

## 4-Methylthio-3-butenyl Isothiocyanate, the Pungent Principle of Radish Root\*

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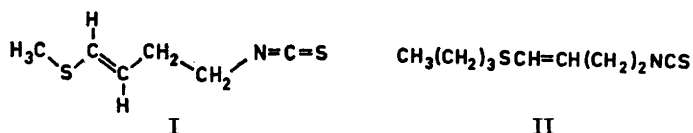
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Previous attempts to identify the pungent principle of radish root are reviewed. The presence in radish of a glucoside undergoing rapid enzymic hydrolysis to an isothiocyanate is demonstrated. The latter is isolated by vapour phase chromatography as a 4:1 mixture of *trans*- and *cis*-4-methylthio-3-butenyl isothiocyanate, identified on the basis of elementary composition, chemical properties, UV-, IR-, 100 Mc NMR-, and mass-spectra.

The unexpectedly facile stereomutation of the radish isothiocyanate and its structural relationship to previously encountered compounds of this class are discussed.

The radish (*Raphanus sativus* L. var. *esculentus* Metzg.\*\*) is a commonly used food relish as well as an old reputed remedy in cures of ailments of the liver and bile tract (*cf.* Ref. 1). Therefore, it is not surprising that many attempts have been made to isolate and characterize the pungent principle of radish, yet without conclusive results thus far. In the present communication evidence is presented to show that the pungent principle in disintegrated radish root is *trans*-4-methylthio-3-butenyl isothiocyanate (I).

More than a hundred years ago, Pless<sup>2</sup> reported that steam distillation of radish root afforded a sulphur-containing oil possessing the taste but



\* Part LV of a series of papers on isothiocyanates. Part LIV: *Acta Chem. Scand.* **19** (1965) 2438.

\*\* Various cultivated forms are commonly encountered such as subvar. *radicula* Pers., a summer form with a spherical hypocotyl, and subvar. *niger* Pers., usually larger and available in various seasonal forms. In the present investigation, both subvarieties have been employed with identical results.

not the odour of fresh radish. Bertram and Walbaum<sup>3</sup> performed a large-scale steam distillation of radish root and obtained an oil of evil smell which did not react with ammonia. At about the same time Moreigne<sup>4</sup> reported on the isolation of a lactone, 'raphanolide', along with a sulphur-containing, nitrogen-free oil, from distillates of radish root. By extracting sliced root material with ether, Gadamer<sup>5</sup> succeeded in obtaining a sulphur-containing oil with the characteristic odour and taste of fresh radish. On treatment with ammonia the oil was converted into a product with properties suggestive of a thiourea-structure, but no further details were reported. Significantly, the same author suggested that the radish root oil was produced by enzymic decomposition of a glycosidic progenitor. Many years later, Heiduschka and Zwergal<sup>6</sup> returned to the problem and performed 150 (!) distillations of a total of 50 kg of fresh radish, thus obtaining two grams of a distilled oil with a pungent odour and radish-like taste. With no chemical documentation whatsoever, save for a reported elemental analysis and molecular weight, these authors suggested the structure (II) for the isolated product. A few years later, Schuphan<sup>7</sup> again provided qualitative evidence for the presence of a non-identified isothiocyanate in distillates from fresh radish material.

In 1948, Schmid and Karrer<sup>1</sup> demonstrated the presence in radish *seed* of a glucoside which on chemical degradation afforded an isothiocyanate, sulphoraphene, to which the structure (III) was assigned, yet without specification as to the geometry of the double bond and the configuration around the



III

chiral sulphur atom.\* Apparently, radish root material was not studied by these authors.

The extensive chromatographic screening of higher plants for isothiocyanate-producing glucosides during the last decade has produced a few results which are pertinent in the present context. In an early paper of this series,<sup>9</sup> a trace of allyl isothiocyanate was reported in radish root.\*\* Schultz and Gmelin<sup>10</sup> in their paperchromatographic survey of numerous crucifer species state that different 'species' of radish root seem to exhibit different patterns. In a certain species ('Schwarzer Winterrettich'), sinalbin and glucotropaeolin were reported to be present. Gmelin,<sup>11</sup> in his survey, lists *R. sativus* root with a content of three non-identified thioglucosides. In a more recent communication from the same group<sup>12</sup> root material of '*R. sativus* L. var. *β nigra* DC.' was again reported to contain three glucosides, two of which were present only in traces. The main constituent possessed an  $R_B$ -value\*\*\* of 1.05–1.08 in butanol:acetic acid:water (4:1:3) and 1.06 in

\* Recently, the (*R*)-configuration has been established for the naturally derived, laevorotatory sulphoraphene.<sup>8</sup>

\*\* Recent attempts in our laboratory to verify the presence of allyl isothiocyanate in distillates of fresh radish have been unsuccessful.

\*\*\* The  $R_B$ -value signifies the  $R_F$ -value relative to that of glucotropaeolin, the thioglucoside producing benzyl isothiocyanate on enzymic hydrolysis.

butanol:pyridine:water (6:4:3), but apparently no structural identification was attempted.\*

It in this laboratory it was found that a methanolic extract of fresh radish roots, prepared without special provisions to minimize enzymic hydrolysis, contained only trace amounts of thioglucoside, as estimated from paper chromatography. The suspicion that a very rapid enzymic decomposition of thioglucosidic progenitors occurred was supported by the detection in these extracts of considerable amounts of free glucose. To overcome the undesired enzymic hydrolysis, radish roots were deep-frozen, disintegrated in the frozen state, and extracted with cold alcohol, resulting in an extract which on paper chromatography proved to contain one thioglucoside, which, after purification by preparative paper chromatography, migrated in butanol:ethanol:water (4:1:4) at a rate corresponding to an  $R_B$ -value of 1.08. Several attempts were made, yet without much success, to produce larger quantities of the pure thioglucoside by this procedure. Obviously, the extremely fast enzymic hydrolysis could not be satisfactorily suppressed when working on a larger scale. Hence, it was found desirable to concentrate the efforts on the isolation and characterization of the isothiocyanate produced during the enzymic decomposition.

After many experiments, conducted under varying conditions of enzymic hydrolysis and extraction, it was finally found that slicing of radish roots directly into hot carbon tetrachloride provided the best yields of the isothiocyanate. Under these circumstances the enzymic hydrolysis apparently proceeds within the few seconds passing after the tissue is injured until the enzymic reaction is arrested by the hot solvent. After careful concentration of the solution, and gaschromatographic purification of the oily residue, an analytically pure specimen of the radish isothiocyanate was obtained,

\* According to Ref. 12 a crystalline acetate of the parent thioglucoside was obtained, but no further details have since appeared.

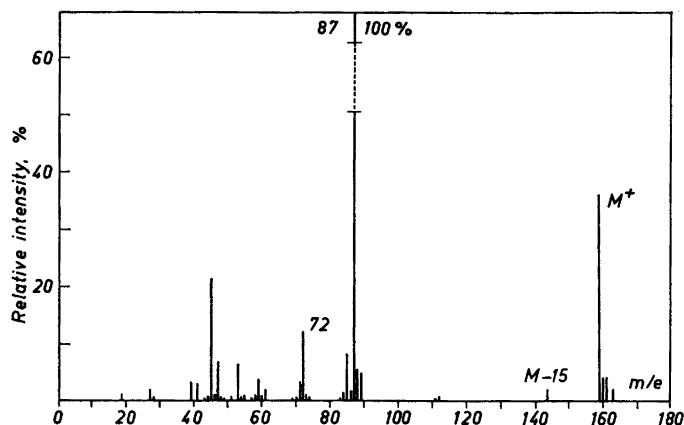


Fig. 1. Mass-spectrum of the radish isothiocyanate representing a 4:1 mixture of *trans*- and *cis*-4-methylthio-3-butenyl isothiocyanates.

possessing the characteristic odour and taste of fresh radish. Its elementary composition,  $C_6H_9NS_2$ , was determined by analysis and mass spectrometry ( $M^+ 159$ ).

The mass spectral fragmentation of the isothiocyanate (Fig. 1) is in concordance with structure (I), or the corresponding *cis*-isomeride. Thus, the base peak ( $m/e$  87) represents the ion  $[CH_3SCH=CHCH_2]^+$  formed by allylic fission; the  $m/e$  72 ion is characteristic for alkyl isothiocyanates unsubstituted in the  $\alpha$ -position,<sup>13</sup> and the absence of a strong ion peak at  $m/e$  61, characteristic for the ion  $[CH_3SCH_2]^+$  derived from *saturated* methylthioalkyl isothiocyanates,<sup>13</sup> supports the presence of a double bond adjacent to the sulphidic grouping. The thioenol ether character is further supported by the facile production of methanethiol upon acid treatment of the isothiocyanate,\* as well as by the UV-spectrum in ethanol, exhibiting a maximum at  $231 m\mu$  ( $\epsilon$  7500) and shoulders at  $246 m\mu$  ( $\epsilon$  6300) and  $206 m\mu$  ( $\epsilon$  3300), characteristic for vinyl sulphides.<sup>15</sup> On treatment with ammonia the radish mustard oil was converted into a thiourea which on paper chromatography in water-saturated chloroform<sup>16</sup> migrated at a rate corresponding to an  $R_{Fk}$ -value<sup>16</sup> of 0.93.

Gaschromatographic analysis of the isolated isothiocyanate indicated that it consisted of a mixture of (I) and the corresponding *cis*-isomeride, with the

\* The isothiocyanate (I) or its glucosidic progenitor, seem likely precursors of the methanethiol previously identified as a product formed in radish root on disintegration.<sup>14</sup>

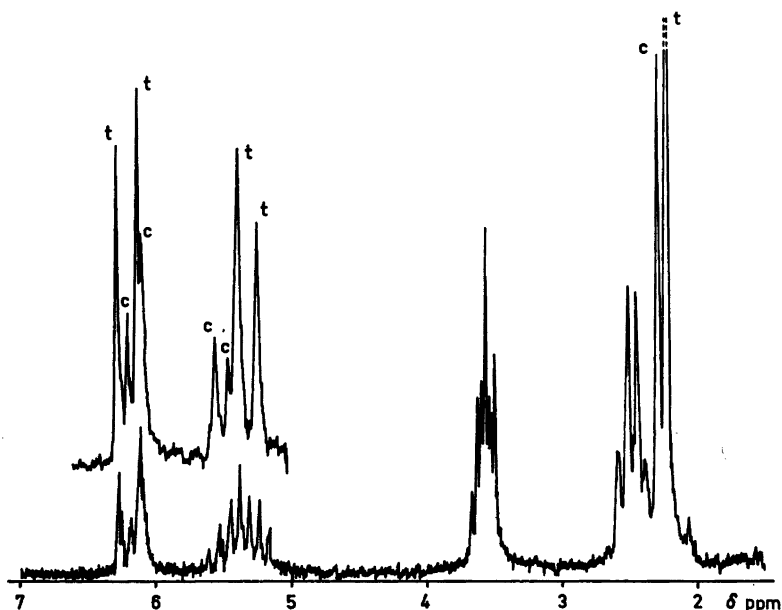
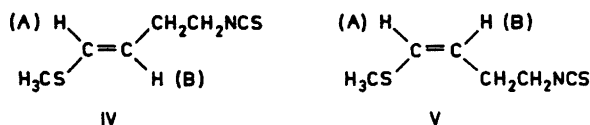


Fig. 2. 100 Mc-NMR spectrum of a 4:1 mixture of *trans*- (IV) and *cis*- (V) 4-methylthio-3-butenyl isothiocyanate. Lower curve: before decoupling. Upper curve: the vinylic proton region after decoupling from the allylic methylene protons.

former as the major component. Detailed studies of the 100 Mc NMR-spectrum of the isothiocyanate fraction fully supported this conclusion (Fig. 2).

The two singlets at  $\delta$  2.23 ppm and 2.29 ppm can be assigned to the  $\text{CH}_2\text{S}$ -protons in the *trans*-(IV) and *cis*-isomeride (V), respectively, in good agreement with the relative chemical shifts previously observed in similar systems.<sup>17</sup>



The complex multiplets at  $\delta$  2.5 ppm and  $\delta$  3.6 ppm arise from the allylic methylene group and its neighbouring  $\text{CH}_2$ -group, respectively. The observed chemical shift of the latter is in agreement with recently reported values for alkyl isothiocyanates.<sup>18</sup> Successive spin-spin decoupling, by double irradiation, of the allylic protons from the vinylic protons (B), (IV, V), and the from adjacent  $\text{CH}_2\text{-N}$ -protons, reduced the pattern at  $\delta$  2.5 ppm to an expected triplet ( $J$  6.5 c/s) and doublet ( $J$  7 c/s), respectively. No change in pattern was observed on irradiation with the frequency of the vinylic (A)-protons (IV, V).

In the vinylic proton region a complex pattern was observed (Fig. 2) which could again be interpreted by decoupling experiments. The most deshielded, one-proton multiplet (at about  $\delta$  6.2 ppm) can be assigned to the (A)-protons in (IV) and (V). On decoupling from the allylic protons two doublets are easily distinguished (Fig. 2), one at  $\delta$  6.14 ppm ( $J$  10 c/s) and another at  $\delta$  6.19 ppm ( $J$  15 c/s), attributable to the *cis*-(V) and *trans*-isomer (IV), respectively. Similarly, the multiplet, centered at  $\delta$  5.4 ppm and arising from the protons (B) in (IV) and (V), on decoupling from the adjacent allylic protons, was reduced to two well-separated doublets at  $\delta$  5.31 ppm ( $J$  15 c/s) and  $\delta$  5.50 ppm ( $J$  10 c/s), assignable to the *trans*-(IV) and *cis*-isomer (V), respectively.\* Estimated from peak areas, the *trans/cis* ratio in the investigated sample appear to be about 4:1.

In order to study the possibility of stereomutation of the naturally derived isothiocyanate during isolation and purification, several experiments were conducted under varying conditions. Vapour phase chromatography under different conditions invariably disclosed the presence of a minor and a major peak, the former possessing the shortest retention time. The IR-spectra of separately collected fractions supported the identification of these as *cis*-(V) and *trans*-4-methylthio-3-butenyl isothiocyanate (IV), respectively, the former possessing a characteristic *cis*-alkene deformation vibration at  $755\text{ cm}^{-1}$ , and the latter a strong *trans*-band at  $945\text{ cm}^{-1}$ .

Separate reinjection of either fraction on v.p.c. columns resulted in the production of virtually the same double peak pattern, suggesting equilibration by stereomutation on the column. Similar rearrangements of propenyl sulphides have previously been discussed by Price and Snyder.<sup>15</sup> These authors con-

\* This assignment is in agreement with that previously made for methyl vinyl sulphide by Hobgood *et al.*,<sup>19</sup> and for this and other  $\alpha,\beta$ -unsaturated methyl sulphides by Bohlmann *et al.*<sup>20</sup>

cluded, partly on the basis of v.p.c. analyses, that the *cis-trans* mixtures when subjected to isomerization either were not isomerized by the catalysts used (mercuric acetate, hydrochloric acid) or were already at equilibrium. In view of the present findings it cannot be excluded, however, that isomerization also in these cases occurred during the analyses. The unexpectedly facile stereomutation may be caused by the ability of the sulphide S-atom to expand its valence shell, thereby diminishing the bond order of the adjacent  $-\text{C}=\text{C}-$  grouping and consequently facilitating stereomutation.

The question therefore naturally arises as to the *cis-trans*-character of the double bond in the genuine thioglucosidic precursor and, consequently, the isothiocyanate produced during the enzymic hydrolysis. In order to study this problem further, attempts were made to purify the isothiocyanate by v.p.c. at lower column temperatures, resulting in poorer separation, but, significantly, also in an increased *trans:cis*-ratio. It therefore seems likely that the genuine glucoside possesses *trans*-configuration, affording on enzymic hydrolysis the *trans*-isothiocyanate (I) which then, during gaschromatographic purification or even on treatment with hot carbon tetrachloride, partly rearranges to the isomeric *cis*-compound.

The radish isothiocyanate constitutes another addition to a long series of methylthio-substituted mustard oils previously encountered as products of enzymic hydrolysis of glucoside progenitors in various Cruciferae.<sup>21-23</sup> The sulphidic S-atom frequently occurs in higher oxidation stages such as sulphoxide or sulphone. In the present case it is interesting that the two isothiocyanates derived from seed and root of radish, respectively, constitute a similar sulphoxide:sulphide pair, suggesting a common biogenetic pathway for the corresponding parent thioglucosides. No evidence is on hand to show that the glucosidic progenitor (glucoraphenin) of the sulphoxide, sulphoraphene (III), is biogenetically derived from the corresponding sulphidic glucoside in radish root although this appears likely.

The new isothiocyanate, described in the present paper, most likely represents the compound marked as 'peak No. 38' in a recent, commercial pamphlet on electron capture detection of volatile components in radish.<sup>24</sup> The parent glucoside almost certainly is the major radish glucoside referred to in the paperchromatographic survey of Schultz and Wagner.<sup>12</sup>

The natural occurrence of a methyl thioenol ether is not without precedents. Thus, *cis*- and *trans*- $\beta$ -methylthioacrylic acid have been reported as esterifying acids in the S-petasitolides A and B from rhizomes of *Petasites officinalis* Moench,<sup>25</sup> and Bohlmann *et al.*<sup>17,26,27</sup> have presented evidence for the presence in Compositae of several acetylenic compounds containing a methyl thioenol ether grouping, probably formed by *in vivo* addition of methanethiol, or its biochemical equivalent, across an acetylenic bond.<sup>29</sup>

## EXPERIMENTAL

*Radish root thioglucoside.* A few fresh radish roots were frozen in a mixture of dry ice and ethanol and, in the frozen state, crushed with a hammer. Further disintegration was achieved in a Waring Blendor. After filtration, the solid was shaken for 15 min with ice-cold 80 % methanol and then removed by filtration. The two filtrates were combined and concentrated to dryness *in vacuo* at room temperature. The residue was dissolved in water and the solution applied as bands to four sheets of Whatman 3 MM paper. After chromatography with the upper layer of a butanol:ethanol:water (4:1:4) mixture as the mobile phase, the glucoside band was located by spraying of edge cuts with ammoniacal  $\text{AgNO}_3$ . The glucoside was eluted with water from the appropriate paper strips and rechromatographed in the same solvent system. One distinct glucoside spot appeared with an  $R_B$ -value of 1.08.

Several attempts to isolate larger quantities of the glucoside were unsuccessful and invariably accompanied by extensive enzymic hydrolysis, revealed through the formation of glucose which was identified by paper chromatography.\*

*Radish root isothiocyanate.* Fresh radish roots (900 g) were rasped into boiling carbon tetrachloride (1 l). The liquid was removed by pressing, dried over anhydrous sodium sulphate, and concentrated to a brown, oily residue (1.5 ml) by distillation of the solvent through a glass column.

A few  $\mu\text{l}$  were applied as a spot on the starting line of a paper chromatogram which was then exposed to gaseous ammonia. The resulting thiourea was chromatographed in water-saturated chloroform<sup>16</sup> yielding one thiourea-spot with an  $R_{Ph}$ -value<sup>16</sup> of 0.93.

The remaining part of the oily material was purified by chromatography on an Aerograph 'Autoprep A-700' instrument, equipped with a non-polar column (5 % silicone elastomer). The starting temperature was 115°, the setting 90, and a stream of 200–250 ml He/min was used. At about 190° a crystalline component emerged, identified as hexachloroethane, probably originating from the solvent. At 220° a total of 72 mg of a brownish oil was collected consisting of essentially pure isothiocyanate. It exhibited conspicuous IR-bands (neat liquid) at: 755 (m), 810 (m), 820 (m), 860 (w), 942 (vs), 970 (m), 1010 (m), 1070 (m), 1190 (w), 1260 (m), 1305 (m), 1340 (vs), 1430 (vs), 1605 (vs), 2090 (vvs), 2170 (vvs), and 2900 (vs)  $\text{cm}^{-1}$ .

Distillation *in vacuo* (cold finger) afforded an analytically pure specimen as a colourless oil. (Found: C 45.16; H 5.66; N 8.71. Calc. for  $\text{C}_6\text{H}_5\text{NS}_2$ : C 45.24; H 5.70; N 8.79). The sample was also employed for determination of UV- and mass-spectra (Fig. 1).

*cis-trans-Isomerism.* In another experiment, 70 mg of the isothiocyanate was obtained from 2 kg of fresh radish by v.p.c. on the 'Autoprep-700'-instrument (same column as above) operated isothermally at 180–182°. On injection of this specimen on an analytical polar column ('F. & M. 500', 200  $\times$  0.5 cm, kieselguhr with trieresyl phosphate, isothermal operation at 140°, injection port 170°, 40 ml He/min), two peaks, A and B, were observed, A having the shortest retention time (14 min). The two fractions were separately collected and their IR-spectra determined. These were virtually coinciding save for a strong band at 753  $\text{cm}^{-1}$  in A and 943  $\text{cm}^{-1}$  in B, indicating their character as *cis*- and *trans*-isomers, respectively.

By rechromatography of the pure *trans*-fraction,  $n_D^{25}$  1.5815, on the same column, the *cis-trans*-mixture with a component ratio of about 1:4 was reestablished. Again, injection of a virtually pure *cis*-fraction resulted in the same 1:4 *cis-trans* pattern.

*Spectra.* Infra-red spectra were determined on neat liquids between KBr discs on a Perkin-Elmer 'Infracord'-instrument. NMR-spectra were determined without solvent on a Varian HR-100 instrument (TMS as internal standard).

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\* The glucosidic progenitor thus seems to be of the usual type.<sup>21</sup> Adopting the semisystematic nomenclature, proposed by Ettliger and Dateo,<sup>28</sup> the parent glucoside anion in radish root therefore can be designated *trans-4-methylthio-3-butenylglucosinolate*.

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