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*iso*Thiocyanates XIX.* L(-)-5-Methylsulphinylpentyl *iso*Thiocyanate, the Aglucone of a New Naturally Occurring Glucoside (Glucoalyssin)

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The isolation of L(−)-5-methylsulphinylpentyl *iso*thiocyanate (VII) from seeds of the crucifer *Alyssum argenteum* Vitm. is described. The mustard oil is liberated from its glucosidic precursor, *glucoalyssin*, on enzymic hydrolysis and characterized as its thiourea derivatives, formed upon reaction with ammonia, aniline and benzylamine. The derivative with the last amine, (I), serves to prove the structure of (VII) by transformation into compounds of established structure. The aniline derivative (IX) is compared with a synthetic sample prepared elsewhere.

The mustard oil of glucoiberin is unambiguously proved to be L(−)-3-methylsulphinylpropyl *iso*thiocyanate (XIII). The configurational relationship of the latter, as well as (VII), with (−)-sulphorophene is pointed out. The natural occurrence of sulphoxides is reviewed.

A paperchromatographic survey of the *iso*thiocyanate glucosides in 12 species of *Alyssum* is presented. They appear to contain glucoalyssin, gluconapin, the glucoside of 4-pentenyl *iso*thiocyanate (glucobrassicanapin) and glucoberteroin in various combinations, together with one or two glucosides containing yet undetermined *iso*thiocyanates.

In a previous paper of this series[1] 5-methylthiopentyl *iso*thiocyanate (V) was proved to be the aglucone of a glucoside (glucoberteroin) *ex* the crucifer *Berteroa incana* (L.) DC. Strong, though admittedly inconclusive evidence further suggested that a second glucoside in the same species was derivable from one of the corresponding stereoisomeric sulphoxides. This surmise has now been put beyond dispute, through the isolation and characterization of L(−)-5-methylsulphinylpentyl *iso*thiocyanate (VII), present in various

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species of the genus *Alyssum* as a glucoside for which the name *glucoalyssin* is proposed *.

Due to insufficient supplies of *Berteroa*, the species *Alyssum argenteum* Vitm.** was selected as a starting material for the isolation of the new *iso*thiocyanate (VII). Paperchromatographic evidence indicated that glucoalyssin is present in *B. incana* as well, the two genera being closely allied with regard to taxonomy ***. *Alyssum* species have been only incidentally quoted as sources of *iso*thiocyanates. Thus, Schultz and Gmelin [2] from paperchromatographic survey work considered sinigrin, glucocochlearin and sinalbin to be present in various species of this genus, in addition to two or three other, less certainly identified or unknown glucosides. In this laboratory, various *Alyssum* species have been subjected to glucoside chromatography by essentially the same method, yet with different conclusions as discussed below.

Glucoalyssin occurs in most *Alyssum* species investigated here (Table 2). In *A. argenteum* Vitm. the glucoside is accompanied by only a trace of glucoberteroin, a fact facilitating the purification considerably; no attempts were made, however, to isolate the glucoside in pure condition. A methanolic extract, partly purified by precipitation of impurities with lead acetate, was subjected to enzymic hydrolysis and the sulphide-*iso*thiocyanate selectively removed by ether extraction. The sulphoxide-mustard oil (alyssin) was then extracted with chloroform and obtained as a colourless, vesicant, *levo*rotatory oil which was used directly for the preparation of solid derivatives. Its rotation value was comparable to those determined by Karrer *et al.* for other sulphoxide-*iso*thiocyanates (Table 1). On reaction with ammonia, benzylamine and aniline, alyssin afforded the corresponding thioureas in good yields. The analytical data left no doubt as to the composition, $C_7H_{13}NOS_2$, for the parent mustard oil. Infra-red spectra strongly indicated the presence of a sulphoxide-grouping in the *iso*thiocyanate, the structure of which was definitely proved through the following series of reactions, starting with the *levo*rotatory benzylthiourea-derivative **** (I).

This compound was transformed into the corresponding benzylurea (II) by a modification of the method of Dixon [5], the reaction probably pro-

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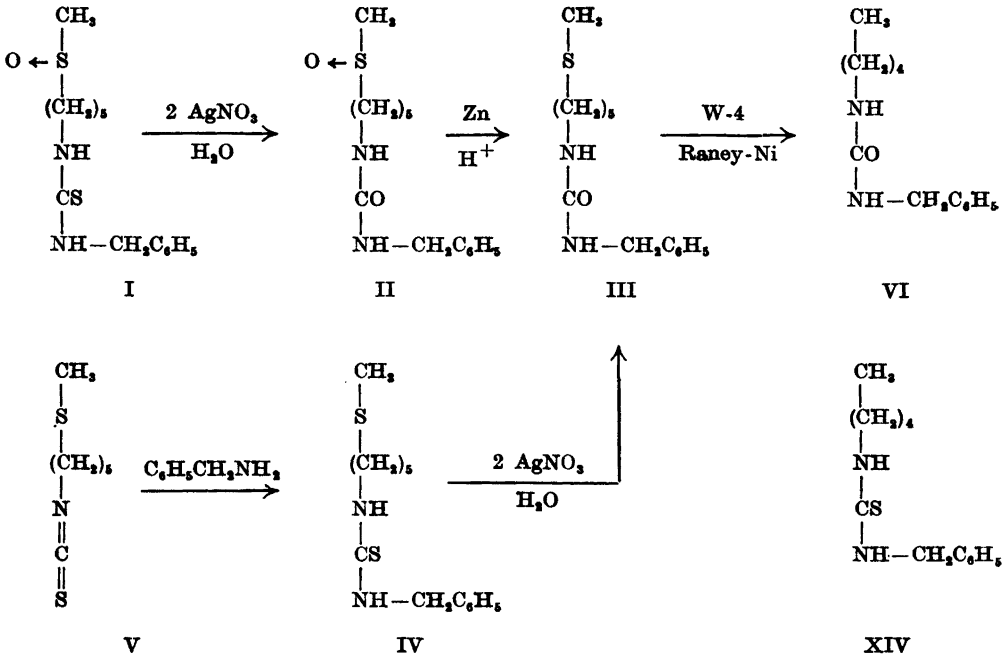
* *Note added in proof:* In a recent paper, submitted to publication a few weeks later than the present communication, Schultz and Wagner [27] describe the isolation from seeds of a species named *Alyssum argenteum* Allioni (*cf.* Table 2) of a crystalline tetraacetate of a glucoside, almost certainly identical with and fortuitously named as our glucoalyssin [28]. The formulation of the corresponding mustard oil as 5-methylsulphinylpentyl *iso*thiocyanate, a structure foreshadowed in a previous paper from this laboratory [1], rests solely on analysis of the acetylated glucoside, infra-red data and analogy to previously recognized *iso*thiocyanates of this type. A non-analyzed preparation of the corresponding thiourea, m. p. 112°, is further reported, yet without optical rotation data.

** The generous gift of a substantial seed sample from E. Benary, Hann.-Münden, Germany, is gratefully acknowledged.

*** *Berteroa incana* DC. was originally described by Linné (1753) as *Alyssum incanum*.

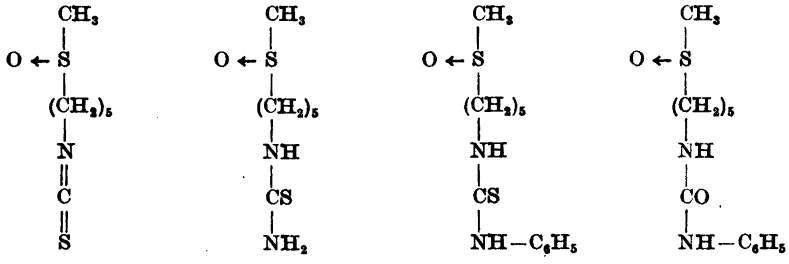
**** For reasons to be discussed in the sequel, (I) is considered to be configurationally related to *levo*-rotatory sulphoraphene, previously proposed by Schmid and Karrer [3] as a configurational reference standard for sulphoxides and arbitrarily allotted the designation L. In this paper, the substances are depicted in conformity with the convention of Karrer *et al.*[4] according to which the oxygen atom is written to the left in the projection formulae of members of the L-series, arranged with the methyl groups at the top.



I II III VI

V IV XIV

ceeding *via* a carbodiimide. Reduction of (II) with zinc dust in acid solution resulted in loss of optical activity, a fact pointing to the sulphoxide-grouping as the sole centre of asymmetry. The identity of the reduced product as 1-benzyl-3-(5′-methylthiopentyl)-urea (III) was established on comparison with an authentic specimen, prepared from the corresponding thiourea (IV) which was, in turn, produced from synthetic 5-methylthiopentyl *iso*thiocyanate [1] (V) and benzylamine. Ultra-violet and infra-red spectra, as well as mixed melting point determination, served as criteria of identity of the two preparations. A specimen of the heretofore unknown 1-benzyl-3-pentylurea (VI), needed in another connection, was produced by hydrogenolysis of (III) with W-4 Raney nickel and also by sulphur exchange of the corresponding thiourea (XIV).



VII VIII IX X

Table 1. Optical rotation data for compounds described in the present paper, (A), together with some previously reported substances of related structures.

Formula	m.p.°	$[\alpha]_D$	$t°$	Solvent	Conc. g/100 ml	Ref.
$CH_3SOCH{=}CHCH_2CH_2NCS$		—108°	18	$CHCl_3$	1.40	7
$CH_3SOCH{=}CHCH_2CH_2NHCSNH_2$	219—220	— 72°	15	H_2O	1.13	7[d]
$CH_3SOCH{=}CHCH_2CH_2NHCSNHC_6H_5$	121	—105°	18	$CHCl_3$	1.03	7
$CH_3SOCH_2CH_2CH_2CH_2NCS$		— 79°	22	$CHCl_3$	1.22	3
$CH_3SOCH_2CH_2CH_2NCS$		— 74°	18.5	C_2H_5OH		6
$CH_3SOCH_2CH_2CH_2NHCSNHC_6H_5$	133—134	— 44°[a]	18	C_2H_5OH		6
$CH_3SOCH_2CH_2CH_2NHCSNHC_6H_5$	137.0—137.5	— 54°	24.5	C_2H_5OH	1.90	A
$CH_3SO(CH_2)_3NHCSNHCH_2C_6H_5$	107.5—108.5	— 51°	24	C_2H_5OH	2.00	A
$CH_3SO(CH_2)_5NCS$		— 9°		C_2H_5OH		6
$CH_3SO(CH_2)_5NCS$[c]		— 69°	24	$CHCl_3$	4.06	A
$CH_3SO(CH_2)_5NHCSNH_2$	106.0—106.5[e]	— 79.5°	24	H_2O	2.00	A
$CH_3SO(CH_2)_5NHCSNHC_6H_5$	123	—19(?)[b]		C_2H_5OH		6
$CH_3SO(CH_2)_5NHCSNHC_6H_5$	126.0—126.5	— 62°	25	C_2H_5OH	2.14	A
$CH_3SO(CH_2)_5NHCSNHCH_2C_6H_5$	103.5—104.0	— 59°	25	C_2H_5OH	1.20	A
$CH_3SO(CH_2)_5NHCONHC_6H_5$	101.5—102.0	— 55°	24	C_2H_5OH	0.76	A
$CH_3SO(CH_2)_5NHCONHCH_2C_6H_5$	105.5—106.5	— 65°	24	C_2H_5OH	0.87	A

[a] *cf.* Foot-note p. 1104. [b] *cf.* Foot-note. [c] Non-analyzed specimen. [d] It has been established in this laboratory that the compound possessing the reported data does not represent authentic sulphoraphene-thiourea. [e] *cf.* Foot-note p. 1101.

The crystalline thiourea-derivatives, (VIII) and (IX), prepared by reaction of alyssin with ammonia and aniline, respectively, possessed properties comparable to those of the above benzyl-derivative. (—)-1-Phenyl-3-(5′-methylsulphinylpentyl)-thiourea (IX), with a specific rotation of —11°, was previously reported by Karrer *et al.*[6] as a reaction product of synthetic (+)-5-methylsulphinylpentyl *iso*thiocyanate (spec. rotat. +4°) and aniline. The corresponding antipodes were reported with the specific rotation values +19°* and —9° for the phenylthiourea and *iso*thiocyanate, respectively.

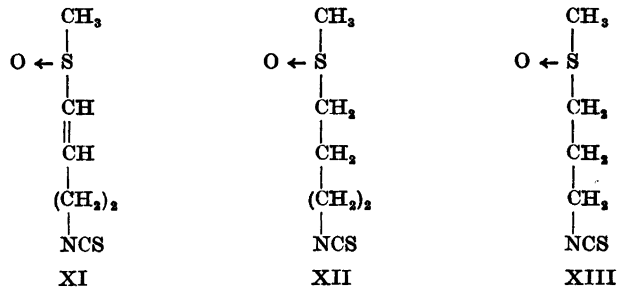
These values differ considerably from those determined in this laboratory for the natural mustard oil (VII) and its derivative (IX) (*cf.* Table 1) and raised doubt as to the stereochemical homogeneity of the formerly described preparations. We are indebted to Professor Karrer for a specimen of the original preparation of the (—)-phenylthiourea which, despite its deviating rotation value, showed hardly any depression of the melting point on admixture with our natural derivative. Moreover, the infra-red spectra of the two specimens were identical save for a few minor details. We are inclined to believe that in-

* A recent redetermination of the specific rotation gave a small negative value (<9°) suggesting that the antipodes had originally been interchanged. In addition, however, a partial racemization must have taken place on storing as estimated from the small numerical value (private communication from Professor Karrer).

completely resolved products account for the numerically low values reported, a suggestion supported by the fact that the rotation data for the natural *iso*thiocyanate and its derivatives are of the same order of magnitude as those found in analogous series (Table 1). In order to extend the number of compounds for comparison purposes, the sulphoxide-phenylthiourea (IX) was also transformed into the corresponding phenylurea-derivative (X).

The first sulphoxide-mustard oil recognized in nature was the *levo*rotatory sulphoraphene (XI), isolated by Schmid and Karrer [7] from seeds of *Raphanus sativus* L. var. *alba* and representing the first natural product in which optical activity was attributable solely to a sulphoxide-grouping. Therefore, (—)-sulphoraphene was proposed [3] as a configurational L-standard for sulphoxides *. The synthetic (—)-dihydroderivative (XII) has been directly related with (—)-sulphoraphene [3] and is consequently to be designated L-sulphoraphane. Comparable rotation values for (—)-3-methylsulphinylpropyl *iso*thiocyanate (XIII), as well as its derivatives afford strong evidence of the relationship also of the latter to the L-series [6]. Schultz and Gmelin [8] suggested the *iso*thiocyanate (XIII) (iberin) as the aglucone of the crystalline glucoside glucoiberin which they isolated from seeds of *Iberis amara* L. They did not succeed in isolating pure iberin and no crystalline derivatives were obtained. The proposed structure (XIII) was based mainly on the composition of the glucoside, infra-red spectra and structural analogy to the long known cheirolin (3-methylsulphonylpropyl *iso*thiocyanate) and sulphoraphene. In order to prove the structure and configurational relationship of iberin we have prepared its crystalline phenylthiourea- and benzylthiourea-derivatives. The former was compared with an authentic, synthetic specimen, generously supplied by Professor Karrer. The natural derivative had the specific rotation —54°, comparable to the reported value [6] (—43.6°) of the synthetic specimen **. Except for this minor deviation in optical rotation, the two products proved to be identical with regard to mixed melting point and infra-red spectra. It is thus established that iberin possesses the structure (XIII) and belongs, as well as alyssin (VII), to the same configurational series as (—)-sulphoraphene. Both are consequently to be designated as L-compounds.

Alyssin (VII) represents a novel addition to the steadily increasing group of naturally occurring sulphoxides. At present it comprises, besides sulpho-



* *Cf.* note added in proof p. 1109.

** A recent redetermination in Professor Karrer's laboratory gave $[\alpha]_D^{22.5}$ —45.4° (*c* 1.76, ethanol) (private communication).

raphene, iberin and alyssin, the active principle of garlic, alliin, which was shown by Stoll and Seebeck [9] to be (+)-S-allyl-L-cysteine sulphoxide; further, the corresponding (+)-S-methyl-L-cysteine sulphoxide, recently recognized as a constituent of cabbage juice [10] and turnip roots [11] by two groups of workers. (+)-Biotin sulphoxide has been isolated from milk residues [12] and the diastereoisomeric (−)-sulphoxide from cultures of *Aspergillus niger* [13]. Again, the so-called β-lipoic acid, isolated from liver residues, has been demonstrated to be a sulphoxide [14].

The recent recognition of so many sulphoxides in nature raises the question as to what extent these compounds shall be regarded as artefacts, formed as a result of secondary oxidation *in vitro*, or rather as species of individual metabolic significance. Although convincing evidence is lacking, various observations point to an independent biochemical role of the sulphoxides.

In Table 2 are summarized the results of paperchromatographic studies of the *iso*thiocyanate glucosides in seeds of several species of the genus *Alyssum*, collected from various botanical gardens *. In every case, paper chromatography of the glucosides in two solvent systems [2], combined with paper chromatography of the thioureas of the corresponding *iso*thiocyanates in several systems (see Experimental), served to make the assignments as correct as possible by this type of approach. Except for two, all species investigated so far contain glucoalyssin ** (A), often in combination with glucoberteroin (D) from which it is probably biogenetically derived by enzymic oxidation. In several species gluconapin (B), a name introduced by Ter Meulen [15], is proved to be present. The *iso*thiocyanate of gluconapin was previously identified as 3-butenyl *iso*thiocyanate in this laboratory [16] and, independently, by Ettlinger and Hodgkins [17], both groups using seed of rape (*Brassica napus* L.) as a starting material. From unpublished survey work, it appears that this mustard oil is one of rather wide distribution. On the paper chromatograms a glucoside (C) was often detectable possessing an R_F-value slightly lower than that of glucoberteroin. The corresponding *iso*thiocyanate is volatile and the glucoside only difficultly distinguishable from glucoerucin, the glucoside containing

* The seed samples have been sowed in the Botanical Garden of the University of Copenhagen with the purpose of controlling their identities. Possible corrections will appear at a later date.

** *Note added in proof:* The formation of glucose and sulphate subsequent to enzymic hydrolysis of glucoalyssin has been established in the present work. This fact, supplemented with the remarkable demonstration by Ettlinger and Lundeen [30] of a revised structural expression for the mustard oil glucosides, lends support to the following formula for glucoalyssin:

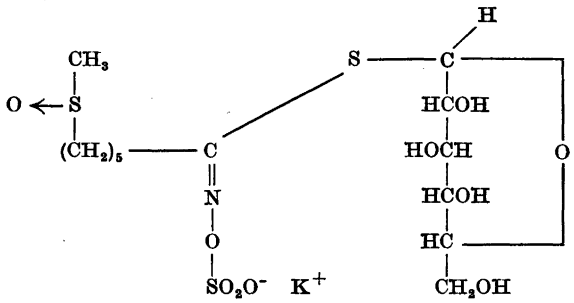


Table 2. iso*Thiocyanate glucoside pattern of seeds of various species of the genus* Alyssum *as determined by paper chromatography.*

Species	A	B	C	D
A. argenteum (All.) Vitm.[b]	+			(+)
A. Bornmuelleri Hausskn.		+		
A. Borzaeanum Nyár.	+			+
A. saxatile L. var. *citrinum*	+	+		+
A. corymbosum Boiss.[a]	+(?)	+		+
A. maritimum Lam.[a,c]	+			
A. montanum L.	+	+		+
A. orientale Ard.	+	+	+	
A. ovirense A. Kern.[a]	+			+
A. saxatile L.	+	+	+	+
A. sinuatum L.	+		+	+
A. alyssoides (L.) L.[a,d]				

A: Glucoalyssin. B: Gluconapin. C: Glucobrassicanapin. D: Glucoberteroin. (+): Traces only. [a] Contains an additional, not yet identified glucoside. [b] Probably identical with the species, less correctly designated as "*A. arg. Allioni*"[20] (=*Lunaria argentea* All.). [c] identical with *Lobularia maritima* Desv. (and *A. Benthamii*[20]). [d] identical with *A. calycinum* L.[20].

4-methylthiobutyl *iso*thiocyanate which we have previously identified in other species [18]. Paper chromatograms of the thiourea corresponding to (C) in special solvent systems, with synthetic 4-pentenylthiourea as a reference substance, leave little doubt, however, that (C) represents a new glucoside containing 4-pentenyl *iso*thiocyanate as the aglucone (glucobrassicanapin). Further evidence of its identity is available in this laboratory and will be presented in a forthcoming communication. It is interesting to note that 4-pentenyl *iso*thiocyanate fits well into the series of allyl and 3-butenyl mustard oil and the formally similar 3-methylthiopropyl [19], 4-methylthiobutyl [18] and 5-methylthiopentyl [1] *iso*thiocyanates. Two additional glucosides are recognized in the *Alyssum* series, the *iso*thiocyanates of which are being further studied.

After this work had been concluded, a paper by Schultz and Wagner [20] appeared, presenting paperchromatographic data for the glucosides of a long series of various plants, including 6 *Alyssum* species, 5 of which are identical with species investigated here. No allocations to specified glucosides are given, but it seems likely that the R_B-values 0.45 and 0.41 in *n*-butanol: acetic acid:water (4:1:3) and *n*-butanol:pyridine:water (6:4:3), respectively, represent glucoalyssin, the values 0.79—0.80 and 0.87—0.95, respectively, gluconapin, 1.06—1.07 (BuOH:HAc:H_2O) the 4-pentenyl *iso*thiocyanate glucoside and 1.16—1.17 (same system) glucoberteroin. Two additional glucosides are listed, one of which, at least, may be identical with one of the still unidentified types observed here (*cf.* Table 2).

The possible significance of the *iso*thiocyanate pattern of the genus *Alyssum* for taxonomical and genetic problems will be discussed in a forthcoming paper.

EXPERIMENTAL

The melting points are uncorrected and determined in capillary tubes in a slowly heated bath. All samples were dried over calcium chloride at room temperature before analysis.

Isolation of (−)-5-methylsulphinylpentyl isothiocyanate (VII). Finely ground seed (200 g) of *Alyssum argenteum* Vitm. was exhaustively extracted with hot 70 % methanol. The filtered extract was freed of methanol by evaporation *in vacuo* to a volume of about 500 ml. Impurities were precipitated by adding excess lead acetate as a 10 % solution. After filtration and removal of excess lead ions as PbS, the filtered solution was again concentrated to *ca.* 500 ml. Solid phosphates were added, in order to buffer the solution at pH 6.6, together with a myrosinase preparation (5 ml), and the mixture was set aside at room temperature for 18 hours. It was then extracted with two 50 ml portions of ether and the extract was discarded. Three extractions with 100 ml portions of chloroform removed all alyssin from the aqueous phase. The organic layer was washed twice with a sodium carbonate solution and finally with water. After drying, the solvent was removed in a vacuum, leaving (VII) as a colourless, vesicant oil (1.01 g) which was used directly for the preparation of the following derivatives. $[\alpha]_D^{24}$ −69° (*c* 4.06, $CHCl_3$).

L(−)-*5-Methylsulphinylpentylthiourea (VIII).* The mustard oil (500 mg) was dissolved in methanol, previously saturated with ammonia at 0°, and the solution kept at room temperature for 24 h in a stoppered flask. After removal of the solvent, a crystalline residue remained which was recrystallized from ethyl acetate, a slight, sticky residue being discarded. After two additional recrystallizations from the same solvent, the thiourea (281 mg) was obtained as colourless compact needles with m.p. 106.0−106.5° (*cf.* foot-note on p. 1101). $[\alpha]_D^{24}$ − 79.5° ± 1.5° (*c* 2.00, H_2O). The ultra-violet spectrum in ethanol was normal, displaying a maximum at 242 mμ (ε 14 200) and a minimum at 222 mμ (ε 3 870). (Found: C 40.28; H 7.60; N 13.29. Calc. for $C_7H_{16}N_2OS_2$: C 40.36; H 7.74; N 13.45).

The thiourea was chromatographed descendingly on Whatman paper No. 1 with the upper layer of the system *n*-butanol:ethanol:water (4:1:4) as the mobile phase, conditions which we previously found useful for certain thioureas [21]. Comparative runs with selected thioureas (Th.) gave the following R_F-values: iberin-Th. 0.44, cheirolin-Th. [22] 0.50, erysolin-Th.[23] 0.53, (VIII) 0.55 and N-methyl-Th. 0.60.

L(−)-*1-Benzyl-3-(5′-methylsulphinylpentyl)-thiourea* (I). From a solution of the mustard oil (VII) (500 mg) in chloroform, containing excess benzylamine, the benzylthiourea (I) crystallized spontaneously after about 5 minutes (616 mg). An analytical specimen was obtained as colourless rhombs after two recrystallizations from aqueous ethanol, m.p. 103.5−104.0°. $[\alpha]_D^{25}$ −58.6° ± 1.0° (*c* 1.20, 96 % ethanol). (Found: C 56.55; H 7.45; N 9.36. Calc. for $C_{14}H_{22}N_2OS_2$: C 56.34; H 7.43; N 9.39). The UV-spectrum in ethanol exhibited a maximum at 242 mμ (ε 14 050) and a minimum at 233 mμ (ε 12 120).

L(−)-*1-Benzyl-3-(5′-methylsulphinylpentyl)-urea (II).* The benzylthiourea (I) (200 mg) was dissolved in a mixture of ethanol (4 ml) and water (5 ml). A solution of silver nitrate (228 mg) in 50 % ethanol (6 ml) was added, resulting in the development of a yellow colour gradually changing to brown. The reaction mixture was heated on the steam bath for 0.5 h and kept neutral by gradual addition of a total of 2.6 ml of 0.5 N NaOH. After filtering through Celite, the solution was taken to dryness *in vacuo* and the benzylurea extracted with hot anhydrous ethanol which was again removed. The crystalline residue was recrystallized from ethyl acetate yielding clusters of small needles (120 mg). An analytical specimen was obtained after a new recrystallization from the same solvent, m.p. 105.5−106.5°. $[\alpha]_D^{24}$ −64.5° ± 1.0° (*c* 0.87, 96 % ethanol). (Found: C 59.30; H 7.51; N 9.63. Calc. for $C_{14}H_{22}N_2O_2S$: C 59.55; H 7.86; N 9.92). The UV-spectrum in EtOH displayed strong end-absorption and low benzyl-group absorption at *ca.* 250 mμ (ε *ca.* 200).

Reduction of (II) to 1-benzyl-3-(5′-methylthiopentyl)-urea (III). The sulphoxide-urea (II) (80 mg) was dissolved in glacial acetic acid (5 ml); this solution had the initial rotation −0.87°. Acid-activated zinc dust (50 mg) and two drops of conc. hydrochloric acid were added and the solution heated on the steam cone. After 4 h, the rotation had

decreased to $-0.07°$, and after standing at room temperature overnight, no optical activity was observable. On evaporation of the solvent and addition of water the reduced benzylurea crystallized (23 mg). Recrystallization from 50 % ethanol afforded the pure compound as rhombic plates, m.p. 86.5—87.5°, alone or in admixture with the synthetic preparation described below. The IR-spectra further served to establish the identity of the two specimens.

1-Benzyl-3-(5'-methylthiopentyl)-thiourea (*IV*). Previously prepared 5-methylthiopentyl *iso*thiocyanate [1] (V) (1.52 g) was gradually added to a solution of benzylamine (1.29 g) in ether (10 ml). Next day, the solvent was removed and the residue recrystallized from aqueous ethanol (1.98 g). An analytical specimen separated as colourless, rhombic plates from the same solvent, m.p. 56.0—57.0°. (Found: C 59.45; H 8.06; N 9.97. Calc. for $C_{14}H_{22}N_2S_2$: C 59.53; H 7.85; N 9.92).

1-Benzyl-3-(5'-methylthiopentyl)-urea (*III*). The benzylthiourea (IV) was transformed into the corresponding benzylurea (III), essentially as described above for the corresponding sulphoxide-derivative (yield 65 %). The reaction product separated from 50 % ethanol as colourless flat needles, m.p. 86.5—87.0°, undepressed on admixture with the specimen prepared above from the natural derivative. (Found: C 62.95; H 8.08; N 10.51. Calc. for $C_{14}H_{22}N_2OS$: C 63.11; H 8.33; N 10.52). The infra-red spectra of the two preparations were identical.

1-Benzyl-3-pentylurea (*VI*). Hydrogenolysis of the methylthio-urea (III) (266 mg) was performed by heating a stirred solution in anhydrous ethanol (30 ml) with a level teaspoon full of freshly prepared W-4 Raney nickel [24] for 2 h at 40°, followed by 2 h at 70°. After filtering, and removal of the solvent, the sulphur-free urea remained (181 mg). It separated from 50 % ethanol as thin platelets, m.p. 93.5—94.5°. (Found: C 70.80; H 9.37; N 12.65. Calc. for $C_{13}H_{20}N_2O$: C 70.88; H 9.15; N 12.72.) No depression was observed on admixture with a specimen produced by treatment of 1-benzyl-3-pentylthiourea with silver nitrate in the usual way.

1-Benzyl-3-pentylthiourea (XIV). A quantitative yield of this thiourea was obtained by reaction of benzyl *iso*thiocyanate with *n*-pentylamine. It separated as colourless, nacreous plates from aqueous ethanol, m. p. 53°. (Found: C 65.95; H 8.51; N 11.80. Calc. for $C_{13}H_{20}N_2S$: C 66.03; H 8.53; N 11.85).

Weller *et al.*[25] report the m. p. 62° for this compound, whereas the value 54° is listed for 1-benzyl-3-*iso*pentylthiourea. A specimen of the latter, prepared in this laboratory, melted at 62°, suggesting an accidental interchange of the two isomerides in the American laboratory. (Found: C 66.15; H 8.58; N 11.79. Calc.: *vide supra*).

L(−)-*1-Phenyl-3-(5'-methylsulphinylpentyl)-thiourea* (*IX*). A solution of the *iso*thiocyanate (VII) (1.00 g) and aniline (2 ml) in chloroform was kept at room temperature for 5 h. On addition of ether an oil separated which rapidly crystallized on scratching. The product separated from chloroform and petroleum ether as small needles (848 mg). An analytical sample was obtained as prisms from aqueous ethanol, m.p. 126.0—126.5° (previously recorded [6] 123°; a mixture melted at 125.0—125.5°). $[\alpha]_D^{25}$ $-61.6° \pm 2.5°$ (*c* 2.14, 96 % ethanol) (*cf.* Table 1). The UV-spectrum in ethanol exhibited a maximum at 247 mμ (ε 14 550), a minimum at 225 mμ (ε 9 000) and a plateau at 260—265 mμ (ε *ca.* 13 100). (Found: C 54.95; H 7.10; N 9.78. Calc. for $C_{13}H_{20}N_2OS_2$: C 54.89; H 7.09; N 9.85). The infra-red spectra of the natural and the synthetic derivative coincided except for a few, very small details.

L(−)-*1-Phenyl-3-(5'-methylsulphinylpentyl)-urea* (*X*). When the above phenylthiourea (IX) (285 mg) was treated with silver nitrate (340 mg) in the usual fashion, the corresponding phenylurea (X) was obtained (138 mg). The pure compound crystallized as thin, rhombic plates from ethyl acetate, m.p. 101.5—102.0°. $[\alpha]_D^{24}$ $-55° \pm 1°$ (*c* 0.76, 96 % ethanol). (Found: C 58.20; H 7.48; N 10.34. Calc. for $C_{13}H_{20}N_2O_2S$: C 58.17; H 7.51; N 10.44). (X) exhibited very intense absorption in the UV-region with maxima at 241 mμ (ε 24 100) and *ca.* 270 mμ (ε 1 700) and a minimum at 265 mμ (ε 1 360). These data are comparable to those reported for similar substances, *e.g.* 1-ethyl-3-phenylurea [25].

L(−)-*1-Benzyl-3-(3'-methylsulphinylpropyl)-thiourea.* A solution of glucoiberin [8] (1.00 g) was dissolved in water (200 ml), 1/15 M phosphate buffer solution of pH 6.6 (50 ml) and a myrosinase preparation (5 ml) were added, and the mixture set aside for 18 h at room temperature. The *iso*thiocyanate (iberin) was extracted with chloroform and the extract divided into two halves, one of which was treated with benzylamine in

the usual way to give a crystalline thiourea (151 mg). An analytical sample separated from ethyl acetate as stout, colourless prisms, m. p. 107.5—108.5. $[\alpha]_D^{24}$ −51° ± 1° (*c* 2.00, 96 % ethanol). (Found: C 53.75; H 6.40; N 10.27. Calc. for $C_{12}H_{16}N_2OS_2$: C 53.30; H 6.71; N 10.36).

L(−)-*1-Phenyl-3-(3'-methylsulphinylpropyl)-thiourea.* The other half of the above chloroform solution of iberin was allowed to react with aniline. The crystalline phenylthiourea (67 mg) separated from water in beautiful, colourless prisms, m.p. 137.0—137.5°. (A synthetic specimen prepared elsewhere [6] had m.p. 134.5—135.0° in the same bath, and a mixture of the two samples melted undepressed at 136.0—136.5°). $[\alpha]_D^{24.5}$ −54° ± 2° (*c* 1.90, 96% ethanol). (Found: C 51.40; H 6.42; N 10.99. Calc. for $C_{11}H_{16}N_2OS_2$: C 51.52; H 6.29; N 10.93). The infra-red spectra of the natural and the synthetic derivative were found to be identical.

Paper chromatography of Alyssum *species.* The seed samples were crushed in a mortar and extracted with hot methanol. Without further purification, these extracts were used for descending paper chromatography in the two solvent systems: (a) *n*-butanol: ethanol: water (4:1:4) and (b) pyridine:amyl alcohol:water (35:30:30), essentially by the method of Schultz and Gmelin [2].

Separate extracts were taken to dryness, dissolved in dilute phosphate buffer (pH 6.6) and hydrolyzed enzymically with myrosinase. The solutions were then extracted with chloroform and transformed into thioureas on treatment with ammonia at room temperature. The concentrated solutions were used for paper chromatography in water-saturated chloroform by our usual method [26], yet modified to the descending technique. Chromatograms in this system, run with suitable authentic reference samples, permitted the unambiguous identification of the thioureas of 3-butenyl and 5-methylthiopentyl *iso*thiocyanates. Also alyssin-thiourea could be clearly distinguished by comparison with an authentic specimen.

In some seed species (*cf.* Table 2) an additional glucoside spot, (C), with an R_F-value slightly lower than that of glucoberteroin, was observed. A corresponding spot appeared in the thiourea-chromatograms with an R_{Ph}-value [26] of *ca.* 0.95, intermediate between those given by benzylthiourea (0.92) and 4-methylthiobutylthiourea (0.99). It was indistinguishable, however, from a reference sample of 4-pentenylthiourea, the synthesis of which will be described in a forthcoming paper. This finding was confirmed in chromatograms, run with the upper layer of the system *n*-heptane:benzene-water (2:9:9) as the mobile phase. In this solvent, the R_F-values of benzyl- and 4-methylthiobutyl-thiourea are depressed to such an extent that they can be clearly differentiated from 4-pentenylthiourea. On the other hand, it is still possible to recognize the latter in the presence of 5-methylthiopentylthiourea. Other evidence (unpublished) is available for the natural occurrence of a glucoside of 4-pentenyl *iso*thiocyanate in nature.

Note added in proof: From a paper by Synge and Wood [10] it appears that an X-ray structural analysis of (+)-S-methyl-L-cysteine sulphoxide has been embarked upon elsewhere with the purpose of relating the sulphoxide configuration directly to that of the *a*-carbon atom, which is known, thus abolishing the need for an arbitrary sulphoxide reference compound.

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