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DISTRIBUTION OF THIOGLUCOSIDES IN DIFFERENT PARTS OF *BRASSICA* PLANTS

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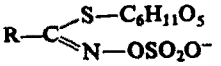
Abstract—Paper- and thin-layer chromatography were used in qualitative investigations on thioglucosides in vegetative parts of *Brassica napus*, *B. campestris* and several forms of *B. oleracea*. The various morphological parts i.e. root, stem, petiole and midvein, lamina excluding midvein, and "head" were analysed separately. Spectrophotometric methods were used for semi-quantitative determinations. It was shown that the thioglucoside pattern may be rather complex. A rape variety which originated from a cross between a turnip rape and marrow stem kale also investigated showed the same pattern as the turnip rape variety. In *B. oleracea* thioglucosides with 4-carbon aglucones are very common while the *B. napus* and *B. campestris* varieties investigated essentially contain thioglucosides with 5-carbon aglucones. A CH_3SO-group often occurs as a terminal substituent in the 4- and 5-carbon aglucone in the thioglucosides of *B. oleracea* and *B. napus–B. campestris* respectively. Glucobrassicin was found in all plants investigated. The variation in thioglucoside pattern within the species *B. oleracea* is fairly wide. The thioglucoside progoitrin seems to be quite absent from the vegetative parts of several *B. oleracea* types.

INTRODUCTION

PRACTICALLY all plants of the family Cruciferae which have been investigated contain thioglucosides.[1] Some of the most valuable cultivated species of this family belong to the genus *Brassica*. Since the thioglucosides on hydrolysis yield split products which are responsible at least for some of the toxic effects of *Brassica* plants[2,3] it is of interest to examine the possibilities of reducing thioglucoside content by plant breeding methods.

In 1956 the general formula (I) for these thioglucosides was established by Ettlinger and Lundeen:[4]



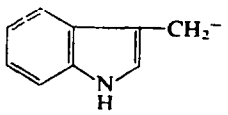
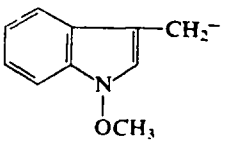
(I)

The chemical nature of R of the thioglucosides found in *Brassica* is shown in Table 1.

The hydrolysis of thioglucosides is catalysed by an enzyme system, myrosinase, which seems to be present in all tissues of thioglucoside-producing plants. To date twelve different thioglucosides are known to occur in *Brassica* (Table 1) which yield split products of various types. Different methods have to be used for the analysis of these products and, in order to find the most suitable methods for use with any plant, it is necessary to know which thioglucosides are present. Comparatively little is known about the distribution of the thioglucosides between various tissues.[1] Therefore it was considered of interest to investigate the thioglucoside content in separated morphological parts of *Brassica* plants. The results might also increase our understanding of the biogenesis and metabolic pathways of these compounds.[1]

[1] A. KJAER, *Progr. Chem. Org. Nat. Prod.* **18**, 122 (1960).
[2] A. I. VIRTANEN, *Umschau* **62**, 129 (1962).
[3] P. LANGER, *Physiol. Bohemoslov.* **13**, 542 (1964).
[4] M. G. ETTLINGER and A. J. LUNDEEN, *J. Am. Chem. Soc.* **78**, 4172 (1956).

TABLE 1. THIOGLUCOSIDES IDENTIFIED IN *Brassica napus*, *B. campestris* OR *B. oleracea*

	Trivial name	Systematic name	Formula I, R =
1.	Sinigrin	Allylglucosinolate ion	$CH_2{=}CHCH_2$
2.	Gluconapin	3-Butenylglucosinolate ion	$CH_2{=}CH(CH_2)_2$—
3.	Glucobrassicanapin	4-Pentenylglucosinolate ion	$CH_2{=}CH(CH_2)_3$—
4.	Glucoibervirin	3-Methylthiopropylglucosinolate ion	$CH_3S(CH_2)_3$—
5.	Glucoiberin	3-Methylsulphinylpropylglucosinolate ion	$CH_3SO(CH_2)_3$—
6.	Glucoerucin	4-Methylthiobutylglucosinolate ion	$CH_3S(CH_2)_4$—
7.	Glucoraphanin	4-Methylsulphinylbutylglucosinolate ion	$CH_3SO(CH_2)_4$—
8.	Glucoalyssin	5-Methylsulphinylpentylglucosinolate ion	$CH_3SO(CH_2)_5$—
9.	Gluconasturtiin	2-Phenylethylglucosinolate ion	$C_6H_5(CH_2)_2$—
10.	Progoitrin	2-Hydroxy-3-butenylglucosinolate ion	$CH_2{=}CH{\cdot}CH{\cdot}CH_2$— (OH)
11.	Glucobrassicin	3-Indolylmethylglucosinolate ion	
12.	Neoglucobrassicin	N_1-methoxy-3-indolylmethylglucosinolate ion	

Since fodder rape and marrow stem kale have attracted an increasing interest as fodder crops in Sweden during the past ten years, these types were included in the present investigation. A variety of forms of *B. oleracea* are used for human consumption. Such horticultural types were also included as they offer good opportunities of investigating the qualitative and quantitative variation within a species. Since *B. napus* has originated from a cross between *B. oleracea* and *B. campestris* and actually contains the sum of chromosomes of the parent species it was also of interest to examine the thioglucosides of *B. campestris*.

The thioglucosides of the seeds of most of these species have been examined by other investigators,[5,6] and this investigation was, therefore, limited to the vegetative parts of the plants. In some cases those parts have been the subject of earlier investigations (see Table 2).

Paper- and thin-layer chromatography were used in the qualitative investigations. For semi-quantitative determinations spectrophotometric methods were applied.

RESULTS

Qualitative Determinations

The chromatographic evidence for presence or absence of a substance should be interpreted with some caution. The quantity of sample which can be applied to the paper is determined by the amount of major substance present and it is often difficult, therefore, to detect minor components. The data obtained in the chromatographic work (Table 2) were compared with previous results outlined in Table 1.

[5] K. A. JENSEN, J. CONTI and A. KJAER, *Acta Chem. Scand.* 7, 1267 (1953).
[6] M. G. ETTLINGER and C. P. THOMPSON, *Final Report to Quarter-master Research Engineering Command. Contr. DA 19-129-QM-1689* (1962).

TABLE 2. QUALITATIVE INVESTIGATIONS OF THIOGLUCOSIDES IN *Brassica napus*, *B. campestris* AND *B. oleracea*

Species	Variety	Part of plant§	Author	Thioglucosides*
Brassica napus L.				
var. oleifera E. & G.	Bronowski	Root	9	2, 3, 5, 9, 10
		Stem	9	2, 3, 10
		Leaf	9	2, 3, 10
		Pod	9	2, 3, 10
	Sv Silona	Root	Present	2 (traces), 3, 7, 8, *9*, 10, 11, *12*
		Stem	Present	2, 3, 7, *8*, *9*, 10, *11*, *12*
		Petiole	Present	2, 3, *8*, 9, 10, *11*, 12
		Lamina	Present	2, 3, *8*, 9, 10, *11*, 12
Brassica campestris L.	Wild turnip	Fresh plants	10	2, 3, 5, 10†
	Chihili	Root	Present	*8*, *9*, 10, 11, *12*
		Stem	Present	3, 7, *8*, 9, 10, *11*
		Petiole	Present	3, *8*, 9, 10, 11, *12*
		Lamina	Present	3, 8, *9*, 10, 11, *12*
var. oleifera E. & G.	Lembke	Root	Present	2, 3, 7, 8, *9*, 10, 11, *12*
		Petiole	Present	2, *3*, 8, 9, 10, 11, *12*
		Lamina	Present	2, *3*, 7, 8, 9, 10, *11*, 12
var. rapifera Metz.	Purple Top Milan Shogoin	Root	26	9
Brassica oleracea L.				
var. silvestris L.	Wild kale from Noli, Italy	Root	Present	1, *2*, 6, 7, 9, 10, *11*, 12
		Stem	Present	1, *2*, 7, 9, 10, *11*, 12
		Petiole	Present	1, *2*, 7, 10, *11*
		Lamina	Present	*2*, 7, 10, *11*, 12
var. acephala DC.	Not reported	—	12	1, 5
var. acephala DC. subvar. plana Peterm. f. viridis	"Marrow stem kale"	Root	Present	1, 5, 7, *9*, 10, 11, *12*
		Stem	Present	*1*, 2 (traces), 5, *9*, 10, *11*, 12
		Petiole	Present	*1*, 5, 10, *11*, 12
		Lamina	Present	*1*, 5, 10, *11*, 12
var. acephala DC. subvar. laciniata L. f. crispa	"Halvhög, mosskrusig"	Root	Present	1, 5, 6, *9*, 11, *12*
		Stem	Present	*1*, 5, 11, *12*
		Petiole	Present	1, *5*, *11*
		Lamina	Present	1, *5*, *11*, 12
	"Garneringskål, vitbrokig"	Root	Present	1, 5, 7, *9*, 10, *11*, 12
		Stem	Present	*1*, 5, 11, *12*
		Petiole	Present	1, *5*, 11, *12*
		Lamina	Present	*5*, *11*, 12

TABLE 2.—*continued*

Species	Variety	Part of plant§	Author	Thioglucosides*
—	Cultivated kale from Tenerife	Root	Present	*1*, 2, 4, 5, 7, 9, 10, 11, *12*
		Petiole	Present	1, *5*, 11, *12*
		Lamina	Present	1, *7*, 11, *12*, one unident.
var. gemmifera Zenker	Not reported	Green parts	13	5
	Not reported	Fresh parts	12	1, 2, 7
	Amager Winter	Root	Present	2, 6, 7, *9*, 10, 11, *12*
		Stem	Present	2, *7*, 9, 10, 11, *12*
		Petiole	Present	2, *7*, 10, *11*
		Lamina	Present	2, *7*, 10, *11*
		Head	Present	*2*, 7, 10, *11*, 12, one unident.
var. italica Plenck	Greenia	Root	Present	*6*, *7*, *9*, 11, *12*
		Stem	Present	*7*, *11*, *12*
		Petiole	Present	*7*, *11*, *12*
		Lamina	Present	1, *5*, 11, *12*
		Edible part	Present	1, *7*, 11, *12*
	Mayflower	Root	Present	1, *5*, 6, 9, 11, *12*
		Stem	Present	1, *5*, *12*
		Petiole	Present	*5*, *11*, 12
		Lamina	Present	1, *5*, 11, *12*
var. botrytis L.	Not reported	Head	5	1
	Large Danish	Root	Present	1, *5*, *9*, 11, *12*
		Stem	Present	*1*, 4, *5*, 9, *11*, 12
		Petiole	Present	*5*, *11*, 12
		Lamina	Present	*1*, 5, *11*, 12
		Head	Present	*1*, 5, *11*, 12
	Erfurtin	Root	Present	1, 5, *9*, 11, *12*
		Stem	Present	*1*, 5, *11*, 12
		Petiole	Present	1, *5*, *11*, *12*
		Lamina	Present	1, *5*, *11*, 12
		Head	Present	*1*, 5, *11*, 12
var. gongyloides L.	Wiener Glas Blue	Root	Present	6, *7*, 9, 11, *12*
		Stem	Present	1 (traces), 6, *7*, 9, *11*, 12
		Petiole	Present	*7*, *11*, 12
		Lamina	Present	*7*, *11*, 12
	Wiener Glas White	Root	Present	*7*, 9, 11, *12*
		Stem	Present	*7*, *12*
		Petiole	Present	*5*, *7*, *11*, 12

		Lamina	Present	*7*, *11*, 12
var. sabauda L.	Not reported	Green parts	13	5, 7
	"Savoy kale"	Root	Present	*1*, 4, 5, 9, 11, *12*
		Stem	Present	*1*, 5, *11*, 12
		Petiole	Present	*1*, 5, *11*, 12
		Lamina	Present	1, *5*, *11*, 12
		Head	Present	*1*, 5, *11*, 12
var. capitata L.	Jersey Queen	Root	26	9
var. capitata f. alba	Not reported	Head	5	1
var. capitata	Bonanza regular and Yellows resistant	Head	7	1, 2, 4, 5
	Harris Resistant	Head	11	1, 2, 4‡
var. capitata f. alba	Ditmarsker	Root	Present	1, 2, 4, 5, 7, *9*, 11, *12*
		Stem	Present	*1*, 5, *11*, 12
		Petiole	Present	*1*, 5, *11*, 12
		Lamina	Present	*1*, 5, *11*, 12
		Head	Present	*1*, 5, *11*
	Amager High	Root	Present	*1*, 5, 9, 10, *11*, 12
		Stem	Present	*1*, 5, 9, 10, *11*, 12
		Petiole	Present	*1*, 5, 10, *11*, 12
		Lamina	Present	1, 2, *5*, 10, *11*, 12
		Head	Present	*1*, 5, 10, *11*
var. capitata f. rubra L.	Not reported	Head	5	1, 2
	Winter A	Root	Present	1, 5, 6, 7, *9*, 10, 11, *12*
		Stem	Present	*1*, 2, 5, 7, 9, 10, *11*, 12
		Petiole	Present	1, 2, 5, *7*, 10, *11*
		Lamina	Present	1, 2, 5, *7*, *11*, 12
		Head	Present	1, 2, 5, *7*, *11*

§ The expression "petiole" refers to petiole and midvein, and the expression "lamina" to lamina excluding midvein.
* Numbers refer to compounds shown in Table 1. Major compounds are italicized.
† Reports also a spot which may be glucoberteroin or gluconasturtiin.
‡ Reports also thioglucosides yielding methyl, *n*-butyl, methylthiomethyl and methylthiobutyl isothiocyanates.

Although Silona rape originated from a cross between Lembke turnip rape and marrow stem kale, its pattern of thioglucosides corresponds entirely to the former parent. Like most other types of *B. oleracea*, marrow stem kale differs from Silona and Lembke in containing the thioglucosides sinigrin and glucoiberin with 4-carbon aglucones. The thioglucoside contents of *B. oleracea* var. gongyloides and var. gemmifera diverge from the common *B. oleracea* pattern in that both varieties contain glucoraphanin (with a 5-carbon aglucone), while var. gemmifera contains two other thioglucosides with 5-carbon aglucones, *viz.* gluconapin and progoitrin. In some cases glucoiberin and glucoraphanin were found together and in var. botrytis, variety Greenia, glucoiberin in the laminae and glucoraphanin in the other parts.

Often a larger number of thioglucosides have been found in the roots than in other parts of the plants. This was not generally due to the fact that the thioglucoside content usually is larger in the root (see Table 3). Gluconasturtiin was found in the roots of all plants investigated where it was often the dominating thioglucoside. This is in good agreement with observations reported in literature.[1]

The thioglucosides sinigrin, gluconapin and glucobrassicanapin, with four, five and six-carbon atoms in the aglucone respectively, differ from glucoiberin, glucoraphanin and glucoalyssin only in that the latter thioglucosides carry a CH_3SO-group on the 4-, 5- and 6-carbon chain. The co-occurrence of sinigrin-glucoiberin, gluconapin-glucoraphanin and glucobrassicanapin-glucoalyssin was very commonly observed and has also been shown by Ettlinger and Thompson in *Brassica* seeds[6] and for sinigrin-glucoiberin by Clapp *et al.* in fresh cabbage.[7]

Of the thiocyanate-yielding thioglucosides, glucobrassicin and neoglucobrassicin, the former seems to be the most widespread and with two exceptions was found in all samples investigated. In most cases it was the dominant compound; in the roots neoglucobrassicin often occurred in larger quantity.

Semi-quantitative Determinations

From Table 3 it is obvious that the rape Silona had a relatively high content of both progoitrin and isothiocyanate-yielding thioglucosides. The turnip rape Lembke contained less progoitrin but much of the latter thioglucosides. These observations correspond to results commonly found in investigations on seeds of rape and turnip rape. The marrow stem kale had comparatively small amounts of these groups of substances. Among the forms of *B. oleracea* only the wild kale, var. gemmifera and var. capitata f. rubra contained considerable amounts of progoitrin. The apparently high contents of this goitrogenic compound in the heads of the two latter subspecies, which are cultivated as food, deserve special attention. Several of the *B. oleracea* forms seem to be quite free from progoitrin in the vegetative parts of full-grown plants. *B. oleracea* var. gongyloides and var. acephala subvar. laciniata f. crispa both contained fairly large amounts of isothiocyanate-yielding glucosides and the latter form, like var. acephala subvar. plana f. viridis, contained large amounts of thiocyanate-yielding glucosides in the laminae. These glucosides were dominant in var. botrytis and sabauda. The wild kale studied and var. gemmifera contained considerable amounts of all three groups of substances. Chihili had a much lower thioglucoside content than the other *B. campestris* variety, Lembke.

[7] R. C. CLAPP, L. LONG, JR., G. P. DATEO, F. H. BISSETT and T. HASSELSTROM, *J. Am. Chem. Soc.* **81**, 6278 (1959).

TABLE 3. CONTENT OF SPLIT PRODUCTS FROM THIOGLUCOSIDES OF *Brassica napus*, *B. campestris* AND *B. oleracea*

Species	Variety	Root			Stem			Petiole			Lamina			Head		
		I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
Brassica napus L.	Sv Silona	40	36	10	50[a]	36	9	22	17	3	18	10	8	—	—	—
Brassica campestris L.	Lembke	98	17	13	—	—	—	20	1	6	41	1	10	—	—	—
	Chihili	21	traces	22	2	1	4	3	1	4	2[b]	1	9	—	—	—
Brassica oleracea L.																
var. silvestris L.	Wild kale from Noli, Italy	36	1	25	32	10	6	31	5	8	29	2	20	—	—	—
var. acephala DC. subvar. plana Peterm. f. viridis	"Marrow stem kale"	16	2	5	2	2	3	traces	0	7	2	0	34	—	—	—
var. acephala DC. subvar. laciniata L. f. crispa	"Halvhög, mosskrusig"	28	0	32	8	0	3	11	0	6	9	0	22	—	—	—
	"Garneringskål, vitbrokig"	87	2	16	14	0	4	2	0	5	8	0	30	—	—	—
—	Cultivated kale from Tenerife	9	7	6	—	—	—	5	0	8	6	0	33	—	—	—
var. gemmifera Zenker	Amager Winter	23	9	7	15	12	2	6	5	2	6	1	5	11	18	15
var. italica Plenck	Greenia	36[c]	0	11	5	0	4	4	0	7	5	0	21	17	0	32
	Mayflower	9	0	7	6	0	3	10	0	4	13	0	20	—	—	—
var. botrytis L.	Large Danish	12	0	9	3[d]	0	4	2	0	4	2	0	19	2	0	8
	Erfurtin	12	0	9	2	0	4	5	0	6	5	0	31	2	0	12
var. gongyloides L.	Wiener Glas Blue	23	0	16	8	0	4	7	0	4	2	0	11	—	—	—
	Wiener Glas White	23	0	13	4	0	4	5[e]	0	3	7	0	14	—	—	—
var. sabauda L.	"Savoy kale"	19	0	13	6	0	4	3	0	3	2	0	18	3	0	13
var. capitata L. f. alba	Ditmarsker	6	0	9	18	0	3	7	0	2	2	0	6	4	0	3
	Amager High	51	1	12	18	1	3	7	1	3	0	0	5	10	1	9
var. capitata L. f. rubra	Winter A	45	7	12	25	11	4	10	traces	3	7	0	5	20	5	5

a—calculated as alyssin; b—calculated as brassicanapin; c—calculated as nasturtiin; d—calculated as iberin; e—calculated as raphanin.
I = isothiocyanates, II = oxazolidinethiones, III = thiocyanate ion.
The figures refer to mg/100 g fresh weight. "Petiole" includes midvein.
The isothiocyanate content was calculated on a basis of the molecular weight of the predominant isothiocyanate (see Table 2).

The larger amounts of isothiocyanate-yielding glucosides in the root are in good agreement with other investigations.[8] The roots have a larger dry matter content than the other vegetative parts of the plant. However, even when the isothiocyanate content was calculated on a dry matter basis, the roots still showed the largest content. Gluconasturtiin, especially, appeared in large quantities in the root.

The content of the isothiocyanate-yielding glucosides was on the average about the same in stems, petioles and laminae. The two latter parts contained less of progoitrin than the stem. The thiocyanate-producing glucosides occurred in about the same quantity in the petioles as in the stems while the content was larger in the laminae, roots and heads.

DISCUSSION

In the investigations of thioglucoside patterns in *Brassica* species, reported in literature, only Trzebny[9] in his investigation of *B. napus* seems to have analysed all the morphological parts of the plant (Table 2)*. However, he did not investigate the thiocyanate-producing glucosides, nor did he separate those giving hydrophilic thioureas. Furthermore he did not find gluconasturtiin in the green parts, and the variety examined was different from that used in the present investigation.

In studies on *B. campestris* Bachelard[10] found glucoiberin as the most hydrophilic glucoside. This difference from the present results may be due to different material being used in the two investigations.

Detailed investigations of thioglucoside pattern of *B. oleracea* material seem to have been made only by Bailey *et al.*[11] and by Clapp *et al.*[7] They found glucoibervirin in the heads of *B. oleracea* var. capitata f. alba, a glucoside found only in the roots of that form in the present investigation. Bailey also reports thioglucosides yielding methyl, *n*-butyl, methylthiomethyl and methylthiobutyl isothiocyanates, which have not been found in the present investigation. The first three of these substances do not seem to be reported in other *Brassica*. Sinigrin which is reported by Gmelin and Virtanen[12] to occur in fresh parts of *B. oleracea* var. gemmifera has not been found in this subspecies in the present study. Procházka *et al.*[13] found both glucoiberin and glucoraphanin in *B. oleracea* var. gemmifera and sabauda. In the present investigation only glucoraphanin was found in gemmifera and glucoiberin in sabauda. These discrepancies may be due to small amounts, or absence, of the missing compounds in the plant material used in this study. Other thioglucosides reported to be present in vegetative parts of the species and forms here studied, have also been found in this investigation (Table 2).

A comparison of the present results with those of Ettlinger and Thompson[6] in their investigation on seed material is of interest but has to be interpreted with some caution, since the varieties were not the same in the two studies. As a rule the same thioglucosides have been found in the seeds as in the vegetative parts of the plant but some exceptions occur. The most prominent difference is that gluconasturtiin occurs in large quantities in the root and often

* Several authors have only examined one thioglucoside in *Brassica* species. In order not to make Table 2 too complicated, these references are excluded. Consequently certain papers demonstrating the presence of a particular thioglucoside in a species for the first time are excluded.

[8] E. P. LICHTENSTEIN, D. G. MORGAN and C. H. MUELLER, *Agr. Food Chem.* **12**, 158 (1964).
[9] W. TRZEBNY, *Pamiętnik Puławski* **8**, 315 (1962).
[10] H. S. BACHELARD and V. M. TRIKOJUS, *Australian J. Biol. Sci.* **16**, 147 (1963).
[11] S. D. BAILEY, M. L. BAZINET, J. L. DRISCOLL and A. I. MCCARTHY, *J. Food Sci.* **26**, 163 (1961).
[12] R. GMELIN and A. I. VIRTANEN, *Acta Chem. Scand.* **14**, 507 (1960).
[13] Ž. PROCHÁZKA, V. ŠANDA and L. JIROUSEK, *Collection Czech. Chem. Commun.* **24**, 3606 (1959).

was found in the other vegetative parts but was absent from the seed or was found there in very small quantities. Gluconapin usually is the major isothiocyanate-producing glucoside in *B. napus* seed while glucoalyssin was predominant in the green parts. Ettlinger and Thompson[6] reported, however, two varieties of rutabaga seed which contained glucoalyssin and not gluconapin. Seed of *B. oleracea* var. capitata f. rubra and var. gemmifera contained much sinigrin but glucoraphanin was more prominent in petioles, laminae and heads. Glucoiberin was found in seed of var. gemmifera but not in the vegetative parts. Seed of *B. oleracea* var. gongyloides contained glucoibervirin and progoitrin which were not present in the vegetative parts. Relatively more sinigrin and glucoiberin were found in the seed than in the rest of the plant parts. *B. oleracea* var. italica contained glucoerucin in the seed and this compound was found in the root but not in the green parts.

As shown (Table 2) up to nine thioglucosides have been found in vegetative parts of some varieties. As these glucosides yield split products with different chemical and physical properties the analytical work may be very complicated in attempts to obtain crop varieties of lower thioglucoside content. For this reason it would be of value to evaluate one method which could be used for all the glucosides present. This method may be based on analysis of the intact glucosides or on one of the split products common to all of them viz. glucose or sulphate. Analysis of glucose would be too tedious, however, because of the purifying process needed to remove glucose from other sources than thioglucosides. All the possibilities here mentioned seem to suggest extraction of the thioglucosides with hot methanol. In species chiefly containing rhodanidogenic glucosides and glucosides yielding volatile isothiocyanates, a combination of thiocyanate analysis according to Aldridge[14,15] and isothiocyanate analysis according to Schwarze[16] may be used.

Since *B. napus* is an allopolyploid of the two species *B. campestris* and *B. oleracea* and the qualitative and quantitative variation in the thioglucoside content appears to be very wide within the two parent species, there should be at least theoretically good possibilities to synthesize a *B. napus* with a low thioglucoside content.

MATERIALS AND METHODS

Materials

Most of the seed material was supplied from Dr. G. Carlsson, Hammenhög. Some varieties were supplied from the Division of Annual Fodder Crops, Svalöv, which also carried out the field work.

All the reagents used for chromatography were of analytical reagent grade.

The following reference substances were obtained from Calbiochem, Los Angeles: glucoiberin, glucotropaeolin and sinalbin. Sinigrin was from Light's, London. Most of the remaining reference substances were gifts from Professor A. Kjaer, Copenhagen, and Professor A. I. Virtanen, Helsinki. A crude preparation of 4-methylsulfinylbutyl thiourea was made from seeds of *Eruca sativa*.

Methods

All experimental material was sown in spring. Plants were harvested at a stage of development most common in agricultural or horticultural practice. At harvest they were divided into different morphological parts. The plant parts were cut into pieces, and after mixing samples of 20 or 40 g were taken for the chemical analysis.

From a quantitative point of view there are certainly some errors in the sampling. According to Schlottman[17] at least thirty plants ought to be harvested in order to get a proper sampling. Smaller numbers of plants were frequently used in this investigation. As the content of thioglucosides also may be different in older and younger parts of the plant,[18] samples of 20 or 40 g may be too small to be fully representative.

[14] W. N. Aldridge, *Analyst* **69**, 262 (1944).
[15] W. N. Aldridge, *Analyst* **70**, 474 (1945).
[16] P. Schwarze, *Der Züchter* **17–18**, 19 (1946).
[17] H. Schlottmann, *Qualitas Plant. Mater. Vegetabiles* **10**, 301 (1963).
[18] R. Gmelin and A. I. Virtanen, *Ann. Acad. Sci. Fennicae* A II (1961).

The thioglucosides were extracted from the samples with boiling methanol of analytical reagent grade for 15 min in order to prevent enzymatic splitting of thioglucosides during extraction.[18] The pieces were then homogenized in the methanol, filtered and the pulp re-extracted with boiling 80% methanol for 10 min. After filtering the pulp was re-extracted a second time.

The combined filtrates were evaporated *in vacuo* at 30° to about 10 ml, and the solution filtered through Hyflo Super Cel, to remove the chlorophyll. The Super Cel was washed with 100 ml of water, and the filtrate was evaporated *in vacuo* at 40° to about 30 ml.

For semi-quantitative determinations the thioglucosides were divided into three groups according to their split products: those giving either isothiocyanates, or the thiocyanate ion, or oxazolidinethiones. Oxazolidinethiones were estimated by spectrophotometric measurement at 248 nm. The isothiocyanates were determined after conversion to thiourea derivatives, which have a λ_{max} at about 250 nm in diethyl ether. The thiocyanate ion was determined according to the method by Aldridge,[14,15] modified by Michajlovskij and Langer.[19]

Oxazolidinethiones and isothiocyanates were analysed essentially according to Langer.[3] The glucosides in an aliquot of the Super Cel filtrate were hydrolysed by a myrosinase preparation at pH 7·0, and the liberated isothiocyanates and oxazolidinethiones extracted with diethyl ether. The isothiocyanates were converted to thiourea derivatives with ammonia and the sum of thiourea derivatives and oxazolidinethiones was determined spectrophotometrically. The quantitative determinations of oxazolidinethiones were made on another aliquot of the ether solution.

The non-volatile isothiocyanates iberin, sulphoraphan and alyssin are more hydrophilic than the volatile ones and are not quantitatively extracted by diethyl ether. For that reason the results for the thioureas will be somewhat low.

A second part of the ether solution of thiourea and oxazolidinethione which was not used for quantitative determinations, was evaporated to dryness and the residue dissolved in ethanol. This solution was used for chromatography of thioureas and oxazolidinethiones.

In order to prepare solutions of the thioglucosides for chromatography 10 ml of the Super Cel filtrate was passed through a column of aluminium oxide (7 g). After washing with water the glucosides were eluated by 1% K_2SO_4. The eluate was evaporated to dryness *in vacuo* and the glucosides dissolved in 100% methanol.

Glucosides were analysed qualitatively on paper chromatography and thioureas on paper and TLC. The glucosides were chromatographed on Schleicher & Schüll 2043 mgl paper, using the top phase of the mixture *n*-butanol:ethanol:water, 4:1:4.[20,21] Two methods were used for the detection of the thioglucoside spots: (a) The papers were dipped into a solution of 400 mg silver nitrate, 10 ml concentrated ammonia and methanol to a total volume of 400 ml.[22] After drying at about 120°, the papers were dipped into the reagent solution again and dried to a dark brown colour. The papers were then dipped into 0·4 N nitric acid and washed with water. The glucosides were seen as grey-blue spots. Glucotropaeolin was used as a marker. (b) The thiocyanate yielding glucosides glucobrassicin and neoglucobrassicin were detected by hydrolysis with myrosinase solution and subsequent spraying with ferric nitrate.[18] Glucobrassicin served as a marker.

Since neoglucobrassicin and free rhodanid which may be present in the sample as an artefact, show similar R_f-values in the chromatography system used, the indole reagent *p*-amino-benzaldehyde[19] was used for detecting the spots in all cases when the presence of free rhodanid was suspected.

The thiourea derivatives were chromatographed on Whatman No. 1 paper. Two systems were used on all samples: (a) Water-saturated chloroform, essentially according to Kjaer and Rubinstein[23] but with the descending technique. The equilibrating period was at least 1 hr and the developing time 70 min. The spots were detected in u.v.-light (325 nm) and by use of Grote's reagent.[24] Phenyl-thiourea was used as a marker. (b) In order to separate the hydrophilic thioureas of iberin, sulphoraphan and alyssin, the thioureas were chromatographed in *n*-butanol:toluene:water, 3:1:1, according to Ettlinger and Thompson.[9] Ascending technique was used. The papers were equilibrated for at least 2 hr and developed for 6 hr. The same detecting methods were used as above. The thioureas of iberin, sulphoraphan and alyssin were used as reference substances besides phenylthiourea.

Frequently the system toluene:acetic acid:water, 5:2:4.[9] was used in order to separate such thioureas which have high R_f-values in water-saturated chloroform.

The thioureas were also analysed on TLC, where the limit of detection is much lower than on paper chromatography. This was especially valuable for detecting vinyl-oxazolidinethione. Silicagel plates were used and the solvent was chloroform:ethanol, 9:1. The spots were detected either with Grote's reagent, or by spraying successively with a solution of starch and a solution containing sodium azide and iodine.[25] In some

[19] N. MICHAJLOVSKIJ and P. LANGER, *Hoppe Seyler's Z. Physiol. Chem.* **312**, 26 (1958).
[20] O.-E. SCHULTZ and R. GMELIN, *Z. Naturforsch.* **8b**, 151 (1953).
[21] A. KJAER and R. GMELIN, *Acta Chem. Scand.* **10**, 1100 (1956).
[22] O.-E. SCHULTZ and R. GMELIN, *Z. Naturforsch.* **7b**, 500 (1952).
[23] A. KJAER and K. RUBINSTEIN, *Acta Chem. Scand.* **7**, 528 (1953).
[24] I. W. GROTE, *J. Biol. Chem.* **93**, 25 (1931).
[25] M. R. ALTAMURA, L. LONG, JR. and T. HASSELSTROM, *J. Biol. Chem.* **234**, 1847 (1959).

cases silicagel GF_{254} was used when preparing the plates and the spots were detected in u.v. light besides by spraying reagents.

For identification purpose, solutions of all the thioglucosides found in *Brassica*, or thiourea derivatives of their split products were run as marker substances when needed, besides the reference substances mentioned above.

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[26] M. A. STAHMANN, K. P. LINK and J. C. WALKER, *J. Agr. Res.* **67**, 49 (1943).