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*iso*Thiocyanates XXXIX *. Glucobenzosisymbrin, a New Glucoside Present in Seeds of *Sisymbrium austriacum* Jacq. **

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As formerly shown, seeds of the crucifer *Sisymbrium austriacum* Jacq. contain three major glycosides (A, B, and C) the first of which, glucosisymbrin, was demonstrated to contain a 2-hydroxy*iso*propyl side-chain of established absolute configuration [1].

In the present paper, glycoside C, designated *glucobenzosisymbrin*, is shown to be a glucoside of the customary type, differing from glucosisymbrin only by containing a benzoyl grouping attached in ester linkage to the side-chain.

The mustard oil (benzosisymbrin), liberated on enzymic hydrolysis of the new glucoside, is characterized as its thiourea derivative. The deduced structure of the latter is confirmed by synthesis of the enantiomeric thiourea. The configuration around the asymmetric carbon atom of the side-chain in glucobenzosisymbrin is shown to be the same as that prevailing in glucosisymbrin. The new glucoside represents the second example of the natural occurrence of *iso*thiocyanate glucosides with benzoylated hydroxy-alkyl side-chains, glucomalcolmiin [6,7] being the first discovered of these.

In a previous communication from this laboratory [1], the distribution of *iso*thiocyanate glycosides in various members of the cruciferous genus *Sisymbrium* was discussed. Particular attention was given to the species *Sisymbrium austriacum* Jacq., a seed extract of which was demonstrated to contain three major glycosides, obviously different from all heretofore described glucosides [2] and designated A, B, and C according to increasing R_B-values [3] on paper chromatograms. It was further shown that compound A, named *glucosisymbrin*, on enzymic hydrolysis afforded (+)-4-methyl-2-oxazolidinethione with the absolute configuration presented in (I), from which a plausible structure could be deduced for the glucoside [1]. It is the purpose of the present paper to report on the chemical structure of glycoside C.

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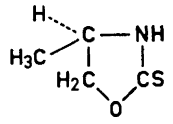
In the course of paperchromatographic studies of methanolic seed extracts of *S. austriacum* Jacq.* it was noticed that glycoside C on treatment with aqueous ammonia was converted into a much more hydrophilic glycoside, indistinguishable from glucosisymbrin on paper chromatography. This observation suggested a chemical relationship between the two. Enzymic hydrolysis of a paperchromatographically purified solution of glycoside C was accompanied by the liberation of *glucose*, whereas acid hydrolysis was employed to demonstrate the formation of sulphate and hydroxylamine. These results indicated that glycoside C was a glucoside of the customary structural type [4]. Consequently, and in view of its chemical character, unveiled in the sequel, the designation *glucobenzosisymbrin* is proposed for glucoside C.

On a preparative scale, it proved advantageous to remove the *iso*thiocyanate (benzosisymbrin) liberated from glucobenzosisymbrin on enzymic hydrolysis by steam distillation, since glucosisymbrin and glycoside B did not give rise to volatile enzymic hydrolysis products.

In view of the limited quantities on hand, no attempts were made to distil the oily *iso*thiocyanate isolated by ether extraction of the aqueous condensate. It was noticed, however, that the crude mustard oil on treatment with alkali was converted into a compound with a positive Grote reaction, which was indistinguishable from (+)-4-methyl-2-oxazolidinethione (I) on paper chromatography. Hence, the observed lability of the glucoside to alkali apparently



I

$C_6H_5COOCH_2CH_2CH_2NCS$

II

resided in the side-chain. The mustard oil was subjected directly to reaction with ammonia in attempts to produce a crystalline thiourea-derivative, suitable for characterization. Paperchromatographic analyses indicated that thiourea formation in aqueous or methanolic solutions of ammonia was accompanied by extensive ammonolysis, whereas treatment with anhydrous ammonia in chloroform under controlled conditions afforded the desired thiourea, yet contaminated by small amounts of an additional thiourea possessing a somewhat higher R_F-value in the solvent system: carbon tetrachloride: 30 % acetic acid (1:1)**. On a preparative scale, 165 g of seeds of *S. austriacum* Jacq. afforded 385 mg of a crystalline *dextro*rotatory thiourea (m.p. 122°) which was subjected to a 60-plate counter-current distribution in order to remove the above-mentioned thiourea contaminant. Thus, a homogeneous specimen was produced, devoid of notable acid or basic properties and possessing the elemental com-

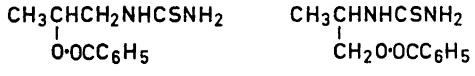
* The present investigation was carried out on seed material propagated in 1959—1960 in *The Botanical Garden of the University of Copenhagen* from the authentic stock employed in our previous studies.

** *Added in proof:* Since this work was submitted, the contamination has been identified as the higher homologue, 1-(1-ethyl-2-benzoyloxyethyl)-thiourea, arising from the glucoside glucobenzsisaustricin [15].

position $C_{11}H_{14}O_2N_2S$. Its ultra-violet absorption spectrum displayed maxima at 238 mμ and 205 mμ, characteristic for thioureas, yet with auxiliary low-extinction shoulders at 273 mμ and 280 mμ, suggestive of the presence of an aroyl grouping. A prominent band at 1 698 cm^{-1} in the infra-red spectrum (in KBr) was accordingly assigned to the C=O stretching mode of an aromatic ester, although this value is slightly below the range commonly quoted for such esters [5]. While reasonably stable towards acid, the new thiourea was readily cleaved in aqueous alkali to *benzoic acid* and a hydroxypropylthiourea, indicating its derivation from one of the isomeric benzoyloxypropyl *iso*thiocyanates.

One of these, *viz.* 3-benzoyloxypropyl mustard oil (II), was previously established in this laboratory as a product of enzymic hydrolysis of the glucoside glucomalcolmiin, encountered in seeds of the crucifer *Malcolmia maritima* (L.) R.Br.[6,7].

The stability of benzosisymbrin-thiourea, together with its optical activity, in fact restricted the possible structural formulations to (III) and (IV). The mustard oil whence (III) derives should be converted into a 5-methyl-2-oxazoli-

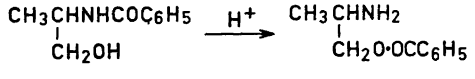


III IV

dinethione upon alkali hydrolysis of the ester grouping, whereas the isothiocyanate corresponding to (IV), subjected to the same treatment, should cyclize to a 4-methyl-2-oxazolidinethione (I). The *dextro*rotatory isomeride of the latter was previously isolated from the same seed material [1]. This fact, together with the abovementioned coinciding spots on paper chromatograms of authentic (I) and alkali-treated crude benzosisymbrin, rendered structure (IV) by far the most likely. A slight, but consistent difference in R_F-values between the 4- and 5-methyl-substituted 2-oxazolidinethiones in several solvent systems lent further support to structure (IV).

This conclusion was finally corroborated by an unambiguous synthesis of the enantiomer of benzosisymbrin-thiourea by a series of reactions providing full information as to its absolute configuration.

In the malcolmiin series it proved feasible to synthesize 3-hydroxypropyl *iso*thiocyanate which could be converted further into malcolmiin upon benzoylation [7]. The greatly enhanced tendency to intramolecular cyclization of aliphatic 2-hydroxy-substituted *iso*thiocyanates, compared to those of the 3-hydroxy-substituted series [7], made a similar approach to the desired 2-benzoyloxy*iso*propyl *iso*thiocyanate impracticable. Efforts were hence directed towards synthesis of the latter from the previously unknown 2-aminopropyl benzoate (VI), accessible as its hydrochloride from N-(2-hydroxy*iso*propyl)-benzamide (V) by acid-induced, intramolecular benzoyl migration. Despite a

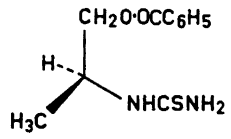


V VI

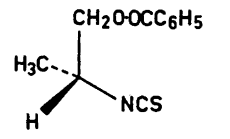
rapidly occurring reconversion of the ester base (VI) to the amide (V), conditions were found under which the rate of reaction of (VI) with thiocarbonyl chloride was sufficiently high, compared to that of the O→N migration, to produce an acceptable yield of the desired *iso*thiocyanate.

In a model experiment, DL-N-(2-hydroxy*iso*propyl)-benzamide (N-benzoyl-DL-alaninol (V)) [8] was transformed into the hydrochloride of DL-2-aminopropyl benzoate (VI) by anhydrous hydrogen chloride in ethanol, conditions similar to those previously employed in analogous reactions (*cf. e.g.* Ref.[9]). Addition of precisely three equivalents of triethylamine to a chloroform solution containing equimolar quantities of the hydrochloride of the racemic base (VI) and thiocarbonyl chloride, resulted in the formation of the racemic 2-benzoyloxy*iso*propyl *iso*thiocyanate which was, in turn, converted into the crystalline racemic thiourea (m.p. 133°) by treatment with ammonia in chloroform under controlled conditions.

Repetition of the above sequence of reactions, starting from optically pure L-alaninol [10], led to the production of *levo*rotatory 1-(2-benzoyloxy*iso*propyl)-thiourea (m.p. 122°) with the absolute configuration depicted in (VII). The synthetic specimen had the same m.p., R_F-value, ultra-violet and infra-red



VII



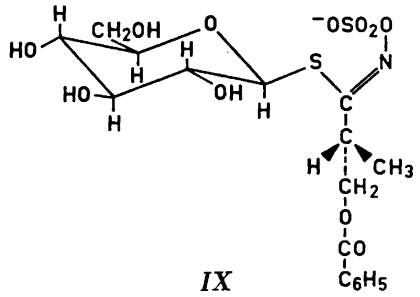
VIII

spectrum as naturally derived benzosisymbrin-thiourea, whereas its optical rotation was *similar in magnitude but opposite in sign* to that of the natural specimen. Recrystallization of a mixture of equal amounts of the natural and synthetic thiourea afforded the racemic compound, which melted undepressed in admixture with synthetic, racemic material. On the likely assumption that the enzyme-induced rearrangement leading to the *iso*thiocyanate proceeds with retention of configuration [4], structure (VIII) can be attributed to benzosisymbrin which therefore constitutes the benzoate of the hypothetic 2-hydroxy-*iso*propyl *iso*thiocyanate, liberated from glucosisymbrin on enzymic hydrolysis and undergoing spontaneous cyclization to (+)-4-methyl-2-oxazolidinethione (I). The establishment of identical absolute configurations in the cyclized and the benzoylated, noncyclizing *iso*thiocyanate, derivable from glucosides occurring in the same plant, strongly supports the view that such cyclizations proceed without configurational changes.

The combined evidence suggests that the genuine glucoside, glucobenzosisymbrin, on basis of the general structural expression of Ettlinger and Lundeen [4], possesses the structure (IX) and thus represents the second example of a natural glucoside of this type containing a benzoate grouping in the side-chain. The finding of benzoates in this series of glucosides is well in line with the frequently observed occurrence of benzoyloxy-substitutes among natural products (*e.g.* populin, coniferyl benzoate, cocaine, aconitine *etc.*). It appears

likely that the benzoyl grouping enters the glucosides at a late stage of their biosyntheses. A possible biosynthetic pathway to the 2-hydroxy*iso*propyl grouping was discussed in a previous paper[1].

The structure elucidation of glucoside B in seeds of *S. austriacum* Jacq. will form the subject of a forthcoming communication.



IX

EXPERIMENTAL

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. Infra-red spectra are determined in KBr pellets on a Perkin-Elmer "Infracord" instrument. Analytical specimens are dried *in vacuo* over calcium chloride at room temperature.

Exploratory paperchromatographic studies. An extract of 1.25 g of finely ground seeds of *Sisymbrium austriacum* Jacq. in 70 % methanol was evaporated to dryness, redissolved in water, filtered through Celite, and concentrated to a volume of 3 ml. This solution was applied to four sheets of filter paper (Schleicher and Schüll 2043 b) and chromatographed for 24 h with the upper layer of the solvent system *n*-butanol:ethanol:water (4:1:4) as the mobile phase. Narrow edge cuts from each sheet were sprayed with ammoniacal $AgNO_3$ in order to locate the three major glycoside bands (A, B, and C, *cf.* Ref.[1]) which were then cut out and eluted with water to a total volume of 2 ml for each glycoside. Control chromatograms of the eluate of glycoside C in the same solvent system gave an R_B-value of *ca.* 1.47, similar to the value 1.42 observed for a sample of glucomalcolmiin [7] run on the same chromatogram *.

Treatment of a 50 μl-portion of the eluate of glycoside C with conc.aqueous ammonia caused its rapid conversion into another glycoside, indistinguishable on the paper chromatogram from glucosisymbrin [1] (glucoside A). More than half of C had disappeared within one hour and after 28 h the conversion was complete.

Another 50 μl-portion of the eluate was buffered with 20 μl of a phosphate solution (pH 6.8) and 5 μl of the usual, cell-free myrosinase solution were added. Next morning, paper chromatography showed that the glycoside had been completely hydrolyzed, and other chromatograms, run in the same solvent system as well as in *n*-butanol:pyridine: water (6:4:3) and developed with the aniline-diphenylamine phosphate sugar reagent, indicated that *glucose* had been liberated during the enzymic fission.

When a 500 μl-portion of glucoside C, from here on designated *glucobenzosisymbrin*, was treated with conc. hydrochloric acid for 2 h at 60°, a hydrolytic fission took place, analogous to that described by Ettlinger and Lundeen [4] for similar glucosides. The forma-

* It has been repeatedly observed in this laboratory that R_B-values of glycosides migrating faster than glucotropaeolin may vary considerably according to the amount of glycoside applied to the paper, the type and orientation of the latter *etc.* This fact explains the observed deviations from previosuly reported R_B-values for glucomalcolmiin (1.36 [6,7]) and glycoside C (1.67 [1]) and indicates that identifications of such glycosides, solely based on R_B-values, are likely to be fallacious.

tion of sulphate and hydroxylamine was demonstrated by precipitation with Ba^{++} and paper chromatography [4], respectively.

Enzymic liberation of benzosisymbrin. Seeds of *Sisymbrium austriacum* Jacq. (165 g) were pulverized and defatted by treatment with three 400 ml-portions of carbon tetrachloride in a Waring blendor. The air-dried seed powder (115 g) was then exhaustively extracted by refluxing for 3 h with three 750 ml-portions of 70 % methanol. The combined filtrates were concentrated *in vacuo* to a dark syrup, foaming being controlled by the addition of small volumes of octanol. The residue was dissolved in water (700 ml) and the solution clarified by filtration through Celite. The dry matter in this solution amounted to 23.5 g.

The solution was now buffered to pH 6.8 by the addition of 50 ml each of 0.2 M solutions of primary and secondary phosphates. Then a few mg of ascorbic acid [11] and a cell-free myrosinase solution (10 ml) were added, and the mixture was set aside at room temperature. After a few hours, secondary phosphate was added to readjust the diminished pH-value to 6.8, and the mixture was left overnight. Next morning, it was steam-distilled and the distillate (about 1 l) was saturated with NaCl and continuously extracted for 24 h with ether. After drying, the solvent was removed through a column and the crude *iso*thiocyanate remained as a faintly yellow oil (675 mg).

The two oxazolidinethiones, deriving from glucosisymbrin [1] and glycoside B, remained unchanged in the enzymic hydrolysis mixture after steam distillation.

Formation of the thiourea derivative of benzosisymbrin. The above crude mustard oil (675 mg) was dissolved in 100 ml of a 2 N solution of anhydrous ammonia in chloroform and left standing at room temperature for 7.5 h when the volatile constituents were removed *in vacuo*. The oily residue crystallized in contact with benzene and was dissolved in this solvent (30 ml). On cooling, colourless prisms separated (385 mg), m.p. 121 – 121.5°. Paper chromatography, run with the lower layer of the solvent system CCl_4:30 % acetic acid (1:1) [12] as the mobile phase, revealed the presence of a minor thiourea (R_{Ph} 2.10) besides that of benzosisymbrin (R_{Ph} 1.50). Since attempts to remove the former by fractional crystallization were fruitless, recourse was taken to a 60-plate counter-current distribution in the same solvent system as employed for paper chromatography. Partial separation was thus achieved and a homogeneous specimen was readily obtained from the appropriate tubes in the Craig-apparatus. Before analysis, the thiourea was recrystallized once from benzene and twice from water, separating from the latter solvent in shiny plates, m.p. 121.0 – 121.5°, $[\alpha]_D^{25}$ + 65.3° (*c* 1.29, 96 % EtOH) (Found: C 55.45; H 5.99; N 11.45; S 13.39. Calc. for $C_{11}H_{14}O_2N_2S$: C 55.44; H 5.92; N 11.76; S 13.46). In 96 % ethanol, the UV-absorption spectrum exhibited a characteristic thiourea pattern, very similar to that of malcolmiin-thiourea[6]: λ_{max} 238 mμ (ε 19 500), λ_{max} 205 mμ (ε 15 000), λ_{min} 216 mμ (ε 11 000), and, in addition, two low-extinction plateaus at 273 mμ and 280 mμ. The infra-red spectrum (in KBr) showed a very strong band at 1 698 cm^{-1}, assigned to the C=O stretching mode of an aromatic ester. Other prominent bands were observed at: 3 350 s, 3 220 s, 3 110 m, 3 020 m, 2 930 m, 1 610 vs, 1 550 vs, 1 485 m, 1 445 s, 1 415 m, 1 385 m, 1 365 m, 1 345 s, 1 315 s, 1 280 vs, 1 170 s, 1 115 vs, 1 070 m, 1 020 m, 963 m, 942 w, 927 m, 858 w, 808 w, 718 vs, 688 w and 675 w cm^{-1} (vs very strong, s strong, m medium, w weak).

Alkali treatment of benzosisymbrin and its thiourea derivative. In consequence of the above-described lability of glucobenzosisymbrin towards alkali, the corresponding steam-volatile, crude mustard oil was exposed to 0.5 N NaOH in 50 % ethanol for 16 h at room temperature. The solution was then acidified and extracted with small volumes of chloroform. The organic phase gave a positive Grote reaction, and on chromatography in benzene:heptane:water (9:2:9), a spot was noticed at exactly the same position as that of a simultaneously chromatographed sample of authentic (+)-4-methyl-2-oxazolidinethione (I), derivable from glucosisymbrin. The isomeric 5-methyl-2-oxazolidinethione [6] could be excluded on basis of paper chromatograms, run with carbon tetrachloride saturated with 30 % acetic acid as the mobile phase [12]. In this solvent system, as well as others, the 4-isomeride migrated at a slightly higher rate than the 5-substituted ring compound.

When a solution of benzosisymbrin-thiourea (42 mg) in ethanol (10 ml) containing 0.1 N NaOH (10 ml) was kept for 24 h at room temperature, an acid could be extracted with ether after acidification. The solid acid was readily identified as *benzoic acid* by means of its infra-red spectrum which was indistinguishable from that of an authentic specimen.

Synthesis of DL-*1-(2-benzoyloxyisopropyl)-thiourea (IV).* DL-Alaninol was prepared by reduction of DL-alanine methyl ester with lithium aluminium hydride according to Karrer *et al.*[13] N-Benzoylation of the amino-alcohol was performed as described by Billman and Parker[8]. The crude DL-N-(2-hydroxy*iso*propyl)-benzamide (V) (3.8 g), m.p. 102—103° (reported: 107—108°[8], 104.5—106°[14]), was refluxed for 1 h with ethanol (40 ml) containing 10 mequiv. of anhydrous HCl per ml. The solution was then concentrated to about 15 ml and ether was added to precipitate the previously unknown *hydrochloride of* DL-*2-aminopropyl benzoate* (VI) (1.08 g), yet heavily contaminated with alaninol hydrochloride, formed by ethanolysis of the benzoate during the reaction, as apparent from paperchromatographic analysis in *n*-butanol:ethanol:water (4:1:4) (spray reagent:ninhydrin). Additional crops from the mother liquor raised the total yield to 1.81 g (40 %). Complete separation of the two hydrochlorides was achieved by a 60-plate countercurrent distribution with *n*-butanol:ethanol:water (4:1:4) as the solvent system. The contents of tubes No. 18—32 afforded the homogeneous ester hydrochloride which was recrystallized twice from anhydrous ethanol:ether mixtures (1:2) before analysis. The salt separated in colourless needles, m.p. 175° (Found: C 55.53; H 6.64; N 6.30; Cl 16.55. Calc. for $C_{10}H_{14}O_2NCl$: Cl 55.68; H 6.54; N 6.50; Cl 16.44). The infra-red spectrum exhibited the expected C=O ester bond at 1 710 cm^{-1}. The procedure employed is analogous to that employed by Philips and Baltzly[9] for analogous intramolecular N→O acyl migrations.

To a stirred solution of the crude ester hydrochloride (500 mg) and thiocarbonyl chloride (276 mg) in chloroform (16 ml), a total of 950 µl of triethylamine (theoretical: 980 µl) was dropwise added in the course of 5 min, resulting in disappearance of the thiophosgene colour. After 2 h at room temperature, the chloroform solution was washed with 1 N HCl and water, dried over Na_2SO_4, and made 2N with regard to ammonia (total volume 60 ml). At the end of 4 h, the solvent was removed and the semi-crystalline residue was dissolved in benzene and treated with charcoal. On cooling, almost colourless prisms separated (141 mg), m.p. 128—129°. An analytical sample of DL-*1-(2-benzoyloxyisopropyl)-thiourea* (IV) was produced on two additional crystallizations, first from benzene and then from water. The colourless prisms melted at 133° (Found: C 55.64; H 6.01; N 11.75; S 13.39. Calc. for $C_{11}H_{14}O_2N_2S$: C 55.44; H 5.92; N 11.76; S 13.46). On paper chromatography in water-saturated chloroform and carbon tetrachloride:acetic acid, the synthetic thiourea was indistinguishable from the optically active thiourea-derivative of benzosisymbrin. The infra-red spectra, however, were, as expected, distinctly different outside the "double-bond region".

Synthesis of (—)-1-(2-benzoyloxyisopropyl)-thiourea (VII). The sequence of reactions described above was employed with minor modifications in the optically active series for the synthesis of the enantiomer of benzosisymbrin-thiourea.

L-Alaninol, $[a]_D^{25}$ + 22.1° (*c* 7.2, 96 % EtOH), was produced by reduction of L-alanine with $LiAlH_4$ in tetrahydrofuran as described[10]. N-Benzoylation proceeded in 88 % yield as reported for the racemic alaninol[8] to give *(—)-N-(2-hydroxyisopropyl)-benzamide* ((—)-N-benzoyl-L-alaninol) separating from benzene in flat needles, m.p. 131.5—132.5°, $[a]_D^{25}$ —2.9° (*c* 7.4, 96 % EtOH)] (Found: C 67.04; H 7.25; N 7.81. Calc. for $C_{10}H_{13}O_2N$: C 67.02; H 7.31; N 7.82). The infra-red spectrum contained the expected C=O amide band at 1 640 cm^{-1}.

A somewhat better yield of a less contaminated product than that produced in the racemic series was obtained in the following way: an anhydrous ethanol solution (50 ml), containing the active N-benzoyl-alaninol (5.0 g) and anhydrous HCl (131 mequiv.), was heated in an open flask on the steam bath for 90 min, when about half of the solvent had evaporated. Addition of ether (100 ml) and cooling overnight yielded the *dextro*rotatory *hydrochloride* of *2-aminopropyl benzoate* (3.26 g, 54 %), including material obtained from the mother liquor. A minor contamination of alaninol hydrochloride, revealed by paper chromatography as above, could be removed by repeated recrystallizations from mixtures of anhydrous ethanol and ether (1:2). A homogeneous specimen exhibited the m.p. 195°, $[a]_D^{25}$ + 21.5° (*c* 1.73, H_2O) (Found: C 55.85; H 6.48; N 6.40; Cl 16.53. Calc. for $C_{10}H_{14}O_2NCl$: C 55.68; H 6.54; N 6.50; Cl 16.44). The infra-red spectrum possessed the expected bands.

The conversion of the aminoester hydrochloride into the *levo*rotatory thiourea (VII) was performed exactly as described above in the racemic series. A product resulted which

after two recrystallizations from benzene and two from water separated in beautiful plates of *(—)-1-(2-benzoyloxyisopropyl)-thiourea* possessing the absolute configuration depicted in (VII), m.p. 122.0—122.8° (Found: C 55.48; H 5.85; N 11.71; S 13.78. Calc. for $C_{11}H_{14}O_2N_2S$: C 55.44; H 5.92; N 11.76; S 13.46). The rotation was $[\alpha]_D^{24}$ —64.3° (*c* 1.28, 96 % EtOH), compared with the value + 65.3° of benzosisymbrin-thiourea. The naturally derived and synthetic samples possessed identical UV-spectra, R_F-values and infra-red curves. Hence, there can be no doubt as to the enantiomeric relationship of the two preparations.

When equal amounts of the two antipodes were mixed and recrystallized from benzene, small prisms separated, m.p. 130°, alone or in admixture with the synthetic racemic compound. The infra-red spectra of the two preparations were identical.

Microanalyses were performed by Messrs. G. Cornali and W. Egger. The authors are grateful to the *Botanical Garden of the University of Copenhagen* for valuable help in propagating the employed seed material.

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