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Note

Ion-exchange chromatography of some selenodiamines

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Recently we reported details of the automated ion-exchange chromatography of some thiodiamines, *i.e.*, cystamine, homocystamine, lanthionamine, homolanthionamine and cystathionamine[1]. Since we had the opportunity to prepare the corresponding selenodiamines, it was of interest to investigate their behaviour on ion-exchange chromatography. In the present note we report the results obtained, which show that the selenodiamines are well separated from each other, and from the thiodiamines, on the amino acid analyzer.

EXPERIMENTAL

Selenocystamine dihydrochloride was obtained from Sigma, St. Louis, Mo., U.S.A. Selenohomocystamine was prepared from 3-bromopropylamine hydrobromide (K and K Labs., Plainview, N.Y., U.S.A.), and selenosulphate in analogy to the methods reported for the synthesis of selenocystamine[2,3]. Selenolanthionamine was prepared from selenocysteamine and 2-bromoethylamine hydrobromide (Fluka, Buchs, Switzerland), selenohomolanthionamine from selenohomocysteamine and 3-bromopropylamine hydrobromide, and selenocystathionamine from selenocysteamine and 3-bromopropylamine hydrobromide. All of the compounds were obtained as dihydrochlorides, and their purity was checked by elemental analysis. Details of these syntheses will be described elsewhere.

Chromatographic analyses were performed on a Bio Cal 200 amino acid analyzer. A short column (0.9 × 12 cm) filled with Aminex A-5 resin (Bio-Rad Lab., Richmond, Calif., U.S.A.) (particle size, 13.5 ± 2 μm) was used. The column temperature was 50°. The buffer flow-rate was 80 ml/h and the ninhydrin flow-rate was 40 ml/h. Macro-cells (light path, 3 mm) were used.

RESULTS AND DISCUSSION

Like the corresponding thiodiamines[1], the selenodiamines could be separated on a short column of the amino acid analyzer which was equilibrated with 1.2 *M* sodium citrate buffer (pH 6.45) and eluted with 0.2 *N* sodium hydroxide. The elution was complete in 85 min, and the selenodiamines were well separated from each other. The elution pattern is shown in Fig. 1. A better separation was obtained when the

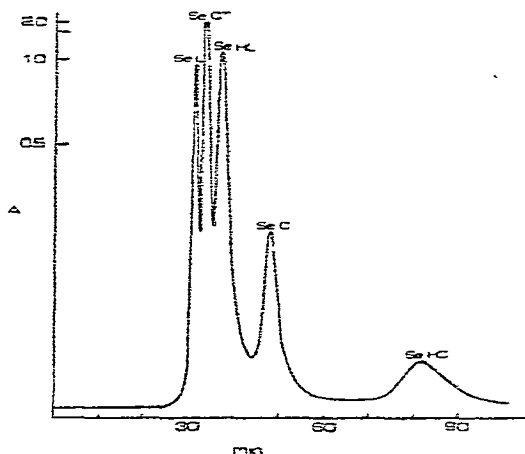

Fig. 1. Elution profile of the selenodiamines obtained on Aminex A-5 with 0.2 *N* NaOH as eluting agent, flow-rate, 80 ml/h. Ninhydrin flow-rate, 40 ml/h. The column (12 × 0.9 cm) was previously equilibrated with 1.2 *M* sodium citrate, pH 6.45. Temperature, 50°. Sample size, 0.5 μmole. Se-L = selenolanthionamine; Se-CT = selenocystathionamine; Se-HL = selenohomolanthionamine; Se-C = selenocystamine and Se-HC = selenohomocystamine.

elution was carried out with 2.35 *M* potassium citrate buffer (0.35 *M* potassium citrate, 2 *M* potassium chloride), pH 5.6) as already exploited for the separation of thiodiamines[1]. The elution profile obtained in these conditions is shown in Fig. 2. Selenohomocystamine, like homocystamine, was not eluted by this buffer; it was obtained when the column was washed with 0.2 *N* NaOH.

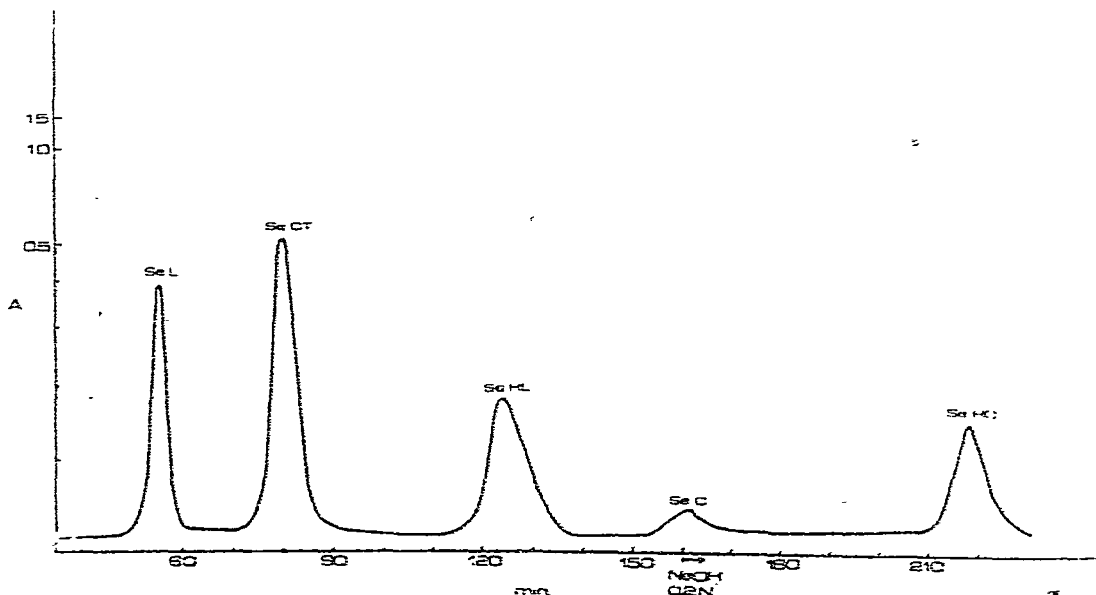

Fig. 2. Elution profile of the selenodiamines obtained using 2.35 *M* potassium citrate buffer (pH 5.6) as eluting agent. At the arrow the buffer was replaced by 0.2 *N* NaOH. All other conditions as in Fig. 1.

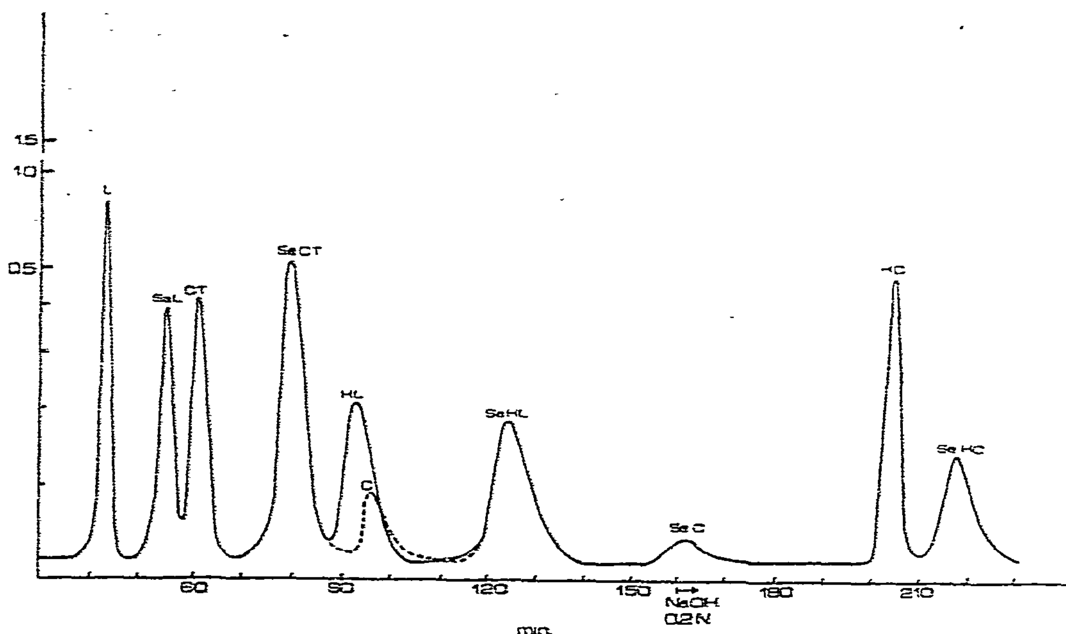

Fig. 3. Elution profile of the selenodiamines and of the corresponding thiodiamines. Experimental conditions as in Fig. 2. L = lanthionamine; CT = cystathionamine; HL = homolanthionamine; C = cystamine and HC = homocystamine. Sample size, 0.5 μmoles. Other abbreviations as in Fig. 1.

Fig. 3 shows the elution profile obtained when the selenodiamines were chromatographed together with the corresponding thiodiamines. A good separation of all of the diamines was obtained, with the exception of cystamine and homolanthion-

TABLE I

ELUTION TIMES, COLOUR CONSTANTS, C_{HW}, AND A_{440}:A_{560} RATIOS

Compound	*Elution time (min)*		C_{HW}***	A_{440}:A_{560}§
	2.35 M Potassium citrate	*0.2 N NaOH*		
Lanthionamine	44	31	29	0.22
Selenolanthionamine	57	31	21	0.27
Cystathionamine	63	33	37	0.19
Selenocystathionamine	82	34	47.8	0.35
Homolanthionamine	93	36	22.3	0.21
Selenohomolanthionamine	124	37	35	0.34
Cystamine	97	41	9.7	0.33
Selenocystamine	163	48	7	0.51
Homocystamine	45*	65	33**	0.32**
Selenohomocystamine	58*	82	14	0.57

* Referred to the change in the buffer on washing with 0.2 N NaOH.

** These values differ from those reported in ref. 1 possibly because of the higher degree of purity of the homocystamine used here.

*** C_{HW} = Peak height × peak width/amount applied (μmoles).

§ A_{440}:A_{560} = The ratio of the absorbances at 440 and 560 nm.

amine. The elution times, the colour constants (C_{HW}) and the $A_{440}:A_{560}$ ratios for the thio- and the selenodiamines are given in Table I.

In conclusion, using 2.35 M potassium citrate buffer (pH 5.6) as eluting agent followed by 0.2 N NaOH, all of the selenodiamines examined are well separated from each other and from the corresponding thiodiamines. All of the selenodiamines are retarded with respect to the corresponding thiodiamines. This behaviour of seleno compared to thio compounds has been already observed for other selenoamino acids such as selenocystine and selenomethionine[4], selenocystathionine and methyl-selenocysteine[5], selenalysine[6] and selenahomolysine[7], carboxymethylselenocysteamine[8], and therefore may be considered to be a general property of the seleno compounds.

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