Chromatographic Evidence on the Occurrence of Thiotaurine in the Urine of Rats Fed with Cystine*

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Rats fed a diet supplemented with L-cystine excrete in the urine taurine, hypotaurine (1-3), and possibly cystamine disulfoxide (4), although the excretion of the last mentioned compound is not certain (3). It was recognized earlier (1) that paper chromatograms of the urine of these rats showed another ninhydrin-positive spot which moved more rapidly than did taurine in collidine-lutidine solvent. The nature of the new compound was not investigated because it did not appear in all chromatograms and because it represented only a small fraction of the sulfur compounds excreted. More recently, an unknown cystine metabolite has been observed by chromatography by Awapara in the organs of rats treated with injections of S^4-cystine (5). We have now accumulated evidence for the identification of the new compound as the thiosulfonate analogue of taurine, aminoethylthiosulfonate (NH_2-CH\_2-CH\_2-SO_2-SH). Following Sörbo (6), the term thiotaurine will be retained for this compound.

EXPERIMENTAL

The following compounds were prepared in this laboratory: cysteine sulfinic acid (7), cysteic acid (8), cysteinesulfonate (9), alaminethiosulfonate (10), cystine disulfoxide (11), cystamine disulfoxide (12), hypotaurine (13), lathionine (14, 15), lathionine sulfoxide (15), lathionine sulfone (16), lathionamine (17), lathionamine sulfoxide (17). Cysteaminesulfonate was prepared by treatment of cysteamine solutions with excess sodium bisulfite before the chromatographic run. The synthesis of thiotaurine will be described in the text. The remaining products were obtained commercially.

Paper chromatography was performed using Whatman No. 4 paper. The solvents were phenol saturated with water and a mixture of equal volumes of collidine and lutidine, saturated with 1 volume of water. In the two-dimensional chromatography the phenol solvent was used first.

Male rats of the Wistar strain weighing 200 to 300 gm. were placed in pairs in metabolic cages and given the basal diet previously described (18). When desired, the animals were given a diet of the same composition supplemented with 6 or 12 per cent of L-cystine. The urine was collected every 24 hours and diluted to 40 ml. with water.

Urinary thiosulfonate was determined by cyamolysis as described by Sörbo (6). This reaction did not give values that were proportional to the amount of urine tested. Better proportionality was obtained by using twice the amount of the reactants employed by Sörbo and a sample of urine not exceeding 3/5 of the 24-hour sample. Nevertheless, the results obtained when this method was applied to urine were only semiquantitative.

RESULTS AND DISCUSSION

Detection of New Compound on Paper Chromatograms—The purpose of the first part of this work was to obtain the reproducible appearance of the new spot on the chromatograms of the urine of rats. It was soon recognized that this spot was invariably present on the chromatograms when \( \gamma \frac{1}{2} \) of the 24-hour urine specimen of two rats receiving the diet containing 6 per cent cystine was spotted on the paper. The compound appears as a ninhydrin-positive elongated spot above the position of taurine. Although the position of this compound in the chromatogram is definite, with this relatively large sample of urine the chromatograms are distorted and somewhat confused, possibly because of the effects of large amounts of taurine, salts, and other compounds.

When the dietary cystine was increased from 6 to 12 per cent, the spot was easily detected with \( \gamma \frac{1}{2} \) and even with \( \gamma \frac{2}{3} \) of the 24-hour sample of urine. Accordingly, most of the work was performed using the higher level of dietary cystine.

Fig. 1 illustrates a typical chromatogram made with \( \gamma \frac{1}{2} \) of the 24-hour urine sample of two rats on the diet containing 12 per cent cystine. The spot marked 3 is the one under study in the present paper; it will be referred to as compound 3 in this paper. Unless the rats were given extra cystine this spot was not seen, even when more urine was used for chromatography.

Elution of Compound 3 and Its Conversion to Taurine—The area presumed to contain compound 3 was cut from 6 chromatograms similar to that illustrated, and the remaining paper was developed with ninhydrin in order to ascertain that most of the compound was removed and that it was not contaminated with taurine. All of the paper fragments were eluted with water, the eluates were pooled, and the resulting solution was divided into two portions. The first was used as a control and was rechromatographed without further treatment. The second was treated with 1 ml. of 30 per cent hydrogen peroxide in the presence of a trace of ammonium molybdate (Dent (19)) and then rechromatographed. The first chromatogram showed compound 3 unchanged, whereas the second showed only taurine. This result suggests that compound 3 is a sulfur derivative of ethylamine which is not identical with taurine.

Cysteamine, cystamine, cystamine disulfoxide, and hypotaurine are all sulfur derivatives of ethylamine easily converted
to taurine by hydrogen peroxide; nevertheless, none of these possesses the chromatographic coordinates of compound 3, a fact that suggests that the sulfur-containing moiety of compound 3 has an unusual structure.

**Determination of Oxidation Level of Sulfur Compounds on Paper Chromatograms**—Procedures for the detection of sulfur-containing compounds on paper chromatograms have occasionally been reported in the literature. By appropriate use of these procedures, together with another devised for that purpose in the present paper, it is possible to establish the degree of oxidation of the sulfur group of an organic sulfur-containing compound found on a paper chromatogram. A summary of these reactions as applied to a group of representative sulfur compounds is seen in Table I. The reactions are performed as follows.

1. **Folin-Marenzi Reaction on Paper**—The dried paper is sprayed with the Folin reagent diluted with 4 volumes of water. The paper, when still wet, is then passed over funnels of concentrated ammonia which has been poured in a large dish. The Folin-Marenzi reaction with bisulfite is performed similarly to the above procedure except that the Folin reagent is diluted with 4 volumes of 10 per cent sodium bisulfite. A positive reaction is shown by the appearance of a blue spot which persists for many days. The reaction is sensitive to 5 µg of cysteine or cystine.

2. **KI + HCl Reaction**—A fresh solution of 20 gm. of KI in 100 ml. of 2 n HCl is sprayed on the dried paper (1). The reaction appears slowly within an hour in the form of a red-brown spot on a yellow background. It is produced by the oxidation of HI to free iodine by some partially oxidized sulfur derivatives which in the same time are reduced to a lower level of oxidation. The limit of sensitivity is 5 µg. of cysteinesulfonic acid or hypotaurine.

3. **Reaction with FeCl₃**—This reaction is specific for the sulfenic group, it is not very sensitive (30 µg. of hypotaurine are required), and does not appear if the paper contains traces of collidine-lutidine. A suitable amount of the suspected compound is eluted from the chromatogram, dried on another paper, and sprayed with a solution of 10 per cent FeCl₃. By this procedure a positive reaction is obtained even after the use of collidine-lutidine solvent and appears as a rusty spot on a yellow background.

4. **HgCl₂ Reaction**—After evaporation of the solvent (preferably strips of paper) is sprayed with a 2 per cent solution of HgCl₂ mixed with an equal volume of 0.1 m sodium acetate buffer of pH 5. After 2 minutes the paper is immersed in a tank of running tap water in such a way that it is completely immersed in a vertical position without touching the walls of the tank. After 15 minutes of thorough washing the paper is immersed in a vessel containing H₂S-saturated water. A black spot appears in a few seconds at the position occupied by a compound able to produce an insoluble complex with Hg.

5. **Iodoplatainate Reaction**—This reaction is used following the procedure indicated by Toennies and Kolb (21).

When the above tests were applied to our chromatograms, compound 3 gave strongly positive reactions only with KI + HCl, HgCl₂, and iodoplatainate reagents; all of the other reactions were clearly negative. As shown by Table I this sequence of reactions is given by the following sulfur compounds: R—SO₂—S—R, R—SO—R, R—SO₃—SH. The first two of these structures could not be ascribed to compound 3 since cystamine disulfide and lanthionamine sulfoxide have different chromatographic coordinates. On this basis it was tentatively concluded that compound 3 was the thiosulfonate analogue of taurine; i.e. thiotaurine.

**Preparation of Thiotaurine**—A sample of synthetic thiotaurine was needed to support the identification of compound 3 as thiotaurine. Thiotaurine had not previously been prepared; its occurrence in a biological medium had been suggested by Sorbo on the basis of studies on transulfuration of hypotaurine (6) in liver homogenates. Since Sorbo had been able to prepare alaminethiosulfonate by dismutation of cysteine disulfides by H₂S (10), we attempted the preparation of thiotaurine by applic-
ing the same reaction to cystamine disulfide. For this purpose, 50 mg. of cystamine disulfide dihydrochloride (12) dissolved in 1 ml. of water were treated for 1 hour with a stream of H₂S. The solution was then aerated for 15 minutes to remove excess H₂S. Paper chromatography in two dimensions showed the presence of taurine, hypotaurine, cystamine, and another spot in the area of spot 3. When this spot was eluted from the paper, the eluted compound gave the reactions described by Sjörbo for a thiosulfonate derivative. A mixed chromatogram made with the urine of rats fed with cystine and the dismutation product of cystamine disulfide by H₂S showed that compound 3 and the thiosulfonate derivative produced in the dismutation reaction exhibited identical chromatographic behavior.

Pure crystalline thiotaurine was prepared as follows. A solution of 1 mmole of hypotaurine in 1 ml. of 0.2 N NaOH was added to 1.5 mmoles of sulfur and refluxed with 20 ml. of ethanol. After boiling for 2 hours the solution was placed at −20°C over-night. The crystalline precipitate obtained was washed with carbon disulfide to remove unchanged sulfur. It was then recrystallized by dissolving it in the minimal amount of water and adding 30 ml. of absolute ethanol. After a day at −20°, 0.7 mmoles of pure crystalline thiotaurine were collected. M.p. was 213-214°.

$$C₇H₁₄O₃NS$$

Calculated: N 9.9, S 45.4
Found: N 9.6, S 45.6

Cyanolysis of the synthetic product gave products consistent with the expected properties of pure thiotaurine. Other details of the preparation and of the properties of the compound are described elsewhere (22). Synthetic thiotaurine was indistinguishable from compound 3 on paper chromatography. Fig. 2 describes a chromatogram of the urine of rats fed cystine added to 50 mg. of synthetic thiotaurine. In this chromatogram, which may be compared with that of Fig. 1, spot 3 is much larger and no separation into two components may be observed in either direction.

Cyanolysis Reaction on Eluted Compound and on Urine—

Gutman (23) has shown that thiosulfonate derivatives yield thiocyanate when treated with cyanide in alkaline medium. This reaction has been used as a qualitative and quantitative method for the estimation of thiosulfonates even in the presence of thiosulfate (6). When compound 3, eluted from the paper by a procedure similar to the one described above, was cyanolysed according to Sjörbo (6), a strong positive reaction was obtained. Furthermore, paper chromatography of the reaction mixture showed that compound 3 was totally converted into hypotaurine by cyanolysis. The release of thiocyanate and hypotaurine by cyanolysis is consistent with the formulation of equation 1.

$$\text{NH}_2-\text{CH}_2-\text{CH}_2-\text{SO}_2-\text{SH} + \text{CN}^- \rightarrow \text{NH}_2-\text{CH}_2-\text{CH}_2-\text{SO}_3\text{H} + \text{SCN}^-$$  (1)

The reaction of cyanolysis was then applied to the urine in order to establish the occurrence of thiotaurine before the

![FIG. 3. Cyanolysis reaction for thiosulfonate (6) applied to 0.5 ml. of the 24-hour urine collection of two rats on a diet with 12 per cent cystine, added with a synthetic sample of pure thiotaurine. The same conditions as in Fig. 1; 50 μg. of thiotaurine have been added to the sample before chromatography.](image)

![FIG. 4. Ion-exchange chromatogram by the method of Stein (23) with the use of citric acid as eluent (2) of the urine of rats on a diet containing 12 per cent of cystine. Fractions analyzed by cyanolysis according to Sjörbo (6). The blank values have been subtracted. ●, 5 ml. of 24-hour urine of two rats; ○, the same sample of urine added with 600 μg. of synthetic thiotaurine.](image)
chromatographic run. Although, as already stated, the procedure when applied to urine is not rigorously quantitative, we obtained consistent data regarding the excretion of a thiosulfonate derivative after ingestion of cystine. The result of one of these experiments is shown in Fig. 3. This finding has particular interest since it excludes the possibility of an artificial origin of thiotaurine during the course of the chromatographic manipulations. The values obtained indicate an excretion of approximately 1 to 2 mg. per day per rat, which is in good agreement with the amount calculated by the visual evaluation of the intensity and area of the chromatographic spot, and with the more precise data obtained by column chromatography.

**Column Chromatography**—When submitted to the Stein technique (24) for the analysis of amino acids as adapted by Cavallini et al. (2) to the determination of the oxidation products of cystine and cystamine, synthetic thiotaurine gives a typical peak which falls in the same area occupied by taurine. Nevertheless, it is possible to detect thiotaurine in the presence of a large amount of taurine when the cyanolysis reaction is used instead of the ninhydrin reaction. Fig. 4 gives the first part of a chromatogram, made by the Stein technique using citric acid-NaCl solution as eluent (2), of 5 ml. of urine of rats fed with cystine. By this very selective technique a compound with the properties of thiotaurine is detected in the urine of rats. The amount of thiotaurine in the sample analyzed indicates an excretion of 1.12 mg. of thiotaurine per rat per day.

Moreover column chromatography permits exact determination of the amount of thiocyanate released by cyanolysis of natural thiotaurine. This determination, deemed necessary to confirm in the natural product the presence of only 1 atom of sulfur removable by cyanide, was made as follows. A sample of natural thiotaurine was obtained by elution of a number of chromatograms. The content of thiotaurine in the pooled eluates was estimated by column chromatography using the ninhydrin reagent and a solution of synthetic thiotaurine as standard. The amount of thiocyanate released by cyanolysis was found by subjecting an aliquot of the eluates to the procedure of Sörbo (6). The following results were obtained: thiotaurine, 1.78 μmoles; SCN⁻ released, 1.70 μmoles. These values indicate that natural thiotaurine contains 1 atom of removable sulfur per mole.

**SUMMARY**

An unknown sulfur-containing compound has been detected in the chromatograms of urine of rats fed a diet supplemented with l-cystine. The compound is readily converted to taurine by hydrogen peroxide. A thiosulfonate structure was suggested by application of a number of reactions devised for determining the level of oxidation of organic sulfur groups. Synthetic thiotaurine, the thiosulfonate analogue of taurine, was indistinguishable from the unknown compound by paper and ion exchange chromatography. These and other properties support the conclusion that the unknown is thiotaurine (aminoethyl-thiosulfonic acid).

**REFERENCES**

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