ACTA CHEMICA SCANDINAVICA 12 (1958) 833—838

*iso*Thiocyanates XXX *. Glucohirsutin, a New Naturally Occurring Glucoside Furnishing (—)-8-Methylsulphinyloctyl *iso*Thiocyanate on Enzymic Hydrolysis

ANDERS KJÆR** and BO CHRISTENSEN

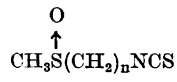
Chemical Laboratory of the University of Copenhagen, Denmark

A survey is presented of the straight-chain, *levo*rotatory ω-methylsulphinylalkyl *iso*thiocyanates (I) formerly recognized as enzymic hydrolysis products of glucosidic progenitors present in various plant species belonging to the family *Cruciferae*. Besides sulphoraphene (II), these mustard oils comprise the compounds I with n = 3, 4, 5, 9 and 10.

The present paper reports the identification of an additional glucoside, *glucohirsutin*, of this general type, present in seeds of *Arabis hirsuta* (L.) Scop. and affording *hirsutin*, the *levo*rotatory 8-methylsulphinyloctyl *iso*thiocyanate (I, n = 8), upon enzymic hydrolysis. The structure of the latter is proved by degradation.

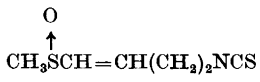
On acid hydrolysis glucohirsutin furnishes hydroxylamine whereas its enzymic cleavage proceeds with liberation of glucose and sulphate in addition to hirsutin. These results establish (VII) as the correct expression for glucohirsutin.

In the course of a systematic search in this laboratory for mustard oil glucosides in seed material of various crucifers, several compounds have been encountered which possess in common the property of furnishing *levo*rotatory *iso*thiocyanates of the general structure (I) on enzymic hydrolysis.



I ***

n = 3: Iberin
n = 4: Sulphoraphane
n = 5: Alyssin



II

n = 8: Hirsutin
n = 9: Arabin
n = 10: Camelinin

* Part XXIX of this series: *Acta Chem. Scand.* **11** (1957) 1423.

** Senior author. Present address: Organic Chemical Laboratory, Royal Veterinary and Agricultural College, Copenhagen V., Denmark.

*** Experimental work is in progress to establish the absolute configuration around the asymmetric sulphur atom. Comparable rotatory data for the homologous *iso*thiocyanates (I), as well as several series of the corresponding thiourea-derivatives, renders it almost certain that all of the parent glucosides, containing a sulphoxide-grouping in the side chain, belong to the same configurational series.

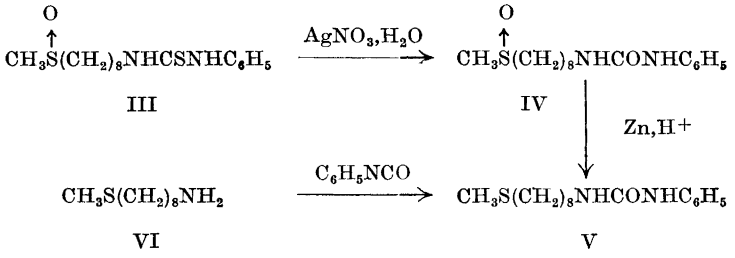
The first recorded compound of this type was sulphoraphene (II), isolated by Schmid and Karrer [1] subsequent to enzymic hydrolysis of a glucoside in seeds of radish (*Raphanus sativus* L. var. *alba*). A few years later, Schultz and Gmelin [2] isolated the crystalline glucoside glucoiberin from seeds of *Iberis amara* L. On enzymic hydrolysis it furnished a mustard oil (iberin) for which the structure (I, n = 3) was advanced, a formulation which has since been unequivocally established in this laboratory [3]. The homologous sulphoxide *iso*thiocyanate, (−)-sulphoraphane (I, n = 4), was synthesized by Schmid and Karrer [4] and has recently been recognized in this laboratory as an enzymic hydrolysis product of a glucoside of rather wide-spread natural occurrence [5]. A previous communication of this series [3] announced the discovery of a new glucoside, glucoalyssin, present in seeds of *Alyssum argenteum* Vitm. and several other *Alyssum* species (*cf.* also Ref.[6]). It was enzymically hydrolyzed to the mustardoil alyssin, identified as (−)-5-methylsulphinylpentyl *iso*thiocyanate (I, n = 5). Seed material of *Arabis alpina* L. served as the source of a formally similar glucoside (glucoarabin), the structure of which appeared from the fact that enzymic hydrolysis resulted in the formation of arabin, unambiguously identified as (−)-9-methylsulphinylnonyl *iso*thiocyanate (I, n = 9) [7,8]. Again, studies of seed extracts of *Camelina sativa* (L.) Crantz resulted in the discovery of glucocamelinin, undergoing enzymic fission to give camelinin which proved to be the next higher homologue, *viz.* (−)-10-methylsulphinyldecyl *iso*thiocyanate (I, n = 10) [9]. In the present paper we wish to extend this class of natural compounds by an additional glucoside, present in seed extracts of *Arabis hirsuta* (L.) Scop. and affording (−)-8-methylsulphinyloctyl *iso*thiocyanate (I, n = 8) upon enzymic hydrolysis.

Paperchromatographic analysis of a methanolic seed extract of *Arabis hirsuta* (L.) Scop. revealed its contents of two major glucosides, travelling in *n*-butanol: ethanol: water (4:1:4) as a single spot with an R_B-value [10] of 0.95 but distinguishable in the solvent system *n*-butanol: pyridine: water (6:4:3) as two spots possessing R_B-values of 0.90 and 0.75. The former glucoside is being further studied at the present, whereas the compound being strongly depressed in the basic solvent system has been identified as a glucoside different from all heretofore recorded species [11]. In accord with general usage in this field we propose the designation *glucohirsutin* for the new constituent which may be identical with an unidentified glucoside, paperchromatographically observed by Schultz and Wagner [10,12] in fresh parts of *Arabis hirsuta* and other *Arabis* species as a spot possessing R_B-values of 0.94 and 0.86 in *n*-butanol:acetic acid:water (4:1:3) and the above pyridine system, respectively.

When the residue from a seed extract of *A. hirsuta* (93 g of seeds) * was subjected to enzymic hydrolysis with a myrosinase preparation in a dilute phosphate buffer at pH 6.7, a chloroform-extractable, non-volatile mustard oil was liberated, derivable from glucohirsutin. The chloroform solution was washed with dilute alkali and water, dried and evaporated to yield the *levo*-rotatory mustard oil (hirsutin). Without further purification this was allowed

* An ample supply of seed material was procured by large-scale cultivation of the plant during the summer of 1957 in the *Botanical Garden of the University of Copenhagen*. We are greatly indebted for the generous assistance of the garden and wish to acknowledge also the good offices of Professor T. Böcher who supplied authentic seed material for sowing.

to react with aniline in chloroform solution to furnish the crystalline *phenylthiourea* (220 mg), $C_{16}H_{26}ON_2S_2$ (III). This *levo*rotatory derivative displayed a strong and characteristic sulphoxide band in the infra-red spectrum. Treatment of the phenylthiourea in aqueous ethanol with silver nitrate, a procedure successfully employed by us in similar cases [3,7,9], resulted in formation of the corresponding *levo*rotatory *phenylurea*, $C_{16}H_{26}O_2N_2S$ (IV), still possessing the sulphoxide-grouping as apparent from its infra-red spectrum. Reduction of the latter compound with zinc dust in acid solution was accompanied by disappearance of the optical rotation as well as the sulphoxide-band in the infra-red spectrum and resulted in production of the crystalline *phenylurea*, $C_{16}H_{26}ON_2S$ (V). This was identified as 1-(8-methylthiooctyl)-3-phenylurea upon critical comparison with an authentic, synthetic specimen produced by reaction of 8-methylthiooctylamine (VI), recently synthesized in this laboratory [8], with phenyl *iso*cyanate. Mixed melting point determination and coinciding infra-red spectra served to establish the identity of the two preparations.



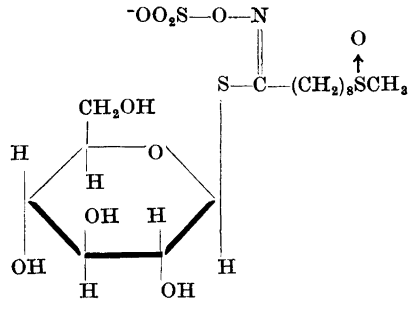
The above sequence of reactions establishes beyond doubt the structure of hirsutin as (−)-8-methylsulphinyloctyl *iso*thiocyanate (I, n = 8).

For further characterization, a separate hirsutin preparation was transformed into the corresponding, *levo*rotatory *8-methylsulphinyloctylthiourea* upon treatment with methanolic ammonia.

Thus far, no attempts have been made to isolate glucohirsutin in pure form, but the following evidence has been adduced to indicate that the glucoside is of the ordinary type. Acid hydrolysis of a paperchromatographically purified specimen of glucohirsutin, conducted according to the directions of Ettlinger and Lundeen [13], resulted in the production of hydroxylamine. The enzymic hydrolysis of a similarly purified glucoside preparation to give hirsutin proceeded with concomitant liberation of glucose and sulphate, a characteristic behaviour of the common mustard oil glucosides. Hence, there can be little doubt that the glucohirsutinate ion, the parent constituent of the employed seed material, possesses the structure (VII), depicted in accord with the recently established general formula for the mustard oil glucosides [13]. In addition to the absolute configuration of the sulphoxide-grouping the stereochemistry around the oxime double bond of (VII) still remains to be clarified.

EXPERIMENTAL

All melting points are uncorrected and determined in capillary tubes in a slowly heated bath. Rotations are measured in a 1 dm tube.



VII

Isolation of hirsutin phenylthiourea (III). A seed sample (93 g) of *Arabis hirsuta* (L.) Scop., produced by cultivation of plants derivable from seed material of wild plants collected near Beograd in Yugoslavia, was finely ground and extracted with one portion (300 ml) of 80 % methanol and two (each of 300 ml) of 70 % methanol. The combined extracts were taken to dryness *in vacuo* and the residue was dissolved in water (200 ml). To the filtered solution were added 1/15 M Na_2HPO_4-(20 ml) and 1/15 M KH_2PO_4-solution (20 ml), together with a cell-free myrosinase preparation (20 ml), and the mixture was set aside at room temperature for 18 h. Next day, it was extracted with three 100 ml-portions of chloroform and the organic layer was washed with 0.2 N NaOH (50 ml) and water. Centrifugation helped to overcome the troublesome emulsion formation at this stage. After being dried over anhydrous Na_2SO_4 the *levo*rotatory solution was concentrated *in vacuo* to a volume of 10 ml whereupon redistilled aniline (1 ml) was added. Next day, the solvent was removed and the residue triturated with ethyl acetate (10 ml) to give the crude, crystalline phenylthiourea derivative. This was dissolved in hot ethyl acetate (100 ml), treated with a little charcoal and the filtered solution concentrated to a volume of 25 ml. On cooling, colourless needles crystallized (117 mg, m. p. 137.5–138.5°). They were combined with additional material (103 mg) of the same m. p., separating from the mother liquor on standing. A specimen for analysis was prepared by two additional crystallizations from the same solvent, m. p. 139° (Found: C 58.90; H 8.18; N 8.65; S 19.81. Calc. for $C_{16}H_{26}ON_2S_2$: C 58.86; H 8.03; N 8.58; S 19.64), $[\alpha]_D^{24}$ −45.0° ± 1.5° ($CHCl_3$, c = 0.8). The infra-red spectrum (in KBr) displayed strong sulphoxide group absorption, apparent as a double band at 986 cm^{-1} and 1 003 cm^{-1}, the former peak being of highest extinction. This split peak character of the sulphoxide band has been frequently observed in this laboratory in spectra of similar compounds in the solid phase and may be attributable to strong hydrogen bonding or even chelation with the substituted thiourea-grouping. The split peak character of the S–O stretching mode around 1 000 cm^{-1} has been observed also by Susi *et al.*[14] in spectra of acids of the general type $RSO(CH_2)_{10}COOH$ in the solid state.

(−)-1-(8-Methylsulphinyloctyl)-3-phenylurea (IV). The above phenylthiourea (191 mg), dissolved in a mixture of ethanol (25 ml) and water (3.5 ml), was treated with a solution of $AgNO_3$ (199 mg) in ethanol, containing a slight amount of water, and the mixture was heated on the steam-bath for about 15 min. During this treatment a total of 1.17 ml of 1 N NaOH was gradually added to maintain neutral reaction. After cooling, precipitated Ag_2S (150 mg) (theoretical amount: 144 mg) was removed by filtration and the filtrate was evaporated to dryness. The residue crystallized in contact with a few ml of water and a pure specimen of the phenylurea was obtained as colourless, rhombic platelets after two recrystallizations from ethyl acetate, m. p. 121°, $[\alpha]_D^{23}$ − 59.7° ± 1.5° (96 % ethanol, c = 1.32) (Found: C 61.87; H 8.32; N 9.05. Calc. for $C_{16}H_{26}O_2N_2S$: C 61.91; H 8.44; N 9.03). The infra-red spectrum (in KBr) was indicative of the presence of a sulphoxide grouping exerting a strong absorption band at 1 005 cm^{-1} with an inflexion near 1 015 cm^{-1}.

Reduction of (IV) to (V). A solution of the sulphoxide-phenylurea (IV) (84 mg) in ethanol (10 ml), containing conc. HCl (0.4 ml), possessed the initial rotation −0.46°. Acid-activated zinc dust (54 mg) was added and the mixture was heated to reflux for 15 min, when the rotation had changed to zero. After filtration, the solution was taken to dryness *in vacuo;* the oily residue crystallized in contact with water to give the crude sulphide-phenylurea (V) (73 mg). Recrystallizations, first from 70 % ethanol and then from ethyl acetate:hexane afforded a pure specimen with m. p. 69°, alone or in admixture with the synthetic sample described below. The two specimens furnished infra-red spectra which were identical throughout the spectral region examined (650−4 000 cm^{-1} cm).

Authentic 1-(8-methylthiooctyl)-3-phenylurea (V). A solution of 8-methylthiooctylamine [8] (268 mg) and phenyl *iso*cyanate (182 mg) in anhydrous xylene (5 ml) was refluxed for 10 min and the solvent then removed *in vacuo*. The crude reaction product (340 mg) was recrystallized from 70 % ethanol and twice from ethyl acetate : hexane to give an analytically pure specimen (175 mg) of the phenylurea, m. p. 70° (Found: C 65.15; H 8.86; N 9.49. Calc. for $C_{16}H_{26}ON_2S$: C 65.29; H 8.90; N 9.52).

(−)-8-Methylsulphinyloctylthiourea. An alkali-washed mustard oil fraction, prepared from 65 g of *Arabis hirsuta* seeds as described above, was treated overnight at room temperature with methanol (25 ml), saturated at 0° with ammonia. Next day, the solution was treated with charcoal and the solvent removed to give an oily residue, which was repeatedly extracted with hot ethyl acetate from which the crystalline thiourea (145 mg) separated on cooling. Two additional recrystallizations from the same solvent afforded an analytically pure specimen of hirsutin-thiourea as colourless prisms, m. p. 88° (Found: C 47.95; H 8.87; N 11.17. Calc. for $C_{10}H_{22}ON_2S_2$: C 47.97; H 8.86; N 11.19), $[\alpha]_D^{23}$ −76° (96 % ethanol, c = 1.0). The IR-spectrum (in KBr) exhibited strong bands at 3 400, 3 280, 3 200, 3 120, 2 930, 2 845, 1 624, 1 575, 1 460, 1 422, 1 342, 1 302, 1 258, 1 230, 1 193, 1 162, 1 070, 1 000 (S−O), 942 and 730 cm^{-1}. On paper chromatography in water-saturated chloroform the thiourea migrated at a rate corresponding to an R_{Ph}-value [15] of 0.84.

Identification of hydrolysis products of glucohirsutin. A band chromatogram of the crude glucoside mixture from seeds of *Arabis hirsuta* was produced by using the upper layer of the solvent system: *n*-butanol:pyridine:water (6:4:3) as the mobile phase. The glucohirsutin-band (R_B-value 0.75), located by spraying two edge-cuts with $AgNO_3$, was eluted with water.

The dry residue was subjected to hydrolysis with conc. HCl for 18 h at room temperature and the concentrated solution chromatographed on paper for hydroxylamine by the method of Bremner [16] with methanol:6 N HCl (7:3) as the solvent system. Spraying with ethanolic picryl chloride revealed a spot of hydroxylamine indistinguishable from that given by an authentic sample of hydroxylamine hydrochloride on the same chromatogram.

Separate band eluates, prepared in the same manner, were subjected to enzymic hydrolysis in unbuffered solution with a sulphate-free myrosinase preparation. Sulphate liberation was revealed by precipitation of $BaSO_4$, and glucose established as a hydrolytic fragment by paper chromatography in *n*-butanol:ethanol:water (4:1:4) followed by spraying with aniline phthalate. Appropriate blanks were checked in all determinations.

The authors are indebted to Dr. R. Gmelin for valuable assistance in the preliminary phases of the present work. Microanalyses were performed in this laboratory by Mr. P. Hansen.

The work is part of investigations supported by *Statens Almindelige Videnskabsfond (The Danish State Research Foundation)* and *Carlsbergfondet (The Carlsberg Foundation)*. A special grant from *Kai Hansens Fond* is gratefully acknowledged.

REFERENCES

1. Schmid, H. and Karrer, P. *Helv. Chim. Acta* **31** (1948) 1017.
2. Schultz, O.-E. and Gmelin, R. *Arch. Pharm.* **287/59** (1954) 404.
3. Kjær, A. and Gmelin, R. *Acta Chem. Scand.* **10** (1956) 1100.
4. Schmid, H. and Karrer, P. *Helv. Chim. Acta* **31** (1948) 1497.

5. Kjær *et al. To be published.*
6. Schultz, O.-E. and Wagner, W. *Z. Naturforsch.* **11b** (1956) 417.
7. Kjær, A. and Gmelin, R. *Acta Chem. Scand.* **10** (1956) 1358.
8. Kjær, A. and Christensen, B. *Acta Chem. Scand.* **11** (1957) 1298.
9. Kjær, A., Gmelin, R. and Boe Jensen, R. *Acta Chem. Scand.* **10** (1956) 1614.
10. Schultz, O.-E. and Wagner, W. *Z. Naturforsch.* **11b** (1956) 73.
11. Kjær, A. *Encyclopedia of Plant Physiology*, Springer-Verlag, Berlin-Göttingen-Heidelberg, 1958, Vol. IX, p. 71.
12. Wagner, W. *Papierchromatographische Analyse der Senfölglucoside, präparative Darstellung ihrer Acetylderivate und ein Beitrag zu ihrer allgemeinen Struktur* (Diss.) University, Tübingen 1956, p. 18.
13. Ettlinger, M. G. and Lundeen, A. J. *J. Am. Chem. Soc.* **78** (1956) 4172.
14. Susi, H., Koenig, N. H., Parker, W. E. and Swern, D. *Anal. Chem.* **30** (1958) 443.
15. Kjær, A. and Rubinstein, K. *Acta Chem. Scand.* **7** (1953) 528.
16. Bremner, J. M. *Analyst* **79** (1954) 198.

Received February 15, 1958.