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Isothiocyanates XLI *. Glucobenzsisaustricin, a New Glucoside Present in Seeds of *Sisymbrium austriacum* Jacq.

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In continuation of previous studies of the isothiocyanate-producing glucosides in seed material of the crucifer *Sisymbrium austriacum* Jacq., a glucoside of the ordinary type, migrating faster on paper chromatography than any of the three formerly identified glucosides [1-3], has been recognized as the benzoic acid ester of glucosisaustricin [3]. The designation *glucobenzsisaustricin* is proposed for the new glucoside.

Enzymic hydrolysis of the glucoside yields a volatile ester isothiocyanate which on alkali treatment is converted into (+)-4-ethyl-2-oxazolidinethione of known absolute configuration [3]. Upon ammonia treatment under controlled conditions, the new isothiocyanate yields a thiourea, which on alkaline fission splits off benzoic acid. These facts establish the relationship of the new glucoside to glucosisaustricin, present in the same seeds.

The production of the O-benzoylated glucosides seems to occur in the seeds during the ripening process.

According to the paperchromatographic pattern, seed extracts of *Sisymbrium austriacum* Jacq. were originally reported to contain two glycosides ('A' and 'B') in the R_B-range 0.3—0.6, besides one glycoside ('C') migrating much faster on the paper ($R_B \sim 1.7$). In addition, faint glycoside spots, tentatively assigned to glucoputranjivin and glucocochlearin, were recorded [1]. Previous communications from this laboratory described the structure identification of 'A', *glucosisymbrin*, containing the 2-hydroxyisopropyl side-chain [1], and *glucobenzosisymbrin*, 'C', the same glucoside esterified with benzoic acid in its side-chain [2]. Glycoside 'B', named *glucosisaustricin*, has since been identified as a glucoside possessing the higher homologous 1-ethyl-2-hydroxyethyl side-chain [3].

Paperchromatographic studies on seed material, propagated in the Botanical Garden of the University of Copenhagen during 1959—1960 from the stock

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used in our previous studies [1], revealed the presence of the three above-mentioned glucosides, and, in addition, a rather faint but distinct glycoside spot with an R_B-value [1] as high as 2.0. (Fig. 1). A desire to complete the glucoside picture of the seed material of *S. austriacum* Jacq. prompted the present investigation. It was established that a paperchromatographically purified solution of the unknown glycoside on treatment with strong hydrochloric acid [4] afforded glucose, hydroxylamine and sulphate ions. Consequently, and in view of its structural relationship to glucosisaustricin outlined in the sequel, the designation *glucobenzsisaustricin* was introduced for the new glucoside.

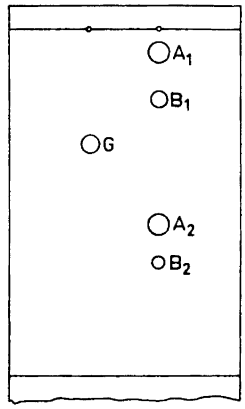

Fig. 1. Paper-chromatographic pattern of the four major isothiocyanate glucosides in ripe seeds of *Sisymbrium austriacum* Jacq.
A_1: glucosisymbrin; A_2: glucobenzosisymbrin;
B_1: glucosisaustricin; B_2: glucobenzsisaustricin.
G: reference sample of glucotropaeolin.
Solvent system: *n*-butanol:ethanol:water (4:1:4)

In analogy with observations made on glucobenzosisymbrin [2], aqueous ammonia effected conversion of the unknown into a much more hydrophilic glucoside, indistinguishable on paper chromatography in two solvent systems from glucosisaustricin, and suggesting a chemical relationship with the latter.

Enzymic hydrolysis of the entire seed glucoside fraction, followed by steam-distillation of the liberated volatile isothiocyanates, gave a mixture of the isothiocyanate, (benzsisaustricin), derivable from the unknown glucoside, and 2-benzoyloxyisopropyl isothiocyanate originating from glucobenzosisymbrin [2].

The limited quantities on hand precluded a fractional distillation of the mixture, in which benzosisymbrin was the predominant constituent, as estimated from paper-chromatography of the corresponding thioureas. The chemical similarity between the two volatile isothiocyanates appeared from their common behaviour towards ammonia. As described in a previous paper of this series [2], non-aqueous conditions are essential to preclude competing ammonolysis of the ester linkage during thiourea formation of benzosisymbrin. A completely analogous behaviour was now noticed in the case of benzsisaustricin and there can be no doubt that the benzosisymbrin-thiourea contamination, referred to

in a previous paper [2] and removable only by counter-current distribution, was, in fact, the thiourea of benzsisaustricin. No attempts were made to isolate a pure specimen of the latter. Proof of its identity was obtained, however, by subjecting the volatile isothiocyanate fraction to treatment with alkali, whereby a mixture of two 2-oxazolidinethiones was produced. One of these was previously established to be (+)-4-methyl-2-oxazolidinethione (sisymbrin), arising from cyclization of a 2-hydroxyisopropyl isothiocyanate which again resulted from alkaline hydrolysis of the benzoate ester linkage in benzosisymbrin [2]. Information as to the second oxazolidinethione was provided by isolation of the compound in pure form from a 180-plate counter current distribution, exactly as described in a previous paper of this series [3]. Critical comparison proved the isolate to be identical with sisaustricin, established in the preceding paper [3] as (+)-4-ethyl-2-oxazolidinethione of known absolute configuration, arising from enzymic fission of glucosisaustricin, present in the same seed material. From the above it appears that glucobenzsisaustricin is an ester of glucosisaustricin.

Information regarding the acid moiety was obtained upon saponification of a paperchromatographic eluate of the thiourea derivative of benzosisaustricin. Chromatography clearly indicated that the esterifying acid is *benzoic acid*, a fact which concludes the structure proof of glucobenzsisaustricin.

In conclusion, the present paper, together with previous communications of this series [1-3], provides complete knowledge as to the structure of the four major isothiocyanate-producing glucosides present in ripe seeds of *Sisymbrium austriacum* Jacq., none of which had formerly been encountered in Nature. No attempts have been made to isolate the glucosides in pure form. As discussed in the preceding communication [3], glucosisymbrin and glucosisaustricin may be biogenetically related to products on the metabolic pathways of the branched chain amino acids valine and isoleucine.

No conclusive evidence is on hand regarding the biosynthetic origin of the two O-benzoylated glucosides. It appears significant, however, that paperchromatographic analysis of a fresh plant of *S. austriacum* Jacq. has shown the roots, flowers and unripe siliques to be entirely devoid of the benzoylated glucosides, but rich in glucosisymbrin and glucosisautricin. The green leaves contain only negligible amounts of glucosides. This observation suggests that enzymic benzoylation of the side-chains of the glucosides occurs during the ripening process. It is hoped that planned experiments will provide further insight into the biosynthesis of this interesting series of related isothiocyanate producing glucosides.

EXPERIMENTAL

Paperchromatographic studies. A methanolic seed extract of ground, mature seeds of *Sisymbrium austriacum* Jacq. (1960 harvest) was chromatographed as previously described [1]. A schematic depiction of the observed pattern is shown in Fig. 1. The present investigation deals with the structure elucidation of glucoside B_2.

An aqueous solution containing only the unknown glycoside was produced by elution of the appropriate zones from four chromatograms, exactly as described for glucobenzosisymbrin [2]. Half of the eluate was left standing for 28 h with conc. ammonia and was then rechromatographed. The unknown glycoside had been quantitatively converted into a new glycoside which on chromatography was indistinguishable from glucosisaustricin [3].

Acid hydrolysis of the unknown glycoside. The remaining half of the above eluate was treated with conc. hydrochloride acid for 2 h at 60°, conditions similar to those employed by Ettlinger and Lundeen [4] in analogous hydrolysis experiments. Paper chromatography indicated the formation during this treatment of hydroxylamine [4] and glucose, whereas liberation of sulphate ions was proved by precipitation as $BaSO_4$. Appropriate blanks were run to preclude contaminations from the chromatography paper. Hence, the new glycoside is of the customary structural type [4] and from here on named *glucobenzsisaustricin.*

Enzymic liberation and chemical characterization of benzsisaustricin. On a preparative scale, the condensate (6 l) from the steam distillation described in the preceding communication [3] was extracted with three 1-l portions of ether. After removal of the solvent through a column, a brown oil remained (3.82 g), which was dissolved in dioxane (120 ml) and treated with 0.2 N NaOH (120 ml) at room temperature for 4 h, at the end of which hydrochloric acid was added to bring the pH to 7. Dioxane and water were removed *in vacuo* and the oily material was dissolved in water (400 ml) to which 1 N NaOH (80 ml) was added. The solution was extracted with ether and the extract discarded. The aqueous solution was readjusted to pH 7 and continuously extracted with ether for 24 h. On removal of the solvent from the dried ether solution, a mixture of two oxazolidinethiones remained as a yellow oil (1.48 g). On comparative paper chromatography in benzene: heptane: water (9:2:9), the two components were indistinguishable from authentic specimens of (+)-4-methyl-2-oxazolidinethione (sisymbrin)[1] and (+)-4-ethyl-2-oxazolidinethione (sisaustricin)[3].

The mixture was dissolved in a total of 40 ml of equal volumes of the upper and lower phase of the above benzene:heptane:water system and introduced into the first two tubes of a Craig counter current apparatus. 180 transfers were made and analysis performed exactly as described in the preceding communication [3]. Again, isolation of the two constituents was achived as previously described [3]. Both sisymbrin (437 mg) and sisaustricin (312 mg) were thus obtained in the pure state. Their identity with (+)-4-methyl-2-oxazolidinethione and (+)-4-ethyl-2-oxazolidinethione, respectively, was secured on critical comparison of the two isolates with authentic specimens [1,3].

Identification of benzoic acid. A mixture of the thioureas of the volatile isothiocyanate fraction was prepared upon treating the latter with anhydrous ammonia under controlled conditions, precisely as previously reported [2]. A solution (240 μl), containing an estimated total of 1.6 mg of the thiourea mixture, was evenly applied to two strips (15 cm broad) of Whatman paper No. 1. Descending chromatography for 3 h in the solvent system: carbon tetrachloride: glacial acetic acid (1:1) resulted in complete separation of the two thioureas. The fastest migrating band was eluted with 96 % ethanol (2 ml), to which 1 N NaOH (2 ml) was added. The solution was kept at 70° for 1 h and then at room temperature overnight. After acidification, the solution was extracted with ether and the ethereal phase assayed by paper chromatography in the solvent system: *n*-butanol: ethanol:water:ammonia (40:40:16:4), along with a blind and a reference sample of benzoic acid [5]. Spraying reagent: 0.2 % ninhydrin in ethanol plus 5 % formic acid [5]. A spot of benzoic acid was noticed in the hydrolyzed sample, indicating the production of this acid during the alkaline hydrolysis of the thiourea. Hence, glucobenzsisaustricin is the O-benzoate of glucosisaustricin [3].

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