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# *iso*Thiocyanates XVI. Glucoconringiin, the Natural Precursor of 5,5-Dimethyl-2-oxazolidinethione

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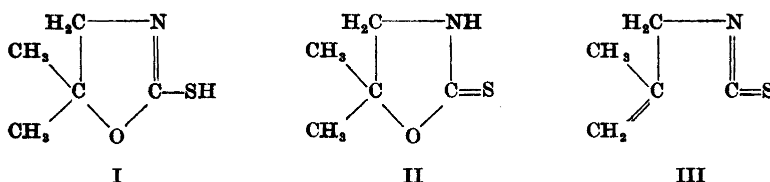
Seed of the crucifer *Conringia orientalis* (L.) Andrz. has been shown to contain a new glucoside, *glucoconringiin*, characterised as a crystalline tetraacetate (IV) and affording glucose, sulphuric acid and 2-hydroxy-2-methyl-propyl *iso*thiocyanate (V) on enzymic hydrolysis. The mustard oil cyclises spontaneously to 5,5-dimethyl-2-oxazolidinethione (II), a fact accounting for the previously reported isolation of (II) from the same seed.

A modified synthesis of the heterocyclic compound is described. A paperchromatographic method has disclosed the presence of glucoconringiin also in three species of the genus *Cochlearia*, further substantiated by the isolation from *Cochlearia officinalis* L. of crystalline 5,5-dimethyl-2-oxazolidinethione after enzymic hydrolysis. Evidence is available of the existence of additional 2-oxazolidinethione-producing glucosides in plants.

In 1938 Hopkins [1] reported the isolation of 5,5-dimethyl-oxazoline-2-thiol (I) from seed of the common Canadian weed *Conringia orientalis* L. (Dumort) *.




The structure of (I) followed from its identity with a synthetic sample, prepared by Bruson and Eastes [2]. On the basis of infra-red studies, Ettlinger [3] later provided evidence in favour of the thione structure (II). It appeared from Hopkins' studies that the heterocyclic compound was formed by enzymic cleavage of a parent glycoside, the aglycone of which was considered to be

* The species should be more correctly designated as *Conringia orientalis* (L.) Andrz. (= *Erysimum orientale* Mill.).

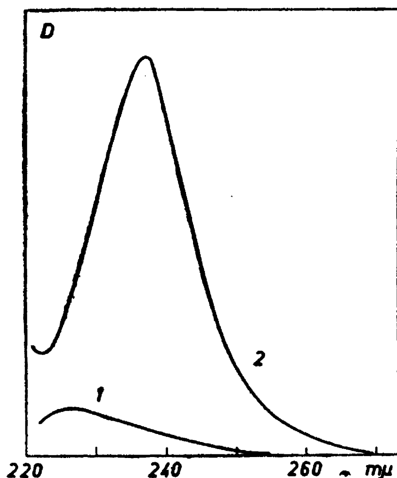



*Fig. 1. Ultra-violet absorption spectra of: 1. an aqueous solution of glucoconringiin; 2. an aqueous solution of the ether-soluble product, (II), formed by enzymic cleavage of glucoconringiin.*

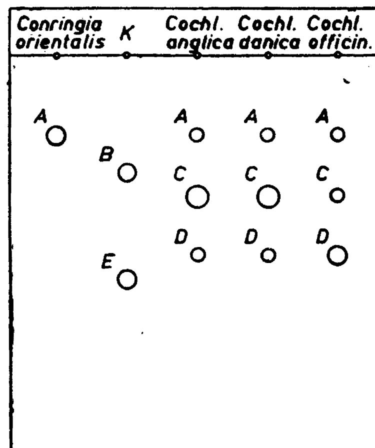


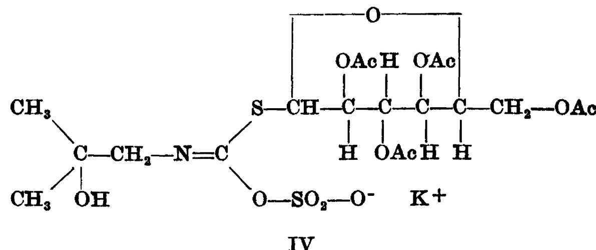

*Fig. 2. Descending paper chromatogram, run in* n*-butanol: acetic acid : water. Sprayed with ammoniacal silver nitrate solution. K: control solution; A: glucoconringiin; B: sinigrin; C: glucoputranjivin; D: glucocochlearin; E: glucotropaeolin.*

2-methylallyl *iso*thiocyanate (III). Addition of water to the *iso*thiocyanate-grouping of the latter, followed by spontaneous cyclisation of the intermediate thiocarbamic acid to (I) was regarded as a likely mode of formation. When synthetic 2-methylallyl *iso*thiocyanate became available in this laboratory [4], it was established that no such tendency to water addition and ring-closure existed.

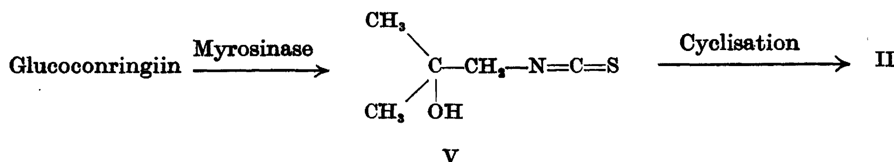
As pointed out in a previous paper of this series [5], seed of *Conringia orientalis* yielded no volatile *iso*thiocyanates on enzymic hydrolysis. Paper chromatography [6] of a seed extract has now, however, disclosed the presence of a single glucoside, giving a spot in the lower $R_F$-region (Fig. 2). The glucoside solution exhibited an absorption spectrum of the usual type (Fig. 1), as known for, *e. g.*, sinigrin [7], a fact precluding the presence of chromophors, such as the heterocyclic ring system (II), in the *Conringia*-glucoside, for which the name *glucoconringiin* is proposed.

Upon enzymic hydrolysis of glucoconringiin an ether-soluble substance arose, with spectroscopical characteristics (Fig. 1) similar to those reported for (II) by Hopkins [1] and Astwood *et al.*[8] The concomitant formation of glucose and sulphate was established. During the summer of 1955, *Conringia orientalis* (L.) Andrz. was cultivated on a larger scale in the Botanical Garden of the University of Copenhagen. The seed material thus obtained was employed for the isolation of further quantities of glucoconringiin, which did not show signs of crystallisation, but could be transformed into a crystalline acetyl-derivative with the composition $C_{19}H_{28}O_{14}NS_2K$. Analytical data indicated the presence of four O-acetyl groupings; on the assumption that these are

located in the glucose moiety, as rendered highly probable by model preparation of the analogous tetraacetyl-sinigrin, and provided sulphuric acid is bound as generally believed in this type of glucosides, the structure (IV) appears to account for the properties of acetylglucoconringiin.




As expected, the hydroxy-grouping of the side-chain escaped acetylation due to its tertiary character. The derivative (IV) was not attacked by myrosinase. Its infra-red spectrum was determined in the solid state and found very similar to that of acetylsinigrin. Probably due to association, no band attributable to the tertiary hydroxy-function was observed in the spectrum of (IV). That acetylation was not accompanied by secondary changes appeared from the fact that (IV) could be smoothly deacetylated to glucoconringiin, the identity of which followed from its rapid enzymic hydrolysis, resulting in the formation of (II). There can be little doubt, therefore, that (II) is to be considered as a secondary product, arising from the initially formed 2-hydroxy-2-methyl-propyl *iso*thiocyanate (V) by spontaneous cyclisation.

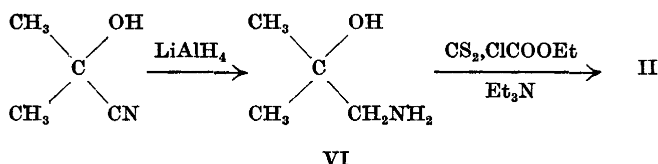



The cyclisation proceeds at a measurable rate. It was spectroscopically established that a partly purified glucoconringiin preparation, subjected to enzymic hydrolysis for four hours at room temperature, afforded the cyclic product in a yield of only about 40 %; about 24 hours were required for the cyclisation to go to completion. From analogous cases it appeared very unlikely that the enzymic hydrolysis is the rate-determining step in the present reaction.

For further studies a synthetic sample of (II) became desirable. As in previously described syntheses [2,9,10] of this compound, 1-amino-2-methyl-2-propanol (VI) was selected as a starting material. Several methods have been described [11,12] for the preparation of this aminoalcohol, which has recently been isolated from the phospholipid fraction of *Neurospora crassa* [13] and established as the amine moiety of sanshoamide, an amide isolated from *Zanthoxylum piperitum* [14]. Whereas Meisenheimer and Chou [15] were unsuc-

cessful in attempts to reduce acetone cyanohydrin catalytically, a mixture of amines, with unstated yield of the desired primary derivative, was formed by high-pressure reduction with Raney-nickel as a catalyst, according to a patent claim [16]. We found that acetone cyanohydrin could be reduced to (VI) with lithium aluminium hydride. Analogous reductions of the cyanohydrins of benzaldehyde [17] and *cyclo*hexanone [18] have been reported previously.

Several methods of converting 1,2-aminoalcohols into 2-oxazolidinethiones have been proposed, all proceeding *via* dithiocarbamates by means of reagents, traditionally employed for converting the latter into *iso*thiocyanates [10,19,20]. The Kaluza method of *iso*thiocyanate synthesis, modified according to Hodgkins and Ettlinger [21], belongs to this type of reaction and has proved to be useful for the preparation of 2-oxazolidinethiones also *. We subjected (VI) to this reaction in a non-aqueous system with triethylamine as a base and obtained (II) in 78 % yield. Several attempts to isolate the intermediate *iso*thiocyanate (V) proved unsuccessful, probably due to the cyclisation being facilitated under basic conditions. The identity of the final product was established by comparison with an authentic specimen of (II), kindly furnished by Dr. Ettlinger.




In the course of our systematic studies on the distribution of *iso*thiocyanates in plants, it was found that oxazolidinethiones could be separated by paper chromatography in the solvent system heptane: *n*-butanol: 90 % formic acid, introduced by Sjöquist [22] for the separation of phenylthiohydantoins. By this technique, oxazolidinethiones were recognised in other plants also. For example, glucoside chromatography disclosed the presence in *Cochlearia anglica* (L.) Asch. & Grb., *C. danica* L. and *C. officinalis* L. of three glucosides (Fig. 2), two of which were recognised as the previously known species glucoputranjivin and glucocochlearin, affording *iso*propyl [23] and *sec*-butyl [5] *iso*thiocyanate, respectively, on enzymic hydrolysis. The third glucoside spot could be attributed to glucoconringiin because of its enzymic cleavage to a compound, indistinguishable from (II) by paper chromatography in the heptane system. Final proof of its identity was provided when a crystalline specimen was obtained from a seed sample of *C. officinalis* L. The natural product was found to be identical with an authentic specimen of (II).

It should be noticed that the structurally analogous *l*-5-vinyl-2-oxazolidinethione has been recognised as a constituent of several species of the important genus *Brassica* [8]. We have spectroscopical evidence that this compound is formed in a similar way from an analogous genuine glucoside of more wide-

* Applied by Dr. Ettlinger in a synthesis of 4-vinyl-2-oxazolidinethione (private communication).

spread occurrence than previously reported and derivable from one of the stereoisomeric 2-hydroxy-3-butenyl *iso*thiocyanates.* Additional natural representatives of the class of oxazolidinethione-progenitors have been discovered and are at present being further studied. An account of the results will form the subject of forthcoming communications.

## EXPERIMENTAL

Melting points are uncorrected and determined in capillary tubes in a glycerol bath, if not otherwise stated. Infra-red spectra were determined in potassium bromide discs on a Beckman IR-2 instrument.

*Detection and nature of glucoconringiin.* Seed of *Conringia orientalis* (L.) Andrz. (1 g) was extracted with hot methanol and the solvent was removed by evaporation. The residue was taken up in water, the solution filtered and a drop of the filtrate used for descending paper chromatography in *n*-butanol:acetic acid:water (4:1:3), essentially as described by Schultz and Gmelin[6]; a single glucoside-spot of glucoconringiin appeared (Fig. 2).

The glucoside solution was purified by exchange on a small column of acidic aluminium oxide, eluted with a 1 % solution of $K_2SO_4$ and a sample withdrawn for ultra-violet absorption measurement (Fig. 1). A few drops of a myrosinase preparation were added to the eluate which was set aside for 24 hours. The now turbid solution was extracted three times with ether; the solvent was removed, the residue dissolved in water (200 ml) and the ultra-violet absorption spectrum determined (Fig. 1). A high-extinction band was observed at 239 mμ, displaced in alkali to 232 mμ, and a minimum at about 222 mμ, both values agreeing well with those previously published [8].

Samples of the aqueous phase were chromatographed for sugars in *n*-butanol:acetic acid:water and in pyridine:amyl alcohol:water. In both solvent systems, the presence of glucose was established upon comparison with control spots. A separate sample of glucoconringiin, which had not been purified by ion exchange, served to establish the presence of sulphate after the enzymic fission.

*Preparation and properties of acetylglucoconringiin* **. *Conringia* seed (100 g) was extracted with two portions (each 500 ml) of hot 70 % methanol and the solution taken to dryness *in vacuo.* The residue was dissolved in water (300 ml) and impurities precipitated by adding excess lead acetate solution. After filtration and removal of excess lead salt as PbS, the solution was again taken to dryness *in vacuo*, leaving glucoconringiin as an almost colourless, glassy mass.

After thorough drying the product was dissolved in a mixture of dry pyridine (25 ml) and acetic anhydride (25 ml) and set aside for 24 hours. Volatile reagents were then removed *in vacuo* and the brown residue dissolved in a large volume of hot ethanol. The solution was treated with charcoal, filtered and chilled. A slight amount of amorphous material was removed and the filtrate concentrated, whereupon the crystalline acetyl-derivative (663 mg) separated. Two recrystallisations from 96 % ethanol, containing a little methanol, afforded an analytical specimen (397 mg) as tiny, colourless needles, m. p. 152° (decomp.) on rapid heating. The acetyl-derivative is easily soluble in water, moderately soluble in methanol and slightly soluble in ethanol. $[\alpha]_D^{24}$ −5.3° ($H_2O$, $c$ = 5.3). (Found: C 37.95; H 4.85; N 2.25; S 10.80; $CH_3CO$ 30.9. Calc. for $C_{19}H_{28}O_{14}NS_2K$: C 38.18; H 4.73; N 2.34; S 10.73; 4 $CH_3CO$ 28.8). The infra-red spectrum had characteristic bands

* *Note added in proof:* After this paper was submitted for publication, Greer [26] announced the isolation of this glucoside, "progoitrin", as a crystalline sodium salt. The composition and provenance of the glucoside "glukorapiferin", recently characterized by Schultz and Wagner [27] as a crystalline pentaacetate, strongly indicate the identity of "progoitrin" and "glukorapiferin".

** According to a personal communication to one of us (R.G.) from Prof. O.-E. Schultz, Tübingen, acetylation has been used in his laboratory for the purpose of characterising *iso*thiocyanate glucosides, *cf.* Ref.[27]

at: 5.67 (very strong, C = O stretching vibration), 5.99 (weak), 7.24 (strong), 7.97, 8.10 (split peak, v. s.), 8.46 (w.), 8.80 (w.), 9.06 (w.), 9.40 (v. s.), 10.10 (w.), 10.92 (medium), 11.12 (m.), 11.45 (w.), 11.95 (w.) and 12.60 $\mu$ (s.).

A solution of acetylglucoconringiin in ethanolic ammonia was kept at room temperature for five days. The solvent was then removed and a sample of the residue subjected to paper chromatography in *n*-butanol:acetic acid:water. A single spot was observed, indistinguishable from that of glucoconringiin. The remaining part of the reaction product was hydrolysed enzymically, and the ether-soluble reaction product was identified as 5,5-dimethyl-2-oxazolidinethione by its absorption spectrum and by paperchromatographic comparison with an authentic sample.

*Acetylsinigrin.* In a model experiment, sinigrin was subjected to acetylation by a similar procedure. Previously, Gadamer [24] reported failure in obtaining a crystalline derivative by treatment of sinigrin with acetic anhydride.

Sinigrin (200 mg) was dissolved in hot pyridine (4 ml), and acetic anhydride (2 ml) added after cooling. After 4 hours, the separation of acetylsinigrin started and was completed by careful addition of ether. The crude product was recrystallised twice from aqueous ethanol and once from methanol to give pure tetraacetylsinigrin as thin, colourless needles (77 mg), m. p. 192—93° (dec.), determined in an electrically heated block. The solubility properties were the same as those of acetylglucoconringiin. $[\alpha]_D^{24}$ $-18.6°$ ($H_2O$, $c$ = 3.6). (Found: C 38.15; H 4.36; N 2.30. Calc. for $C_{18}H_{24}O_{13}NS_2K$: C 38.24; H 4.28; N 2.48). The infrared spectrum had conspicuous bands at 5.67 (v. s., C = O stretching vibration), 6.00 (w.), 7.24 (s.), 7.76 (s.), 7.97 (v. s. —C—O-vibration), 8.62 (w.), 9.39 (v. s.), 10.43 (w.), 10.64 (w.), 10.84 (w.), 11.20 (medium) and 12.60 $\mu$ (s.).

*1-Amino-2-methyl-2-propanol* (VI). To a well-stirred solution of $LiAlH_4$ (9.1 g) in dry ether (200 ml) was slowly added a solution of acetone cyanohydrin (8.9 g) in dry ether (100 ml). The reaction was performed in a nitrogen atmosphere, and completed upon heating to reflux temperature for 30 minutes. Then water (10 ml) and 15 % NaOH (25 ml) were cautiously added, the precipitate filtered off and thoroughly washed with ether, and the ether solution dried over KOH. The ether was removed and the colourless, viscous amine (4.6 g, 50 %) distilled, b. p. 150°, in agreement with several literature values. No attempts were made to raise the yield by systematic variations of the conditions. It was found, however, that reduction in the presence of $AlCl_3$, a recently introduced modification in the reduction of ordinary nitriles [25], gave a much lower yield (25 %), due to cleavage of the cyanhydrin. In this case, a rather large proportion of pinacol was also isolated as a secondary product.

*5,5-Dimethyl-2-oxazolidinethione* (II). The above amine was converted into (II) by a recently published method of *iso*thiocyanate synthesis [21]. To a stirred solution of (VI) (4.6 g) in dioxan (7.5 ml) and triethylamine (7.5 ml), kept at $-10°$, carbon disulphide (3.1 ml) was slowly added and the solution allowed to come to room temperature. After cooling again, ethyl chlorocarbonate (5.6 ml) was added very slowly, and the mixture again brought to room temperature. Triethylammonium chloride was removed by filtration and washed with a little dioxan. A solution of triethylamine (7.5 ml) in chloroform (15 ml) was then added and the solution heated shortly to 50°. After removal of all volatile constituents at 50° and 12 mm, crystalline (II) remained (5.31 g, 78 %). A pure sample was obtained as colourless platelets by recrystallisation, first from ether and then from benzene, m. p. 107°, alone or in admixture with an authentic specimen, kindly furnished by Dr. Ettlinger.

*Isolation of (II) from seed of* Cochlearia officinalis *L.* When a seed sample of *C. officin.* L. was assayed by the method described for the determination of 5-vinyl-2-oxazolidinethione in plant tissues [8], a content of about 45 mg per 100 g of seed was found.

500 g of seed were employed for isolation of the heterocyclic compound. With minor modifications, the procedure was the same as that used for the isolation of the vinylderivative [8]. The mainly crystalline, crude product (100 mg) was purified by recrystallisation, first from ether and then from benzene. Thus, colourless flat prisms were obtained (22 mg), m. p. 105°. A mixture of this compound and the synthetic specimen of (II) melted at 106°. (Found: C 46.05; H 7.25; N 10.18. Calc. for $C_5H_9ONS$: C 45.78; H 6.91; N 10.68). As further proof of identity, the infra-red spectra of the two products were determined and found to coincide. The spectral pattern was essentially the same as that previously reported for (II) [3].

Microanalyses were performed in this laboratory by Mr. P. Hansen. We wish to thank the *Botanical Garden of the University of Copenhagen* for the cultivation of *Conringia orientalis* (L.) Andrz. on a larger scale, and for various seed samples.

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