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A New Thioglucoside, (*R*)-2-Hydroxy-3-butenylglucosinolate from *Crambe abyssinica* Seed*

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ABSTRACT: The major thioglucosidic constituent of seed from *Crambe abyssinica* Hochst ex R. E. Fries was isolated and shown to be a salt of (*R*)-2-hydroxy-3-butenylglucosinolate. This thioglucoside differs only in stereochemical configuration from the glucorapiferin (progoitrin) which occurs in a number of related plants in the mustard family. On enzymatic hydrolysis the new thioglucoside is converted to the enantiomer of goitrin, a physiologically active degradation product of glucorapiferin.

An example is thus provided of the occurrence in nature, in closely related species, of substances differing only in configuration at a single asymmetric center.

Seed from *Crambe abyssinica* Hochst ex R. E. Fries contains relatively large amounts of thioglucosides, including one or more that yields oxazolidinethione-type compounds on mustard myrosinase hydrolysis (VanEtten *et al.*, 1965). Thioglucoside hydrolysis to yield oxazolidinethiones has also been demonstrated to occur microbiologically (Greer, 1962). Since defatted *Crambe* seed meal may be used as a component of animal feeds and since oxazolidinethiones are known to possess physiological activity, it seemed desirable to investigate the thioglucosides of *Crambe*. Kjaer (1960) reviewed the occurrence of thioglucosides in the crucifer and closely related botanical families, and he showed the considerable diversity of their aglycons. We deal with the isolation and chemical characterization of the principal thioglucoside of *Crambe* seed.

Experimental

Crude Thioglucoside Preparation. Mature *Crambe* seed was obtained from a 1961 crop grown in Texas. The separation of thioglucosides from the crude extract of seed meal was similar to that employed by Kjaer *et al.* (1956). After removal of the pericarp the seed was flaked, the flakes were pentane-hexane extracted at room temperature, and the meal was ground to pass a 100-mesh screen. A 165-g sample of air-dried meal was shaken for 1 hour with 1500 ml of 80% (v/v) acetone-water. The solids were then removed in a continuous-type centrifuge. The residue meal was reextracted with two 750-ml volumes of the solvent. The combined aqueous acetone extracts were concentrated *in vacuo* at 45° to a volume of about 300 ml. After standing at refrigerator temperature overnight the precipitate that formed was removed by centrifugation. A 36 × 3.5-cm column was prepared from a water suspension of 300 g of acidic alumina (Camag) and washed with 1–2 liters of water. The supernatant extract was passed through the anionotropic column followed by a water wash of 2–3 liters. The thioglucosides were eluted with a 1% K_2SO_4 solution. Those eluate fractions (20-ml volumes) which contained thioglucosides were combined. The thioglucoside-containing fractions were

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detected by thin-layer chromatography on silica gel (Merck) essentially as described by Stahl and Kaltenbach (1961), using ethyl acetate–65% aqueous 2-propanol (53:47) as developing solvent. The spots were detected with either anisaldehyde–sulfuric acid (Stahl and Kaltenbach, 1961) or alkaline silver nitrate (Trevelyan *et al.*, 1950). Usually the fractions combined were numbers 10 through 30. Fractions higher than 30 generally contained orange-brown colored material and only small amounts of thioglucosides. The combined fractions were lyophilized, and the dried material was triturated with absolute methanol and filtered to remove inorganic sulfate (Gmelin and Virtanen, 1959). The filtrate was concentrated to near-dryness under vacuum at 49° and dried under vacuum over phosphorus pentoxide. The nearly white hygroscopic crude glucoside weighed 7–8 g and analyzed (*vide infra*) as 90 ± 5% pure when calculated as the potassium salt of glucorapiferin (progoitrin). Analysis also showed that less than 5% of the thioglucosides formed volatile isothiocyanates.

The noncrystalline product gave $[\alpha]_D^{25}$ −25° (*c*, 3.4; water) and had the same paper chromatographic mobility as glucorapiferin chromatographed with the upper phase of the solvent system butanol-ethanol-water (4:1:4).

In this isolation procedure, determinations of the thioglucoside content were followed by measuring the products formed after hydrolysis with mustard myrosinase prepared according to Schwimmer (1961). The oxazolidinethione formed by the enzyme at *p*H 6.0–7.0 was estimated in a manner similar to that described by Astwood *et al.* (1949). The liberated sulfate was estimated as described by VanEtten *et al.* (1965) and the volatile isothiocyanates by the method of Wetter (1955). Some loss of thioglucoside occurred by enzyme conversion during the aqueous acetone extraction. Additional losses were incurred in subsequent purification steps. The total amount of thioglucoside recovered in isolation was estimated as 60% of that initially present as determined by the sulfate method.

Preparation of Thioglucoside Pentaacetate. After the crude *Crambe* thioglucoside (2.6 g) was dissolved in 32 ml of dry redistilled pyridine, 32 ml of redistilled acetic anhydride was added. After 24 hours at room temperature, the solution was decanted to separate it from a small amount of solids and stored in a refrigerator for 3 days. Removal of volatiles under reduced pressure at 50° left a crystalline mass. Recrystallization from 95% ethanol yielded 3.2 g.

Anal. Calcd for $C_{21}H_{28}O_{15}NS_2K$ (637): C, 39.6; H, 4.4; N, 2.2; S, 10.1; K, 6.1; acetyl, 33.8. Found: C, 39.6; H, 4.6; N, 2.1; S, 10.3; K, 6.3; acetyl, 32.9. Optical rotation $[\alpha]_D^{25}$ −14.8 and −15.1° (two preparations) (*c*, 1.7; water).

Isolation of 2,4-Pentadienoic Acid from Acid Hydrolysis of Thioglucoside. The crude thioglucoside (2.4 g), dissolved in 15 ml of 6 N hydrochloric acid, was allowed to stand at room temperature overnight and then heated in a boiling-water bath for about 45 minutes. The hydrolysate was centrifuged and the supernatant was extracted three times (2–3 volumes each extraction) with pentane-hexane. The combined extracts were concentrated to near dryness and sublimed *in vacuo* at 35–40°. Yield, 53 mg of white hygroscopic crystals which were removed from the sublimation apparatus in a dry atmosphere, mp 53–56°.

The infrared spectrum in carbon disulfide included absorption at wavelengths, in microns, as follows: 3–4 (short-chain free acid), 5.95 (carbonyl), 9.97 (conjugated dienone), and 10.82 (terminal unsaturation). The material was estimated to be 85% pure by gas chromatography, with a thermal conductivity detector. The major component had an equivalent chain length (ECL, Miwa *et al.*, 1960) of 7.5 on a 200 × 0.6-cm glass column packed with 20% LAC-2R-446 (Cambridge Industries Co., Inc.)[1] polyester on 60/80 mesh Celite (Johns-Manville).

Anal. Calcd for $C_5H_6O_2$ (98): C, 61.2; H, 6.1; neutral equivalent, 98. Found: C, 61.5; H, 7.1; neutral equivalent, 90. Ultraviolet analysis: λ_{max} 248 mμ (isooctane); λ_{max} 244 mμ (ethanol, ε 21,500). Reported for 2,4-pentadienoic acid (mp 71–73°) (Jones *et al.*, 1954) λ_{max} 242 mμ (ethanol, ε 24,800).

The unsaturated acid was hydrogenated with palladium-on-charcoal catalyst by the method of Ogg and Cooper (1949). Hydrogen uptake was 1.8 moles/mole compared with the theory of 2 moles for 2,4-pentadienoic acid. After removal of catalyst, gas-liquid chromatography of the hydrogenated product gave a retention time identical to that of *n*-valeric acid run under the same conditions. A *p*-bromophenacyl derivative of the hydrogenated acid and also of *n*-valeric acid were prepared by the micro method of Stodola (1963). Each *p*-bromophenacyl derivative gave a melting point of 71–72°, which showed no depression of melting point when the two were mixed. X-Ray patterns of the two derivatives were identical.

Identification of Glucose and Sulfate as Products of Enzymatic Hydrolysis. The liberation of sulfate from the myrosinase hydrolysis was determined quantitatively by titration with 0.005 M barium perchlorate. No free glucose was found in the glucoside preparation, but it was readily detected and identified, following treatment with myrosinase, by thin-layer chromatography according to Stahl and Kaltenbach (1961). The sugar was further identified by its migration on paper in butanol-ethanol-water (4:1:4), upper phase, and detection of the spot by spraying the chromatogram with a mixture of glucose oxidase, peroxidase, and *o*-dianisidine which is specific for D-glucose (Glucostat reagent; Worthington Biochemical Corp., Freehold, N.J.). Appropriate blanks of enzyme and buffer preparations used for the hydrolysis were run on the same chromatograms.

Isolation of (R)-5-Vinyloxazolidine-2-thione[2] *from*

[1] The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

[2] The (*R*) and (*S*) terms are defined in Cahn *et al.* (1956).

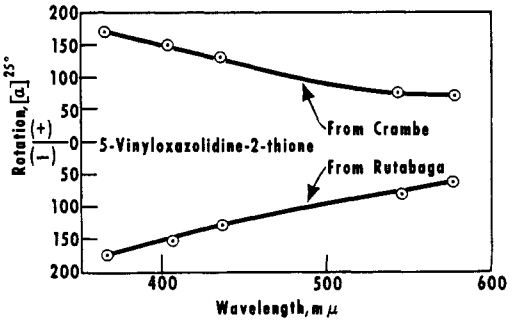


FIGURE 1: Rotatory dispersion curves: (upper) (*R*)-5-vinyloxazolidine-2-thione from *Crambe* (*c*, 1.06% MeOH); (lower) (*S*)-5-vinyloxazolidine-2-thione from rutabaga (*c*, 1.02% MeOH).

Crambe. The glucoside preparation (6 g) was dissolved in *p*H 7.0 buffer (384 ml 0.2 N sodium hydroxide plus 600 ml 0.2 M potassium dihydrogen phosphate diluted to 1200 ml) to which was added 600 mg mustard myrosinase prepared according to Wrede (1941). The buffered solution was held at 37° for 4 hours and the resulting hydrolysate was extracted with three 1200-ml volumes of chloroform. The combined extracts were dried over anhydrous sodium sulfate, filtered, and concentrated to a yellow oil. The oil was suspended in 25–30 ml of warm water, and after chilling the compound slowly crystallized. Yield of crude crystalline product, 1.2 g. The compound was recrystallized to a constant melting point of 47–48°. Synthetic (*R*)-5-vinyloxazolidine-2-thione melted at 50–51° (Ettlinger, 1950).

Anal. Calcd for C_6H_7ONS (129): C, 46.5; H, 5.4; N, 10.8; S, 24.8. Found: C, 46.7; H, 5.5; N, 10.7; S, 24.2. Optical rotation $[\alpha]_D^{25}$ +75.1° (*c*, 2.50; chloroform).

A nuclear magnetic resonance spectrum of the compound was run in deuteriochloroform with tetramethylsilane as internal standard.

Isolation of (S)-5-Vinyloxazolidine-2-thione (Goitrin) from Rutabaga Seed. The source of goitrin was seed from *Brassica napobrassica* (Mill), American purple rutabaga, from which the seed coat (10% by weight) had been removed. Isolation was carried out as described for the thioglucoside and the enantiomer from *Crambe*. From 165 g of meal 5.2 g of the crude thioglucoside was obtained. Enzymatic hydrolysis of 4 g gave 0.67 g of crude goitrin. After recrystallization the melting point was 47–48°; reported 50° (Astwood *et al.*, 1949).

Anal. Calcd for C_6H_7ONS (129): C, 46.5; H, 5.4; N, 10.8; S, 24.8. Found: C, 46.7; H, 5.6; N, 10.6; S, 24.3. Optical rotation $[\alpha]_D^{25}$ −76.8° (*c*, 2.0; chloroform); reported for goitrin (Astwood *et al.*, 1949) $[\alpha]_D^{25}$ −70.5° (*c*, 2.0; methanol).

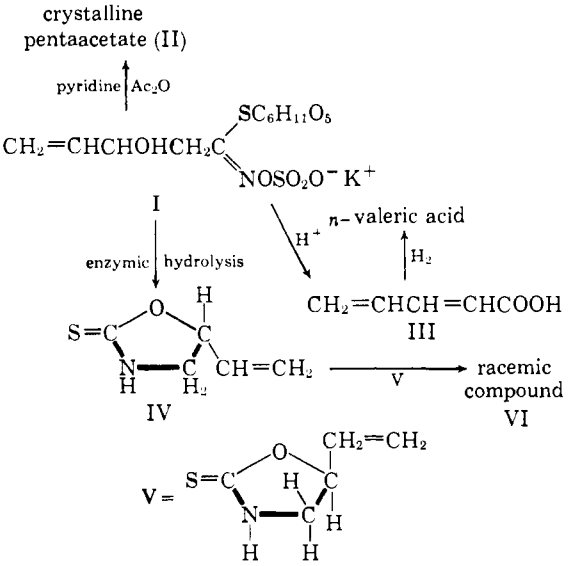
Comparison of (R)-, (S)-, and Racemic 5-Vinyloxazolidine-2-thione. Equal weights of the compounds from *Crambe* and rutabaga seeds were dissolved in acetone. The crystalline product formed on evaporation of the solvent had a melting point of 61–62°. Melting point for synthetic *dl* racemate 64–65° (Ettlinger, 1950). Optical rotations over the wavelengths 365–580 mμ for the two compounds are given in Figure 1.

Infrared spectra in potassium bromide disks and X-ray patterns for each compound were identical. The infrared spectra and the X-ray patterns for the racemate obtained from the two isolates were identical to those from an authentic sample of the synthetic racemate. Their infrared absorption and X-ray patterns showed small differences from those of the *d* and *l* isomers.

Melting points obtained with a Fisher-Johns melting point apparatus were uncorrected. Ultraviolet spectra were obtained with a Cary recording instrument; infrared spectra with a Perkin-Elmer Model 137 Infracord; and the nuclear magnetic resonance spectrum with a Varian Associates, Model A60 NMR[3] spectrometer. Rotatory dispersion measurements were obtained with an O. C. Rudolph and Sons Model 200 spectropolarimeter adapted to a Bellingham and Stanley polarimeter with quartz optics and a Hanovia Model SH mercury lamp with a Bausch & Lomb monochromator. The instrument measured optical rotations at specific wavelengths over the range 313–580 mμ. Gas chromatograms were obtained on a Burrell Kromotog Model K5.

Results

The *Crambe* thioglucoside (compound I) obtained from defatted seed appeared to be different from the known progoitrin since it could not be crystallized when appropriate solutions were seeded with authentic samples of that compound. Yet all analytical data on the pentaacetate (compound II) derived from it were



in agreement with those of progoitrin pentaacetate except for the optical rotation. Thioglucoside I was readily converted by mustard myrosinase hydrolysis to

[3] Abbreviation used in this work: NMR, nuclear magnetic resonance.

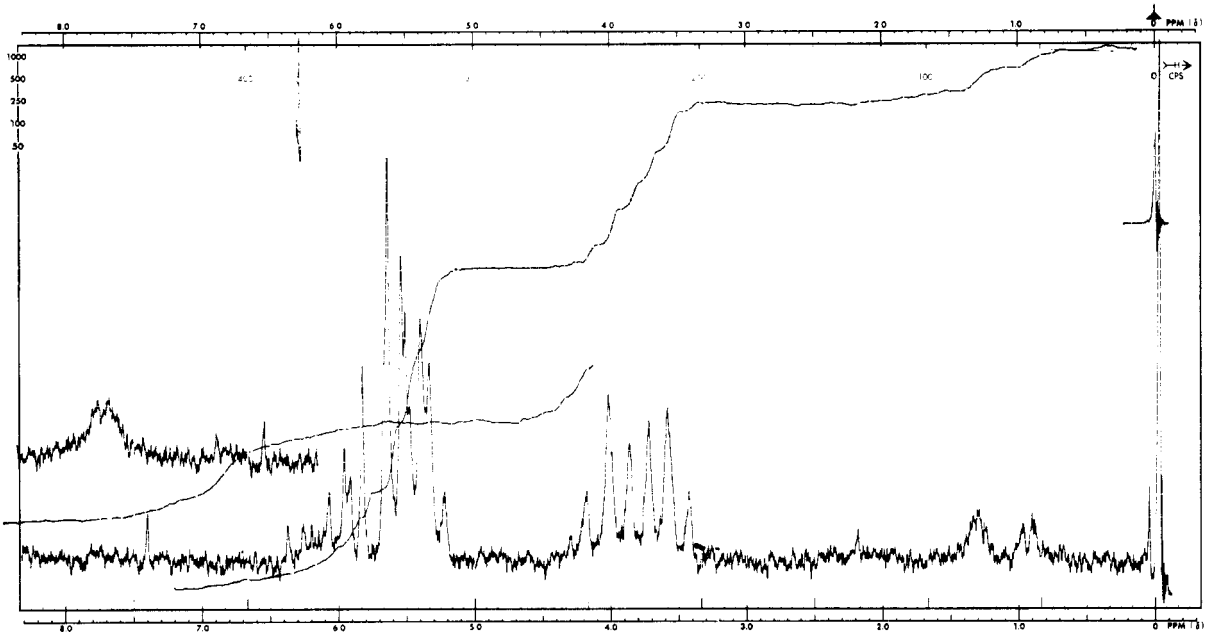


FIGURE 2: NMR spectrum of (*R*)-5-vinyloxazolidine-2-thione in $CDCl_3$; internal standard, $(CH_3)_4Si$.

an oxazolidinethione (compound IV), not identical with the known (*S*)-5-vinyloxazolidine-2-thione (compound V) (goitrin), although again alike in all analytical data with the exception of optical rotation.

Two structural possibilities for compound IV merited consideration. It might have been a positional isomer of compound V having substitution in the 4 position or the enantiomer of compound V.

Evidence for the vinyl group in the 5 position on the oxazolidinethione was obtained by the isolation of 2,4-pentadienoic acid (compound III) from mineral acid hydrolysis of the thioglucoside. The ultraviolet absorption of the acid was in agreement with that for conjugation in a straight-chain compound in contrast to conjugation in a branched chain (Nair and Adams, 1961). Identification of the hydrogenated product with *n*-valeric acid by gas chromatography and by X-ray patterns of their *p*-bromophenacyl derivatives established the identity of the acid. Substitution of the vinyl group in the 4 position on the oxazolidinethione would require that the hydroxyl of the thioglucoside be on a branch chain; for example, see Kjaer and Christensen (1959). The reactions involved during the hydrolysis assume dehydration and hydrolysis of an intermediate β-hydroxynitrile. Analogous acids without a hydroxyl group have been isolated from the mineral acid hydrolysis of other thioglucosides, such as vinylacetic acid from sinigrin and *p*-hydroxyphenylacetic acid from sinalbin, by Ettlinger and Lundeen (1956) as part of their proof of the structure of thioglucosides.

The NMR spectrum (Figure 2) of compound IV, when compared with spectra of related compounds reported by Kjaer and Thomsen (1962), is also in accord with formulation of the vinyl group in the 5 position on the oxazolidinethione ring. Absence of significant absorption at 4–5 δ (the range for the (5)-CH_2O multiplet) and the appearance of absorption at 3–4 δ (the range of the (4)-CH_2 protons) clearly demonstrates that the oxazolidinethione is substituted in the 5 position.

Since compound IV had an optical rotation equal in magnitude and opposite in sign to V, its unequivocal characterization as the enantiomer of compound V was made by comparison of properties with authentic V isolated from enzymic hydrolysis of rutabaga seed thioglucoside. The agreement of the mixed melting point of the two compounds with that of the synthetic racemate reported by Ettlinger (1950), which in this case melts higher than the *d* and *l* forms, the optical rotatory dispersion curves (Figure 1), the identical infrared absorption curves, the identical X-ray patterns, and the identical X-ray pattern of the racemate formed from the two compounds with that of an authentic synthetic *dl*-5-vinyloxazolidine-2-thione confirm their identity as optical isomers. Physical constants established by synthesis of *d*-, *l*-, and the racemate of 5-vinyloxazolidine-2-thione by Ettlinger (1950) aided in the identification.

The prior establishment of the absolute configuration of the (*S*)-5-vinyloxazolidine-2-thione (goitrin) by Kjaer *et al.* (1959), named according to the system of Cahn *et al.* (1956), provides a ready assignment of the absolute configuration of the compound from *Crambe* seed as (*R*)-5-vinyloxazolidine-2-thione.

Myrosinase hydrolysis of the thioglucoside also formed sulfate ion and D-glucose. On the assumption that no change in configuration occurs about the asymmetric carbon atom containing the hydroxyl in the side chain during the enzymatic conversion and cyclization to form a ring compound, the thioglucoside I from *Crambe abyssinica* seed differs from glucorapiferin

(progoitrin from rapeseed) only in the configuration about the carbon atom with the hydroxyl group. Ettlinger and Lundeen (1956) suggest that retention of configuration probably occurs during the intramolecular rearrangements accompanying the enzymic reaction on thioglucosides. Kjaer and co-workers (Kjaer *et al.*, 1959; Kjaer and Christensen, 1959, 1962) in their research on glucosisymbrin, glucosisaustricin, and progoitrin also state that the same configuration probably prevails in the side chain of the parent thioglucosides as in the enzymatically formed oxazolidinethiones.

Discussion

Following convention, the thioglucoside newly characterized in this work might have been called glucocrambin. However, in order to show better the interrelationships among naturally occurring thioglucosides and their degradation products, we choose not to coin a new trivial name. Rather, the new thioglucoside may be termed *epi*-glucorapiferin or *epi*-progoitrin and the derived (*R*)-5-vinyloxazolidine-2-thione may be referred to as (*R*)-goitrin, to associate them with the structurally related well-known compounds of biochemical interest derived from rapeseed. We have found very useful the nomenclature proposed by Ettlinger and Dateo (1961) by which the glucoside may be referred to as a salt of the (*R*)-2-hydroxy-3-butenylglucosinolate ion.

The name (*R*)-goitrin also seems appropriate for the (+)-5-vinyloxazolidine-2-thione because of its apparent biological activity. Greer (1962) reports synthetic *dl*-goitrin to have the same antithyroid activity as the *l* isomer alone, as measured by uptake of radioactive iodine by the thyroid. Since crystalline progoitrin also has antithyroid activity when assayed by the same method (Greer and Deeney, 1959), one might expect *epi*-glucorapiferin from *Crambe* also to be active.

Both (*S*) and (*R*) spatial configurations have been found in naturally occurring thioglucosides of *Cruciferae* and closely related plant families and in oxazolidinethiones derived enzymatically from those thioglucosides (Kjaer and Gmelin, 1957, 1958; Kjaer *et al.*, 1959). Goitrin has been isolated from the seed of turnip, cabbage, kale, and rape by Astwood *et al.* (1949) and was first shown by Kjaer *et al.* (1959) to have the (*S*) configuration. In view of such prior studies, isolation of the (*R*) compounds *epi*-glucorapiferin and (*R*)-goitrin in the present work is not surprising. Yet to our knowledge this isolation represents the first instance where the stereochemically dissimilar compounds found were structurally identical except for configuration about a single asymmetric carbon atom. Earlier examples involved the presence of different hydrocarbon radicals as well as relationship to a different stereochemical series. Thus another example is added to the growing list of compounds occurring in nature as enantiomers, such as (+)- and (−)-borneol, (+)- and (−)-camphor, (+)- and (−)-lactic acid, and (+)- and (−)-pinene, and the D-amino acids from microorganisms (Greenstein and Winitz, 1961) and from a plant (Kjaer and Larsen, 1963).

A possible biogenetic relationship between the naturally occurring thioglucosides and the α-amino acids was first suggested by Kjaer (1954) and subsequently discussed in several papers from his laboratory, including his excellent review on the thioglucosides (1960). Evidence to support such a relationship between mustard oil glucosides and either amino acids or their keto acid analogs as precursors has recently been provided by Benn (1962) and by Underhill *et al.* (1962). However, many biosynthetic questions appear unresolved as to the agreement on this possible origin from amino acids. A precursor which would account for the presence in nature of both glucorapiferin and its epimer may be related to optically active forms of the compound $CH_2{=}CH{-}CHOH{-}CH_2{-}CHNH_2COOH$. In the absence of experimental evidence, it is tempting to speculate that lysine may be implicated at some stage of these biosynthetic transformations.

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