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CHROMATOGRAPHIC SEPARATION OF SELENOHYPOTAURINE, SELENOTAURINE, SELENOHOMOHYPOTAURINE AND SELENOHOMOTAURINE

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SUMMARY

By either paper or ion-exchange chromatography the two seleninic compounds selenohypotaurine and selenohomohypotaurine, and the two selenonic compounds selenotaurine and selenohomotaurine may be all separated from each other.

On paper chromatography seleninic derivatives may be separated from the corresponding sulphinic compounds, while selenonic compounds show R_F values similar to those of the corresponding sulphonic derivatives. These two latter types of compounds may be differentiated, however, since selenonic compounds liberate iodine from HI, while sulphonic compounds do not.

Also by automated ion-exchange chromatography seleninic derivatives are well separated from the analogous sulphinic compounds, while selenonic compounds are eluted together with the corresponding sulphonic compounds.

INTRODUCTION

In the course of a recent study on the alkaline degradation of selenocystamine[1], we used ion-exchange chromatography for the separation of selenohypotaurine from selenotaurine. It was observed that, although selenotaurine behaved like taurine, selenohypotaurine was more strongly adsorbed on a sulphonated resin than was hypotaurine, an indication that in this respect the seleninic and sulphinic compounds differ from each other more than do selenonic and sulphonic compounds.

It seemed interesting to study the chromatographic behaviour of higher homologues of selenohypotaurine and selenotaurine (*i.e.*, selenohomohypotaurine and selenohomotaurine) and to compare these compounds with the corresponding sulphur ones, both to confirm the observed differences between seleninic and sulphinic derivatives and to devise methods for the separation and identification of these sulphur and selenium compounds.

In this paper we report on the separation, by paper and ion-exchange chroma-

tography, of the following compounds:

selenohypotaurine	R–SeO_2H	hypotaurine	R–SO_2H
selenotaurine	R–SeO_3H	taurine	R–SO_3H
selenohomohypotaurine	R'–SeO_2H	homohypotaurine	R'–SO_2H
selenohomotaurine	R'–SeO_3H	homotaurine	R'–SO_3H
		thiotaurine	R–SO_2SH
		homothiotaurine	R'–SO_2SH

where $R = H_2NCH_2CH_2$– and $R' = H_2NCH_2CH_2CH_2$–.

MATERIALS AND METHODS

The following compounds were prepared by methods described previously: selenohypotaurine and selenotaurine[1], hypotaurine and thiotaurine[2], homohypotaurine, homothiotaurine and homotaurine[3]. Taurine was a commercial product. Selenohomotaurine was prepared from selenohomocystamine[4] by oxidation with hydrogen peroxide in the presence of ammonium molybdate, as in the preparation of selenotaurine from selenocystamine[1]. All these compounds were in crystalline form, and their purities were checked by elemental analysis.

We were unable to obtain selenohomohypotaurine as a pure solid. It was prepared in solution, together with traces of selenohomotaurine, by treating selenohomocystamine with the stoichiometric amount of hydrogen peroxide.

Paper chromatography was performed on sheets of Whatman, No. 1 paper, with use of the solvents specified later.

Ion-exchange chromatography was performed on a Bio-Cal 200 amino acid analyzer. The long (54 × 0.9 cm) and short (12 × 0.9 cm) columns were filled, respectively, with Aminex A-6 and Aminex A-5 resins (particle size, 13.5 ± 2 μm; Bio-Rad Labs., Richmond, Calif., U.S.A.); the column temperature and the flow-rates of buffer and ninhydrin solutions are specified later. The standard macro cells of the instrument (light path 3 mm) were used.

RESULTS AND DISCUSSION

Paper chromatography

Table I shows the R_F values of the test compounds in three common solvent systems. All the compounds gave a typical colour with ninhydrin; some of them gave specific reactions as indicated.

It can be seen that the two seleninic compounds were well separated from the two selenonic compounds with either phenol or butanol–acetic acid as developing solvent. For the separation of the two seleninic acids (and the two selenonic acids) from each other, the best solvent was 2,4,6-trimethylpyridine–2,6-dimethylpyridine (1:1).

Compared with their sulphur analogues, selenonic acids showed R_F values similar to the sulphonic acids in all the solvents tested; however, seleninic derivatives could be separated from the sulphinic derivatives with phenol.

It is noteworthy that the selenonic compounds can be differentiated from their sulphonic analogues by spraying the chromatograms with a 20% solution of potassium iodide in 2 *M* hydrochloric acid[5]: selenonic acid compounds (like seleninic and sulphinic acids) liberate iodine on the paper, whereas sulphonic acid compounds do not.

All the seleninic and sulphinic acids gave a positive reaction with iodoplatinate[6],

TABLE I

PAPER CHROMATOGRAPHY OF TAURINE AND RELATED COMPOUNDS

The solvent systems used were: A, water-saturated phenol in the presence of ammonia vapour; B, butanol–acetic acid–water (4:1:5), upper phase; C, 2,4,6-trimethylpyridine–2,6-dimethylpyridine (1:1), water-saturated.

Compound	*R_F values in system*			*Behaviour in reaction with*		
	A	*B*	*C*	*KI in HCl**	*Folin–Marenzi reagent***	*Iodoplatinate*
Selenohypotaurine	0.78	0.23	0.14	+	−	+
Selenohomohypotaurine	0.91	0.20	0.27	+	−	+
Selenotaurine	0.52	0.11	0.21	+	−	−
Selenohomotaurine	0.52	0.11	0.12	+	−	−
Hypotaurine	0.68	0.20	0.19	+	−	+
Homohypotaurine	0.75	0.22	0.15	+	−	+
Taurine	0.49	0.16	0.30	−	−	−
Homotaurine	0.55	0.16	0.26	−	−	−
Thiotaurine	0.49	0.25	0.57	+	+	+
Homothiotaurine	0.55	0.22	0.49	+	+	+

* A 20% solution of potassium iodide in 2 *M* hydrochloric acid.
** Folin–Marenzi reagent with added hydrogen sulphite[8].

with which sulphonic and selenonic compounds did not react. Thiotaurine and homothiotaurine could be differentiated from all the other compounds because they were the only ones to give a positive reaction with the Folin–Marenzi reagent[7,8]; moreover, they showed the highest R_F values in the 2,4,6-trimethylpyridine–2,6-dimethylpyridine system.

Thus, paper chromatography, in conjunction with the indicated specific reactions, may be a useful first approach to the separation and identification of the compounds cited.

Ion-exchange chromatography

Fig. 1 shows the elution profile of the four selenium compounds from the long column of the amino acid analyzer operated at 50° and at a buffer flow-rate of 80 ml/h. Selenotaurine and selenohomotaurine are eluted with 0.1 *M* citric acid–0.2 *M* sodium chloride[9]; selenohypotaurine and selenohomohypotaurine are eluted with 0.2 *M* sodium citrate buffer of pH 4.25. It can also be seen from the separation of hypotaurine and homohypotaurine that seleninic acid compounds are highly retarded with respect to the corresponding sulphinic acids. However, the selenonic acids are not separated from sulphonic acids; in fact, selenotaurine and selenohomotaurine emerge at almost the same time as taurine and homotaurine. Moreover, selenotaurine and selenohomotaurine are not well separated from each other. Therefore, the fully oxidized selenium and sulphur compounds cannot be separated from each other. In the same position also are eluted thiotaurine and homothiotaurine (which, however, may be differentiated by cyanolyzing them before the chromatography[9]).

A better separation of selenotaurine from selenohomotaurine was achieved by operating the column at 30° and with a buffer flow-rate of 60 ml/h, as shown in the inset of Fig. 1.

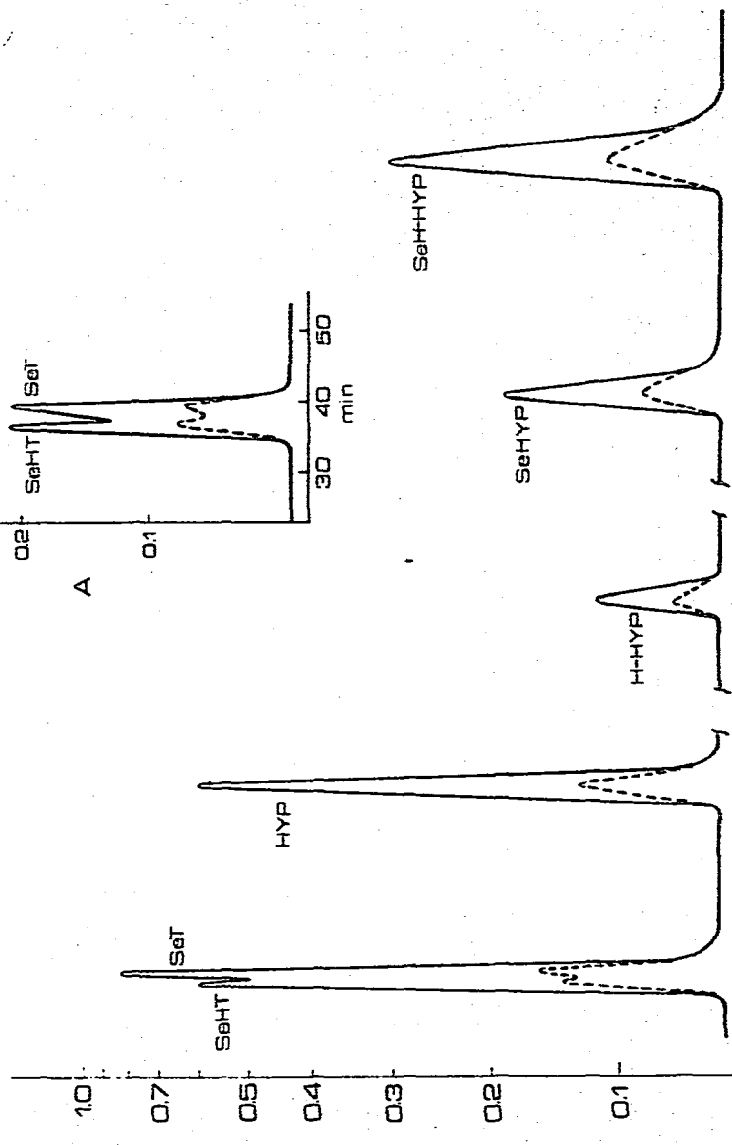

Fig. 1. Elution profile from the long column (54 × 0.9 cm) of the amino acid analyzer operated under the conditions specified in Table II, with 0.5 μmole of each compound applied. SeHT = selenohomotaurine; SeT = selenotaurine; HYP = hypotaurine; H-HYP = homolypotaurine; SeHYP = selenohypotaurine; SeH-HYP = selenohomohypotaurine. Taurine, homotaurine, thiotaurine and homothiotaurine are eluted in the same position as selenotaurine. Full line = absorbance at 560 nm; broken line = absorbance at 440 nm. In set: separation of SeHT and SeT with the column temperature at 30°, a buffer flow-rate of 60 ml/h and a ninhydrin flow-rate of 30 ml/h.

As selenohypotaurine and selenohomohypotaurine had long elution times from the long column, attempts were made to separate them on the short column. Fig. 2 shows their elution profile when they are loaded on the short column after

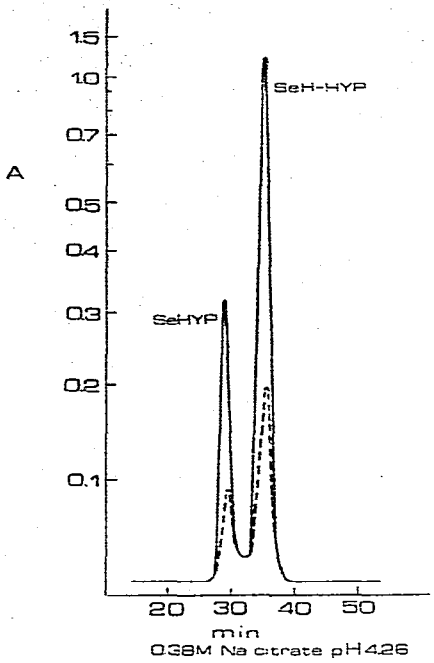

Fig. 2. Elution profile of SeHYP and SeH-HYP from the short column (12 × 0.9 cm) of the amino acid analyzer operated under the conditions specified in Table II, but with an eluting buffer of 0.38 *M* sodium citrate of pH 4.26.

TABLE II

ION-EXCHANGE CHROMATOGRAPHY OF TAURINE AND RELATED COMPOUNDS

Column size, 54 × 0.9 cm; resin, Aminex A-6; column temperature, 50°; buffer flow-rate, 80 ml/h; ninhydrin flow-rate, 40 ml/h. Elution schedule: 0–60 min, 0.1 *M* citric acid–0.2 *M* NaCl; 60–140 min, 0.2 *M* sodium citrate (pH 3.25), 140–340 min, 0.2 *M* sodium citrate (pH 4.25).

Compound	*Elution time, min*	C_{HW}*	A_{440}/A_{560}**
Selenohomotaurine	27	7	0.2
Selenotaurine	28	7.5	0.2
Taurine	28	8	0.2
Thiotaurine	28	7.5	0.2
Homotaurine	28	8.2	0.2
Homothiotaurine	28	8	0.2
Hypotaurine	55	18	0.2
Homohypotaurine	132	16	0.4
Selenohypotaurine	291	10.5	0.4
Selenohomohypotaurine	324	—***	0.3

* C_{HW} = peak height × peak width/amount applied (μmoles).
** A_{440}/A_{560} = ratio of absorbances at 440 and 560 nm.
*** Not calculated, as impure compound used.

equilibration with the eluent (0.38 *M* sodium citrate buffer of pH 4.26). Under these conditions, none of the other compounds is retained on the column, so that chromatography on the short column is more suitable for rapid analysis of selenohypotaurine and selenohomohypotaurine.

In Table II are reported the elution times, the colour constants (C_{HW}) and the ratios of the absorbances at 440 and 560 nm of all the compounds examined.

In conclusion, automated ion-exchange chromatography allows the separation from each other of the four selenium compounds, and, moreover, the seleninic acids are well separated from the analogous sulphinic acids. However, under our conditions, it was not possible to separate selenonic from sulphonic compounds; to differentiate between these two classes of compounds, the reaction with potassium iodide in hydrochloric acid on paper chromatograms may be useful.

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