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**Preparation and Properties of Glucoconringin, the Precursor of the Thyrostatic 5,5-Dimethyl-2-Oxazolidinethione**

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The mustard oil glucoside glucoconringin is present according to Kjær *et al.* in several *Cochlearia* spec. which are rather common in the Finnish flora. In the course of investigations with goitrogens in plants it seemed therefore of interest to isolate this glucosidic precursor of the thyrostatic and probably also goitrogenic 5,5-dimethyl-2-oxazolidinethione.

Some years ago Kjær *et al.* and Schultz and Wagner prepared and characterized glucoconringin as its tetraacetyl derivative from seeds of *Conringia orientalis* (L.) Andr. and showed that the deacetylated glucoside which they obtained in amorphous form is split by myrosinase into sulphate, glucose and 5,5-dimethyl-2-oxazolidinethione. This heterocyclic compound, isolated and identified by Hopkins twenty years ago from *C. orientalis* seeds, is formed by spontaneous cyclization from an intermediate 2-hydroxy-2-methylpropyl iodothiocyanate. Its thyrostatic effect was established by Astwood *et al.* and was found comparable to that of (-)-5-vinyl-2-oxazolidinethione (goitrin).

The purpose of this paper is to report briefly the method for preparation and some properties of crystalline glucoconringin, which unlike its tetraacetyl derivative has the advantage of being split readily by myrosinase and is therefore more suitable for physiological investigations.

Glucoconringin was prepared by ion exchange on Dowex 2-X 4 of an extract from seeds of *Conringia orientalis* (L.) Andr. Upon elution with K₂SO₄ solution it was obtained as a colourless syrup which crystallized upon long standing in the refrigerator. Two recrystallizations from 90 % ethanol yielded pure glucoconringin as anhydrous potassium salt in white, short needles. Elementary analysis agreed with the composition C₁₁H₁₆NO₉S₂K. F 185°C (decomp., uncorr.), [α]ᵢ²₀ᵦ = -10.87° in H₂O.

In agreement with earlier results, the formation of glucose, sulphate and 5,5-dimethyl-2-oxazolidinethione could be observed during enzymatic cleavage. The enzymatic process could be followed by UV spectroscopy: with increasing cleavage the maximum absorbance shifts from 230.5 μ to 240 μ (ε = 15 400), the maximum of 5,5-dimethyl-2-oxazolidinethione.

Spectrophotometric assay of the enzymatic cleavage of glucoconringin: 0.0155 g of glucoconringin was dissolved in 2 ml of a mixture of equal parts of myrosinase solution and phosphate buffer pH 6.8. In certain intervals 0.1 ml portions were taken off, diluted to 50 ml with water and the UV absorption measured between 220 μ and 260 μ against an equal blank dilution of myrosinase solution and phosphate buffer solution.

<table>
<thead>
<tr>
<th>t (min)</th>
<th>UV maximum (μ)</th>
<th>Optical density (ε)</th>
<th>Mol. ext.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>230.5</td>
<td>0.245</td>
<td>6 700</td>
</tr>
<tr>
<td>20</td>
<td>233</td>
<td>0.258</td>
<td>7 140</td>
</tr>
<tr>
<td>90</td>
<td>235</td>
<td>0.310</td>
<td>8 560</td>
</tr>
<tr>
<td>180</td>
<td>237</td>
<td>0.430</td>
<td>11 900</td>
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<tr>
<td>270</td>
<td>238</td>
<td>0.516</td>
<td>14 250</td>
</tr>
<tr>
<td>360</td>
<td>239</td>
<td>0.562</td>
<td>15 600</td>
</tr>
</tbody>
</table>

* based on the initial substrate concentration.

By treatment of glucoconringin with hydrochloric acid hydroxylamine was formed which was detected by the method of Blom. These results indicate that glucoconringin is of the same structural type as the other known mustard oil glucosides, and has the following structure (p. 1719).

85 g of finely ground seeds of *Conringia orientalis* (L.) Andr. were defatted by petro-

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leum ether and extracted with two 11 portions of 70 % methanol. The combined extracts were concentrated in vacuo to 300 ml and treated with a solution of lead acetate in order to precipitate undesired plant material. After separation of Pb-ions by H₄S the straw yellow filtrate was shortly boiled in vacuo until all H₂S had disappeared. The solution was filtered through a short column with 5 g of Al₂O₃. Merek and was then passed slowly through a column containing 10 ml Dowex 2-X 4 (chloride-form, 200 mesh). The effluent was free of glucoside and was discarded. After washing the resin with 200 ml of water the glucoside was eluted by 0.1 M K₂SO₄ solution. Fractions of 20 ml each were collected. The fractions 3—18 containing glucocorinogin as determined by the anthrone method and myrosinase test were brought to dryness in vacuo. The white residue was triturated with 3 portions of 30 ml CH₃OH at 40—50°C. The filtered methanolic solution left after evaporation in vacuo a colourless syrup which was dissolved in a small volume of hot 90 % ethanol. After cooling the glucoside separated partly as a viscous oil which crystallized after standing several weeks in the refrigerator. Two recrystallizations from 90 % ethanol yielded 585 mg glucocorinogin in short, white needles. F 168°C (decomp., uncorr.) [α]D -10.87° (c = 3.68; in water). UV-maximum in H₂O 230.5 μ (λ = 6 720), minimum 208 μ (λ = 3 750). (Found: C 30.81; H 4.55; N 3.52; S 14.93. Calc. for C₁₁H₁₂NO₃S₂K: C 30.75; H 4.69; N 3.27; S 14.93.)

The identification of the products formed by enzymatic cleavage was performed in the same way as described by Kjær et al., for the crude glucocorinogin obtained from tetraacetyl-glucocorinogin. From 300 ml of glucocorinogin 82 mg of crude 5,5-dimethyl-2-oxazolidinethione were obtained which gave after two recrystallizations from benzene 32 mg of pure 5,5-dimethyl-2-oxazolidinethione, melting at 107°C alone or in mixture with a synthetic sample. Glucose was determined by paper chromatography in three solvent systems; sulfate was determined as BaSO₄.

For the detection of hydroxylamine as a product of hydrolysis with strong acids 10 mg of glucocorinogin were dissolved in 1 ml conc. hydrochloric acid and the solution was brought to dryness on the water bath. The residue was taken up in 2 ml of water. After addition of about 200 mg of Na acetate the solution was further treated according to the procedure described by Blom.

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5. Blom, I. Ber. 59 (1926) 121.

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2',5'-Dihydroxyterphenyl-2,2'-dicarboxylic Acid Dilactone

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Ozonolysis of 2-methylnaphthoquinone yields methylphenylglyoxal-o-carboxylic acid which has a cyclic lactol structure (I) rather than the free carboxyl structure (II).

\[ \text{HO} - \text{COCOCOCH₃} \]

\[ \text{COCOCOCH₃} \]

By briefly heating an alkaline solution of the acid a condensation product C₉H₄O₄ was formed in low yield. This product was sparingly soluble in most organic solvents and the melting point was very high (420—422°C) without noticeable decomposition. Dissolution could be effected by ethanolic but not aqueous sodium hydroxide and