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Glucosinolates in Seeds of *Arabis hirsuta* (L.) Scop.: Some New, Naturally Derived Isothiocyanates

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Seeds of the crucifer *Arabis hirsuta* (L.) Scop. contain, in addition to the previously reported 8-methylsulphinyloctylglucosinolate (Ia), a second major glucosinolate which, on enzymic hydrolysis, affords (+)-5-(*p*-methoxyphenyl)-2-oxazolidinethione, possessing (*S*)-configuration (VI), as established by circular dichroism measurements. The parent glucosinolate, most likely possessing the structure (Ie) (with (*R*)-configuration in the side chain) has not previously been encountered in Nature.

A minor, more hydrophilic glucoside produces, on enzymic hydrolysis, a new isothiocyanate, identified by spectroscopic methods as (*R*)-8-methylsulphinyl-3-oxooctyl isothiocyanate. Most likely, the parent glucosinolate has the structure (If).

A small, lipophilic glucosinolate fraction produces a mixture of isothiocyanates, shown by mass spectrometry to contain 7-methylthioheptyl, 8-methylthiooctyl (II), and 8-methylthio-3-oxooctyl (III) isothiocyanate, none of which have previously been reported as products of enzymic hydrolysis of naturally occurring glucosinolates.

In 1958, 8-methylsulphinyloctylglucosinolate (Ia) ** was described as one of the two major glucosinolates in seeds of *Arabis hirsuta* (L.) Scop. (family Cruciferae), whereas no conclusion was reached as to the identity of the second major compound.[3] We have returned to the problem and now present evidence for the structure of the latter, and, in addition, for several minor glucosinolates in the same seeds.

The original studies were carried out on material propagated froms seeds collected in the wild in Yugoslavia,[3] whereas the seeds employed in the present investigation represent the offspring of a single plant, collected in 1961 on

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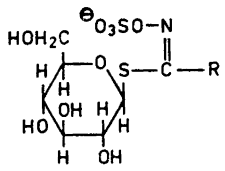
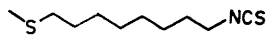
** The chiral sulphoxide group was subsequently shown to possess (*R*)-configuration by chiroptical correlation [1] with a configurationally established sulphoxide.[2]





(II)

(Ia); R: $-(CH_2)_8\overset{O}{\overset{\blacktriangledown}{S}}CH_3$

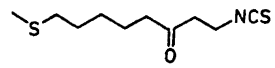
(Ib); R: $-(CH_2)_8SCH_3$

(Ic); R: $-(CH_2)_7SCH_3$

(Id); R: $-(CH_2)_2CO(CH_2)_5SCH_3$

(Ie); R: $-CH_2CH(OH)C_6H_4OCH_3(p)$

(If); R: $-(CH_2)_2CO(CH_2)_5\overset{O}{\overset{\blacktriangledown}{S}}CH_3$



(III)

the Danish island Bornholm.* Chromatographic analyses indicated qualitative coincidence with regard to glucosinolates in the two seed collections. As previously reported,[3] paper chromatography of a seed extract in two solvent systems served to establish its contents of (Ia) and another major glucosinolate. Besides the latter, two observed, but previously unreported minor glucosinolate fractions, one considerably more, and one less lipophilic than the major compounds, will be discussed in the present paper.

Since attempts to separate the individual glucosinolates on a preparative scale were of no avail, a purified glucosinolate mixture was subjected to enzymic hydrolysis with a crude myrosinase preparation, and the chloroform-soluble hydrolysis products were separated, by chromatography on silica gel, into four fractions, numbered (1) – (4) according to the order in which they emerged from the column (see Experimental).

(1) The most lipophilic fraction was very small and contained, according to mass spectroscopic analysis, a mixture of isothiocyanates, convertible, on reaction with ammonia, into a mixture of lipophilic thioureas. By repeated chromatography of the isothiocyanate-containing mixture, a minute, homogeneous fraction was obtained which possessed IR- and mass spectra indistinguishable from those of an authentic specimen of 8-methylthiooctyl isothiocyanate (II).[4,5] Again, the corresponding thiourea possessed spectroscopical characteristics identical with those of authentic 1-(8-methylthiooctyl)-thiourea.[4] Most likely, the isothiocyanate derives from small amounts of 8-methylthiooctylglucosinolate (Ib), present in the lipophilic glucosinolate fraction, but not previously encountered in Nature. Mass spectrometric data of certain fractions strongly suggested the presence also of trace amounts of 7-methylthioheptyl isothiocyanate, supposedly originating from 7-methyl-

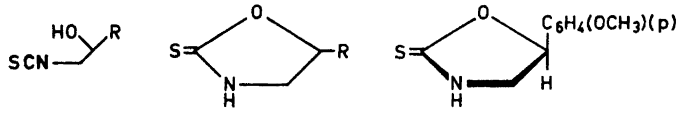
* A herbarium voucher has been deposited in the Botanic Museum of the University of Copenhagen.

thioheptylglucosinolate (Ic), new as a natural compound, but representing the reduced counterpart of 7-methylsulphinylheptylglucosinolate, which was recently reported as a constituent of seeds of *Sibara virginica* (L.) Rollins, in which it occurs along with 8-methylsulphinyloctylglucosinolate.[6]

Another part of the lipophilic isothiocyanate fraction exhibited strong carbonyl absorption in IR and gave mass spectroscopic data suggesting that its structure was 3-oxo-8-methylthiooctyl isothiocyanate (III), probably arising again by enzymic hydrolysis of a parent glucosinolate, (Id), the sulphoxide of which most likely gives rise to a corresponding sulphoxide isothiocyanate described below.

Additional lipophilic isothiocyanates were present in the enzymically hydrolysed seed extracts, but in quantities so minute that they escaped chemical identification.

(2) The second fraction emerging from the column consisted mainly of a crystalline, optically active compound with UV-absorption data characteristic of 2-oxazolidinethiones (V), the frequently observed, spontaneous cyclisation products of enzymically produced 2-hydroxy-substituted isothiocyanates



(IV) (V) (VI)

(IV). Closer examination of the purified compound, $C_{10}H_{11}O_2NS$, including evaluation of its UV, IR, NMR, and mass spectrometric data, revealed its identity as the previously unknown (+)-5-(*p*-methoxyphenyl)-2-oxazolidinethione (V, R = (*p*)-$CH_3OC_6H_4$), almost certainly deriving from the corresponding 2-hydroxy-2-(*p*-methoxyphenyl)-ethyl isothiocyanate, which, in its turn, results from enzymic hydrolysis of the second major seed glucosinolate, most likely possessing the structure (Ie). The close analogue, 5-phenyl-2-oxazolidinethione (V, R = C_6H_5), has previously been isolated from enzymically hydrolyzed plant extracts, both as the *levo*rotatory (from *Barbarea* species [7] and *Reseda luteola* L.[8]) and *dextro*rotatory enantiomer (from *Sibara virginica* (L.) Rollins [6]). Absolute configurations were assigned to the phenyl-substituted ring compounds several years ago,[8] and a comparison of the circular dichroism curve of the *dextro*rotatory *p*-methoxyphenyl derivative with those of the (−)(*R*)- and (+)(*S*)-isomers of the phenyl substituted compound unambiguously established the (*S*)-configuration (VI) for the (+)-5-(*p*-methoxyphenyl)-2-oxazolidinethione. Consequently, the chiral side-chain of the glucosinolate (Ie), whence (VI) derives, possesses (*R*)-configuration.

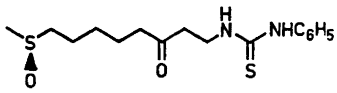
The enantiomeric purity of the isolated 5-(*p*-methoxyphenyl)-2-oxazolidinethione is unknown; the highest molecular rotation measured in methanol on any sample from several isolations was +59°, contrasted with +127° for the presumably pure enantiomer of the corresponding 5-phenyl-derivative. The facile racemization of the *p*-methoxyphenyl substitute, observed, *e.g.*, during recrystallization, along with its propensity to undergo oxidation to strongly

*levo*rotatory products, possibly disulphides, has precluded the isolation of a product of convincing sterical purity. To what extent, therefore, the parent (*R*)-glucosinolate (Ie) is accompanied by its (*S*)-epimer in the seeds remains an open question.

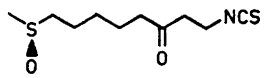
In order to substantiate the assigned structure (V, R = (*p*)-$CH_3OC_6H_4$), a racemized, naturally derived preparation was compared with a synthetic specimen, produced by reaction of (±)-2-amino-1-*p*-methoxyphenyl-ethanol [9] with thiocarbonyl chloride in the presence of triethylamine. M.p., mixed m.p., IR, UV, NMR, and mass spectra served to establish the identity of the synthetic and the naturally derived specimen.

(3) The third fraction eluted from the column consisted of virtually homogeneous (*R*)-8-methylsulphinyloctyl isothiocyanate, arising from (Ia), one of the two major glucosinolates in *A. hirsuta*,[3] and characterized as the previously described (−)(*R*)-1-(8-methylsulphinyloctyl)-3-phenylthiourea, formed upon reaction of the isothiocyanate with aniline.[3]

(4) The least lipophilic fraction contained, according to IR-data, a sulphoxide isothiocyanate, possessing, in addition, one or more carbonyl groups (strong absorption at 1718 cm^{-1}). Upon reaction with aniline, the isothiocyanate was converted into a crystalline, *levo*-rotatory phenylthiourea, $C_{16}H_{24}N_2O_2S_2$, corresponding to the composition $C_{10}H_{17}NO_2S$ for the isothiocyanate, confirmed by mass spectrometry. The mass and NMR-spectra of the phenylthiourea served to establish its structure as (VII). Consequently,



(VII)



(VIII)

the parent and previously unknown isothiocyanate possesses the structure (VIII); the (*R*)-configuration is inferred from the optical rotation, the sign and magnitude of which is very similar to that of the closely analogous (*R*)-1-(8-methylsulphinyloctyl)-3-phenylthiourea mentioned above and previously described.[3] The glucosinolate, whence (VIII) most likely derives, hence possesses the structure (If) and appears on the chromatograms of seed extracts as the least lipophilic glucosinolate. Ketone functionality is not without precedent within the class of naturally occurring glucosinolates, inasmuch as this includes 4-[10] and 5-oxo-heptyl,[11] as well as 5-oxooctyl [12] side chains, but the structure of (If) is the first example of the combination of a terminally positioned methyl-sulphur and a keto function. 3-Hydroxy-5-methylsulphinylpentyl isothiocyanate (most likely with (*R*)-configuration at the *S*-atom), representing a reduced counterpart of (*R*)-3-oxo-8-methylsulphinyloctyl isothiocyanate (VIII), has recently been established as a product of enzymic hydrolysis of a natural glucosinolate.[13]

No attempts have been made to establish the biosynthetic pathways leading to the various glucosinolates, (Ia) – (If), indirectly proved present in seeds of *Arabis hirsuta* through the chemical identity of the enzymically liberated isothiocyanates. From our present knowledge (*cf.* Ref. 14), it seems likely,

however, that phenylalanine (or, possibly, tyrosine) is involved in the biosynthesis of (Ie); and methionine, or close relatives, in the natural production of the other glucosinolates (Ia – Id, If).

The character of the glucosinolates described here is interestingly similar to that of the glucosinolates recently reported in *Sibara virginica* (L.) Rollins,[6] in keeping with the close affinity between the genera *Arabis* and *Sibara*. To what extent the combined occurrence of 2-hydroxy-2-arylethyl and ω-methylthioalkyl (or *S*-oxides) side-chains, preferably with unbranched C_8- (or C_7-) arrangements, is characteristic for a certain, well-defined collection of taxa remains to be established.

EXPERIMENTAL

Melting points are uncorrected and determined in capillary tubes in an electrically heated bath, when not otherwise indicated. Mass spectra were recorded on a Perkin-Elmer model 270 mass spectrometer (ionizing potential 70 eV). Rotations were measured on a Perkin-Elmer model 141 polarimeter, circular dichroism curves on a Roussell-Jouan Dichrographe instrument. Infra-red spectra were recorded on a Perkin-Elmer Infracord; UV-spectra on a Perkin-Elmer 402 UV-spectrophotometer, and NMR-spectra on Varian A-60 or Varian HR-100 instruments.

Paper chromatographic analyses. Paper chromatography in the solvent systems (A) (butanol:ethanol:water, 4:1:4) and (B) (butanol:pyridine:water, 6:4:3) was performed as previously described.[6] R_B-values[6] of 0.95 in solvent (A), and 0.90 and 0.75 in solvent (B), for the two major glucosinolates were previously reported[3] and confirmed for the present seed material.* In addition, however, two minor glucosinolate spots were observed on the chromatograms, one with the R_B-values 0.46 (in (A)) and 0.54 (in (B)), and another with the values 1.43 (in (A)) and 1.16 (in (B)).

Extraction, enzymic hydrolysis, and chromatography. In a typical experiment, finely ground seed material (350 g) was defatted by cold extraction with petroleum ether (1.5 l). The seed material was subsequently extracted with two boiling 1 l portions of 70 % methanol. The combined extracts were filtered through Hyflo-Supercel and freed of methanol by evaporation *in vacuo*. Thr aqueous solution (about 500 ml) was passed slowly through a column of Dowex 1 x 1 ion exchange resin (75 ml) in the chloride form. The column was rinsed with water, and the glucosinolates were eluted by passing a 7 % K_2SO_4-solution (2 l) through the column. Paper chromatography revealed the presence of glucosinolates in fractions Nos. 1 – 13 (each fraction 125 ml). These were combined and evaporated to dryness *in vacuo*. The residue was digested with hot 85 % ethanol; filtration removed large quantities of inorganic salt. The ethanol solution was evaporated to dryness, and the residue was dissolved in a citrate buffer (pH 6.7) (250 ml), to which a trace of ascorbic acid and a cell-free myrosinase solution (15 ml) were added. After standing for 3 h at 37°, the mixture was extracted with several portions of chloroform. The combined extracts were dried over Na_2SO_4, carefully concentrated to a small volume (about 7 ml), and chromatographed by passage through a column of silica gel (100 g), deactivated with 15 % of water. The column was eluted with ethyl acetate (500 ml), chloroform (150 ml), and chloroform containing 2 % of ethanol (350 ml); 15 ml fractions were collected. According to TLC chromatography, the fractions were divided into groups: (1 – 2), fractions Nos. 2 – 10; (3), fractions Nos. 45 – 49; and (4); fractions Nos. 52 – 57. On repeated chromatography on silica gel, with chloroform as eluent, group (1 – 2) was divided into group (1), fractions Nos. 5 – 7; and group (2), fractions Nos. 9 – 13.

* The seeds employed in the present study were produced by large-scale cultivation in 1964 – 1965 of plants, stemming from seeds of a single plant, collected in 1961 near Jon's chapel on the Danish island Bornholm.

Chemical properties and characterization of the fraction groups (1)–(4)

Group (1). The oily residue (about 100 mg) was rechromatographed on a silica gel column, with benzene, containing increasing amounts of chloroform, as eluent. The *most lipophilic* fraction (about 20 mg) gave IR- and mass spectra in keeping with its composition as predominantly *8-methylthiooctyl isothiocyanate* (II), with *7-methylthioheptyl isothiocyanate* as a minor contaminant. Mass spectra of both compounds have previously been reported.[5] On treatment with methanolic ammonia, a thiourea mixture was produced. Separation by preparative TLC (in EtOAc) gave a product which was recrystallized to give 1-(8-methylthiooctyl)-thiourea (4.7 mg), m.p. 71–72°, alone or in admixture with a slightly higher melting authentic specimen.[4] IR- and mass-spectra of the two specimens were indistinguishable. An aliquot of the *least lipophilic* fraction from the TLC separation was treated with methanolic ammonia, and the resulting, thiourea-containing fraction was subjected to preparative TLC chromatography. A minute amount of a thiourea was isolated, possessing IR- and mass spectroscopic data identical with those of an authentic specimen of 1-(7-methylthioheptyl)-thiourea.[4]

The remaining part of the *least lipophilic* fraction was likewise treated with methanolic ammonia, and the resulting thiourea mixture was chromatographed on a silica gel column, chloroform, with increasing amounts of ethanol (up to 3 %), serving as eluent. The least lipophilic fraction afforded a quantity of a partly crystalline thiourea, insufficient for rigorous purification, but serviceable for spectroscopic characterization as 1-(8-methylthio-3-oxooctyl)-thiourea, derived from the isothiocyanate (III). The IR-spectrum exhibited strong absorption at 1710 cm^{-1}, due to a C=O-group, in addition to the general thiourea absorption, and the MS was supporting the proposed structure: *m/e* 248 (M^+), 230 (base peak, $M-H_2O$), 217, 215 ($M-SH$), 201 ($M-CH_3S$), 183 ($230-CH_3S(?)$), 172, 142, 141, 125, 124, 103 (C=O-α-cleav.), 102, 100, and 87.

Group (2): On evaporation of the fractions Nos. 9–13, spontaneous crystallization of the residue (200 mg) occurred. On repeated recrystallizations, performed at room temperature by dissolving the products in ethyl acetate and adding ether, a crystalline fraction was eventually obtained, m.p. 111–112° (4°/min; block; capillary introduced at 100°); $[\alpha]_D^{22}+23°$ (*c* 1.1, $CHCl_3$), $[\alpha]_D^{22}+28°$ (*c* 0.5, MeOH). (Found: C 57.52; H 5.40; N 6.57; S 15.20. Calc. for $C_{10}H_{11}O_2NS$: C 57.37; H 5.30; N 6.69; S 15.32.) UV: λ_{max} 232 (infl., log ε 4.03), 245 (log ε 4.28), 275 (log ε 3.23), and 281 nm (log ε 3.16), in EtOH. The mass spectrum contained characteristic fragments at *m/e* 209 (M^+), 152, 134, and 121. The combined evidence suggested that the isolate was (+)-*5-(*p*-methoxyphenyl)-2-oxazolidinethione* (V, R=(*p*)-$CH_3OC_6H_4$), fully confirmed by the NMR data (in $CDCl_3$, 100 MHz): δ 7.9 (1H, NH, br), δ 7.35 (2H, d, *J* 11, arom.). δ 6.95 (2H, d, *J* 11, arom.), δ 5.85 (1H, appar. t, benzylic H), δ 4.12 (1H, appar. t, $>CH_AH_B$), δ 3.84 (3H, s, OMe), and 3.75 (1H, appar. t, $>CH_AH_B$) ppm. The circular dichroism curve was determined in acetonitrile solution: $[\theta]_{229}-66\,200$; $[\theta]_{247}+38\,400$; and $[\theta]_{287}+3500$. The corresponding curves for (*S*)5-phenyl-2-oxazolidinethione (and the antipodal (*R*)-isomer) showed the following extremes in acetonitrile: $[\theta]_{219}-39\,000$ (+37 700); $[\theta]_{248}+28\,500$ (−29 700); and $[\theta]_{289}+3300$ (−3100).

During the repeated recrystallizations, small amounts of strongly levorotatory, slightly soluble products were removed by filtration. These fractions, presumably oxidation products, were not further studied.

On careful addition of ether to the ethyl acetate solutions, it was possible to make virtually racemic modifications separate selectively, due to their lower solubility. In a typical experiment, the crystalline fraction, $[\alpha]_D+2.7°$ ($CHCl_3$), had m.p. 128–129°, (4°/min, capillary introduced at 110°), alone, or in admixture with a synthetic, racemic specimen (*vide infra*). Solution spectra, as well as the MS, were indistinguishable from those of the optically active material.

A synthetic specimen of (±)-*5-*(p-*methoxyphenyl)-2-oxazolidinethione* was obtained in the following way. The oxalate of (±)-2-amino-1-*p*-methoxyphenylethanol [9] (3 g) was dissolved in 2 N NaOH (15 ml), and the free amino alcohol (2.2 g) was obtained after extractions with chloroform and ether. It was dissolved in chloroform (50 ml), containing triethylamine (2.7 g). To the cooled and stirred solution, thiocarbonyl chloride (1.44 g), dissolved in chloroform (25 ml), was slowly added. Ten minutes after the addi-

tion was complete, the chloroform solution was washed with five 25 ml portions of water. After drying and evaporation of the solvent, the semisolid residue was recrystallized from ethanol to give a slightly yellow product (1.2 g). An analytical specimen of (±)-5-(*p*-methoxyphenyl)-2-oxazolidinethione, m.p. 127–128° (4°/min, capillary introduced at 110°), was obtained after two additional recrystallizations from ethanol. (Found: C 57.42; H 5.41; N 6.72; S 15.31.) λ_{max} 232 (infl., log ε 4.20), 245 (log ε 4.35), 275 (log ε 3.37), and 281 nm (log ε 3.26), in EtOH.

Group (3). The oily residue (300 mg) consisted mainly, according to IR and MS, of 8-methylsulphinyloctyl isothiocyanate (IR: 2100, 2180 cm^{-1} (NCS), 1045 cm^{-1} (SO), MS: *m/e* 233 (M^+), 218, 217, 216, 170 ($M-CH_3SO$), 72 (CH_2NCS)). For further characterization, a fraction of the isothiocyanate was treated with aniline as previously described.[3] A crystalline phenylthiourea was obtained, indistinguishable, on critical comparison, from an authentic specimen of (*R*)-1-(methylsulphinyloctyl)-3-phenylthiourea.[1-3]

Group (4). On evaporation of fractions Nos. 52–57, an oily residue remained (150 mg). According to IR and MS, it consisted mainly of a sulphoxide isothiocyanate (M^+: *m/e* 247), containing, in addition, a carbonyl function (IR 1718 cm^{-1}). Without further purification, part of the isothiocyanate was reacted with aniline in chloroform (overnight at room temperature) to give a crystalline phenylthiourea, which was recrystallized twice from ethyl acetate before analysis, m.p. 126–127°, $[\alpha]_D^{22}$ −39.5° (*c* 1.0, abs. EtOH). (Found: C 56.30; H 7.22; N 7.98. Calc. for $C_{16}H_{24}N_2O_2S_2$: C 56.46; H 7.11; N 8.23.) IR: 1710 cm^{-1} (C=O) and 1000, 1015 cm^{-1} (SO). The NMR-spectrum (in $CDCl_3$, 100 MHz) helped to clarify the structure as (R)-*1-(8-methylsulphinyloctyl)-3-phenylthiourea* (VII) (the configuration assigned solely on the basis of the D-line rotation): δ 8.05 (1H, br. $-NHC_6H_5$), δ 7.2–7.5 (5H, m, C_6H_5), δ 6.8 (1H, br., $-NH-CS$), δ 3.9 (2H, q, *J* 6, $-CH_2NH-$), δ 2.85 (2H, t, *J* 6, $-COCH_2CH_2NH-$), δ 2.65 (2H, t, *J* 5, $-SOCH_2-$), δ 2.58 (3H, s, CH_3SO-), δ 2.45 (2H, t, *J* 7, $-CH_2CH_2CH_2CH_2CO-$), and δ 1.3–1.9 (6H, m, $-SOCH_2CH_2CH_2CH_2-$).

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