

***iso*Thiocyanates IV. A Systematic Investigation of the Occurrence and Chemical Nature of Volatile *iso*Thiocyanates in Seeds of Various Plants**

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More than a hundred years have elapsed since allyl *isothiocyanate* was first isolated from seeds of black mustard. Since then, numerous investigations have served to establish the wide-spread occurrence of glycosidically bound *isothiocyanates*, especially in plants belonging to the family *Cruciferae*. The older results have been previously reviewed^{1,2}; here it suffice to mention that only four steam-volatile *isothiocyanates* with well-established structures were known as constituents of the crucifers when the present studies were initiated, *viz.* allyl-, (+)-*sec*-butyl-, benzyl- and β -phenylethyl *isothiocyanate*. In a previous paper³ we added to this series *isopropyl isothiocyanate* while the preceding communication reported the isolation of 3-butenyl *isothiocyanate* from seeds of various *Brassicæ*.

Older investigations have suffered from the disadvantage of requiring large amounts of biological material, thereby rendering only the most common and abundant seeds accessible for detailed studies. Microchemical tests have been reported (*cf.* Ref.⁴) but they do not allow to discern between individual *isothiocyanates*.

Recently we described a paperchromatographic technique⁵ by which minute amounts of thioureas can be separated and identified. This method, permitting the use of as little as one gram of seeds, has now been applied to a systematic scanning of a large number of seeds for their contents of volatile *isothiocyanates*. In a few cases of special interest the fresh plants or parts thereof have been investigated also. The species studied in the present communication belong to a great extent to the Scandinavian flora, the major part being crucifers. Owing to their special interest the results from similar studies within the important genus *Brassica* have been separately presented. Qualitative and quantitative estimates of the volatile *isothiocyanates* have been made throughout, while the interesting *isothiocyanates*, which are not volatile with steam, form the subject of future work.

EXPERIMENTAL

Enzymic liberation and distillation of isothiocyanates

The individual seed sample (*ca.* 2 g) was ground in a small hand mill and then immediately added to a hot mixture of ligroin (b.p. 60–100°) (10 ml) and ethanol (5 ml) and refluxed for half an hour. This treatment denatured the myrosinase present in the seeds and removed much fatty material. In preliminary experiments, where the treatment with hot ligroin-ethanol was substituted with a continuous, cold extraction in a Soxhlet apparatus, an undesired and often complete enzymic degradation took place, resulting in removal of the liberated isothiocyanates into the solvent. The partially extracted sample was then ground to a fine powder in the mill and exhaustively defatted by refluxing with a mixture of petroleum ether and ethanol (3 : 1). After filtration and air-drying the total weight loss (fat plus minor amounts of water) was determined. The values found are presented in Table 2.

The finely divided material was then suspended in 20 ml of distilled water; 0.2 ml of a cell-free myrosinase preparation⁶ was added and the mixture allowed to stand at room temperature for 18 hours in a glass-stoppered vessel. Next day, the contents were distilled in a rapid stream of steam and the distillate (200–300 ml) collected in a receiver containing 20 ml of concentrated ammonia. After standing overnight the transformation into thiourea was complete and the clear solution was evaporated to dryness *in vacuo* at a bath temperature not exceeding 50°. The often oily residue was dissolved in absolute ethanol and properly diluted before the spectrophotometrical evaluation described below. After being measured, the solution was again taken to dryness *in vacuo* and then, on basis of the found contents, dissolved in enough 96 % ethanol to make a *ca.* 1 % solution. This was applied to a paper strip and chromatographed as previously described⁶. The results obtained are summarised in Table 2.

In a few cases the seeds could not be effectively removed from the siliques in which they were located. Therefore, these samples (Table 2, Nos. 3, 11, 19 and 29) were only qualitatively investigated for their contents of volatile isothiocyanates in the seed-containing siliques.

In cases, where fresh plants or parts thereof were studied, the biological material was homogenised in a Waring blender, kept for about half an hour at room temperature, distilled and further processed as described above.

Spectrophotometric evaluation of isothiocyanate contents

According to the paperchromatographic results the following volatile isothiocyanates were encountered during the present investigation: methyl-, allyl-, isopropyl-, *sec*-butyl-, 3-butenyl-, benzyl- and β -phenylethyl isothiocyanate. The ultraviolet absorption spectra of synthetic samples of the corresponding thioureas were carefully measured in anhydrous ethanol solutions. A characteristic feature of all the spectra is a maximum at 243 $m\mu$ (ϵ 12–13 000). The molecular extinction values at 220 $m\mu$, 240 $m\mu$ and 260 $m\mu$ are tabulated in Table 1.

Table 1. Absorption data for various thioureas in ethanol solution.

N-Substituted thiourea	ϵ_{220}	ϵ_{240}	ϵ_{260}	K
Methylthiourea	3 750	12 510	1 680	1.28
Allylthiourea	5 100	12 570	2 690	1.45
isoPropylthiourea	4 020	12 230	2 270	1.34
<i>sec</i> -Butylthiourea	3 900	11 990	2 180	1.34
3-Butenylthiourea	3 930	12 150	2 160	1.33
Benzylthiourea	10 020	12 140	3 260	2.20
β -Phenylethylthiourea	9 280	12 270	2 730	1.96

It was apparent from the absorption curves of the "natural" thioureas that some nonspecific absorption, due to unknown volatile by-products, was superimposed upon the true thiourea spectra. On the rather likely assumption that the extinction of the impurities increases linearly in the range 220–260 $m\mu$, a corrected extinction value at 240 $m\mu$, e_{240} , could be calculated in the following way.

When E denotes the observed extinctions, k' and k'' the ratios between the extinction values at 260 $m\mu$ and 220 $m\mu$, respectively, to those at 240 $m\mu$ of the pure substances (calculated from Table 1), the following expressions are obtained:

$$E_{240} = e_{240} + a \quad (1)$$

$$E_{220} = k'e_{240} + a + b \quad (2)$$

$$E_{260} = k''e_{240} + a - b \quad (3)$$

where $a + b$ and $a - b$ represent the non-specific extinctions at 220 $m\mu$ and 260 $m\mu$, respectively. From the equations (1)–(3), the following expression for the corrected extinction at 240 $m\mu$ is easily derived:

$$e_{240} = \left(E_{240} - \frac{E_{220} + E_{260}}{2} \right) K$$

where

$$K = \frac{1}{1 - \frac{k' + k''}{2}}$$

The individual correction factors (K) are tabulated in Table 1. From the corrected extinction values the amount of thiourea and consequently of isothiocyanate can readily be calculated. In Table 2 the found contents of volatile isothiocyanates are given in mg per 100 g of untreated (fat-containing) seeds.

All ultraviolet absorption measurements were carried out on a Beckman model DU quartz spectrophotometer in 1 cm cells.

DISCUSSION

The current studies of the distribution of volatile isothiocyanates in seeds and other plant materials were undertaken for several reasons. A general interest in the phytochemical metabolism of sulphur made an extended knowledge of the occurrence and chemical nature of the isothiocyanates desirable. Furthermore, it seemed of interest to attempt to find a possible correlation between the isothiocyanate contents in various species and the traditional use of the latter in a number of drugs and old remedies.

From the experimental results, presented in the tables, some general conclusions may be drawn. First it should be noted that mixtures of two volatile isothiocyanates often are encountered, though no consistency seems to exist with regard to the preferred combinations of the individual isothiocyanates. Surprisingly often, however, isopropyl and *sec*-butyl isothiocyanate are found in the same species. It seems rather incomprehensible that the widely distributed isopropyl derivative has hitherto escaped notice within the *Cruciferae*. The reason might be found in previous failures to recognise this isothiocyanate in admixture with the closely related *sec*-butyl derivative.

It is noteworthy that quite a few of the seed samples proved to be devoid of volatile isothiocyanates (Table 3). The seeds of *Lepidium perfoliatum* L., *Lepidium Menziesii* DC., *Berteroa incana* (L.) DC., *Eruca sativa* Mill. and *Turritis glabra* L. gave paperchromatographic results which warranted a closer

Table 2. Contents of volatile isothiocyanates in seeds, determined by paper chromatography.

No.	Species	% Fat	R _F	isoThiocyanate(s)	mg % ^c
<i>Cruciferae</i>					
1.	<i>Barbarea arcuata</i> (Opiz.) Reichb. ^d	27	{0.02 1.11	{Methyl β-Phenethyl ^b	20 70
2.	<i>Barbarea intermedia</i> Bor. ^d	29	1.11	β-Phenethyl	43
3.	<i>Cakile maritima</i> Scop. ^d	—	0.27	Allyl	—
4.	<i>Cardamine graeca</i> L. ^d	15	{0.62 0.91	{3-Butenyl ^b Benzyl	591
5.	<i>Cheiranthus cheiri</i> L.	21	{0.02 0.39	{Methyl isoPropyl ^b	7 11
6.	<i>Cheiranthus maritimus</i> ^d	21	{0.02 0.71	{Methyl sec-Butyl ^a	123
7.	<i>Cochlearia anglica</i> (L.) Asch. & Grb. ^d	31	{0.41 0.73	{isoPropyl ^b sec-Butyl	282 326
8.	<i>Cochlearia danica</i> L.	26	{0.44 0.76	{isoPropyl ^b sec-Butyl	220 254
9.	<i>Cochlearia officinalis</i> L.	35	{0.41 0.76	{isoPropyl sec-Butyl ^b	254 292
10.	<i>Coronopus didymus</i> (L.) Sm.	23	0.91	Benzyl	154
11.	<i>Crambe maritima</i> L.	—	0.26	Allyl	—
12.	<i>Diplotaxis muralis</i> (L.) DC. ^d	30	0.27	Allyl	500
13.	<i>Draba borealis</i> DC. ^d	23	{0.74 0.96	{sec-Butyl Benzyl (?)	770
14.	<i>Draba incana</i> L.	23	{0.27 0.59	{Allyl 3-Butenyl ^a	690
15.	<i>Erucastrum gallicum</i> (Willd.) O. E. Schulz ^d	32	{0.27 0.59	{Allyl ^b 3-Butenyl	34 36
16.	<i>Erysimum cheiranthoides</i> L. ^d	39	0.27	Allyl	8
17.	<i>Erysimum Perofskianum</i> Fisch. et Mey.	33	{0.02 0.96	{Methyl Benzyl (?)	179
18.	<i>Hutchinsia alpina</i> R.Br. ^d	20	{1.09 1.21	{β-Phenethyl (?) ^a	430
19.	<i>Isatis tinctoria</i> L.	—	0.59	3-Butenyl	—
20.	<i>Lepidium densiflorum</i> Schrad. ^d	25	0.91	Benzyl	203
21.	<i>Lepidium sativum</i> L.	27	{0.90 1.12	{Benzyl β-Phenethyl (?) ^a	942
22.	<i>Lepidium virginicum</i> L. ^d	21	0.89	Benzyl	762
23.	<i>Lunaria annua</i> L. ^d	27	{0.40 0.73	{isoPropyl ^c sec-Butyl ^a	488
24.	<i>Matthiola annua</i> R.Br.	25	{0.03 0.43	{Methyl ^a isoPropyl ^a	
25.	<i>Matthiola fenestralis</i> (L.) R.Br. ^d		0.03	Methyl ^a	
26.	<i>Nasturtium officinale</i> R.Br.	34	1.09	β-Phenethyl	558
27.	<i>Raphanus sativus</i> L. var. <i>alba</i> D.C.	46	{0.02 0.40	{Methyl ^a isoPropyl	4
28.	<i>Raphanus sativus</i> L. var. <i>radicula</i> Pers.	41	0.26	Allyl	3
29.	<i>Rapistrum perenne</i> (L.) All. ^d	—	0.61	3-Butenyl	—
30.	<i>Sinapis alba</i> L.	35	0.39	isoPropyl ^a	3
31.	<i>Sisymbrium sophia</i> L. ^d	30	0.27	Allyl	30
32.	<i>Sisymbrium strictissimum</i> L.	30	{0.40 0.73	{isoPropyl ^a sec-Butyl	158
33.	<i>Thlaspi arvense</i> L.	35	0.27	Allyl	418

<i>Resedaceae</i>					
34.	<i>Reseda Ungronette odorata</i>	40	0.03	Methyl	7
<i>Tropaeolaceae</i>					
35.	<i>Tropaeolum majus</i> L.	15	0.90	Benzyl	970
36.	<i>Tropaeolum majus nanum</i> ^d	14	0.89	Benzyl	977
37.	<i>Tropaeolum peregrinum</i> (<i>canariense</i>) ^d	29	{0.41 0.74	{ <i>iso</i> Propyl ^b	37
				{ <i>sec</i> -Butyl	43
<i>Capparidaceae</i>					
38.	<i>Cleome arabica</i> L. ^d	26	0.03	Methyl	30
39.	<i>Gynandropsis gynandra</i> (L.) Briq. ^d	27	0.03	Methyl	251

^a traces only; ^b predominant; ^c shown by isolation (cf. Ref. ⁹); ^d not previously investigated for isothiocyanates; ^e calculated as the appropriate isothiocyanate.

investigation of their isothiocyanate contents. The results of these studies now in progress will be presented at a later date.

Many of our results confirm earlier findings whereas other necessitate a revision of statements in the literature. In this connection it should be mentioned that Schultz and Gmelin ^{7,8} very recently described a paperchromatographic technique for the separation and identification of glycosides in a large number of crucifers. Their method has no limitation as to the nature of the glycosides responding to the separation procedure and therefore provides evidence also of the presence of glycosides containing non-volatile isothiocyanates. However, their R_f -values of the individual glycosides seem to fall within a very narrow range. This fact renders the unambiguous recognition of the spots somewhat difficult. To this circumstance we attribute certain inconsistencies between their results and ours. Space does not permit a detailed discussion of the divergencies which, however, are easily noticed upon comparison of the experimental results in their publication and the present one.

The detection of 3-butenyl and benzyl isothiocyanate in seeds of *Cardamine graeca* (2,4) * was surprising in view of the previously established presence of *sec*-butyl isothiocyanate in fresh parts of *C. amara* L.⁹ and *C. pratensis* L.¹⁰. Seeds of the wallflower (*Cheiranthus cheiri*: 2,5) have formerly been known as a source of the non-volatile cheiriline ¹¹, but no previous indication of the presence of other isothiocyanates in this species seems to be on record. From *Cochlearia danica* (fresh plants) Blanksma ¹⁰ isolated *sec*-butyl isothiocyanate whereas Urban ¹² reported the presence of the same compound in seeds of *C. officinalis*. On this background it was unexpected to find *isopropyl* isothiocyanate to be present in all three species of *Cochlearia* investigated (2,7—9), in two of them as the predominant constituent. The detection of benzyl isothiocyanate in seeds of *Coronopus didymus* (2,10) confirms recent studies by McDowall *et al.*¹³. In the literature ⁸ seeds of *Crambe maritima* (2,11) are reported to contain a glucoside of unknown structure. Evidence is now provided for the isothiocyanate therein being the allyl derivative. A number of repre-

* In the following, the two figures in brackets refer to the number of the table and species, respectively.

Table 3. Species of seeds found devoid of volatile isothiocyanates by paper chromatography.

<i>Cruciferae</i>	
1. <i>Alyssum alyssoides</i> L. ^a	10. <i>Lepidium heterophyllum</i> (DC.) Benth. ^a
2. <i>Arabis alpina</i> L. ^a	<i>Resedaceae</i>
3. <i>Bunias erucago</i> L.	11. <i>Reseda lutea</i> L.
4. <i>Camelina sativa</i> (L.) Crantz ^a	<i>Phytolaccaceae</i>
5. <i>Capsella bursa pastoris</i> (L.) Medic.	12. <i>Petiveria alliacea</i> L. ^a
6. <i>Conringia orientalis</i> (L.) Andr. ^a	<i>Euphorbiaceae</i>
7. <i>Erophila verna</i> (L.) Chev. ^a	13. <i>Euphorbia dentata</i> Michx. ^a
8. <i>Hesperis matronalis</i> L.	14. <i>Euphorbia exigua</i> L. ^a
9. <i>Iberis amara</i> L.	15. <i>Euphorbia graeca</i> Boiss. et Sprunn ^a

^a not previously investigated for isothiocyanates.

representatives of the genus *Draba* were formerly studied ^{7,8}. In the present work two species (2,13—14) were found to be particularly rich with regard to volatile isothiocyanates but with different types predominating.

It is interesting to notice the presence of volatile isothiocyanates in seeds of various *Erysimum* species (2,16—17) because those have hitherto been considered as containing only the non-volatile isothiocyanates cheiriline ¹⁴ and erysoline ¹⁵. In roots ^{4,7} and seeds ⁸ of *Isatis tinctoria* (2,19) the presence of allyl and *sec*-butyl isothiocyanate has previously been suggested. We found no indication of the presence of either of these, but repeatedly obtained evidence of 3-butenyl isothiocyanate being present in the seeds. Water-cress seeds (*Nasturtium officinale*: 2,26) have not been previously studied with regard to their contents of volatile isothiocyanates. It was not surprising, however, to find β -phenylethyl isothiocyanate being the sole constituent in view of its well-established occurrence in the fresh plants ¹⁶. The presence of isopropyl isothiocyanate, though only in very small amounts, in seeds of white mustard (*Sinapis alba*: 2,30) was rather unexpected because this material is generally believed to be homogenous as to its glucoside, containing the non-volatile *p*-hydroxybenzyl isothiocyanate. The two species of the genus *Sisymbrium* (2,31—32) furnish an illustration to the often encountered phenomenon that closely related species may contain completely different isothiocyanates.

The occurrence of β -phenylethyl isothiocyanate in the essential oil from roots of *Reseda odorata* has been known for many years ¹⁷. Therefore, it was rather surprising to find only slight amounts of presumably methyl isothiocyanate in the seeds, whereas *R. lutea* (3,11) proved completely devoid of volatile mustard oils.

Another striking example of the lacking consistency regarding the isothiocyanate contents within a certain botanical group is furnished by the seeds of *Tropaeolum peregrinum* (2,37) which contain two volatile isothiocyanates. Both of these are, however, different from the long known benzyl isothiocyanate from *Tr. majus* (2,35). The high mustard oil contents in species of the family *Capparidaceae* are interesting and merit further consideration.

The absence of volatile isothiocyanates in seeds of *Alyssum alyssoides* (3,1) and *Arabis alpina* (3,2) is not in accord with literature reports ⁸ on the

Table 4. Contents of volatile isothiocyanates in fresh plants or parts thereof as determined by paper chromatography.

Species	Part(s)	isoThiocyanate(s)	mg %
<i>Cochlearia anglica</i> L. ^c	Leaves, stems and flowers	isoPropyl ^a	11
<i>Armoracia rusticana</i> G., M. & Sch. (Horseradish)	Root	{ Allyl ^b β-Phenethyl	126
<i>Raphanus sativus</i> L. var. <i>radicula</i> Pers. (Radish)	Root	Allyl	~ 1
<i>Capsella bursa pastoris</i> (L.) Medic.	Leaves, stems and flowers	None	.
<i>Sisymbrium luteum</i> (Maxim.) O. E. Schulz ^c	Flowers, siliques	None	
»	Stems, leaves	None	

^a cf. Ref. 3; ^b predominant; ^c not previously investigated for isothiocyanates.

presence of mixtures of glucosides containing different isothiocyanates, mostly non-volatile though, in seeds of related species. We have not been able to confirm older suggestions ¹⁸ that *Capsella bursa pastoris* should contain traces of allyl isothiocyanate. Neither seeds (3,5) nor fresh plant material (Table 4) gave even the slightest trace of volatile mustard oil. Hopkins ¹⁹ isolated from seeds of *Conringia orientalis* the heterocyclic compound .2-mercapto-5,5-dimethyl-oxazoline and suggested α -methallyl isothiocyanate as a possible biogenetic precursor to the heterocyclic substance. We did not, however, obtain any evidence of the presence of α -methallyl or any other volatile isothiocyanate in our paperchromatographic studies.

On account of the isolation by Puntambekar ²⁰ of three volatile isothiocyanates from an Indian plant belonging to the family *Euphorbiaceae*, a series of seeds from plants of this family was investigated (3,13—15). None of these contained detectable quantities of mustard oils.

Many problems in connection with the occurrence of isothiocyanates in nature remain to be solved. The distribution of isothiocyanates within the individual plant, the biosynthesis of the mustard oils and their rôle in the sulphur metabolism are only examples of problems to which we hope to return in future communications.

SUMMARY

Paper chromatography has been used for systematic, qualitative studies of the contents of volatile isothiocyanates in numerous seed samples from plants belonging to the *Cruciferae*, *Resedaceae*, *Tropaeolaceae*, *Capparidaceae*, *Phytolaccaceae* and *Euphorbiaceae*.

The method has been combined with a spectrophotometrical evaluation of the amounts of isothiocyanates present.

The results presented in the tables have been discussed in the light of previous findings.

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