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The Synthesis and Some Properties of the Thiosulfonate Analogue of Glutathione (γ-L-Glutamyl-L-3-thiosulfoalanyl-glycine)

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γ-L-Glutamyl-L-3-thiosulfoalanyl-glycine has been synthesized from glutathione disulfide. The compound was weakly active as a sulfur donor for rhodanese and had no inhibitory action on glutathione reductase.

Owing to their possible natural occurrence, the thiosulfonate analogues of naturally occurring thiol compounds are of biochemical interest. Thiosulfonates have thus been shown to participate in certain enzyme-catalyzed reactions [1–3] as substrates or products. Furthermore, after the administration of cystine [4] to rats, the thiosulfonate analogue of cysteamine (thiotaurine) is excreted in the urine. Among the thiosulfonates related to naturally occurring thiol compounds, only the analogues of cysteamine [5,6] and of cysteine [7,8] have been synthesized. The present paper describes the synthesis of the thiosulfonate analogue of glutathione.*

The compound was prepared from GSSG, which had first been oxidized with hydrogen peroxide-formic acid (*cf.* Ref. 10) to give the thiolsulfonate ester.

$$GSSG + 2H_2O_2 \rightarrow GSO_2SG + 2H_2O$$

The latter was treated with ammonia to give the sulfinate according to Ref. 11.

$$3GSO_2SG + 4OH^- \rightarrow 4GSO_2^- + GSSG + 2H_2O$$

The sulfinate was finally converted to the thiosulfonate with ammonium polysulfide.[5]

$$GSO_2^- + (NH_4)_2S_n \rightarrow GSO_2S^- + (NH_4)_2S_{n-1}$$

* The nomenclature of the sulfur oxy-acids derived from naturally occurring thiol compounds is often ambiguous or incorrect, as pointed out by Savige and MacLaren.[9] If the "prefix system" of these authors is used, the correct name for the thiosulfonate analogue of glutathione should be γ-L-glutamyl-L-3-thiosulfoalanyl-glycine. In this paper the abbreviations GSSG, GSO_2SG, GSO_2H, GSO_2SH, and GSO_3H will be used for the disulfide, thiolsulfonate ester, sulfinic acid, thiosulfonic acid, and sulfonic acid derived from glutathione (GSH).

An alternative would be a direct conversion of GSO_2SG to GSO_2SH by hydrogen sulfide [7] according to

$$2GSO_2SG + H_2S \rightarrow GSO_2SH + GSO_2H + GSSG$$

However, the crude GSO_2SG was found to be contaminated with GSO_3H which is very difficult to separate from GSO_2SH (or GSO_2SG), whereas the separation of GSO_3H from GSO_2H could be easily achieved. It should be added that GSO_2H has previously been synthesized by Calam and Waley [12] through dismutation of GSSG with silver ions, but as the product obtained by this reaction may be contaminated with silver ions, which strongly inhibit enzymes, we developed the synthetic route outlined above.

As other thiosulfonates have been shown to function as sulfur donors in transsulfuration reactions catalyzed by rhodanese,[1,5] the ability of GSO_2SH in this respect was investigated. The compound was thereby found to be only 1.7-fold as active as thiosulfate and thus, in comparison to thiotaurine or ethanethiosulfonate,[5] a poor substrate for rhodanese. This may be due to steric hindrance by the comparatively bulky peptide chain of GSO_2SH or to the fact that the latter at neutral pH contains two negative charges in the molecule (as in the case of thiosulfate) whereas the more active thiosulfonates contain only one negative charge.

Thiosulfonates show a structural resemblance to disulfides and may act as inhibitors for enzymes using the corresponding disulfides as substrates. GSO_2SH was tested as an inhibitor for glutathione reductase as this enzyme is inhibited by S-sulfoglutathione ($GSSO_3H$),[13] another glutathione derivative with structural similarities to glutathione disulfide. However, at 7×10^{-4} M concentration, GSO_2SH had no significant effect on glutathione reductase when the latter was assayed with GSSG as substrate between 6×10^{-5} M and 3×10^{-3} M concentrations. Neither was GSO_2SH active as a substrate for glutathione reductase.

EXPERIMENTAL

Materials. Glutathione disulfide (the ethanol complex, containing 80 % GSSG) was obtained from C. F. Boehringer & Soehne, Germany. Crystalline beef liver rhodanese was prepared according to Sörbo [14] and beef liver glutathione reductase according to Racker.[15]

Methods. Nitrogen analysis of GSO_2SH was carried out by the Kjeldahl method, as it was found that determinations by the Dumas method consistently gave too low values. Cyanide-labile sulfur was determined according to Sörbo [5] and total sulfur by the Microanalytical Laboratory of the University of Uppsala. The ability of GSO_2SH to serve as a sulfur donor for rhodanese was studied in the test system previously [5] described. Glutathione reductase activity was assayed spectrophotometrically according to Racker.[15]

Synthesis. GSSG, 1.84 g (2.4 mmole), was dissolved in 15 ml formic acid, containing 0.30 ml conc. hydrochloric acid, and hydrogen peroxide (0.75 ml of a 30 % solution) was then slowly added in 0.05 ml portions with efficient stirring. The solvents were then removed at room temperature in a rotary evaporator and the residue was dissolved in a few ml of water and concentrated to a syrup in order to remove any remaining hydrogen peroxide or solvent. This step was repeated once. The residue was dissolved in a few ml of water and the pH adjusted to about 6 with conc. ammonia. The solution was concentrated in a rotary evaporator and the crude GSO_2H was dissolved in 3 ml 1 M HCl. This solution was applied to a column (3 × 15 cm) of Dowex 50 W – X 12 (100–200 mesh) in the hydrogen form and eluted with water. GSO_3H appeared in the first 50 ml of effluent and the sulfinate was then recovered in the following 200 ml of eluate. This

fraction was taken to dryness in a rotary evaporator and the residue dissolved in 4 ml of 1 M ammonia. To this was added an ammonium polysulfide solution, prepared by treating a suspension of 260 mg of sulfur in 2.5 ml of conc. ammonia with hydrogen sulfide until all sulfur had dissolved. The mixture was left at room temperature for 30 min and was then concentrated in a rotary evaporator. The residue was suspended in 15 ml of 0.1 M ammonia and undissolved sulfur removed by centrifugation. The clear solution was then taken to dryness at room temperature, a glassy residue being thereby obtained. This was dried *in vacuo* over silica-gel to give an initially amorphous product which gradually changed into a crystalline mass. The latter contained 2.0 mmole of thiosulfonate determined as cyanide-labile sulfur. Paper chromatography in 4 different solvent systems (acetone-water (3:1) R_F 0.77, phenol-water (4:1) R_F 0.04, 2,4-lutidine-water (3:1) R_F 0.06 and butanol-acetic acid-water (4:1:1) R_F 0.04) showed that the product contained only one ninhydrin- and iodoplatinate-positive component. Paper electrophoresis at pH 1.9 (formic acid-acetic acid-water) also showed only one ninhydrin-positive component, which migrated towards the anode with the same mobility as GSO_3H, but, in contrast to the latter, was iodoplatinate-positive, as expected for a thiosulfonate. If the product was treated with potassium cyanide, paper electrophoresis showed the formation of a ninhydrin- and iodoplatinate-positive component, which migrated as GSO_2H. These data were consistent with GSO_2SH being the product, but elementary analysis and determination of ammonium ions [16] showed that a mixture of the monoammonium and diammonium salts of GSO_2SH had been obtained. The product could nevertheless be used for the experiments with rhodanese and glutathione reductase (its thiosulfonate content was assayed as cyanide-labile sulfur). In order to obtain a product with satisfactory values for elementary analysis, the barium salt of GSO_2SH was prepared (*cf.* Ref. 17). An equimolar amount of barium acetate was added to a solution of the mixed ammonium salt of GSO_2SH, after which the barium salt was precipitated with 5 volumes of ethanol, this product then being reprecipitated from water-ethanol and dried *in vacuo* over silica-gel. (Found: N 7.5; S 11.1; Cyanide-labile S 5.5. Calc. for $C_{10}H_{15}N_3O_8S_2Ba \cdot 4H_2O$: N 7.3; S 11.1; Cyanide-labile S 5.5). The infra-red spectrum of the compound in a KBr-pellet showed strong bands at 8.44 and 9.48 μ, falling in the range of those observed for the S—O stretching modes in other thiosulfonates [18] and in GSO_3H.[12]

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