and UV Method

extraction method	vicine + convicine, % (dry basis)[a] titanium method[b]	UV method[c]
2% Cl_3AcOH (Higazi and Read, 1974)	0.832 ± 0.026[a,d]	0.970 ± 0.018[c]
0.1 N NaOH (Pitz and Solsulski, 1979)	0.841 ± 0.034[a]	0.967 ± 0.018[c]
ethanol-water (3:1) (Olsen and Anderson, 1978)	0.524 ± 0.010[b]	0.945 ± 0.016[c]
ethanol-water (3:2) (Olaboro, 1979)	0.651 ± 0.012[b]	0.958 ± 0.010[c]

[a] Mean ± SE based on six determinations. [b] Values are expressed as the sum of vicine and convicine, based on the vicine standard curve. [c] Values are expressed as the sum of vicine and convicine, based on $\epsilon = 16.3 \times 10^3$ at 273.5 nm for vicine. [d] Means not sharing a common superscript letter are significantly different at $P < 0.01$.

glycoside (with coefficients of determination (r^2) of 0.998. The regression coefficient for isouramil (0.0054) was significantly higher ($P < 0.05$) than that of divicine (0.0049). Calculation of molar absorptivities on the basis of standard curves are listed in Table I. It is evident that molar absorptivity for the divicine–titanium complex is approximately 10% lower than that for the isouramil–titanium complex. For comparison molar absorptivities of the corresponding glycosides, vicine and convicine, at 273.5 and 271 nm, respectively, are included in Table I. It is interesting to note that a similar difference in magnitude between the molar absorptivity of these glycosides at these wavelengths was evident which parelleled the corresponding titanium–aglycon complexes. This is particularly important as the determination of vicine and convicine by the UV method of Collier (1976) is based on absorbance at 273.5 nm. The molar absorptivities of vicine and convicine by using the Folin and Ciocalteau phenol reagent are also listed in Table I. It is evident that there is a 32% difference between the molar absorptivities for vicine and convicine when the phenol reagent is used.

The data obtained for extraction of vicine and convicine from fababean flour using four different extractants are summarized in Table II. All values represent the sum of vicine and convicine and are expressed as vicine equivalents. By use of the titanium procedure 2% Cl_3AcOH and 0.1 N NaOH appeared to be more efficient extractants compared to the corresponding ethanol–water systems. The values obtained by using the UV method, however, were not significantly ($P < 0.05$) different among the four extractants. This discrepancy may be due to the suppressed formation of the aglycone–titanium complex by ethanol, resulting in lower absorbance readings. Preliminary investigations, however, indicated that small amounts of ethanol did not interfere with the formation of the titanium complex.

The amount of vicine equivalent (vicine plus convicine) in fababean flour, fababean protein concentrate, and fababean starch is shown in Table III. The crude extracts, extracted according to Scheme I, consistently yielded higher results than the corresponding purified extracts either by UV or by titanium methods. The alumina treatment significantly ($P < 0.01$) lowered the readings due to 8–18% of the materials in the extract being absorbed. Significantly ($P < 0.01$) higher results were obtained by using the UV method compared to the titanium method which suggested the UV method measured materials absorbing at 273.5 nm which were neither vicine or convicine. Neither vicine or convicine materials accounted for 13–14% in the purified extracts based on the difference between the UV and titanium methods. Jamalian (1978) reported at least three classes of nitrogenous compounds with similar properties to vicine (ϵ_{max} at 277–278 nm) were present in the nonprotein fraction of the Cl_3AcOH extract of broad beans. Studies reported by Hoehn et al. (1980) clearly demonstrated the presence of $TiCl_4$-reacting components other than vicine and convicine which were present in the crude extract of fababean which could be eliminated by treatment with alumina. It is well documented that nucleosides and nucleotides occurring widely in biological systems show strong absorbance in the UV range: 250–280 nm. Studies carried out on a range of nucleosides and nucleotides revealed that these components did not interfere with the titanium complexes. This is particularly important as they are not absorbed in alumina and are present in the purified extract. It is therefore apparent that the titanium method provides a more accurate measure of vicine equivalents via their aglycons than the UV procedure. The recovery of vicine/convicine following the extraction process was 100 ± 2% (five determinations), thus indicating no losses occurred during the extraction period. The reproductability of the titanium method was examined on fababean flour. Ten determinations were performed, yielding a mean of 0.833% vicine equiv with a coefficient of variation amounting to 4.32%. By use of the Folin–Ciocalteu phenol reagent (Higazi and Read, 1974), the amount of vicine equivalents determined for the purified fababean extract was 0.528 ± 0.024% (Table III). On the basis of the difference in molar absorptivities between vicine and convicine by using the phenol reagent, a lower reading (vicine equivalent) was anticipated. However, when the vicine:convicine ratio is

Table III. Vicine and Convicine Content of Fababean Flour, Protein Concentrate, and Starch by the Titanium Reagent Method and UV Method

sample	vicine + convicine, % (dry basis)[a] UV method[b]	titanium method[c]	phenol reagent[c]
fababean flour			
crude extract	1.024 ± 0.036[a,d]	0.908 ± 0.045[g]	
purified extract	0.940 ± 0.026[b]	0.816 ± 0.034[h]	0.528 ± 0.024
fababean protein concentrate			
crude extract	2.258 ± 0.065[c]	1.920 ± 0.070[i]	
purified extract	1.863 ± 0.085[d]	1.607 ± 0.039[j]	
fababean starch			
crude extract	0.778 ± 0.014[e]	0.664 ± 0.034[k]	
purified extract	0.658 ± 0.021[f]	0.566 ± 0.029[l]	

[a] Mean ± SD based on five determinations. [b] Values are expressed as the sum of vicine and convicine, based on $\epsilon = 16.3 \times 10^3$ at 273.5 nm for vicine. [c] Values are expressed as the sum of vicine and convicine, based on the vicine standard curve. [d] Means ± SE not followed by the same superscript letter are significantly different at $P < 0.01$.

assumed to be 2.8:1 in fababean flour (Pitz and Sosulski, 1979), the results obtained were much lower than expected (0.748% calculated on the basis of the ratio of vicine: convicine and their respective molar absorptivities). While this difference cannot be adequately explained, the possible interference by other compounds cannot be ruled out.

The absorbance spectrum of the extracted fababean flour sample with the titanium reagent (20% $TiCl_4$ in concentrated HCl) was characteristic of the aglycons of vicine/convicine with a maximum at 480 nm (Figure 2). The colored complex was stable for several hours and permitted readings to be taken during that time interval without any loss in colored complex. This stability of the titanium complex is a distinct advantage over the colorimetric procedure of Higazi and Read (1974) which requires readings to be taken 30 min following the addition of the Folin Ciocalteu reagent to samples. The molar absorptivities for the aglycons of vicine and convicine with titanium reagent are quite close compared to the phenol reagent. Thus the titanium method measures more accurately vicine and convicine in terms of vicine equivalents than does the Higazi and Read (1974) procedure. The absence of interference by other compounds such as nucleosides and nucleotides, which are not absorbed by alumina, is a distinct advantage of the titanium method over the UV procedure described by Collier (1976).

This study establishes the titanium procedure as a simple and reliable colorimetric method for measuring vicine and convicine via their aglycons in fababean samples.

ACKNOWLEDGMENT

We are grateful to Stacey Johnson for technical assistance.

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Received for review December 8, 1980. Revised manuscript received August 3, 1981. Accepted August 18, 1981.

Influence of Methods of Pesticide Application on Subsequent Desorption from Soils

Bruce T. Bowman* and Walter W. Sans

The amounts of fensulfothion [*O,O*-diethyl *O*-[*p*-(methylsulfinyl)phenyl] phosphorothioate] and its related sulfide [*O,O*-diethyl *O*-[*p*-(methylthio)phenyl] phosphorothioate] desorbed from three mineral soils with distilled water were greater when the compounds were spiked on the soil with either of two organic solvents (hexane–acetone, 95:5 v/v, or pure acetone) than when they were initially adsorbed from aqueous solution. For muck soil, the method of applying the two insecticides did not greatly alter their subsequent desorption behavior.

Desorption processes in soil–water systems have been traditionally studied by initially adsorbing the compound from aqueous solution and then successively desorbing it with distilled water increments (hereafter referred to as the "equilibrium adsorption method") (Bowman, 1979; Swanson and Dutt, 1973). Recently, Sharom et al. (1980) spiked (fortified) soil adsorbents with hexane solutions of insecticides (at 5 μg/g) and subsequently evaporated the solvent prior to adding distilled water to initiate the first desorption cycle. This approach offers the advantage of easily treating soil adsorbents with equal insecticide concentrations, facilit .g comparisons of desorption tendencies among different insecticides on various soil adsorbents. In contrast, the equilibrium adsorption method requires prior knowledge of the adsorption isotherm in

Table I. Properties of Soil Adsorbents

adsorbent	% sand	% silt	% clay	% organic matter	pH[a]
Plainfield sand	91.5	1.5	7	0.7	6.9
Big Creek sediment	71	22	7	2.3	6.5
Bondhead sandy loam	77	15	8	3.9	6.9
muck soil	52	34	14	36.7	6.3

[a] Measured in 0.01 M $CaCl_2$ (20 mL/10 g of soil).

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0021-8561/82/1430-0147$01.25/0