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*iso*Thiocyanates XIV. 5-Methylthiopentyl *iso*Thiocyanate, a New Mustard Oil Present in Nature as a Glucoside (Glucoberteroin)

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Seeds of the crucifer *Berteroa incana* (L.) DC. appear to contain three *iso*thiocyanate glucosides (A, B and C) as estimated by paper chromatography. On enzymic hydrolysis, one of these, *viz.* (C), for which the name *glucoberteroin* is suggested, affords a volatile *iso*thiocyanate, $C_7H_{13}NS_2$, different from all previously known natural mustard oils. Its thiourea-derivative has been prepared and demonstrated to be identical with an authentic specimen of N-(5-methylthiopentyl)-thiourea (II), synthesized *via* the previously unknown 5-methylthiopentyl *iso*thiocyanate (I) in an unambiguous series of reactions. Evidence is presented of the identity of the second glucoside (A) as the sulphoxide (VIII), corresponding to glucoberteroin, whereas the chemical nature of the third glucoside (B) remains to be elucidated. Glucoberteroin seems to be rather widely distributed in nature.

In a previous paper of this series [1] seeds of the crucifer *Berteroa incana* (L.) DC. were mentioned as the source of a steam-volatile *iso*thiocyanate, meriting further investigation.

Recently, Schultz and Gmelin [2,3] reported the appearance of three glucoside spots in paper chromatograms of methanolic extracts of seeds of *B. incana*. The spot of lowest R_F-value, belonging to their "Gruppe II", was considered to represent a glucoside of unknown nature, whereas the other two spots were attributed to sinalbin and glucotropaeolin, respectively. The same general pattern was obtained on repetition here (Fig. 1), although the interpretation needed thorough revision. This became evident when the seed material was submitted to enzymic hydrolysis and a characteristic radish-like smell developed, due to the liberation of an *iso*thiocyanate, which could be removed by steam-distillation and transformed into a thiourea upon treatment with ammonia. Paper chromatography in chloroform demonstrated the presence of only one thiourea, possessing an R_{Ph}-value [4] of 1.13. This finding immediately excluded the presence of benzylthiourea (R_{Ph} 0.92) and conse-

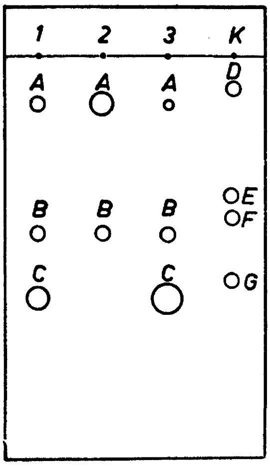


Fig. 1. Descending paper chromatogram in n-*butanol : acetic acid : water (4 : 1 : 3) of : 1. a freshly prepared extract of seeds of* Berteroa incana *(L.) DC.; 2. a two-week old extract of the same seeds; 3. the aged extract after short treatment with a hot solution of* $Na_2S_2O_4$*; K. a control solution.*
A : sulphoxide of glucoberteroin (VIII); B : unknown glucoside; C : glucoberteroin; D : glucoiberin[3]*; E : sinigrin; F : sinalbin and G : glucotropaeolin.*

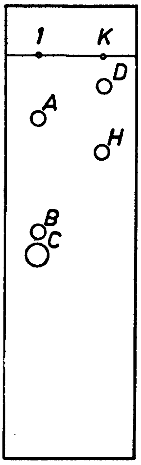


Fig. 2. Descending paper chromatogram in pyridine : amyl alcohol : water (35 : 35 : 30) of 1. a freshly prepared extract of seeds of B. incana; *K. a control solution. A, B, C and D : the same as in Fig. 1.; H : glucocheirolin, applied as an extract of seeds of* Cheiranthus cheiri *L.*

quently also of glucotropaeolin. Furthermore, paperchromatographic data proved the volatile *iso*thiocyanate to be different from all previously known, natural members of this class. From a larger seed sample, cultivated during the summer of 1954 in the Botanical Garden here, it was possible to isolate the thiourea of the new *iso*thiocyanate in pure, crystalline form. It was devoid of optical activity and analyses indicated the composition $C_7H_{16}N_2S_2$. Consequently, it could be inferred that the new *iso*thiocyanate, $C_7H_{13}NS_2$, possessed a sulphur-containing side-chain.

Recently we demonstrated by isolation the presence in nature of 3-methylthiopropyl[5] and 4-methylthiobutyl[6] *iso*thiocyanate, both genuinely occurring in seed materials as glucosides, *viz.* glucoibervirin and glucoerucin, respectively. In analogy we wish to suggest the name *glucoberteroin* for the parent glucoside of *B. incana* (L.) DC., giving rise to the $C_7H_{13}NS_2$-mustard oil on enzymic hydrolysis. It appeared reasonable to regard the latter as a methylthio-derivative also, not least on account of its radish-like smell being so similar to that of the other methylthioalkyl *iso*thiocyanates. The rather limited amounts of thiourea on hand did not permit a direct structure proof by degradation. Hence, it was assumed as a working hypothesis that the C_7-*iso*thiocyanate could be a further member of the straight-chain methylthio-series, *viz.* the

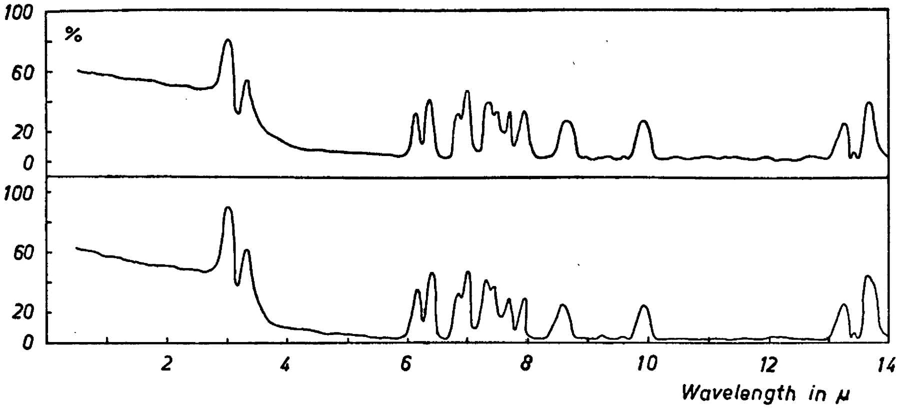


Fig. 3. Infrared spectra determined on a Beckman IR-2 instrument in potassium bromide discs. Upper curve : thiourea, derived from the volatile iso*thiocyanate of* Berteroa incana *seeds. Lower curve : synthetic N-(5-methylthiopentyl)-thiourea (II). Ordinate: Absorption in per cent.*

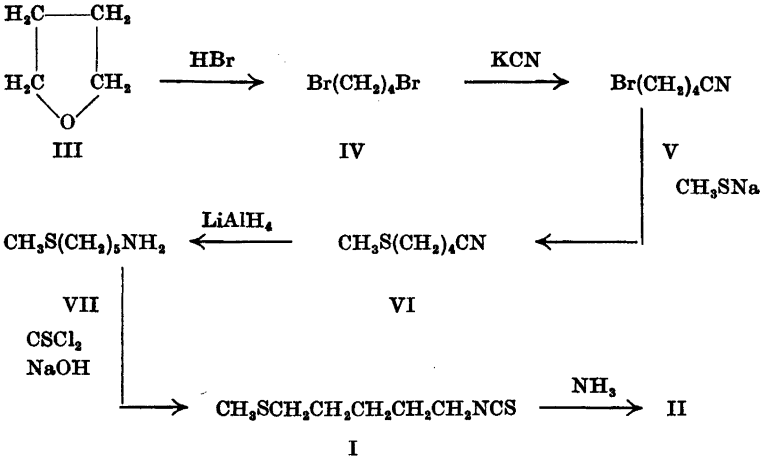
previously unknown 5-methylthiopentyl *iso*thiocyanate (I); a synthetic approach was undertaken to test this supposition.

$CH_3SCH_2CH_2CH_2CH_2CH_2NCS$ I

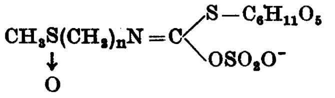
$CH_3SCH_2CH_2CH_2CH_2CH_2NHCSNH_2$ II

Tetrahydrofuran (III) was transformed into 1,4-dibromobutane (IV) [7] and the latter in turn into 5-bromovaleronitrile (V) with potassium cyanide [8]. Reaction of (V) with sodium methylmercaptide afforded in quantitative yield 5-methylthiovaleronitrile (VI) which was smoothly reduced with lithium aluminium hydride to 5-methylthiopentylamine (VII), characterized as its derivative with 2,4-dinitrochlorobenzene. The amine appears to have been used by Karrer *et al.*[9] for further syntheses with no record, however, of experimental or physical data. Thiocarbonyl chloride and alkali [10] reacted with (VII) to the high-boiling 5-methylthiopentyl *iso*thiocyanate (I), possessing a characteristic radish-like smell, undiscernible from that of enzymically hydrolyzed extracts of seeds of *B. incana*. The *iso*thiocyanate was finally transformed into the thiourea (II) by reaction with ammonia. The identity of the latter with the thiourea derived from the natural *iso*thiocyanate, established by paper chromatography, mixed melting point determination and comparison of infrared spectra (Fig. 3), proved beyond doubt the presence in nature of 5-methylthiopentyl *iso*thiocyanate (I).

It was incidentally noted that storage of seed extracts of *B. incana* was accompanied by the disappearance of glucoberteroin in the paper chromatograms (Fig. 1). This observation provided the clue to identification of the glucoside (A). A suspicion, that the latter might be the sulphoxide (VIII), corresponding to glucoberteroin, was confirmed by paper chromatography in pyridine : amyl alcohol : water (Fig. 2), a solvent system which has proved very useful for the purpose of distinguishing between glucosides, possessing



sulphoxide- and sulphone-groupings in their side-chains. An R_F-value lower than that of glucocheirolin, containing the short methylsulphonylpropyl-side chain, excluded the identity of the Berteroa-glucoside (A) as a sulphone. On the other hand, the presence of a rather long sulphoxide-containing chain in the molecule was suggested through its location on the paper, definitely above that of glucoiberin [3] (IX) (Fig. 2).



$$\underset{\underset{O}{\downarrow}}{CH_3S}(CH_2)_nN=C\begin{matrix}\diagup S-C_6H_{11}O_5\\ \diagdown OSO_2O^-\end{matrix}$$

IX : n = 3

VIII : n = 5

Further support for the above interpretation was provided in a more direct way. When an aged methanolic extract, devoid of glucoberteroin, was subjected to reduction with sodium dithionite and chromatographed, a strong spot of glucoberteroin appeared at the expense of the sulphoxide-derivative (VIII) (Fig. 1). This result leaves little doubt as to the occurrence of (VIII) even in freshly prepared seed extracts of *B. incana*. Although genuinely present in plant material, (VIII) is most likely to consider as a secondary product, formed by oxidation *in vivo* of glucoberteroin.

The third glucoside (Fig. 1, B), appearing in the chromatograms at about the site of sinalbin, is being further studied at the present. No colour is produced on its exposure to diazotized sulphanilic acid, a reaction previously shown to be diagnostic for the presence of sinalbin [3]. On enzymic hydrolysis the glucoside affords a non-volatile *iso*thiocyanate.

The occurrence of glucoberteroin is by no means confined to *B. incana*. So far, we have proved it to be present also in seeds of *Lunaria rediviva* L. as well as in numerous species of *Alyssum*. An account of the distribution of glucosides within the latter genus will be presented in a forthcoming communication.

EXPERIMENTAL

All melting points are uncorrected and determined in capillary tubes in a water-bath. Paper chromatography was performed on Whatman paper No. 1 by the descending technique in a constant temperature room.

Isolation of the volatile iso*thiocyanate as thiourea.* Seeds of *Berteroa incana* (L.) DC. (27.2 g) were crushed and defatted with a mixture of ligroin and ethanol by our usual procedure [1]. The dried seed powder (22.1 g) was suspended in water (150 ml), to which was added a myrosinase-solution (1.5 ml), and the mixture was left at room temperature for 16 hours. The radish-smelling *iso*thiocyanate was removed by steam distillation and collected in conc. ammonia (100 ml). Next day the solution was concentrated to dryness *in vacuo*, leaving a colourless oil which soon crystallized (121 mg). The product was recrystallized from 30 % ethanol and separated as colourless, flat prisms (78,5 mg). Another recrystallization, this time from ethyl acetate and pentane, afforded an analytical specimen (58.5 mg) as nacreous platelets, m.p. 67°. The ultraviolet spectra were determined in both water and 96 % ethanol and represented typical thiourea-absorption ($\lambda_{\max}^{H_2O}$ 237 mμ (ε 12 500); $\lambda_{\max}^{EtOH}$ 243 mμ (ε 13 700)). A solution of the thiourea (13.9 mg) in EtOH (1.02 ml) did not give any observable optical rotation. (Found : C 43.55; H 8.17; N 14.38; S 33.06. Calc. for $C_7H_{16}N_2S_2$: C 43.71; H 8.39; N 14.57; S 33.35).

Paper chromatography of the thiourea in chloroform as usual [4] showed it to be homogenous with an R_{Ph}-value of 1.13, easily discernible from β-phenylethylthiourea (R_{Ph} 1.06), possessing the highest R_{Ph}-value of all thioureas derived from previously recognized, natural *iso*thiocyanates.

Syntheses

1,4-Dibromobutane (IV). This compound was prepared from tetrahydrofuran (III) and hydrogen bromide, essentially as described by Wilson [7]; b.p. 77.5° at 11 mm. Yield 77 %.

5-Bromovaleronitrile (V). The nitrile was prepared from a large excess of the dibromide (783 g) and potassium cyanide (47.1 g), as reported by Leonard and Wildman [8]. We found the reaction to be complete after 5 hours heating, where previously 24 hours were prescribed. B.p. 114—116° at 12 mm. Yield 46.4 g.

5-Methylthiovaleronitrile (VI). Methyl mercaptan (16 g) was slowly added to a solution of sodium (7.0 g) in dry methanol (175 ml). To this solution was added in one portion 5-bromovaleronitrile (46.4 g) dissolved in methanol (100 ml). The solution was refluxed for 7 hours and the methanol removed by distillation. Water (50 ml) was added to dissolve the salt and the solution repeatedly extracted with ether (100 ml). The extract was dried, the ether removed by distillation and the residue distilled *in vacuo* as a colourless liquid with a characteristic smell. Yield 33.7 g (91 %); b.p. 112—114° at 8 mm. (Found : C 55.70; H 8.57; N 10.42. Calc. for $C_6H_{11}NS$: C 55.76; H 8.58; N 10.84).

5-Methylthiopentylamine (VII). Finely powdered lithium aluminium hydride (11.4 g) was dissolved in dry ether (250 ml) and the solution placed in a three-necked flask, provided with magnetic stirring, a separatory funnel, reflux condenser and a nitrogen inlet. In a slow stream of nitrogen, the nitrile (VI) (25.8 g), dissolved in ether (40 ml), was dropwise added over 30 minutes to the well-stirred reaction mixture. This was finally refluxed for 30 minutes in order to complete the reduction, and then cooled in an ice-bath. From the funnel were carefully added water (13 ml), 15 % sodium hydroxide (13 ml) and water again (45 ml). The precipitate was removed on a sintered glass filter and thoroughly washed with fresh portions of ether. The pooled ether solutions were dried over pellets of KOH, the ether slowly removed by means of a short Vigreux-column and the resulting amine distilled *in vacuo*. The colourless liquid distilled at 88° and 8 mm; n_D^{25} 1.4852. (Found : C 53.75; H 11.23; N 10.38. Calc. for $C_6H_{15}NS$: C 54.10; H 11.34; N 10.51). The yield was 20.3 g (76 %).

2,4-Dinitro-N-(5'-methylthiopentyl)-aniline. For the purpose of characterization a derivative was prepared from the above amine and 2,4-dinitrochlorobenzene in the usual way. Beautiful yellow needles were obtained upon recrystallization from methanol, m.p. 40°. (Found : C 47.96; H 5.83; N 13.98. Calc. for $C_{12}H_{17}O_4N_3S$: C 48.16; H 5.73; N 14.04).

5-Methylthiopentyl iso*thiocyanate* (I). To a suspension of the amine (VII) (13.3 g) in water (75 ml) was slowly added a solution of thiocarbonyl chloride (12.7 g) in chloroform (100 ml), followed by 0.2 N NaOH (100 ml). The reaction proceeded rapidly under evolution of heat and after shaking for 15 minutes the dark-coloured layers were separated and the aqueous phase extracted with two additional portions of chloroform. The organic layer was dried and the solvent removed by distillation. The resulting *iso*thiocyanate distilled as a colourless oil at 155° and 10 mm; n_D^{25} 1.5422. Yield 12.3 g (70 %). The compound had a very penetrating smell of radish. (Found : C 48.25; H 7.51; N 8.09; S 36.48. Calc. for $C_7H_{13}NS_2$: C 47.97; H 7.48; N 7.99; S 36.58).

N-(5-Methylthiopentyl)-thiourea (II). A mixture of the *iso*thiocyanate (2.64 g), conc. ammonia (20 ml) and ethanol (20 ml) was left standing at room temperature for 48 hours. The now homogenous solution was evaporated to dryness *in vacuo* and the crystalline residue recrystallized from ethyl acetate and pentane (2.03 g, 70 %). An additional recrystallization from chloroform and pentane afforded the thiourea as colourless, rhombic plates of m.p. 67.5°, alone or in admixture with the above derivative, obtained from the volatile *iso*thiocyanate of *B. incana*. (Found : C 43.70; H 8.22; N 14.35. Calc. for $C_7H_{16}N_2S_2$: C 43.71; H 8.39; N 14.57). The infrared spectra of the two products were determined in potassium bromide discs and proved to be identical (Fig. 3). Paper chromatography likewise served to confirm the identity of the two thioureas.

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