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Received for review March 19, 1979. Accepted October 2, 1979.

Sinalbin and Other Glucosinolates in Seeds of Double Low Rape Species and *Brassica napus* cv. Bronowski

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The content of glucosinolates in seeds of some double low rape cultivars has been investigated. Using a newly developed method, the glucosinolates have been isolated, separated, and determined semiquantitatively. The identification has been performed by chromatography, high-voltage electrophoresis, ^{1}H- and ^{13}C-NMR spectroscopy, and by gas chromatography–mass spectroscopy. Sinalbin (4-hydroxybenzyl glucosinolate) is one of the dominating glucosinolates in seeds of cv. Erglu rape, cv. Tower, cv. Candle, and some other double low rape species, whereas it is not found in appreciable amounts in seeds of other double low rape cultivars and *Brassica napus* cv. Bronowski. The presence of relatively high concentrations of sinalbin and indolylmethyl glucosinolates in seeds of some double low rape cultivars is previously unreported. This is briefly discussed in relation to the methods used for identification and semiquantitative estimation of glucosinolates and in relation to glucosinolate catabolism and the nutritional value of rape seeds.

The seeds of some cruciferous plants (e.g., rape) are economically important as they are used both as food and plant oil sources. High contents of erucic acid in the oil and of glucosinolates in the meal are the reason for some unfavorable qualities. Therefore, a lot of plant breeding efforts have been placed in the production of rape cultivars (double low cultivars), giving seeds with a relative low content of glucosinolates and erucic acid compared to that found in other varieties of rapeseed commonly used.

The problems concerning glucosinolates in food are related both to the total amount, to the type of glucosinolates present, and to the products produced from them by autolysis or other degradation. All glucosinolate-containing plants also seem to contain β-thioglucosidases, EC 3.2.3.1. (myrosinases), in a separate compartment (Bjørkman, 1976). When the plant is crushed the thioglucosides are hydrolyzed by the liberated enzymes, and during the autolysis process a Lossen-type rearrangement may occur with the formation of isothiocyanates (Ettlinger and Lundeen, 1956). Within some cruciferous plants autolysis leads to other products such as thiocyanates (Gmelin and Virtanen, 1962), cyanides and amines (Saarivirta, 1973), or oxazolidinethiones and cyanoepithiolkanes (Daxenbichler et al., 1977). In strong acid solution glucosinolates are hydrolyzed to the corresponding carboxylic acids (Ettlinger and Lundeen, 1956), and in strong alkaline solution some glucosinolates are transformed to amino acids in a Neber-type rearrangement (Friis et al., 1977) (Figure 1).

Although about 80 different glucosinolates have been identified, much is unknown about autolysis and glucosinolate catabolism. However, it is well known that some glucosinolates are more easily transformed to thiocyanates and the thiocyanate ion than others (Gmelin and Virtanen, 1962; Lüthy and Benn, 1977; Nielsen et al., 1979). If glucosinolates are transformed to the thiocyanate ion, they will escape detection by the methods commonly used for glucosinolate analysis since these methods are based on the identification of common hydrolysis products (Srivastava and Hill, 1975; Wetter and Youngs, 1976).

It is known that the thiocyanate ion is produced in rapeseed meal during thioglucosidase hydrolysis (Srivastava and Hill, 1975; McGregor, 1978), and it is a well-known fact that glucosinolates influence the nutritional value of rapeseed meal (Josefsson, 1975).

The present study is a continuation of our previous work on the isolation (Nielsen et al., 1979; Olsen and Sørensen, 1979) and catabolism of glucosinolates (Dalgaard et al., 1977). It forms part of a study on the nutritional value of different double low rape cultivars, and the purpose of this study is isolation of the total pool of glucosinolates from rapeseed meal, followed by identification and quantitative estimations of the isolated glucosinolates, including the previously unknown precursors of the thiocyanate ion, which is known to be the major glucosinolates in seeds of some double low rape species (McGregor, 1978).

EXPERIMENTAL SECTION

Plant Material. Seeds of *Brassica napus* L. cv. Bronowski were purchased from State Research Station, DK-4000 Roskilde, Denmark. Seeds of the common rape, *Brassica napus* L. cv. Gulle, and the double low rape cultivars, *Brassica napus* L. cv.: Erglu, DP 075, DP 076, DP 525, DP 540, DP 666, DP 724, DP 941, and DP 2/12 were obtained from Danish Plant Breeding, Boelshøj, DK-4660 St. Heddinge, Denmark. Seeds of *Sinapis alba* L. were obtained from Trifolium-Silo A/S, DK-2630 Tåstrup, Denmark. *Brassica napus* L. cv. Tower and *Brassica campestris* L. cv. Candle were obtained from Professor L. D. Campbell, Department of Animal Science, University of Manitoba, Winnipeg, Canada.

General Methods and Instrumentation. ^{1}H NMR spectra were determined in D_2O solution at 60 MHz and chemical shifts are given here in ppm downfield from sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate

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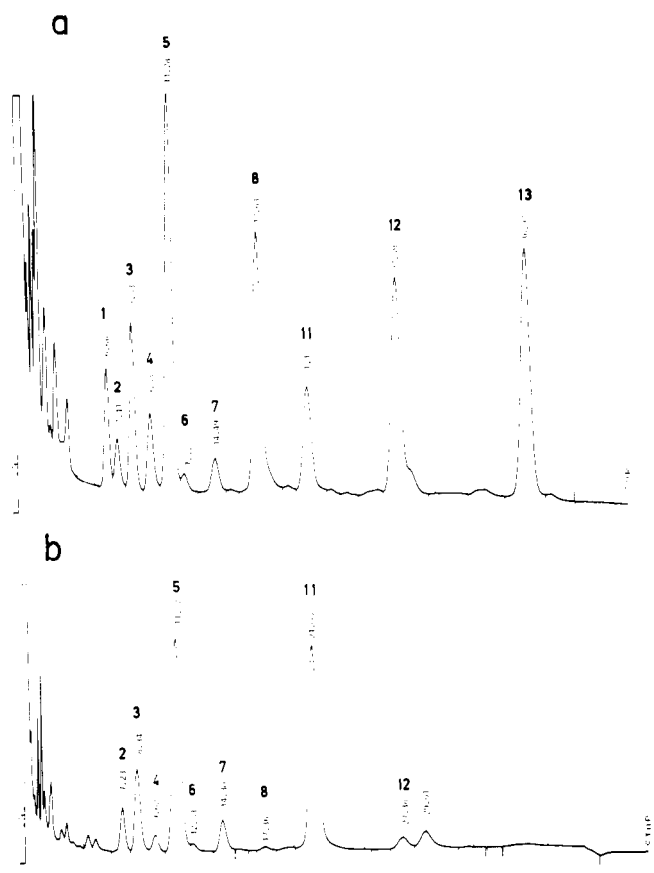
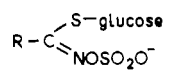


GLUCOSINOLATE



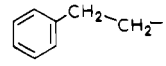
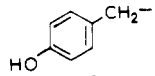
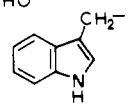
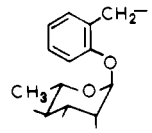
	R GROUP
(1) GLUCOCAPPARIN	CH_3-
(2) SINIGRIN	$CH_2=CH-CH_2-$
(3) GLUCONAPIN	$CH_2=CH-(CH_2)_2-$
(4) GLUCOBRASSICANAPIN	$CH_2=CH-(CH_2)_3-$
(5) PROGOITRIN	$CH_2=CH-\underset{\underset{OH}{|}}{CH}-CH_2-$
(6) NAPOLEIFERIN	$CH_2=CH-CH_2-\underset{\underset{OH}{|}}{CH}-CH_2-$
(7) GLUCOTROPAEOLIN	
(8) GLUCONASTURTIIN	
(9) GLUCORAPHANIN	$CH_3-SO-(CH_2)_4-$
(10) GLUCOALYSSIN	$CH_3-SO-(CH_2)_5-$
(11) SINALBIN	
(12) GLUCOBRASSICIN	
(13) o-(α-L-RHAMNOPYRANOSYLOXY)-BENZYLGLUCOSINOLATE	

Figure 1. GLC chromatograms of Me_3Si derivatives of glucosinolates. Peak numbers refer to the glucosinolates listed in the figure and the retention times (t_r, min) are shown below the peak numbers. The reference glucosinolates for chromatogram a have been isolated from different plants. Chromatogram b shows the results obtained with glucosinolates isolated from *Brassica napus* L. cv. Tower and the internal standards sinigrin and glucotropaeolin.

used as internal standard. ^{13}C-NMR spectra were recorded at 22.63 MHz on a Bruker HX 90E instrument using the pulse technique with Fourier transformation. Chemical shifts are given in ppm downfield from Me_4Si and dioxane was used as internal standard [$\delta(Me_4Si) = \delta(\text{dioxane}) + 67.4$ ppm]. Gas chromatography (GLC) was performed on a Pye Unicam GCV chromatograph equipped with a Hewlett-Packard 3380 A Integrator and a flame ionization detector. The carrier gas was nitrogen. Gas chromatography–mass spectrometry (GLC–MS) was performed on a Pye Unicam 104 gas chromatograph in conjunction with an AEJ MS 3074 mass spectrometer (70-eV ionizing energy). The carrier gas was helium.

GLC conditions used for separation and identification of isothiocyanates and oxazolidinethiones were as follows: a glass column, 3.0 m × 4 mm i.d. packed with 3% OV-17 silicon on Chromosorb WHP 80/100 mesh, was programmed from 70 °C for 2 min, followed by an increase of 10 °C/min to the final temperature of 280 °C. The injection port was 280 °C, the flame ionization detector was maintained at 280 °C, and the carrier gas was 40 mL/min.

GLC conditions used for separation and identification of Me_3Si derivatives of carboxylic acids and glucosinolates were as follows: a glass column, 1.5 m × 4 mm i.d. packed with 3% OV-1 silicone on Chromosorb WHP 80/100 mesh, programmed from 70 °C for 1 min, followed by an increase of 5 °C/min to the final temperature of 280 °C for the carboxylic acid derivatives and programmed from 200 °C and a temperature increase of 2 °C/min to the final temperature 280 °C for the glucosinolate derivatives. Other conditions were as above.

Paper chromatography (PC) was performed by the descending technique on Whatman No. 1 paper or Schleicher and Schüll 2043 b paper, and the following solvents were used: (solvent 1) 1-butanol–acetic acid–water (12:3:5); (solvent 2) 2-propanol–water–concentrated ammonia (8:1:1); (solvent 3) 1-butanol–pyridine–water (6:4:3), and (solvent 4) 1-butanol–ethanol–water (4:1:4). High-voltage electrophoresis (HVE) was carried out on Whatman 3 MM paper using a flat plate unit and the following systems: (I) buffer pH 1.9, acetic acid–formic acid–water (4:1:45), 2 h at 3.2 kV and 90 mA; (II) buffer pH 3.6, pyridine–acetic acid–water (1:10:200), 2 h att 3 kV and 90 mA; (III) buffer pH 6.5, pyridine–acetic acid–water (25:1:500), 50 min at 5 kV and 90 mA. Glucosinolates were detected with silver nitrate (Schultz and Wagner, 1956), phenols with Paulys reagent (Jatzkewitz, 1953), and carboxylic acids with the aniline–xylose reagent (Smith, 1958).

Isolation of the Glucosinolates by Ion-Exchange Chromatography, Preparative HVE, and Preparative PC. The seeds (500 g) were ground in a mill, the meal falling directly into boiling methanol–water (3 L, 7:3) to ensure inactivation of the myrosinase. Further homogenization was performed with an Ultra-Turrax homogenizer. The homogenate was boiled for 2–3 min and filtered. After filtration, the residue was washed twice with 50 mL of methanol–water (7:3). The combined filtrates were evaporated to dryness, redissolved in 500 mL of water, and extracted with 3 × 1 L of diethyl ether. The water phase was concentrated in vacuum (to about 15 mL) and transferred to a column of Amberlite IR-120 (H^+, 2.5 × 90 cm). After flushing with water (21 mL fractions were

collected at 150 mL/h), the glucosinolate-containing fractions (5–27) were detected by spot test, pooled, neutralized with 1 M KOH, concentrated in vacuum (to about 10 mL), and applied to a column of Ecteola-Cellulose (purchased from E. Merck, Darmstadt) (AcO^-, 2.5 × 80 cm). Fractions (14 mL) were collected at 40 mL/h. After washing with water (fractions 1–62), the column was eluted with 1 M pyridine. The glucosinolate containing fractions (69–92) and (96–122) were taken to dryness and further purified by preparative HVE in buffer system (I), followed by preparative PC separation in the solvents described above according to the R_f values for the compounds (Kjaer and Jensen, 1956; Olsen and Sørensen, 1979).

Myrosinase Catalyzed Hydrolysis of Glucosinolates and GLC–MS Analysis of the Hydrolysis Products. Enzymatic conversion of glucosinolates to isothiocyanates was performed as described previously (Nielsen et al., 1979). Myrosinase was isolated from *Sinapis alba* L. by the method described previously (Appelqvist and Josefsson, 1967).

Hydrolysis of Glucosinolates in 6 M HCl and GLC–MS Analysis of the Carboxylic Acid Me_3Si Derivatives. The procedure is an adaption of the method described previously (Olsen and Sørensen, 1979). Glucosinolates (5 mg) were dissolved in 2.5 mL of 6 M HCl and hydrolyzed for 4 h at 60 °C, followed by 18 h at 25 °C. The reaction mixture was taken to dryness and stored in a dessiccator containing $CaCl_2$. The residue was then mixed with 250 μL of DMF (dimethylformamide, silylating grade) and 250 μL of BSTFA (*N,O*-bis(trimethylsilyl)trifluoroacetamide) and left at 60 °C for 30 min.

Analytical Determination of Glucosinolates in Rape Seeds by GLC Determination of the Me_3Si Derivatives of Isolated Glucosinolates. Seeds (2 g) were added to boiling methanol–water (7:3, 75 mL) and homogenized with an Ultra-Turrax homogenizer, after which the homogenate was boiled for 2–3 min, cooled, and filtered. To complete the extraction, the residue was boiled twice with methanol–water (7:3, 37-mL portions) for 2–3 min, cooled, and filtered. The combined filtrates were evaporated to a small volume (less than 5 mL), 1 mg of glucotropaeolin and 1 mg of sinigrin were added to the solution as internal references. The solution was then transferred to a column of Amberlite IR-120 (H^+, 1.6 × 40 cm) connected in series to a column of Ecteola-Cellulose (AcO^-, 2.6 × 20 cm). The columns were rinsed with water (500 mL), leaving the glucosinolates on the Ecteola column from which they were eluted with 1 M pyridine (400 mL). The pyridine eluate was evaporated to dryness and purified by preparative HVE in buffer system (I), followed by ion-exchange chromatography on the Ecteola column as described above. Evaporation of the pyridine eluate left a residue, of which one-half was subjected to acid hydrolysis while the other was stored in an dessiccator prior to silylation with 250 μL of BSTFA and 250 μL of DMF for 1 h at 120 °C.

The preparative HVE of the glucosinolates followed by ion-exchange chromatography may be omitted when the glucosinolates are detected directly as the Me_3Si derivatives, but the sequence is necessary previous to acid hydrolysis of the glucosinolates to ensure that the carboxylic acids detected are originally derivatives of the glucosinolates.

RESULTS AND DISCUSSION

Investigations of the glucosinolates in seeds of double low rape cultivars by HVE and traditional GLC analysis (Nielsen et al., 1979) revealed that not all the glucosinolates present in appreciable amounts were detected by GLC of the isothiocyanates produced by myrosinase-catalyzed hydrolysis. Satisfactory analytical determination of the glucosinolates require that these compounds are separated from other plant constituents. This has been performed by the previously described ion-exchange chromatography method (Olsen and Sørensen, 1979) which employs a volatile eluant (1 M pyridine) so as to avoid high salt concentrations in the evaporated glucosinolate-containing fractions (Schultz et al., 1953; Danielak and Borkowski, 1969; Wetter and Dyck, 1973) as well as strongly acid and alkaline conditions, which may cause glucosinolate degradation (Ettlinger and Lundeen, 1956; Friis et al., 1977). The weakly basic Ecteola anion-exchange column has functional groups with pK_a values of about 7.5 and retains the glucosinolates quantitatively. The problems regarding the elution of glucosinolates from anion-exchange resins (Van Etten and Daxenbichler, 1977) are not encountered since the charge on the Ecteola column is removed by the 1 M pyridine eluant, thereby releasing the glucosinolates in a few fractions without separation of the individual compounds. However, some aromatic compounds have a tendency to adsorption and they are therefore eluted somewhat later, as is the case for sinalbin and indolylmethyl glucosinolates.

Further purification and separation of the different glucosinolates are obtained by preparative PC (see Experimental Section) and HVE in buffer system I (Olsen and Sørensen, 1979). The glucosinolates were identified by NMR spectroscopy (Olsen and Sørensen, 1979). PC and HVE investigations of the pyridine eluates from the Ecteola column revealed that seeds of the investigated double low rape species contained gluconapin (3-butenyl glucosinolate), glucobrassicanapin (4-pentenyl glucosinolate), and progoitrin (2-hydroxy-3-butenyl glucosinolate) in appreciable amounts. These compounds have been identified by ^{1}H-NMR spectroscopy after isolation by preparative HVE and PC. Glucoraphanin (4-methylsulfinylbutyl glucosinolate) and glucoalyssin (5-methylsulfinylpentyl glucosinolate) isolated by preparative HVE and PC from seeds of Erglu rape and identified by their ^{1}H-NMR spectra with the characteristic methylsulfinyl signals (Dalgaard et al., 1977) are present in these seeds in low concentrations. Napoleiferin (2-hydroxy-4-pentenyl glucosinolate) and gluconasturtiin (2-phenylethyl glucosinolate) are also present in cv. Erglu rape seeds in relatively low concentrations (vide infra).

The ^{13}C-NMR spectrum of sinalbin isolated from seeds of cv. Erglu rape exhibited signals at 82.3, 72.8, 77.9, 70.1, 81.1, and 61.8 ppm for the carbon atoms C(1′)–C(6′) in the thioglucose part, at 38.4 ppm for the benzylic carbon atom, at 127.6, 130.4, 116.8, 155.7, 116,8, and 130.4 ppm for the carbon atoms C(1)–C(6) in the aromatic part, and at 163.7 ppm for the glucosinolate carbon atom. Comparison of the ^{13}C chemical shift values with previously reported values (Olsen and Sørensen, 1979) confirms the sinalbin structure.

Sinalbin is one of the dominating glucosinolates in seeds of cv. Tower and cv. Erglu and it is present in appreciable amounts in some of the other double low rape species investigated, although not in all of them. This compound is easy to detect with Paulys reagent on the paper after HVE and PC. An as yet unidentified compound which reacts with Paulys reagent yielding a color different from that obtained with sinalbin is present in most of the rape species investigated. This unidentified compound (vide infra) is unstable in both acid and base and has mobilities relative to sinalbin in HVE, buffer system I–III: 0.81, 0.78, and 0.70, respectively. Correspondingly the mobilities in PC relative to sinalbin in solvent 1, 2, 3, and 4 are 0.98,

Table I. Identification and Concentration (Micromoles of Glucosinolate/Gram of Seed) of Glucosinolates in the Seeds of Rape (*Brassica napus* L.) and Some Double Low Rape Cultivars[a] Determined by GLC Analysis of Isothiocyanates[b] and Oxazolidinethiones[c]

glucosinolate	t_r, min	$M^+/m/e$ base peak	Gulle rape[e]	Tower[e]	Candle[f]	Erglu[e]	DP-075[e]	DP-076[e]	DP-525[e]	DP-540[e]	DP-666[e]	DP-724[e]	DP-2/12[e]
gluconapin	8.7[b]	113/72	14.80	2.82	2.63	2.08	3.93	0.81	1.76	1.12	0.89	0.57	0.73
glucobrassicanapin	10.2[b]	127/99	7.50	0.93	2.03	1.10	0.48	0.51	0.89	0.44	0.69	0.31	0.47
progoitrin	19.0[c]		38.88	4.50	3.00	3.72	2.78	0.72	1.57	3.53	1.72	0.98	2.07
napoleiferin	20.2[c]		7.74	0.78	0.90	0.30	0.02	0.02	0.11	0.02	0.02	0.02	0.02
gluconasturtiin	16.7[b]	163/91	3.15	0.65	0.21	0.62	0.09	0.09	0.09	0.10	0.14	0.06	0.16
glucoraphanin			ND[d]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
glucoalyssin			ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
sinalbin			ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a] For the origin of the seeds, see the Experimental Section; the internal standards sinigrin and glucotropaeol are used in calculations of the glucosinolate amounts; (t_r) – ($M^+/m/e$ base peak) for allyl isothiocyanate and benzyl isothiocyanate were (6.9 min) – (99) and (15.3 min) – (149/91), respectively. [b] Isothiocyanate. [c] Oxazolidinethione. [d] Not detected by this method (see the Results and Discussion section. [e] *B. napus* L. [f] *B. campestris* L.

Table II. Concentration (Micromoles of Glucosinolate/Gram of Seed) of the Quantitatively Important Glucosinolates in the Seeds of Rape (*Brassica napus* L.) and Some Double Low Rape Cultivars Determined by GLC Analysis of the Me_4SI Derivatives[a]

glucosinolate[b]	t_r, min	Gulle rape[d]	Tower[d]	Candle[e]	Erglu[d]	DP-075[d]	DP-076[d]	DP-525[d]	DP-540[d]	DP-666[d]	DP-724[d]	DP-941[d]	DP-2/12[d]	Bronowski[d]
gluconapin	8.3	13.50	2.63	2.40	2.00	3.81	1.17	1.51	1.30	0.93	0.86	1.18	0.92	1.05
glucobrassicanapin	9.7	5.78	0.64	1.72	1.09	0.51	0.39	0.73	0.33	0.81	0.49	0.59	0.78	0.91
progoitrin	10.9	39.94	7.77	3.27	4.80	5.08	1.99	2.03	4.87	2.10	1.48	2.46	2.46	1.53
napoleiferin	12.1	5.98	0.29	0.85	0.46	0.04	0.06	0.24	0.29	0.40	0.18	0.22	0.19	0.32
gluconasturtiin	17.4	1.06	0.13	0.77	0.52	0.16	0.07	0.14	0.04	0.24	0.11	0.14	0.07	0.12
sinalbin	20.9	ND[c]	9.46	0.26	3.18	0.11	ND	ND	ND	0.25	0.10	0.37	ND	0.04

[a] For the origin of the seeds, see the Experimental Section; the internal standards sinigrin and glucotropaeolin are used in calculations of the glucosinolate amounts; t_r for the Me_3Si derivatives of sinigrin and glucotropaeolin were 7.3 and 14.4 min, respectively. [b] An unidentified peak appeared at t_r 29.1 min for all of the investigated seed samples. [c] Not detected. [d] *B. napus* L. [e] *B. campestris* L.

0.93, 0.91, and 0.96, respectively.

Table I gives the content of glucosinolates in seeds of some rape species. The concentration of the individual glucosinolates has been determined by GLC analysis of the corresponding isothiocyanates and oxazolidinethiones using a modification of the previously described method (Nielsen et al., 1979) with sinigrin (allyl glucosinolate) and glucotropaeolin (benzyl glucosinolate) added as internal references. This method does not permit estimation of some of the aromatic glucosinolates, e.g., sinalbin and glucobrassicin (3-indolylmethyl glucosinolate) because of the instability of the isothiocyanates produced from them (Gmelin and Virtanen, 1962; Nielsen et al., 1979; McGregor, 1978) and the method is also unqualified to glucosinolates with hydrophilic side chains (Olsen and Sørensen, 1979). Furthermore, it is well known that some glucosinolates produce other products than isothiocyanates (Figure 1) by treatment with myrosinase (Nielsen et al., 1979; Olsen and Sørensen, 1979).

The content of sinalbin and other aromatic glucosinolates in seeds of cv. Erglu rape and cv. Tower accounts for more than 30% of the total amount of glucosinolates as determined from ^{1}H-NMR spectra of the glucosinolates isolated by preparative HVE in buffer system I. The presence and amount of sinalbin has also been determined by GLC–MS analysis of *p*-hydroxyphenylacetic acid produced by acidic hydrolysis of the glucosinolate (Ettlinger and Lundeen, 1956; Olsen and Sørensen, 1979). However, this method cannot be used in the determination of 3-indolylmethyl glucosinolates owing to their instability in the acid conditions during the hydrolysis.

Table II shows the results obtained by GLC analysis of the Me_3Si derivatives of glucosinolates (Underhill and Kirkland, 1971; Olsson et al., 1977; Thies, 1978) isolated from seeds of different rape species by ion-exchange chromatography, preparative HVE, and finally anion-exchange chromatography on the Ecteola column. Sinigrin and glucotropaeolin have been used as internal references (see Experimental Section). It has been shown by NMR spectroscopy that this purification procedure yields nearly pure glucosinolates. Only peaks corresponding to the glucosinolates appear in the GLC chromatograms, and the identities of the peaks have been confirmed by reference compounds.

The preparative HVE in buffer system I results in a very efficient purification of the glucosinolates since only few plant constituents have a negative net charge at this pH. This procedure assures that the peaks in the GLC chromatograms represent the corresponding glucosinolates and not the desulfoglucosinolates which may occur in the same plant as the glucosinolates (Underhill and Kirkland, 1971). However, we have shown that it is possible to perform the analysis on the less pure glucosinolate fraction from the Ecteola column without the preparative HVE step and the following ion-exchange purification. Differences in loss of the individual glucosinolates under the acidic conditions in buffer system I have therefore been avoided. The results presented in Table II have also been obtained by this method.

Very little information on glucosinolate catabolism is available, but the production of different glucosinolate autolysis products as shown in Figure 1 is known to depend on both the structure of the individual glucosinolates and the presence of other plant constituents than myrosinase (Olsen and Sørensen, 1979, and references cited therein). The production of the thiocyanate ion from glucosinolates in rapeseed meals has been reported previously (Srivastava and Hill, 1975; McGregor, 1978), and the antinutritional effect of glucosinolates in rapeseed is well known (Lo and Bell, 1972; Lo and Hill, 1972; Josefsson, 1975; Srivastava

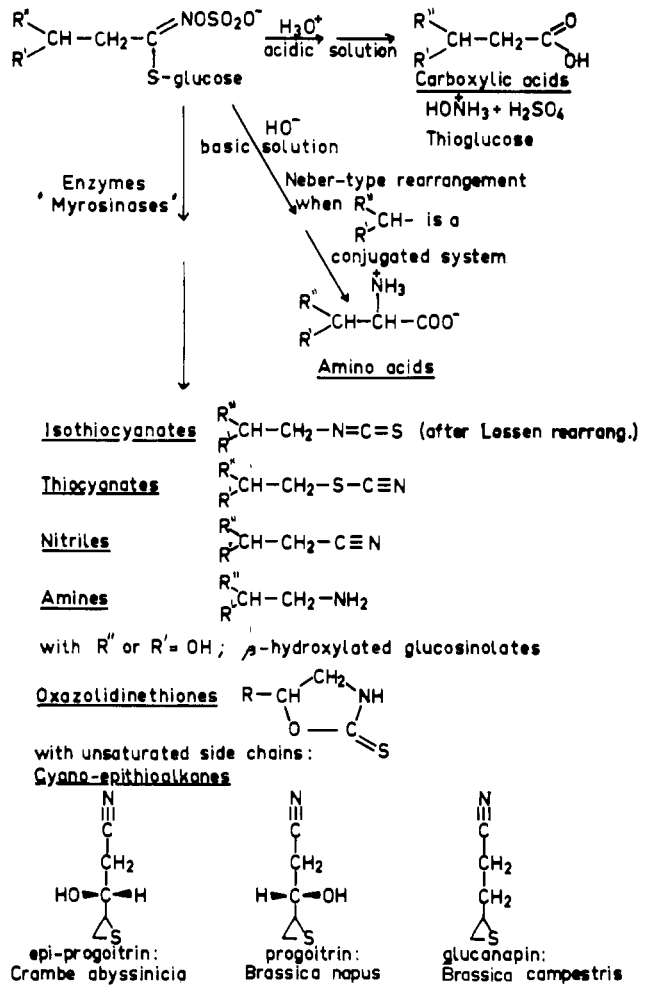


Figure 2. Enzymatic and nonenzymatic degradation of glucosinolates.

et al., 1975).

The use of the meal from the new double low rapeseed as animal feed is limited both by its total content of glucosinolates and by the relative amounts of different glucosinolate types. Thus it is known that oxazolidinethiones, the thiocyanate ion, and isothiocyanates affect the thyroid gland (Van Etten and Wolff, 1973) and it has been reported that degradation products from sinalbin are harmful to animals (Josefsson, 1970). Liver damage (liver hemorrhage) is observed among poultry fed rapeseed meal (Smith and Campbell, 1976; Marangos and Hill, 1976; Papas et al., 1979) and increased weight of the thyroid gland and liver has been observed among pigs fed meal from cv. Erglu rapeseed (Petersen and Schultz, 1976).

The results presented in Tables I and II shows that only some glucosinolates are detectable by GLC analysis of the products produced during the myrosinase-catalyzed hydrolysis. The results show also that in some cases (e.g., seed of cv. Tower) only 50% of the glucosinolates in seeds of these new rape cultivars are determined by GLC analysis of isothiocyanates and oxazolidinethiones, and that the latter is underestimated. The problems concerning the quantitative glucosinolate analysis of new double low rapeseed cultivars by use of the previously described methods have been reported recently (McGregor, 1978). It is thus evident that these methods are unreliable with reference to the analysis of new rape varieties, although they are very suitable for the investigation of common rapeseeds (e.g., Gulle) with a high glucosinolate content. The common rapeseeds do not contain appreciable amounts of sinalbin or other glucosinolates which escape detection by these methods, as is the case for some of the new double low rape species. Glucosinolate analysis based on isolation of the glucosinolates by the newly developed ion-exchange method and GLC of the Me_3Si derivatives permits detection of these compounds as revealed by the results presented in Table II. Furthermore, compounds with hydrophilic side chains are easily detected, e.g., o-(α-L-rhamopyranosyloxy)benzyl glucosinolate, which is present in high concentration in Resedaceous plants (Olsen and Sørensen, 1979).

In Figure 2 is shown a comparison of the GLC chromatograms of the Me_3Si derivatives of glucosinolates isolated from different plants and used as reference compounds (a) and of the Me_3Si derivatives of glucosinolates isolated from seeds of *B. napus* L. cv. Tower including the internal reference compounds sinigrin and glucotropaeolin (b). This method not only permits detection of the glucosinolates previously known to be present in rapeseeds, but sinalbin and glucosinolates with hydrophilic side chains (Olsen and Sørensen, 1979) are also easily detected by this method. The peak with t_r 29.2 min represents presumably the unidentified compound described above. This compound has some properties in common with an indolylmethyl glucosinolate and such a compound has recently been proposed to be present in seeds of double low rape species (McGregor, 1978).

Furthermore, our results indicate that the peak at t_r 27.6 min represents glucobrassicin or, less likely, neoglucobrassicin (Figure 2). The previously unknown glucosinolate precursors of the thiocyanate ion in seeds of the new rape varieties have been discussed in previous works (Srivastava and Hill, 1975; McGregor, 1978). As revealed by the results presented here, these precursors are undoubtedly sinalbin and to some extent the above-mentioned, not yet identified indolylmethyl glucosinolates. The quantitative results obtained by our GLC analysis of the Me_3Si derivatives of glucosinolates (Table II, Figure 2) are thus in agreement with the newly reported results for *B. napus* cv. Tower and *B. napus* cv. Bronowski. Sinalbin, glucobrassicin, and neoglucobrassicin are reported to be lacking or to occur only in trace amounts in the seeds of *B. napus* species (Kjaer and Jensen, 1956). These three compounds are in fact difficult to detect by the traditionally applied methods (Nielsen et al., 1979), but not by the method described in this paper.

Of the serious problems in quantitative glucosinolate analysis are the risk of myrosinase-catalyzed degradation during the homogenization procedure and the instability of the glucosinolates at extreme pH values, e.g., the ion-exchange resin. Furthermore, some glucosinolates containing aromatic side chains have a tendency to adsorb to ion-exchange resins and other column materials resulting in delayed elution from the columns, e.g., sinalbin and 3-indolylmethyl glucosinolates.

ACKNOWLEDGMENT

The authors are greatly indebted to the persons and institutions mentioned above for their gift of seed samples. Thanks are due to K. Schaumburg, the University of Copenhagen, for help with the NMR spectra. Support from the Danish Natural Science Research Council and the Danish Agricultural and Veterinary Research Council for the mass spectrometer is gratefully acknowledged.

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Received for review May 7, 1979. Accepted August 27, 1979.

Trypsin Inhibitor from *Lathyrus sativus* Seeds: Final Purification, Separation of Protein Components, Properties, and Characterization

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The trypsin inhibitor fraction LSTI-B″ from *Lathyrus sativus* seeds was purified to homogeneity as shown by gel filtration, sodium dodecyl sulfate ($NaDodSO_4$) gel electrophoresis, and analytical ultracentrifugation, all showing that the molecular weight was 22 000. The sedimentation coefficient, $S_{20,w}$, was 2.0S and the isoelectric point was at pH 6.2 ± 0.2. No self-aggregation could be noticed by running column chromatography in Sephadex G-75 eluted with 4 M guanidine hydrochloride and also 8 M urea. The specific activity of LSTI-B″ was 1900 trypsin units inhibited/mg of protein with a 108-fold purification. The N-terminal amino acid of the protein was found to be glycine. The percent nitrogen in the protein was 15.38. When LSTI-B″ was subjected to gel electrophoresis in the absence of $NaDodSO_4$ in Tris–glycine buffer system at pH 8.3 five major protein bands could be demonstrated. The nonaggregation nature of the bands could be demonstrated by subjecting the fraction to gel electrophoresis in the absence of $NaDodSO_4$ with increasing gel concentration. The five proteins were taken to be "charge isomers"; they could be separated on a DEAE–cellulose column where a gradient was established between pH 9.0 and 6.0. The properties of these isomers are presented.

The presence of a trypsin inhibitor in *Lathyrus sativus* seeds was reported earlier by Roy and Rao (1971). Its partial purification, characterization, and biological properties were also reported in earlier communications (Roy and Rao, 1971; Roy, 1972a,b). The partially purified protein fraction IV(A) was shown to have five distinct protein components as demonstrated by differences in mobilities in polyacrylamide gel electrophoresis in the absence of sodium dodecyl sulfate ($NaDodSO_4$) (Roy, 1972a). This report deals with attempts to further purify and separate the active protein components, all having trypsin inhibitory property, in order to elucidate their physicochemical and biological properties.

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EXPERIMENTAL SECTION

Materials. Sephadex G-75 (for gel filtration), CM-Sephadex (C-50), and blue dextran 2000 were obtained from Pharmacia (Uppsala, Sweden), DEAE–cellulose chromedia (DE 11) medium fiber powder, nominal capacity 1.0 mequiv min^{-1}/g^{-1} obtained from Whatman Ltd. (England); trypsin, 213 units/mg (twice crystallized), and ovalbumin (twice crystallized) from Worthington Biochemical Corporation (Freehold, NJ); trypsin (about 20 000 fluid-gross unit/g), 1-fluoro-2,4-dinitrobenzene (FDNB), and glycine from E. Merck (Germany); vitamin-free casein from Nutritional Biochemicals Corporation (Cleveland, OH); ribonuclease (bovine, pancreas), myoglobin crystals, salt-free (lyophilized) from Schwarz/Mann (Orangeburg, NY); bovine serum albumin, β-mercaptoethanol (MCE), dansyl chloride, coomassie brilliant blue-R, hemoglobin,

0021-8561/80/1428-0048$01.00/0