ACTA CHEMICA SCANDINAVICA 10 (1956) 26—31

*iso*Thiocyanates XV. *p*-Methoxybenzyl *iso*Thiocyanate, a New Natural Mustard Oil Occurring as Glucoside (Glucoaubrietin) in *Aubrietia* Species

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Fresh plants and seeds of various species of the crucifer genus *Aubrietia* have been demonstrated to contain a new glucoside, *glucoaubrietin*, enzymically hydrolysed to sulphuric acid, glucose and *p*-methoxybenzyl *iso*thiocyanate. This mustard oil, which has not been previously recognised in nature, has been identified by comparison of the crystalline derivatives, formed upon reaction with ammonia and benzylamine, with authentic specimens of *p*-methoxybenzylthiourea (I) and *N*-(*p*-methoxybenzyl)-*N'*-benzylthiourea (II).

In the course of studies of *iso*thiocyanates in plants it was incidentally noticed that fresh parts of the crucifer *Aubrietia hybrida* hort. possessed a sharp, anis-like taste which made a closer investigation desirable. Paper chromatography [1] revealed the presence of a single glucoside in fresh parts of various species of the genus *Aubrietia*, a hitherto unrecognised source of mustard oils. With *n*-butanol:acetic acid:water (4:1:3) as a solvent system, the *Aubrietia*-glucoside appeared as a spot possessing an R_F-value slightly above that of glucotropaeolin, the well-known glucoside containing benzyl *iso*thiocyanate. This location, in conjunction with the spectroscopical characteristics discussed below, precluded the identity of the *Aubrietia*-compound with any of the previously recognised glucosides. In accordance with common usage, the name *glucoaubrietin* has been adopted for the new glucoside.

Ultra-violet absorption spectra of various mustard oil glucosides in aqueous solutions have been determined here. Provided strongly absorbing groups are absent from the aglucone side chains the glucosides display a single maximum at 228 mμ (ε *ca.* 8 000) as seen from the examples in Fig. 1. A partly purified specimen of glucoaubrietin exhibited maximum absorption at about the same wave-length but had in addition a broad band of medium intensity at about 270 mμ (Fig. 2). This pattern was retained after enzymic hydrolysis (Fig. 2), indicating unusual absorption properties of the corresponding *iso*thiocyanate.

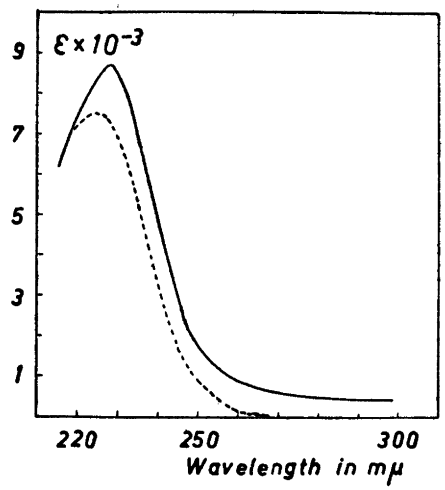


Fig. 1. Ultra-violet absorption spectra of aqueous solutions of : ———— *sinigrin;* — — — — *glucoiberin*[1].

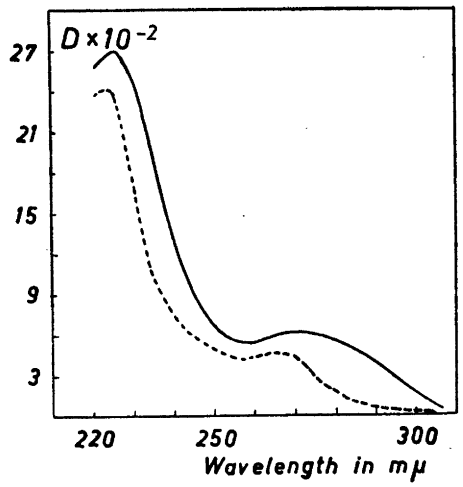


Fig. 2. Ultra-violet absorption spectra of: ———— *a partly purified extract of the glucoside of fresh parts of* Aubrietia columnae *Guss.;* — — — — *the corresponding crude* iso*thiocyanate. Solvent: water.*

The absorption curve suggested that the latter was of aromatic character, most likely substituted by one or more auxochromic groups. The spectral similarity with compounds such as *p*-hydroxy- and *p*-methoxy-benzylpenicillin [2], tyrosine [3], the cresols [4] *etc.* was striking. No colour appeared on spraying the chromatograms with diazotised sulphanilic acid [1], a fact precluding the presence of free aromatic hydroxy- and amino-groups in the glucoside. During the enzymic action, the concomitant formation of sulphate and glucose was established, the latter by paper chromatography in two solvent systems. Hence, glucoaubrietin appears to be a glucoside of the usual type. No attempts were made to crystallise or further characterise the genuine glucoside.

Whole, fresh plants of *Aubrietia deltoidea* DC. served as the starting material for the provision of further quantities of the glucoside. A methanolic extract was partly purified by percolation through aluminium oxide and then submitted to enzymic hydrolysis. The liberated *iso*thiocyanate was isolated by ether extraction and without further purification transformed into well-crystallising thioureas by reaction with ammonia and benzylamine. Analyses of both derivatives proved the presence of one methoxy-group and established the composition C_9H_9ONS for the parent *iso*thiocyanate. The ultra-violet absorption spectrum of the simple thiourea in 96 % ethanol exhibited a maximum at 245 mμ (ε 14 000), displaced in water to 237 mμ (ε 15 900), and an auxiliary band at 275 mμ (ε 1 900), practically unchanged in aqueous solution. In the present case, the high-extinction band could be regarded as diagnostic of an aralkyl-substituted thiourea, because the only alternative, a substituted arylthiourea, should possess quite different absorption data. For example,

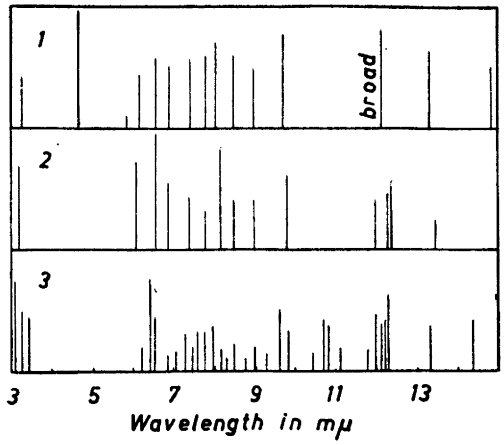
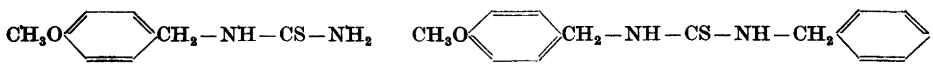


Fig. 3. Schematic presentation of the infra-red absorption spectra of: 1. p-*methoxybenzyl* isothiocyanate *(neat liquid, 0.1 mm layer); 2.* p-*methoxybenzylthiourea (I) (KBr pellet); 3.* N-(p-*methoxybenzyl*)-N'-*benzylthiourea (II) (KBr pellet).*

phenylthiourea in 96 % ethanol exhibits a maximum at 266 mμ (ε 15 400) with an inflection at 245 mμ (ε 10 000) and minimum at 227 mμ (ε 8 000), changed in water to a split peak at 248 and 240 mμ (ε 14 700 and 15 700). Hence, the mustard oil of glucoaubrietin must be one of the isomeric methoxybenzyl *iso*thiocyanates. In the literature [5,6] *p*-methoxybenzylthiourea is recorded with the m. p. 133—135°, sufficiently close to the m. p. 137° of the *Aubrietia*-thiourea to warrant a synthesis of an authentic specimen for comparison purposes.

p-Methoxybenzylamine was prepared in 34 % yield by reduction of anisaldoxime with zinc and acetic acid, according to Sunagawa *et al.*[7], or, more conveniently, from anisaldehyde by a Leuckart-reaction in 23 % yield as described by Lewis [8]. The amine was transformed into *p*-methoxybenzyl *iso*thiocyanate in 75 % yield by the Kaluza-Hodgkins synthesis, successfully employed recently by Ettlinger and Lundeen [9] for the preparation of the corresponding *m*-isomeride. The *iso*thiocyanate was formerly described by v. Braun and Deutsch [10] as a colourless liquid boiling over a rather wide range and suffering extensive decomposition on distillation at 16 mm. Here, the mustard oil appeared as a colourless, constant-boiling oil of agreeable odour and a burning taste, resembling that of fresh *Aubrietia* plants.



I II

By reaction with ammonia and benzylamine the synthetic mustard oil afforded the corresponding thioureas, (I) and (II). Analyses, melting points, mixed melting points and infra-red spectra served to establish the identity of the two *Aubrietia*-thioureas with the authentic specimens. The infra-red

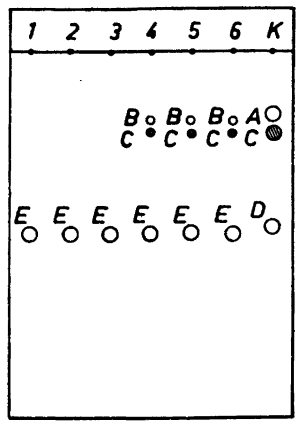


Fig. 4. *Schematic presentment of descending paperchromatographic analyses of the glucosides in various* Aubrietia *species. Solvent system:* n-*butanol:acetic acid:water (4:1:3).*

1. A. deltoidea *DC.*
2. A. hybrida *hort.*
3. A. columnae *Guss.*
4. A. deltoidea *DC (seed).*
5. A. erubescens *Griseb. (seed).*
6. A. intermedia *Heldr.* et *Orph. (seed).*
K. a control solution.
A. sinigrin; B. unknown glucoside; C. sinalbin;
D. glucotropaeolin; E. glucoaubrietin.

spectra are schematically reproduced in Fig. 3. It has thus been proved that *p*-methoxybenzyl *iso*thiocyanate represents an addition to the rather extensive series of naturally occurring *iso*thiocyanates.

Besides its occurrence in fresh plants of *Aubrietia deltoidea* DC., *A. hybrida* hort. and *A. columnae* Guss., glucoaubrietin has been demonstrated to be present in seeds of *A. deltoidea* DC., *A. erubescens* Griseb. and *A. intermedia* Heldr. *et* Orph. In the seed samples, however, the glucoside is accompanied by the *p*-hydroxybenzyl *iso*thiocyanate glucoside and an unidentified glucoside, the latter two being present in minor amounts only (Fig. 4). Glucoaubrietin therefore appears to be a rather constant and characteristic constituent of the genus *Aubrietia*. Its possible occurrence in other genera is being investigated at present. The detection of *p*-methoxybenzyl *iso*thiocyanate in nature is not altogether surprising in view of the long recognised existence of *p*-hydroxybenzyl *iso*thiocyanate (*cf.* ref. 11) as the aglucone of the glucoside sinalbin which occurs in white mustard and a few other plants. Biological methylation of aromatic hydroxy-groups is a reaction too well-known in phytochemistry to need further comments.

At the conclusion of the present work, the authors were informed that Ettlinger and Lundeen [9] have recently established the occurrence of the isomeric *m*-methoxybenzyl *iso*thiocyanate in seed of the American plant *Limnanthes douglasii* R.Br., belonging to the family *Limnanthaceae*. The latter finding is remarkable because of the rare occurrence of *m*-disubstituted aromatic compounds in nature.

EXPERIMENTAL

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

Paper chromatography of Aubrietia *species.* Methanolic extracts of whole, fresh plants and seeds of various *Aubrietia* species were applied to Whatman paper No. 1 and chromatographed in *n*-butanol:acetic acid : water (4:1:3) by the descending technique. The chromatograms were developed as previously described [1] and the results are schematically presented in Fig. 4. Whereas extracts of the fresh plants gave only one glucoside-spot, the seed samples contained minor amounts of two additional glucosides. One of these (C in Fig. 4), appeared to be identical with the glucoside containing *p*-hydroxybenzyl *iso*thiocyanate. Like an authentic specimen of the latter, the *Aubrietia*-compound C developed a red colour on spraying the chromatogram with diazotised sulphanilic acid [1]. The nature of the other minor glucoside, (B), is unknown.

Properties of glucoaubrietin. A specimen of fresh parts of *Aubrietia columnae* Guss. (1 g) was extracted three times with boiling methanol. The solvent was removed *in vacuo* and the residue taken up in water (20 ml). Insoluble material was removed by filtration and the filtrate passed through a column of acid-washed aluminium oxide (2 g). After the column had been washed with water the glucoside was eluted with 1 % K_2SO_4-solution[1].

A small fraction of the eluate was used, after appropriate dilution, for the determination of the ultra-violet spectrum, while the remaining part was subjected to enzymic hydrolysis by adding a drop of a cell-free myrosinase preparation. After some hours, the reaction mixture was extracted with ether, the solvent removed and the residue dissolved in water. Again, the ultra-violet absorption spectrum of this solution was determined. The original aqueous phase was chromatographed in 1) *n*-butanol:acetic acid:water (4:1:3) and 2) pyridine:amyl alcohol:water (30:35:30), in both solvent systems accompanied by glucose as a reference substance. The R_F-values were 0.24 and 0.42, respectively, the same as of glucose in the two systems.

A separate plant extract, which was not further purified, served to establish the presence of sulphate after enzymic hydrolysis of the glucoside.

Crystalline derivatives of the Aubrietia *isothiocyanate.* It was established in preliminary experiments that the *iso*thiocyanate, corresponding to glucoaubrietin, was volatile with steam. The steam distillation, however, was accompanied by a considerable loss of material for reasons which are not quite clear. Therefore, it was preferred to extract the free mustard oil by ether after enzymic hydrolysis.

Fresh, whole plants of *Aubrietia deltoidea* DC. (1.2 kg) were repeatedly extracted with a total of 5 l of methanol. After removal of the latter, the residue was taken up in water (1 l) and filtered. The filtrate was divided into four parts and each was allowed to flow slowly through a column of acid-washed aluminium oxide (35 g). The percolates were pooled, a few ml of a myrosinase solution was added and the mixture set aside overnight. Next day, the *iso*thiocyanate was collected by ether extraction (3 × 100 ml) and the ether solution was divided into two parts.

Thiourea. To one of these was added a solution of ammonia in ethanol. After 12 hours, the solvents were removed and the partly oily residue was treated with charcoal in hot water and filtered. On cooling, the solution deposited colourless needles (49 mg). The product was recrystallised from water (3.5 ml) for analysis (43 mg), m. p. 137.5—138.5°. Paper chromatography of the thiourea in chloroform gave a spot with an R_{Ph}-value [12] of 1.03. The ultra-violet and infra-red spectra were determined. (Found: C 55.10; H 6.02; N 13.93; S 15.90; OCH_3 15.90. Calc. for $C_9H_{12}ON_2S$: C 55.05; H 6.17; N 14.27; S 16.33; OCH_3 15.83).

Benzylthiourea. To the other half of the ether solution was added an excess of benzylamine. Next day, the solvent was removed and the residue treated with water, containing a drop of hydrochloric acid. The partly crystalline product was recrystallised three times from 50 % ethanol to give colourless, nacreous platelets (66 mg), m. p. 105°. (Found: C 67.25; H 6.24; N 9.87; S 11.04; OCH_3 10.94. Calc. for $C_{16}H_{18}ON_2S$: C 67.10; H 6.34; N 9.78; S 11.20; OCH_3 10.83). The ultra-violet and infra-red spectra of the disubstituted thiourea were determined.

Synthesis of p-*methoxybenzyl* iso*thiocyanate.* *p*-Methoxybenzylamine was prepared by reduction of anisaldoxime with zinc and acetic acid, according to Sunagawa *et al.*[7], who

reported a yield of 75 %. Our yields were considerably smaller (30—40 %) and it was therefore preferred to prepare the amine by the Leuckart-reaction proposed by Lewis [8]. The stated yield (23 %) was obtained. The amine was distilled *in vacuo*, b. p. 115° at 12 mm.

The synthesis of the *iso*thiocyanate was performed exactly as described by Ettlinger and Lundeen [9] for the corresponding *m*-isomeride and proceeded in 75 % yield. The mustard oil appeared as a colourless liquid of a faint odour, but possessing a sharp and aromatic taste, b. p. 133° at 0.7 mm; n_D^{25} 1.5935. (Found: C 60.30; H 5.21; N 7.98; S 17.86. Calc. for C_9H_9ONS: C 60.31; H 5.06; N 7.82; S 17.89).

p-*Methoxybenzylthiourea* (I). The synthetic mustard oil was allowed to react with ammonia in dilute ethanol overnight. The resulting thiourea was obtained as clusters of colourless needles after recrystallisation from water, m. p. 138° alone or when mixed with the *Aubrietia*-thiourea above. (Found: C 55.20; H 6.15; N 14.24. Calc. for $C_9H_{12}ON_2S$: C 55.05; H 6.17; N 14.27.) The infra-red spectrum was found identical with that of the *Aubrietia*-thiourea.

N-*(p-Methoxybenzyl)*-N'-*benzylthiourea* (II). An ether solution of the mustard oil and benzylamine was left standing at room temperature overnight. Next day, the crystals were collected and recrystallised from 50 % ethanol. The benzylthiourea separated as colourless plates, m. p. 105°, alone or in admixture with the *Aubrietia*-derivative. (Found: C 67.00; H 6.33; N 9.82. Calc. for $C_{16}H_{18}ON_2S$: C 67.10; H 6.34; N 9.78). Again, the infra-red spectrum served to establish the identity of the compound with the benzylthiourea, derived from the *Aubrietia* mustard oil.

The authors wish to thank the *Botanical Garden of the University of Copenhagen* for placing at our disposal all the plant materials employed in the present investigation. Microanalyses were made in this laboratory by Mr. P. Hansen. The work is part of investigations supported by *Statens Almindelige Videnskabsfond (The Danish State Research Foundation)* and *Carlsbergfondet (The Carlsberg Foundation)*.

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Received October 25, 1955.