isoThiocyanates XII. 3-Methylthiopropyl isoThiocyanate (Ibervirin), a New Naturally Occurring Mustard Oil

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The previously unknown 3-methylthiopropyl isothiocyanate (II) has been synthesized and its occurrence in glucosidic form in nature has been demonstrated. Paper chromatography has indicated the simultaneous presence in seeds of Iberis sempervirens L. of glucoiberin, glucocerin and the new glucoside, for which the name glucoiberin is suggested. From this seed material a mixture of 3-methylthiopropyl- and 4-methylthiobutyl-thioureas has been obtained after enzymatic hydrolysis, steam distillation and treatment with ammonia. The mixture has been quantitatively separated by a 100-transfer countercurrent distribution and the individual compounds identified on comparison with synthetic specimens.

In a previous paper of this series 1 4-methylthiobutyl isothiocyanate (I) was recognized as a constituent of glucoiberin, a new naturally occurring mustard oil glucoside. We now wish to adduce experimental evidence for the presence in nature of a structurally related glucoside, provisionally termed glucoiberin, which gives rise to 3-methylthiopropyl isothiocyanate (II) on enzymatic degradation.

\[
\begin{align*}
\text{CH}_3\text{SCH}_2\text{CH}_2\text{CH}_2\text{NCS} & \quad \text{CH}_3\text{SCH}_2\text{CH}_2\text{CH}_2\text{NCS} \\
\text{CH}_3\text{SCH}_2\text{CH}_2\text{CH}_2\text{NCS} & \quad \text{CH}_3\text{SCH}_2\text{CH}_2\text{CH}_2\text{NHC\textsubscript{2}}\text{SNH}_2 \\
\text{I} & \quad \text{II} \\
\text{III} & \quad \text{IV}
\end{align*}
\]

Seeds of Iberis sempervirens L. were previously investigated for mustard oil glucosides by paper chromatography 2. Revised results 3 indicated the presence of three glucosides one of which possessed an \( R_f \)-value considerably lower than those of the other two. Upon repetition here, a similar paperchromatographic pattern was observed. From its behaviour in various solvent

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systems the spot of low $R_F$-value could safely be attributed to glucoiberin, a glucoside containing methylsulphonylpropyl isothiocyanate (III), recently characterized by Schultz and Gmelin $^3,4$. The glucoside chromatograms did not render possible, however, a correspondingly unambiguous allocation of structure to the two glucosides of higher $R_F$-values.

When seeds of Iberis sempervirens L. were crushed and treated with water and myrosinase a characteristic radish-like smell soon developed, ascribable to enzymatic liberation of volatile mustard oils which could be removed by steam distillation. Upon treatment of the distillate with ammonia a mixture of two thioureas was produced as inferred from paper chromatography according to our usual method $^5$. In chloroform-water as solvent system the two compounds possessed $R_{PA}$-values of 0.82 and 0.99, respectively. They appeared to be present in the ratio 3:2 as roughly judged from the chromatograms. The highest $R_{PA}$-value coincided with the value of the thiourea derived from 4-methylthiobutyl isothiocyanate (I), the radish-smelling oil which we recently showed to be present in seeds of various crucifers $^1$. The other spot fell within the range of sec-butylthiourea which at first glance would seem to be an attractive possibility in view of the well-established occurrence in many plants of glucooestharin, a glucoside yielding sec-butyl isothiocyanate on enzymatic cleavage. This possibility could be excluded, however, when it was found that sec-butylthiourea travelled on the paper at a much higher rate than the unknown thiourea in heptane:n-butanol:formic acid, a solvent system $^6$ which has often proved to be useful also for our purpose. A distillation experiment on seeds of Iberis amara L., the source of glucoiberin $^3,4$, demonstrated volatile isothiocyanates to be absent. Hence, (III) can be excluded as responsible for thiourea spots originating from the volatile fraction.

In view of the occurrence in the seed sample of glucoiberin, producing (III) on enzymatic cleavage, the simultaneous existence of the reduced form (II) appeared to be a reasonable assumption. The hitherto unknown mustard oil was synthesized from 3-methylthiopropylamine and thioacetyl chloride and transformed into 3-methylthiopropylthiourea (IV). Upon paper chromatography in various solvent systems the latter was indistinguishable from the thiourea of the seed distillate possessing an $R_{PA}$-value of 0.82.

On the assumption that (I) and (II) represented the volatile isothiocyanates of I. sempervirens the partition ratios for their thioureas between water and chloroform were determined and found to be 0.47 and 1.35, respectively. With these values a calculation indicated that a 100-transfer counter-current distribution would render possible a practically quantitative separation of the two thioureas, permitting their individual isolation and identification (Fig. 1). This was born out experimentally when the thiourea-mixture from the distillation of 50 g of seeds was distributed in a Craig-apparatus. From plates Nos. 22—39 were isolated pure 4-methylthiobutylthiourea, identified on comparison with an authentic specimen. The contents of the vessels Nos. 50—60 afforded chemically homogenous 3-methylthiopropylthiourea (IV), identical with the synthetic sample. Paper chromatography disclosed the presence of small amounts of an additional thiourea in plates with the serial numbers 65—71. Due to its presence in extremely small amounts this third constituent escaped analytical detection before fractionation. Only after enrichment during the

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Fig. 1. Theoretical 100-plate distribution curves in water: chloroform of 1) 4-methylthio-
butylthiourea with a partition coefficient of 0.47, and 2) 3-methylthiopropylthiourea (IV) 
with a coefficient of 1.35.

counter-current distribution could its identity as a thiourea-derivative of the 
elsewhere encountered 3-butenyl isothiocyanate be chromatographically 
verified.

The recognition of 3-methylthiopropyl isothiocyanate as a natural con-
stituent completes a series of mustard oils, differing only in the oxidation stage 
of the sulphur atom, viz.:

\[
\begin{align*}
\text{CH}_3\text{SCH}_2\text{CH}_3\text{CH}_2\text{NCS} & \quad \text{Iberirin} \\
\text{CH}_3\text{SCH}_2\text{CH}_3\text{CH}_2\text{NCS} & \quad \text{Iberin}^3,^4 \\
\downarrow & \\
\text{O} & \\
\uparrow & \\
\text{O} & \\
\downarrow & \\
\text{CH}_3\text{SCH}_2\text{CH}_3\text{CH}_2\text{NCS} & \quad \text{Cheirolin}^5
\end{align*}
\]

These types of isothiocyanates have been encountered mainly in the genera 
Iberis, Erysimum and Cheiranthus. A survey of the contents of isothiocyanate 
glucosides in various species of the two first genera has been made and will be 
published separately. A pronounced tendency to spontaneous oxidation of 
methylthio-derivatives to sulphone has been observed throughout the cur-
rent studies. Consequently, the question arose to what extent the previously 
recognized sulphone and sulphoxide were artefacts, formed during isolation. 
Glucoberin was reported with a specific rotation of $-55.3^\circ$ whereas the cor-
responding value for sinigrin is $-17.6^\circ$. From these data it can be inferred that

a considerable contribution to the total rotation resides in the sulphoxide-grouping in the side chain of glucoiberin. Here it should be remembered also that sulphoraphen (4-methylsulphinyl-3-butenyl isothiocyanate), isolated by Schmid and Karrer 9 from seeds of Raphanus sativus L., possessed a considerable specific rotation. Hence, at least in some cases the sulphoxides and sulphones appear to be present as genuine plant constituents, very likely formed, however, by enzymatic oxidation of the corresponding sulphides during transportation and storage within the living cells. These problems are at present being further studied.

EXPERIMENTAL

All melting points are uncorrected and determined in capillary tubes in a water-bath.

For chromatographic work redistilled solvents, boiling within 0.5°, have been employed.

Paper chromatography of glucosides. A methanolic extract of crushed seeds of Iberis sempervirens L. was applied to a strip of Schleicher and Schüll 2043 b paper and developed by the descending technique with n-butanol : acetic acid : water (4:1:3) for 4—6 hours. After spraying with silver nitrate and heating 4,3, three distinct spots appeared one of which was of low Rf-value and indistinguishable from glucoiberin, simultaneously run as a control substance. The two other spots appeared within the region of glucococchelin and glucotropaeolin, respectively. A parallel run in pyridine : amyl alcohol : water (30:36:30) served to confirm the identity of the glucoiberin-spot.

Another seed sample was treated with myrosinase, steam-distilled and the volatile isothiocyanates of radish-like smell transformed into thioureas according to our usual method 8. Descending paper chromatography in chloroform : water 8 on Whatman paper No. 1, followed by spraying with Grote's reagent revealed two spots with Rf-values of 0.82 and 0.99. The latter coincided with the spot of 4-methylthiobutyliothiourea 1 as well in this solvent system as in heptane : n-butanol : formic acid (20:15:20) 4. A provisional interpretation of the 0.82-spot as due to sec-butylthiourea required revision when it was found that the butyl-derivative possessed an Rf-value of 1.15, compared to the value 0.51 for the Iberis-spot, when both were run in the formic acid system.

Synthesis of 3-methylthiopropyl isothiocyanate (II) and thiourea (IV). To a cooled solution of thioacetyl chloride (8.2 g) in chloroform (60 ml) was added a solution of 3-methylthiopropylamine 11 (7.2 g) in water (45 ml). 100 ml of 1.4 N NaOH were slowly added with vigorous shaking and after half an hour the chloroform layer was separated, dried and the solvent removed in vacuo. Distillation under diminished pressure afforded the isothiocyanate, b. p. 120.5—122.5°/12 mm. Redistillation gave an analytically pure product as a colourless liquid with a pronounced smell of radish (5.5 g, 54 %); b. p. 118°/7 mm. (Found: C 40.35; H 6.86; N 9.64. Calc. for C6H12NS2: C 40.77; H 6.16; N 9.51).

The isothiocyanate was dissolved in ethanol and excess of conc. aqueous ammonia was added. After 4 days at room temperature the reaction mixture was concentrated in vacuo, leaving an oil which rapidly crystallized. The thiourea (IV) was recrystallized twice from water containing a little ethanol, and finally from ethyl acetate and pentane as thin, hexagonal plates; m. p. 66—67°. (Found: N 16.98; S 39.08. Calc. for C6H11N2S2: N 17.06; S 39.03).

Counter-current distribution. A portion (50.2 g) of finely ground seeds of Iberis sempervirens L. was refluxed with a mixture of ethanol (100 ml) and ligroin (200 ml, b. p. 60—100°) for 20 minutes and the extraction repeated with petroleum ether (250 ml) and ethanol (50 ml) for 15 minutes. The dried powder was then treated with water (200 ml) and a colloid-free myrosinase-preparation (2.0 ml) and left at room temperature overnight. The volatile isothiocyanates were removed in a stream of steam and collected in a receiver containing 25 % ammonium (125 ml). After standing at room temperature for 24 hours the solution was taken to dryness in vacuo (bath temperature not exceeding 50°). The yellow, viscous residue (909 mg) was brought into solution in chloroform by the addition of some ethanol.

The solution was transferred to plates Nos. 0—2 of an all-glass Craig counter-current apparatus, preloaded with water-saturated chloroform in the 100 plates, each holding 40 ml of both the lower and upper phase. 100 transfers were made; at the end of the

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distribution the fractions were collected individually and each five concentrated and investigated paper chromatographically. Aside from a very small overlapping in plates Nos. 40—45 a quantitative separation was achieved in agreement with the calculated curves of Fig. 1. The contents of plates Nos. 22—39 were pooled and concentrated to dryness in vacuo, giving a semi-crystalline mass (275 mg). Two recrystallizations from ethyl acetate and petroleum ether afforded thin, colourless plates (116 mg); m. p. 51°, alone or in admixture with authentic 4-methylthiobutylthiourea. (Found: C 40.34; H 7.80; N 15.85. Calc. for C₈H₁₄N₂S₂: C 40.42; H 7.91; N 15.72). Plates Nos. 50—60 were pooled and worked up in a similar way. The crude product (229 mg) was recrystallized twice from ethyl acetate and petroleum ether and gave shiny, rhombic plates (150 mg); m. p. 67° alone or when mixed with the synthetic 3-methylthiopropylthiourea (IV) above. (Found: C 36.26; H 7.17; N 17.22. Calc. for C₁₂H₁₄N₂S₂: C 36.56; H 7.37; N 17.06).

Paper chromatography in chloroform of the residues from plates Nos. 65—71 disclosed the presence of very small amounts of an additional thiourea with an Rₚₜₐₜ-value of 0.62. Its identity as 3-butenylthiourea is regarded as established by this value.

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REFERENCES


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