Rapid Isolation and Purification of 1-Cyano-2-Hydroxy-3-Butene (Crambene) from *Crambe abyssinica* Seed Meal Using Immiscible Solvent Extraction and High-Performance Liquid Chromatography

Thomas E. Niedoborski,[†,||] Barbara P. Klein,[†,‡] and Matthew A. Wallig*[,‡,§]

Department of Food Science and Human Nutrition, Division of Nutritional Sciences, and Department of Veterinary Pathobiology, College of Agricultural, Consumer and Environmental Sciences and College of Veterinary Medicine, University of Illinois at Urbana-Champaign, 2001 South Lincoln Avenue, Urbana, Illinois 61802

1-Cyano-2-hydroxy-3-butene (**crambene**) is a nitrile found in cruciferous vegetables that causes significant upregulation of quinone reductase and glutathione S-transferases in vivo and in vitro, making it a likely candidate as a cancer chemopreventive compound. To investigate further the putative anticarcinogenic mechanisms of crambene, a compound of the highest possible purity is vital. Therefore, a rapid and effective method of purification of crambene is necessary to continue studies of its beneficial health effects. A rapid method to isolate and purify natural crambene from either *Crambe abyssinica* (crambe) seed or commercially processed crambe seed meal was developed using immiscible solvent extraction followed by high-performance liquid chromatography. Use of this methodology eliminated the need for time-consuming and relatively inefficient column chromatography, improved extraction efficiency, and resulted in higher purity than previously used methodologies. Elimination of trace amounts of fatty acid residues, unachievable with previous methodologies, also was accomplished.

Keywords: *Crambene; 1-cyano-2-hydroxy-3-butene; immiscible solvent extraction; crambe; cruciferous vegetables; chemoprotection, anticarcinogenic; high-performance liquid chromatography*

INTRODUCTION

Cruciferous vegetables contain compounds associated with protection against cancer. Researchers have demonstrated that a healthy diet including as little as 10 g/day of cruciferous vegetables such as broccoli, kale, or cabbage can reduce the risk of cancer (*1*−*3*). The distinct modes of action responsible for this chemoprotective effect have been linked to the breakdown of glucosinolates, phytochemicals common to all cruciferous plants. Although there are many different glucosinolates, and their concentrations vary widely among cruciferous vegetables varieties depending on genetic and environmental factors (*4*, *5*), most break down to either isothiocyanates or nitriles when the plant is injured, harvested, processed, or eaten. The isothiocyanates (e.g., sulforaphane) are particularly potent chemoprotective compounds against cancer; they have been shown to slow the growth of or kill tumor cells in in vitro model systems (*6*) and induce the expression of detoxification enzymes important in carcinogen inactivation in rodents (*7*, *8*). The degree of increased activity of these detoxification enzymes via enhanced expression has been correlated directly to potency for inhibiting carcinogenisis (*9*).

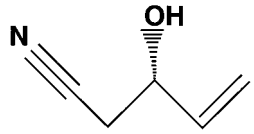

Figure 1. Chemical structure of 1-cyano-2-hydroxy-3-butene (crambene).

One of these glucosinolate breakdown products, S1-cyano-2-hydroxy-3-butene (CAS no. 6071-81-4), also known as 2-hydoxybut-3-enyl cyanide and crambene (*10*, *11*), was first identified in 1966 (*12*), and since then has been implicated as one of the possible contributors to the anticancer effect (Figure 1). Crambene is a potent inducer of "phase II" detoxification enzymes, including certain glutathione S-transferases and quinone reductase, which are important enzymes associated with conjugation and elimination of reactive chemical intermediates and carcinogens (*11*, *13*, *14*). Furthermore, mixtures of individual cruciferous breakdown products that include crambene have shown synergistic activity with regard to induction of these two protective detoxification enzymes (*11*, *15*). In these mixtures, crambene was shown to be a major contributor to the effect. Crambene as a chemoprotectant is relatively unique in that it is a nitrile rather than an isothiocyanate, the type of compound typically associated with anticarcinogenesis. To define the role of a compound such as crambene in protection against cancer in the most precise way possible, purity of the compound under study is of utmost importance so that the contribution of each component can be assessed accurately.

* Corresponding author. Tel: 217-244-0138. Fax: 217-244-7421. E-mail: m-wallig@uiuc.edu.
[†] Department of Food Science and Human Nutrition.
[‡] Division of Nutritional Sciences.
[§] Department of Veterinary Pathobiology.
[||] T. E. Niedoborski's current address is: Kraft Foods, 801 Waukegan Road, Glenview, IL 60025.

10.1021/jf001366y CCC: $20.00
Published on Web 06/26/2001

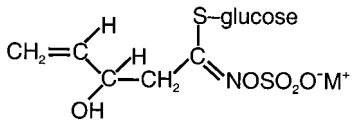

Figure 2. Chemical structure of *epi*-progoitrin, the precursor molecule of crambene.

Crambene is derived from (2S)-hydroxy-3-butenyl glucosinolate, also known as *Epi*-progoitrin (Figure 2). *Epi*-progoitrin is the counterpart of (2R)-hydroxy-3-butenyl glucosinolate, or progoitrin. *Epi*-progoitrin is the predominant glucosinolate in *Crambe abyssinica*, the Mediterranean kale, whereas progoitrin is the major glucosinolate in rapeseed (*Brassica napa*). Upon hydrolysis of *Crambe abyssinica* meal, e*pi*-progoitrin will break down to several nitrile compounds, including highly reactive epithionitiriles as well as crambene (*16, 17*), whereas in commercially processed meal, only crambene is found (*18*). Breakdown of *epi*-progoitrin yields the S form of crambene, whereas breakdown of progoitrin yields the R-form (*12*). Both forms have equivalent biological effects (*19*). Very little of another potential hydrolysis product, the isothiocyanate, which cyclizes to form 5-vinyloxazolidine-2-thione (goitrin), is not present in either hydrolyzed or commercially processed meal, except under certain experimental conditions (*20*).

Several methods have been used in previous purifications of bioactive breakdown products, but these processes have been very time-consuming and characterized by low yields (<20%) with residual colored contaminants (*12, 21*). Rapid and efficient isolation procedures are needed to enable more extensive in-depth research using the potentially beneficial compound. Furthermore, many existing standard biologically active cruciferous compounds that have been utilized in previous studies are not entirely pure, and most are in very short supply. Rapid and efficient methods need to be developed to consistently isolate highly purified standard compounds for future lab animal, human cancer cell, and human clinical studies. Synthesis of these compounds is possible; however, synthesis requires several highly toxic substances, and final products from these reactions still contain toxic residues and require further purification (*22*).

The methodology described in this paper was developed to eliminate several time-consuming steps, eliminate residual pigments, and rapidly purify gram quantities of crambene. A similar method was used to isolate sulforaphane (1-isothiocyanato-4-[methylsulfinyl] butane) and sulforaphane nitrile (5-[methylsulfinyl] pentanenitrile) from autolyzed broccoli seed meal (*23*).

MATERIALS AND METHODS

Crambe abyssinica cv. Meyer (crambe) seed and meal were from the former National Sun Industries (Enderlin, ND) grown at the North Dakota State University Agricultural Experiment Station in 1991 and 1993. The meal had been processed commercially using a prepress solvent extraction on hulled seed, which extracts oil with steamed hexane from a cakelike solid of pressed seed.

All water used was reagent grade, purified using a Photronix reagent-grade water system. All chemicals for extractions were purchased from Fisher Chemicals (Fisher Scientific, St. Louis, MO). Sodium chloride (NaCl) and sodium sulfate (Na_2SO_4) were reagent grade; methylene chloride (CH_2Cl_2) was reagent grade for crude extractions and Optima grade for gas chromatography (GC) and GC/mass spectroscopy (MS) analysis. Hexane and methanol (UV cutoff 205 nm) were high-performance liquid chromatography (HPLC) grade. Ultrahigh purity gases and compressed air for GC analysis were purchased from S. J. Smith Welding Company (Champaign, IL). Crambene standards were USDA standards provided by G. F. Spencer (NCAUR/ARS/ USDA, Peoria, IL).

Grinding Crambe Seed. Seeds were ground in a Tekmar A-10 analytical mill for ~10 s with tap-water cooling to the unit. Ground crambe seed was passed through a Tyler Standard Screen Scale 10-mesh screen to remove larger unground material, which was then reground and passed through the screen again. The recovered material, primarily whole or halved seeds, was reground and combined with the more finely ground seed meal.

Defatting of Ground Seed Meal. Ground seed meal (300−400 g) was added to approximately 2 L of hexane and extracted for a minimum of 4 h per extraction. Hexane was separated the from seed meal by filtering through Whatman no. 4 or no. 1 filter paper. Defatting was repeated 3 times. The hexane/oil phase was concentrated on a Büchi RE-111 vacuum rotary evaporator with water-bath temperature at 60−70 °C. After the final extraction, the defatted seed meal was allowed to air-dry overnight at room temperature in a ventilation hood.

Glucosinolate Analysis. Samples were prepared by grinding seed in a Tekmar A-10 analytical mill for 10 s with tap-water cooling to the unit, passing the ground seed through a Tyler Standard Screen Scale 60-mesh screen, and defatting 3 times in hexane, all followed by air-drying overnight at room temperature (22 °C). For HPLC analysis, 0.2 g of each sample was placed in an ultracentrifuge tube. The HPLC method of Kushad et al. (*4*), in which desulfonated forms of glucosinolates are quantified, was used for determining *Epi*-progoitrin concentrations. This methodology is based on a method modified from the EU standard procedure for analysis of glucosinolates in rapeseed (*24*).

Acidic pH Hydrolysis of Crambe Seed Meal. Approximately 200 g of defatted crambe seed meal (~200 g) was used for analysis. As maximum nitrile breakdown product formation was desired, an acidic environment was created for autolysis by adding 400 g of water, and followed by addition of concentrated HCl dropwise while stirring with a glass stir rod until the pH reached 3.0. The initial autolysis mixture contained approximately 33% crambe meal, 65% H_2O, and ~2% concentrated HCl. The mixture was then hydrolyzed at room temperature (22−25 °C) for 24 h. After 24 h, the mixture was stirred and the pH was recorded. The hydrolyzed meal was then extracted immediately.

An alternate source for extraction was commercially processed crambe meal (derived from seed grown at the North Dakota State University Agricultural Experiment Station) that had been previously defatted, by prepress solvent extraction using steam, at the former National Sun Corporation (Enderlin, ND) in 1991 and 1993. This meal was dry, and it was much easier to extract than wet hydrolyzed seed meal. GC analysis determined that the meal had undergone autolysis at some point in time and was rich in crambene and devoid of goitrin.

Crude Extraction of Autolyzed Crambe Seed Meal. To each hydrolyzed sample, NaCl and Na_2SO_4 were added in the weight proportion of 1:1:0.75 (autolyzed meal/NaCl/Na_2SO_4). The salts were mixed well with the autolyzed meal before the addition of excess CH_2Cl_2. Salted meal was mixed with an excess of CH_2Cl_2, stirred well, and kept at room temperature for a minimum of 5 h. To separate the solvent from the meal, the slurry was filtered through Whatman no. 4 filter paper. To eliminate residual H_2O from the filtrate, excess Na_2SO_4 was added to the filtrate and the mixture was stirred for 15 min. To prevent evaporative loss of crambene, the resulting extract was concentrated on a rotary evaporator at a water bath temperature no higher than 32 °C. The meal was extracted twice more for an equivalent amount of time for each extraction. The extract was then sparged with nitrogen before storage. The final extract was a viscous, dark-colored, oily residue. This extract was diluted approximately 10-fold with CH_2Cl_2 and analyzed by GC to detect the presence of the desired compounds.

Immiscible Solvent Cleaning/Extraction. A 3-fold excess of degassed H_2O was added to a weighed amount of the concentrated (dried) crude breakdown product extract at room temperature (20−24 °C), and the mixure was swirled gently to suspend the crude extract. Hexane was added in great excess (~100:1 water/crude extract v/v) to the crude extract, and the mixture was swirled gently and decanted. This washing of the aqueous layer was repeated 5−6 times until the hexane layer remained clear. The hexane extracts were kept and analyzed by GC to determine whether any compounds of interest had been extracted into the hexane.

To analyze the aqueous phase, a sample (2 mL) was mixed with anhydrous Na_2SO_4 (which was added to to absorb the aqueous phase); then 100 mL of CH_2Cl_2 was added to dissolve the compounds that were in the aqueous phase. After separating the CH_2Cl_2 from the Na_2SO_4, the CH_2Cl_2 fraction was concentrated to a golden oily residue on a rotary evaporator with water-bath temperature between 20 and 32 °C. The concentrate was dissolved in a measured volume of solvent and then analyzed by GC to determine purity and concentration of breakdown products in the aqueous layer. At this stage, crambene was ~95% of the total compounds detectable by GC.

Gas Chromatography. The volatile glucosinolate breakdown products were analyzed by GC using flame ionization detection (FID) and a Hewlett-Packard 5890 gas chromatograph with a Hewlett-Packard 7673A automatic sampler and controller. A megabore DB-17 (30 m, 0.53 mm i.d., 1.0 μm film thickness) capillary column (J & W Scientific, Inc., Folsom, CA) with a 50% phenyl−methyl deactivated 1 m × 0.53 mm guard column (Restek) was used for the separation of the breakdown products. The carrier gas was ultrahigh-purity helium (99.999%) and the other make-up gases were ultrahigh-purity nitrogen and hydrogen.

Prior to injection on the auto sampler, samples were stored at room temperature in amber silanized-glass crimp-top vials. A 1- or 2-μL sample was injected onto the column through a J&W double-gooseneck injection liner with splitless on-column injection. Initial temperature was 80 °C for 2 min, then ramped at 10 °C/min to 280 °C and held for 4 min. All data were collected and recorded on a Spectra Physics SP4200 computing integrator.

HPLC Compound Isolation. HPLC was used to further purify the crambene after the immiscible solvent wash step. The separation utilized a Waters System Interface Module controlled with the Waters Millenium 32 chromatography manager program. The injector was a model U6K universal liquid chromatography injector with a 2.0-mL injection loop. The solvent was delivered by two Waters 501 HPLC pumps. The column was a Waters Prep Nova-Pak (19 m × 300 mm, 60 A, 6 μm) HR C-18 column. Compound separation was monitored with a Waters R401 differential refractometer followed by a Waters 486 UV detector set at 254 nm.

The solvent system for crambene purification consisted of an 80:20 mixture of H_2O and methanol pumped at 9.0 mL/min for 11 min. The column was cleaned by pumping pure methanol at 5.0 mL/min with a second HPLC pump while the first continued to pump the 80:20 solvent at 9.0 mL/min. After running both pumps for 5 min (pumping a total of 14 mL/min), the second pump was shut down and the first continued to run to reequilibrate the system for the next sample. Fractions were collected when peaks were detected. A peak from crambene was evident with the UV detector just before the crambene entered the RI detector where it elicited a strong RI change. Crambene was isolated from the system in a single one minute fraction after ~9 min of elution time. Each sample run time, with appropriate column washing to achieve a stable baseline between runs, was approximately 25 min.

GC−MS and NMR Analysis. These run parameters were similar to the those of the GC analysis in order to compare the compounds' elution profiles with the DB-17 megabore 0.53 μm capillary column. However, a DB-5 capillary column was used for the GC/MS separation. Following are the operating conditions for the GC/MS 70-VSE instrument: acceleration potential of 8 kV, 70 eV ionization potential with 100 μA of emission current, ion source temperature at 200 °C, and transfer line temperature at 290 °C. The purified crambene was dissolved in D_2O in a 5-mm NMR tube and analyzed by ^1H spectrum. A Unity 500 MHz narrow bore spectrometer was used.

Table 1. Effects of pH on Yield of Crambene from Hydrolyzed Crambe Seed

condition	pH*i*	pH*f*	avg. pH	crambene yield (% of control)
control 35.0 g seed 70.0 g H_2O	5.05	5.05	5.05	100
acid I 35.0 g seed 65.0 g H_2O 5.0 g concd HCl	1.30	1.30	1.30	75
acid II 35.0 g seed 68.5 g H_2O 1.7 g concd HCl	2.70	3.10	2.90	167
base I 35.0 g seed 67.0 g H_2O 3.0 g 1 N NaOH	6.40	5.70	6.05	60
base II 35.0 g seed 64.0 g H_2O 6.0 g 1 N NaOH	8.60	6.40	7.50	75

RESULTS AND DISCUSSION

Glucosinolate Analysis. Crambe seed defatted in the laboratory and commercially defatted meal were analyzed for glucosinolate content. For the seed, each batch was ground and defatted with excess hexane, reground, passed through a 100-mesh screen, and defatted again with excess hexane to remove all excess oil contaminants prior to HPLC analysis. In both cases, *epi*-progoitrin was the predominant glucosinolate, being 80% (60 μmol/g) and 88% (71 μmol/g) of the total glucosinolates in both hydrolyzed and commercially processed seed meals, respectively (data not shown). This is concordant with proportions of *epi*-progoitrin in crambe meal used in feeding studies (*25*). The high *epi*-progoitrin concentrations indicate that either crambe seed itself or processed meal are good sources for crambene in relatively concentrated form.

Varied pH of Hydrolysis. The yield of crambene from crambe seed autolyzed at varying pH levels is shown in Table 1. Five different hydrolysis systems were chosen to correlate pH with crambene production. Each sample was hydrolyzed for 24 h, frozen, and lyophilized before extraction and analysis. The pH change recorded after 24 h of autolysis showed the pH generally shifting toward the control pH, as described in Table 1. This demonstrates an evident buffering capacity of the seeds themselves. Crambe hydrolysis at different pH levels showed maximum crambene production at pH 2.9. This compares favorably with hydrolysis conditions utilized previously to form nitrile compounds (*12*, *26*). A batch of hydrolyzed crambe was processed in parallel with these samples but was not lyophilized. NaCl was added in excess (~40% by mass) to the nonlyophilized sample which was then extracted with excess CH_2Cl_2 for a period of several days. A higher amount of crambene was extracted from this sample than from the lyophilized samples, as determined by comparison of GC peak areas in equally diluted samples. Therefore, to aid in CH_2Cl_2 extraction, crambe was autolyzed at a pH near 3.0, and NaCl was added to bind excess water.

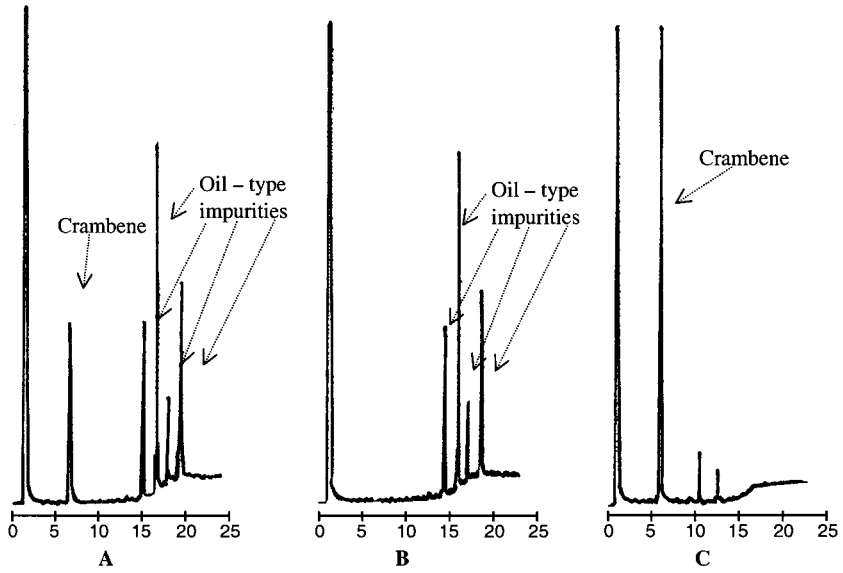

Figure 3. Immiscible solvent wash GC tracings. The (**A**) concentrated crude extract, after being resuspended in H_2O and washed with organic solvent, yielded two phases. A depiction of the GC analyses of the (**B**) organic phase and the (**C**) aqueous phase shows the impurities removed and the increase in crambene concentration, respectively.

Solvent Extraction. Six different solvents were tested for extraction of crambene from equal amounts of crambe meal. Each solvent was tested with and without NaCl addition because previous researchers described higher crambene solubility in some solvents in the presence of salt. Solvents tested included methylene chloride, ethyl acetate, methanol, chloroform, acetone, and hexane. A 100-mL portion of each solvent was added to 10 g of autolyzed crambe meal with and without addition of 5 g of NaCl. After overnight extraction, the solvent was separated from the meal and tested by GC without solvent concentration to check the crambene solubility in equal volumes of each solvent. The largest amount of crambene was extracted by methanol, followed by methylene chloride. The other three solvents did not extract detectable amounts of crambene. This led to further method development using dilutions of methanol because this solvent can be used for food-grade extraction of crambene.

Oil Contaminant Studies. Crude extracts from commercially defatted, as well as benchtop defatted, crambe meal had consistent contaminant peaks on GC runs. In an attempt to identify these components, oil was pressed out from fresh crambe seed. The pressed oil, after analysis by GC, had the same peaks as the contaminant in the crude extract as depicted in Figure 3. To remove these primary contaminants, several modifications of immiscible solvent washes were tested, primarily using different proportions of H_2O in the washes to monitor completeness of crambene extraction. After the final hexane wash, the aqueous sample volume was measured and recorded. Samples were chilled to ~4 °C and filtered through a 0.45-μm membrane filter to ensure removal of any particulates. The membrane filter was washed with a volume of methanol to bring the total sample solvent ratio equal to that of the HPLC solvent system (80:20 H_2O/MeOH) for final purification.

Crude extracts from commercially defatted crambe meal had higher initial crambene purity than extracts from the autolyzed seed meal (30−40% compared to 10−25%). The primary contaminants in the meal extracts were residual fatty acids that remained after commercial defatting. When the dry meal was pre-washed with hexane, some crambene was also extracted. Extraction with CH_2Cl_2 and concentration before proceeding with the immiscible solvent extraction to remove the fatty acids avoided loss of crambene and produced yields of ~99% of calculated maximum yield (i.e., 71 μmol/g of *epi*-progoitrin in the meal should theoretically yield 6.89 mg/g of crambene). The polar hydroxyl group on crambene is responsible for its high solubility in the aqueous phase compared to that of the nonpolar organic phase, which removes nonpolar contaminants that are extracted by CH_2Cl_2. H_2O was added to the concentrated crude extract at 3:1 (water/extract) and hexane was used in great excess (~100-fold volume) through a sequence of 3−5 washes/sample. Attempts to use H_2O more sparingly led to higher loss of crambene in the organic phase. This may be due to saturation of the aqueous phase with polar compounds. The saturation point was estimated as 20 mg of crambene per mL of H_2O. This was determined by collecting 1 g of purified crambene from 10 mL of aqueous phase purified by 5 combined HPLC separations.

The concentrated crude extract, after being resuspended in H_2O and washed with organic solvent (hexane), yielded two phases (Figure 3). The immiscible solvent wash of concentrated crude extract increased crambene purity by over 70% and removed most organic solvent soluble impurities before the final HPLC purification. This method is more rapid than previous gravity elution column purification. Yield was calculated to be 65.6% of the crambene present in the crude extract prior to the immiscible solvent wash step.

The possibility of washing the concentrated crude extract with aqueous methanol (20%) to prepare for immediate HPLC extraction was explored. The aqueous methanol phase had a deeper yellow color than the H_2O used alone for the wash. Purified crambene is very pale yellow, so the excessive yellow color probably was attributed to pigment that was more soluble in aqueous methanol than in H_2O alone. Aqueous methanol samples did not show an increase in crambene concentration, so it was decided to dilute the H_2O washes containing crambene with methanol to approximately the same mobile-phase proportion used for HPLC purification.

HPLC. Crambene purification was achieved by optimizing H_2O and methanol proportions and flow rates

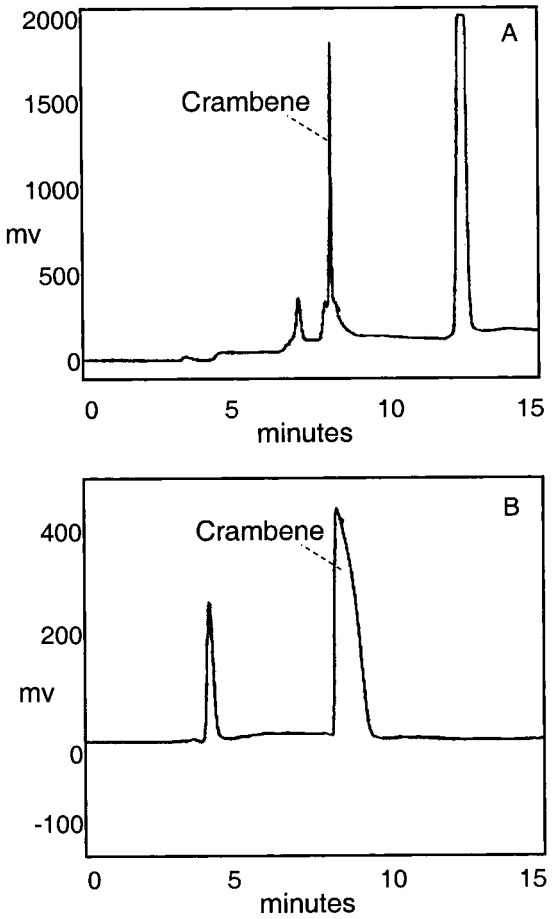

Figure 4. HPLC Results of crambene purification detection. (**A**) UV absorbance at 254 nm and (**B**) RI diffraction (atten. = 16×). After injections, the aqueous phase from an immiscible solvent wash of crambene is separated by HPLC as seen above. Surprisingly, crambene appears to have a strong UV absorbance.

to obtain sufficient separation in a short elution time for the complete sample. Optimum separation was achieved using the gradient run described previously in the methods section. Crambene was purified, and the column was rapidly cleaned for the next sample. The developed gradient HPLC separation is completed in 25 min with crambene, which elutes at ~9 min. Compounds were detected by ultraviolet absorbance and refractive index detection in sequence. The elution profile from a normal run of an aqueous phase from an immiscible solvent wash of crambe can be seen in Figure 4. Calculated yield after HPLC was 62.6% of the crambene in the water extract after immiscible solvent extraction. Therefore, using crambe seed meal as an example, 71 μmol of *epi*-progoitrin/g meal should yield 71 μmol of crambene, corresponding to 6.89 mg crambene/g meal. Under optimal hydrolysis conditions, ~99% yield was obtained, or 6.82 mg crambene/g meal. Immiscible solvent washing resulted in a yield of 4.47 mg crambene/g meal and HPLC purification in a final yield of 2.80 mg crambene/g meal, or 40.6% of the theoretical maximum.

GC/MS Electron Impact (EI) and NMR. Semipurified and purified samples were analyzed by GC/MS. The compounds analyzed by electron impact ionization had fragmentation patterns (data not shown) consistent with those reported by Spencer and Daxenbichler (*27*). NMR analysis of the puriified crambene sample produced results that also were consistent with those previously reported (*12, 28*) and did not show the presence of compounds other than crambene.

Significance of Crambene. Crambe (*Crambe abyssinica)* has been used since the mid 1960s as an oil and feed source; however, it is considered a relatively new industrial oilseed crop. Concerns about the high concentrations of glucosinolates present in crambe has limited the amount of defatted meal that is allowed in animal feeds. *Epi*-progoitrin, one of the two parent glucosinolates that can break down to crambene, is found in very high concentrations in crambe meal and accounts for >80% of total glucosinolates in the meal (*28*−*30*). Therefore, crambe seed or meal can serve as a highly concentrated source for crambene. *Epi*-progoitrin is present in edible cruciferous vegetables as well, although at lower concentrations and in variable concentrations, depending on species and variety (*4*).

Isolation of natural forms of crambene from crambe seed meal was achieved using immiscible solvent wash methodology with subsequent HPLC purification. These methods are simple and more efficient than previously published processes and yield compounds of higher purity than previous standards. Furthermore, modifications of this same methodology have been used to isolate other glucosinolate breakdown products, such as sulforaphane and sulforaphane nitrile, from cruciferous vegetables (*31*).

ABBREVIATIONS

1-Cyano-2-hydroxy-3-butene, crambene; *Crambe abyssinica*, crambe; 5-[methylsulfinyl] pentanenitrile, sulforaphane nitrile; (1-isothiocyanato-4-[methyl-sulfinyl] butane, sulforaphane; (*2S*)-2-hydroxylbut-3-enyl-gluosinolate, *epi*-progoitrin; (*2R*)-2-hydroxylbut-3-enyl-glucosinolate, progoitrin; 5-vinyloxazolidine-2-thione, goitrin.

ACKNOWLEDGMENT

The authors acknowledge the assistance of Anna Louisa Peters in sample preparation and analysis.

LITERATURE CITED

(1) Graham, S. Results of case-control studies of diets and cancer in Buffalo. *Cancer Res.* **1983**, *43*, 2409−2413.
(2) Wattenberg, L. W. In *Inhibition of carcinogenesis by nonnutrient constituents of the diet*. Waldron, K. W., Johnson, I. T., Eds.; The Royal Society of Chemistry: London, England, 1993; pp 12−24.
(3) Kohlmeier, L.; Su, L. Cruciferous vegetable consumption and colorectal cancer risk: meta-analysis of the epidemiological evidence. *FASEB J.* **1997**, *11*, A369 (2141).
(4) Kushad, M. K.; Brown, A. F.; Kurilich, A. C.; Juvik, J. A.; Klein, B. P.; Wallig, M. A.; Jeffery, E. H. Variation of glucosinolates in vegetable crops of *Brassica oleracea*. *J. Agric. Food Chem.* **1999**, *47*, 1541−1548.
(5) Ciska, E.; Martyniak-Przybyszewska, B.; Kozlowska, H. Content of glucosinolates in cruciferous vegetables grown at the same site for two years under different climatic conditions. *J. Agric. Food Chem.* **2000**, *48*, 2862−2867.
(6) Nastruzzi, C.; Cortesi, R.; Esposito, E.; Menegatti, E.; Leoni, O.; Iori, R.; Palmieri, S. In vitro antiproliferative activity of isothiocyanates and nitriles generated by myrosinase-mediated hydrolysis of glucosinolates from seeds of cruciferous vegetables. *J. Agric. Food Chem.* **2000**, *48*, 3572−3575.
(7) Fahey, J. W.; Zhang, Y.; Talalay, P. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 10367−10372.

(8) Gerhäuser, C.; You, M.; Liu, J.; Moriarty, R. M.; Hawthorne, M.; Mehta, R. G.; Moon, R. C.; Pezzuto, J. M. Cancer chemoprotective potential of sulforamate, a novel analogue of sulforaphane that induces phase 2 drug-metabolizing enzymes. *Cancer Res.* **1997**, *57*, 272−278.
(9) Zhang, Y.; Talalay, P. Anticarcinogenic activities of organic isothiocyanates: chemistry and mechanisms. *Cancer Res. (Suppl.).* **1994**, *54*, 1976s−1981s.
(10) Leoni, O.; Felluga, F.; Palmieri, S. The formation of 2-hydroxybut-3-enyl cyanide from (*2S*)-2-hydroxybut-3-enyl glucosinolate using immobilized myrosinase. *Tetrahedron Lett.* **1993**, *34*, 7967−7970.
(11) Staack, R.; Kingston, S.; Wallig, M. A.; Jeffery, E. H. A comparison of the individual and collective effects of four glucosinolate breakdown products from Brussels sprouts on induction of detoxification enzymes. *Toxic. Appl. Pharmacol.* **1998**, *149*, 17−23.
(12) Daxenbichler, M. E.; VanEtten, C. H.; Wolff, I. A. (*S*)- and (*R*)-1-cyano-2-hydroxy-3-butene from myrosinase hydrolysis of epi-progoitrin and progoitrin. *Biochemistry* **1966**, *5*, 692−697.
(13) March, T.H.; Jeffery, E. H.; Wallig, M. A. The cruciferous nitrile, crambene, induces rat hepatic and pancreatic glutathione S-transferases. *Toxicol. Sci.* **1998**, *42*, 82−90.
(14) Nho, C. W.; Jeffery, E. H. Crambene, a nitrile from cruciferous vegetables produces upregulation of quinone reductase that is ARE-dependent. *FASEB J.* **1999**, *13*, A918.
(15) Wallig, M. A.; Kingston, S.; Staack, R.; Jeffery, E. H. Induction of rat pancreatic glutathione S-transferase and quinone reductase activities by a mixture of glucosinolate breakdown derivatives found in Brussels sprouts. *Food Chem. Toxicol.* **1998**, *36*, 265−273.
(16) Galletti, S.; Bernadi, R.; Leoni, O.; Rollin, P.; Palmieri, S. Preparation and biological activity of four epiprogoitrin myrosinase-derived products. *J. Agric. Food Chem.* **2001**, *49*, 471−476.
(17) Daxenbichler, M. E.; VanEtten, C. H.; Wolff, I. A. Diastereomeric episulfides from *epi*-progoitrin upon autolysis of crambe seed meal. *Phytochemistry* **1968**, *7*, 989−996.
(18) Kloss, P.; Jeffery, E.; Tumbleson, M.; Zhang, Y.; Parsons, C.; Wallig, M. Studies on the toxic effects of crambe meal and two of its constituents, 1-cyano-2-hydroxy-3-butene (CHB) and epi-progoitrin, in broiler chick diets. *Br. Poultry Sci.* **1996**, *37*, 971−986.
(19) Kore, A. M.; March, T. H.; Davis, M. A.; Jeffery, E. H.; Wallig, M. A. Toxicity, distribution and elimination of intravenous cyanohydroxybutene (CHB). *FASEB J.* **1991**, *5*, 1571.
(20) Kloss, P.; Jeffery, E.; Tumbleson, M.; Parsons, C.; Johnson, L.; Reuber, M.; Wallig, M. Efficacy of feeding gluoscinolate-extracted crambe meal to broiler chicks. *Poultry Sci.* **1994**, *73*, 1542−1551.
(21) Wallig, M. A.; Kore, A. M.; Crawshaw, J.; Jeffery, E. H. Separation of the toxic and glutathione-enhancing effects of the naturally occurring nitrile, cyanohydroxybutene. *Fundam. Appl. Toxicol.* **1992,** *19*, 598−606.
(22) Das, N. B.; Torssell, K. B. G. Silyl nitronates, nitrile oxides and derived 2-oxazolines in organic synthesis. Functionalization of butadiene, a novel route to furans and 2-isoxazolines as an alternative to aldol-type condensation. *Tetrahedron* **1983,** *39*, 2247−2253.
(23) Niedoborski, T. E.; Wallig, M. A.; Klein, B. P. Purification of cruciferous bioactive breakdown products. *Program Book of Abstracts, Annual Meeting of the Institute of Food Technologists*; Institute of Food Technologists: Chicago, IL, 1999; p 15, 11A-9.
(24) Warthelet, J. P.; Wagstaffe, P. J.; Boeke, A. The certification of the total glucosinoalte and sulfur contents of three rapeseeds (colza). *Comm. Eur. Communities* [Rep.] EUR 13339 EN, **1991**, 67−70.
(25) LeDoux, D. R.; Belyea, R. L.; Wallig, M. A.; Tumbleson, M. E. Effects of feeding crambe meal upon intake, gain, health and meat quality of broiler chicks. *Anim. Feed Sci. Technol.* **1999**, *76*, 227−240.
(26) Kore, A. M.; Spencer, G. F.; Wallig, M. A. Purification of the w-(methylsulfinyl)alkyl glucosinolate hydrolysis products: 1-isothiocyanato-3-(methylsulfinyl)propane, 1-isothiocyanato-4-(methylsulfinly)butane, 4-(methylsulfinyl)butanenitrile, and 5-(methylsulfinyl)pentanenitrile from broccoli and *Lesquerella fendleri*. *J. Agric. Food Chem.* **1993**, *41*, 89−95.
(27) Spencer, G. F.; Daxenbichler, M. E. Gas chromotography−mass spectrometry of nitriles, isothiocyanates, and oxazolidinethiones derived from cruciferous glucosinolates. *J. Sci. Food Agric.* **1980,** *31*, 359−367.
(28) Daxenbichler, M. E.; Peters, J. E.; Wolff, I. A.; Booth, A. N. Seed meal from *Crambe abyssinica*. *J. Agric. Food Chem.* **1965**, *13*, 24−27.
(29) VanEtten, C. H.; Daxenbichler, M. E.; Peters, J. E.; Wolff, I. A.; Booth, A. N. Seed meal from *Crambe abyssinica*. *J. Agric. Food Chem.* **1965**, *13*, 24−27.
(30) Fenwick, G. R.; Heaney, R. K.; Mullin, W. J. Glucosinolates and their breakdown products in food and food plants. *CRC Crit. Rev. Food Sci. Nutr.* **1983,** *18*, 123−201.
(31) Matusheski, N. V.; Wallig, M. A.; Juvik, J. A.; Klein, B. P.; Ksuhad, M. M.; Jeffery, E. H. Preparative HPLC method for the purification of sulforaphane and sulforaphane nitrile from *Brassica oleracea*. *J. Agric. Food Chem.* **2001**, *49* (4), 1867−1872.

Received for review November 15, 2000. Revised manuscript received May 10, 2001. Accepted May 17, 2001. This research was supported in part by the Illinois Council on Food and Agricultural Research.

JF001366Y