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Isothiocyanates XL *. Glucosisaustricin, a Novel Glucoside Present in Seeds of *Sisymbrium austriacum* Jacq.

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Structure determinations of two glucosides in seed extracts of *Sisymbrium austriacum* Jacq. have been described in two preceding papers [1,2]. The present communication reports on the chemical structure of a third glucoside, *glucosisaustricin*, from the same source, characterized by undergoing enzymic hydrolysis to glucose, sulphate and (+)-4-ethyl-2-oxazolidinethione (sisaustricin), the latter arising from cyclization of 1-ethyl-2-hydroxyethyl isothiocyanate, initially formed by the enzymic hydrolysis.

The structure and absolute configuration of sisaustricin, (VI), are established by synthesis and stereochemical correlation with 2-aminobutyric acid. The absolute configuration of optically active 2-amino-1-butanol is determined.

The biosynthetic origin of the isothiocyanate producing glycosides with branched side-chains is discussed and a possible relationship to the metabolism of valine and isoleucine is suggested.

Previous communications of this series dealt with structure elucidations of glucosisymbrin [1] and glucobenzosisymbrin [2], two glucosides occurring in seed extracts of the crucifer *Sisymbrium austriacum* Jacq. Paperchromatographic studies, however, indicated the presence in the same seed material ** of an additional glycoside, previously referred to [1] as glycoside B.*** It is the purpose of the present paper to report on the structure determination of this novel glycoside.

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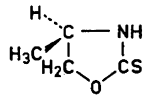
** The present investigation was carried out on seed material propagated in 1959–1960 in *The Botanical Garden of the University of Copenhagen* from the authentic stock employed in our previous studies.

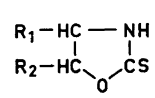
*** Only this additional glycoside, (B), was reported in the previous communication [1]. However, more recent investigations have disclosed the presence in the same extracts of minor amounts of a fourth glycoside, with an R_F-value still higher than that of glucobenzosisymbrin [2]. A subsequent paper [4] reports on the structure of this glycoside.

Paper chromatography of a methanolic seed extract of *S. austriacum* Jacq., followed by elution of the appropriate band, furnished a solution of glycoside B which was subjected to acid hydrolysis according to Ettlinger and Lundeen [3]. The presence of glucose and hydroxylamine in the hydrolysate was demonstrated by paper chromatography, and concomitantly liberated sulphate by precipitation as barium sulphate. Hence, glycoside B is a glucoside of the conventional type [3] for which we propose the name *glucosisaustricin*.

For preparative purposes, a methanolic extract of defatted seed material was subjected to enzymic hydrolysis by myrosinase in a citrate buffer. Steam distillation served to remove the volatile ester isothiocyanates, originating from glucobenzosisymbrin [2] and glucobenzsisaustricin [4]. In a previous paper [1], evidence was presented for the probable structure of *sisaustricin* ('THIOX B'), the aglucone of glucosisaustricin, as a substituted 2-oxazolidinethione. Consequently, the unknown compound must be searched for in the steam-distilled residue, together with (+)-4-methyl-2-oxazolidinethione (sisymbrin) (I), the latter originating from glucosisymbrin [1]. A 180-plate counter-current distribution in the solvent system benzene:heptane:water (9:2:9) was utilized to separate the two aglucones, both of which could be isolated in pure form as low-melting, crystalline products. The sisymbrin fraction possessed all the properties previously recorded for a specimen prepared by fractional crystallization [1]. Sisaustricin, m.p. 33—34°, had the elemental composition C_5H_9ONS which together with its ultra-violet and infra-red spectra strongly indicated its character as a higher homologue of sisymbrin. These data, in conjunction with the established optical activity, admitted of only three substitution patterns for sisaustricin, *viz.* 4,5-dimethyl (II), 5-ethyl (III), and 4-ethyl (IV).



I



II. $R_1 = R_2 = CH_3$

III. $R_1 = H$; $R_2 = CH_3CH_2$

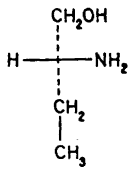
IV. $R_1 = CH_3CH_2$; $R_2 = H$

A racemic modification of (II), with unknown stereochemistry at C_4 and C_5, was prepared during the present work. Whereas none of the optically active isomers have formerly been described, racemic modifications of (III)[5] and (IV)[6,7] were recorded in the literature and both were synthesized for comparison purposes in this laboratory. Considerable deviations in melting points and solid phase infrared spectra between racemates and enantiomers have frequently been noticed in the present series (*cf. e.g. Ref.*[1]), a fact which makes comparisons of melting points and IR-spectra of limited diagnostic value. However, careful comparative paper-chromatographic studies * indicated that sisaustricin was in all probability the *dextro*rotatory enantiomer of (IV), a surmise for which experimental support was obtained by the following syn-

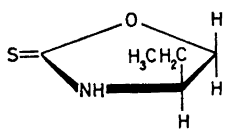
* In no case have differences in R_F-values been noticed between racemates and enantiomers in the 2-oxazolidinethione series.

thesis of (+)-4-ethyl-2-oxazolidinethione, proceeding by a route which provides full information as to its absolute configuration.

(−)-2-Amino-1-butanol was prepared by resolution of the racemic aminoalcohol with (+)-tartaric acid as described by Radke *et al.*[8] The *levo*rotatory enantiomer was further converted into (+)-4-ethyl-2-oxazolidinethione by the procedure employed by Rosen [6] for the preparation of the corresponding racemic compound. The synthetic specimen proved to be identical with sisaustricin with regard to sign and magnitude of rotation as well as all other physical criteria. Furthermore, its absolute configuration was established on basis of the following chemical correlation: (−)-2-amino-1-butanol was converted into its *levo*rotatory acid oxalate, whereas the 2-amino-1-butanol obtained by lithium aluminium hydride reduction of configurationally known L-2-aminobutyric acid [9] yielded the enantiomeric, *dextro*rotatory acid oxalate, derivable from (+)-2-amino-1-butanol. Consequently, (−)-2-amino-1-butanol has the absolute configuration shown in (V)* whereas the naturally derived (+)-sisaustricin possesses the absolute configuration depicted in (VI)**. It is noteworthy that



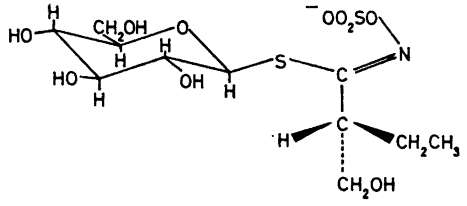
V



VI

the homologous, *dextro*rotatory, 4-substituted 2-oxazolidinethiones, sisymbrin and sisaustricin, belong to the same configurational series. It has been previously established in this laboratory that in the isomeric (+)-5-ethyl-2-oxazolidinethione the substituent is located below the ring plane in the same depiction [11].

The structure elucidation of sisaustricin furnishes the clue to the structure of glucosisaustricin, the parent glucoside whence it derives. On the likely assumption that the enzymic hydrolysis, as well as the subsequent ring-closure of the initially formed 1-ethyl-2-hydroxyethyl isothiocyanate, proceeds with retention of configuration, the parent glucoside ion possesses the structure (VII), depicted in analogy with the general structure of similar glucosides [3].



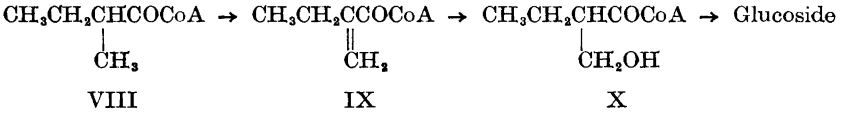
VII

* (*R*)-2-Amino-1-butanol in the specification system of Cahn *et al.*[10]
** (*R*)-4-Methyl-2-oxazolidinethione in the same specification.

The following communication [4] reports on the occurrence of O-benzoylated glucosisaustricin in the same seed material.

Unpublished considerations suggest that energy-rich esters, RCOOX, may function as biogenetic precursors in the *in vivo* synthesis of isothiocyanate glucosides containing the side chains R. In the case of glucosisymbrin and glucosisaustricin, the biosynthesis of such esters of 2-methyl- and 3-methylhydracrylic acid, or their biological equivalents, therefore has to be accounted for.

The catabolism of valine as a possible source of the 2-methyl substitute was discussed previously in this series [1]. In analogy, (X) may conceivably arise from an analogous degradation of isoleucine. It is generally agreed that the metabolic pathways of valine and isoleucine run closely parallel both in animals and various microorganisms, partly catalyzed by the same enzyme systems. The frequently observed, simultaneous appearance in plants of isothiocyanate glucosides containing the isopropyl and sec-butyl side-chains [12], as well as their β-hydroxylated derivatives, therefore lends support to the idea that intermediates on the catabolic pathway of the branched amino acids may function as biogenetic precursors of the isothiocyanate glucosides with branched side-chains *. Thus, glucosisaustricin may originate from the coenzyme A ester of 2-methylbutyric acid (VIII), an accepted intermediate in isoleucine catabolism in mammalian tissue, by the following route:



VIII IX X

This highly speculative scheme deviates from the usually accepted pathway in animal tissue by the postulation of the 2-ethylacrylic ester (IX) as an intermediate, furnishing (X) upon hydration, rather than the isomeric tiglyl ester, normally functioning as an intermediate in the further degradation of (VIII) to the coenzyme A esters of acetic and propionic acid [14].

The possibility that the above transformations occur subsequent to incorporation of (VIII) into thioglucosidic linkage should, of course, not be overlooked. It is intended to subject the above biosynthetic schemes to experimental trials.

EXPERIMENTAL

When not otherwise indicated melting points are determined in capillary tubes in an Anschütz-Hershberg apparatus equipped with fully immersed thermometers. Rotations are measured in a 1 dm tube. All infra-red spectra are determined in KBr pellets on an 'Infracord' instrument. Analytical specimens are dried *in vacuo* over calcium chloride at room temperature.

* It is of interest in this connexion that Butler and Butler [13] demonstrated that products from the valine and isoleucine metabolism are involved in the biosynthesis of the cyanogenetic glucosides, lotaustralin and linamarin, derivable from the cyanohydrins of methyl ethyl ketone and acetone, respectively, and occurring together in *Trifolium repens* L.

Acid hydrolysis of glucosisymbrin and 'glycoside B'. A methanolic seed extract of *Sisymbrium austriacum* Jacq. was prepared and chromatographed on paper as previously reported [2]. The bands containing glucosisymbrin and 'glycoside B'[1] were cut out and individually eluted with water. Both solutions were treated with conc. hydrochloric acid for 2 h at 60° to accomplish hydrolytic fission according to Ettlinger and Lundeen [3]. The presence of glucose and hydroxylamine in the hydrolyzed solutions was established on paper chromatography [3], whereas the liberation of sulphate ions was proved by precipitation as barium sulphate. Appropriate blanks were employed to exclude contaminations from the paper.

Consequently, both glucosisymbrin and 'glycoside B'[1] are of the customary type [3], the latter henceforth designated *glucosisaustricin.*

Enzymic production and isolation of sisaustricin. A portion (1 110 g) of dry seeds of *S. austriacum* Jacq. was milled, defatted and extracted with 70 % methanol, exactly as described previously [2]. The extract was concentrated to a syrup which was taken up in water (3 l), and the solution was filtered. The volume was increased to 5.6 l and 1 M sodium citrate (430 ml) was added. The pH-value was then adjusted to 6.8 by addition of solid citric acid, and a cell-free, crude myrosinase solution (120 ml) was added. After having been kept for 18 h at room temperature, at the end of which the pH-value had decreased to 6.1, the enzymically hydrolyzed solution was steam-distilled to remove volatile isothiocyanates. A total of 6 l of distillate was collected.

The steam-distilled residue was concentrated to a brown suspension (800 ml) which was continuously extracted with ether for 48 h. The ether phase, including some solid material, was then extracted with one 1-l and two 0.5-l portions of 0.2 N NaOH. Several hours were required for the emulsions to break. After adjustment of the pH-value to 7 with conc. HCl the aqueous solution was concentrated *in vacuo* to a volume of 0.5 l, and the brown suspension was percolated with ether for 24 h. The procedure of extraction with 0.2 N NaOH, neutralization, and percolation with ether was repeated. The now almost colourless ether solution was dried over calcium chloride and concentrated to a yellowish oil, essentially consisting of a mixture of sisymbrin and sisaustricin.

The oily material was distributed over the first four tubes (each holding 20 ml) of a Craig-apparatus preloaded with the lower phase of the solvent system benzene:heptane: water (9:2:9). After 180 transfers, paperchromatographic analysis indicated that complete separation of the two oxazolidinethiones had been achieved.

On concentration of the contents of tubes Nos. 10-34, a sligthly yellow oil was obtained which crystallized spontaneously (2.84 g). Two recrystallizations from ether afforded pure *sisymbrin,* m.p. 65°, $[\alpha]_D^{17}$ +21° (*c* 0.7,EtOH), indistinguishable from the previously isolated specimen [1] as estimated from infra-red spectra and mixed melting point determination.

The contents of tubes Nos. 42—68 were likewise concentrated to a yellowish oil which crystallized on prolonged cooling (1.34 g). The product was dissolved in ether at 0°, the solution was filtered, one third volume of pentane was added, and the solution cooled to —80°. Colourless prisms separated slowly, and repetition of this recrystallization procedure afforded a pure specimen of *sisaustricin,* (674 mg), m.p. 32—33° (uncorr., water-bath), $[\alpha]_D^{26}$ +46.3° (*c* 1.4, 96 % EtOH). No depression of the m.p. was observed on admixture with the synthetic specimen described in the sequel. Again, the infra-red spectrum was identical with that of the synthetic material and exhibited conspicuous bands at 3 120 vs, 2 930 s, 1 510 vs, 1 455 m, 1 440 w, 1 398 m, 1 375 w, 1 330 s, 1 270 s, 1 255 s, 1 175 vs, 1 115 w, 1 095 w, 1 065 m, 1 018 w, 970 s, and 915 s cm^{-1}.

The ultra-violet spectrum of sisaustricin (in 96 % EtOH) was characteristic of 2-oxazolidinethiones, and exhibited λ_{max} 244 mμ (ε 17 800), λ_{max} 203 mμ (ε 4 000) and λ_{min} 218 mμ (ε 350).

Paper chromatography of sisaustricin and isomerides. Small, but consistent differences were noticed on paper chromatography in two solvent systems of sisaustricin and the two racemic isomerides (II and III), whereas the naturally derived compound was indistinguishable from the racemic 4-ethyl-substitute (IV). In Table 1, the R_{Ph}-values, *i.e.* the ratio of the distances travelled by the said compound and (±)-5-phenyl-2-oxazolidinethione [15], are presented. The compounds were revealed as blue spots on spraying with Grote's reagent.

Table 1.

Substituents	Formula	R_{Ph}-Value	
		System A[a]	System B[b]
4,5-Dimethyl	II	0.56	0.41
(±)-5-Ethyl	III	0.62	0.47
(±)-4-Ethyl	IV	0.66	0.55
Sisaustricin	VI	0.66	0.55

[a] Benzene:heptane:water (9:2:9)
[b] Carbon tetrachloride:30 % acetic acid (1:1) [16].

4,5-Dimethyl-2-oxazolidinethione (II). The 3-amino-2-butanol required for the synthesis of (II) was obtained by reduction of diacetyl monoxime (5 g) with lithium aluminium hydride (5 g) in ether (100 ml). After reaction and hydrolysis of excess hydride with small amounts of water, the solid hydroxides were extracted in a Soxhlet apparatus with ether for 24 h. The amino-alcohol (2.3 g) distilled at 110—125°/15 mm as a colourless liquid. Without further purification, it was converted into 4,5-dimethyl-2-oxazolidinethione by means of thiocarbonyl chloride and triethylamine in chloroform solution by our standard procedure [1]. A low yield (400 mg) of a solid product was thus obtained, which separated from ethyl acetate-pentane mixtures as colourless prisms, m.p. 107° (Found: C 45.92; H 6.96: N 10.51. Calc. for C_5H_9NOS: C 45.77; H 6.92; N 10.68). λ_{max}^{EtOH} 244 mμ (ε 17 000), 203 mμ (ε 3 600), λ_{min}^{EtOH} 218 mμ (ε 700). The infrared spectrum displayed the expected bands.

The sharp melting point suggests that the synthetic specimen is homogeneous but no attempts were made to decide whether it represents the *cis-* or *trans*-isomeride.

(±)-*4-Ethyl-2-oxazolidinethione.* This compound was prepared as a model, essentially as described by Rosen [6]. M.p. 72-3°; lit.values: 74-5° [6], 72.8-73.2° [7]. The considerable difference in melting point between the racemic compound and the (+)-enantiomer (33°) is noteworthy.

(±)-*5-Ethyl-2-oxazolidinethione.* Propionaldehyde cyanohydrin [17] was reduced with $LiAlH_4$ to give a 70 % yield of (±)-1-amino-2-butanol, b.p. 74.5°/9 mm. The amino-alcohol, on reaction with thiocarbonyl chloride and triethylamine in chloroform solution [1], furnished a 25 % yield of (+)-5-ethyl-2-oxazolidinethione, which was recrystallized from ethyl acetate-pentane mixtures, m.p. 86.5°. Clapp and Watjen [5] reported the m.p. 84.6—85.2° for a specimen prepared by a different method.

(−)-*2-Amino-1-butanol and its acid oxalate.* Essentially as described by Radke *et al.*[8], racemic 2-amino-1-butanol was resolved by means of (+)-tartaric acid to give a monohydrated acid tartrate of the *levo*rotatory amino-alcohol, $[\alpha]_D^{24}$ + 10.9° (*c* 5.7, H_2O), m.p. 71—3°, with loss of water, resolidification, and final melting at 103—104°. Lit.data [8]: $[\alpha]_D^{23}$ +10.5° (*c* 5, H_2O), m.p. 103°. The amino-alcohol was set free by means of CaO[8] and distilled, b.p. 76°/8 mm, $[\alpha]_D^{26}$ −9.9° (neat) (reported[8]: $[\alpha]_D^{26}$ −9.92°).

On addition of a solution of the *levo*rotatory amino-alcohol (400 mg) in ethanol (7 ml) to a solution of oxalic acid dihydrate (650 mg) in ethanol (7 ml), the acid oxalate separated as colourless needles, which were recrystallized from ethanol, m.p. 141—142°, $[\alpha]_D^{27}$ −9.0° ± 1° (*c* 3, H_2O). (Found: C 40.14; H 7.33; N 7.79. Calc. for $C_6H_{13}NO_5$: C 40.21; H 7.31; N 7.82).

Synthesis of (+)-4-ethyl-2-oxazolidinethione. The *levo*rotatory amino-alcohol (2.0 g) was converted into the *dextro*rotatory 4-ethyl-2-oxazolidinethione by the method designed by Rosen [6] for the synthesis of the racemic isomeride. On careful recrystallization of the crude, semi-solid reaction product from ether-pentane at −80°, a colourless product (1.20 g) was obtained. Another recrystallization from the same solvent system afforded a

pure specimen as colourless prisms, m.p. 32.5—33.5° (uncorr., water bath), $[\alpha]_D^{26}$ +49.2° (*c* 1.2, 96 % EtOH). (Found: 45.85; H 7.05; N 10.79. Calc. for C_5H_9NOS: C 45.78; H 6.91; N 10.68). No depression of the m.p. was observed on admixture with the naturally derived sisaustricin. Again, ultra-violet and infra-red spectra of the two specimens coincided.

In connexion with the present work it was noticed that a synthetic specimen of (+)-4-ethyl-2-oxazolidinethione, which had been stored at 0° for one year, had undergone some change. This parallels observations made earlier in this laboratory on other substitutes of the same series. A characteristic feature of the change is an increased absorption at 1 640 cm^{-1}, as well as the formation of varying amounts of ether-insoluble material. We attribute this change to the formation of disulphides, arising from oxidation of the ring system in its thio-enolized form. Recrystallization readily removes the secondary products.

Absolute configuration of 2-amino-1-butanol and 4-ethyl-2-oxazolidinethione. Correlation of (−)-2-amino-1-butanol, and hence of sisymbrin, with configurationally known substances was achieved by reduction of L-2-aminobutyric acid * (1.8 g) with lithium aluminium hydride (2.0 g) in tetrahydrofuran (30 ml) by a procedure analogous to that employed by Vogel and Pöhm [18] for reduction of the racemic amino acid. The resulting amino alcohol (390 mg) was distilled, b.p. 80—82°/10 mm, and without further characterization transformed into its acid oxalate as described above for the antipode. Colourless needles were obtained after recrystallization from ethanol, m.p. 141—142°, $[\alpha]_D^{27}$ +8.2° ± 1° (*c* 3, H_2O) (Found: C 40.20; H 7.29; H 7.96. Calc. for $C_6H_{13}NO_5$: C 40.21; H 7.31; N 7.82). The infra-red spectra of the antipodal acid oxalates were identical. By the above transformations, sterical correlation has been achieved between the naturally derived (+)-sisaustricin and D-2-aminobutyric acid.

Microanalyses were performed by Mr. G. Cornali. The assistance of E. Vesterager, M.Sc., in part of the synthetic work is acknowledged. The authors are very grateful to the *Botanical Garden of the University of Copenhagen* for valuable assistance in propagating the employed seed material.

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* A preparation with $[\alpha]_D$ +20.5° (*c* 4.0, 6 N HCl), purchased from California Corp. for Biochemical Research, Los Angeles 63, was employed.