was collected from the dispenser in 2-ml aliquots. The results are shown in Fig. 4. As may be seen from this graph, the mixing between liquids (corresponding to buffers) was negligible. Much more mixing would be expected within a column during the actual elution.

Comments

The machine is easily adaptable so that fewer or more buffers can be handled, as desired.

Another feature of the machine is that with one modification it could serve as a gradient type elution apparatus. Letting the reservoir (Fig. 2) be flask A and installing flask B equipped with a stirring bar below flask A, the result would then become an automatic gradient type elution system.

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Received August 14th, 1961

Ion-exchange chromatography of some uncommon amino acids

During studies involving the isolation and identification of substances from the blood plasma and urine of patients with disease[1,2], the authors found it expedient to determine the chromatographic behavior of several compounds. The detailed reports by SPACKMAN, STEIN AND MOORE[3], and by HAMILTON AND ANDERSON[4], contain information relative to several "non-protein" amino acids. Such amino acids are not likely to be encountered in hydrolysis of natural proteins. However, the synthesis of peptides with uncommon amino acids leads to the possibility that their products of hydrolysis will contain unusual compounds. In addition, the list of compounds being identified in human blood plasma and urine is increasing. Previously unidentified or incompletely described compounds may appear in urine or blood plasma, especially in various disease states. Determination of the R_F of several compounds by ion-exchange column chromatography is expensive in time and materials, particularly if synthesis is involved. Accordingly, we wish to record our experiences.

Experimental

Column chromatography using sulfonated polystyrene cation-exchange resins[5], with the automatic recording method of SPACKMAN, STEIN AND MOORE[3]*, was used

* Phoenix Precision Instrument Co., Philadelphia, Pa., U.S.A.

throughout this study. The 150 × 0.9 cm column with temperature change from 30° to 50° and buffer change from pH 3.25 to 4.25 at 11.67 hours was used for acidic and neutral compounds. The 50 cm column with temperature change from 30° to 50° at 14 hours and pH 4.26 buffer throughout was used for basic compounds. These systems are recommended for chromatography of physiological fluids and generally give better separation of unusual compounds than do the more rapid methods recommended for protein hydrolysates[3]. In most instances the instruments were operated with the recommended pre-runs. However, with the 50 cm column, many determinations were made without pre-run to eliminate the possibility that a compound might fail to be recorded because it was eluted at column volume. Nevertheless, all volumes of elution presented herein were corrected back to the recommended pre-runs of 12 ml for the 150 cm column, and 40 ml for the 50 cm column, measured from the time that the buffer pumps were activated. That is, the recording chart was started with zero ml after 24 min for the 150 cm column and after 80 min for the 50 cm column.

The amount of color developed by a compound reacting with the ninhydrin reagent varies from one instrument to another, depending on many factors, particularly the size of the absorption cell. In addition, some of the specimens were not absolutely pure as noted in the table. Therefore, these factors need to be determined experimentally by each investigator and are not reported here.

Results

The locations of compounds tested are indicated in Table I. The "effluent ml" indicated are ml from the zero point on the chart to the top of the recorded peak. The location in ml from the appearance of the pH 4.25 buffer "front" on the 150 cm column is also indicated because this may be a point of convenience to other workers and also bears upon the discussion below. In most cases the compounds in question were not analyzed to determine their exact nature or their purity. However, most were purchased from reliable manufacturers who supply an analysis with their products, and most proved to be chromatographically pure. It was necessary to synthesize some of the compounds.

Discussion

The chromatographic behavior of most of the amino acids tested in this study might be predicted to some degree from theoretical considerations or from knowledge of the behaviour of structurally similar compounds. For example, it was entirely predictable that L-2,4-diaminobutyric acid, containing one —CH_2— less than ornithine, would be eluted somewhat before ornithine. Similarly, the thio-ethers of cysteine are eluted later with increasing molecular weight of the aliphatic group. However, the location of complex compounds with several reactive groups, *e.g.*, L-2-thiol-histidine, *allo*-hydroxy-D-proline, could only be determined experimentally.

It is especially interesting that several diamino-dicarboxylic compounds emerge close to the buffer front from pH 3.25 to 4.25 on the 150 cm column. These include cystine, *meso*-cystine, cystathionine, L-djenkolic acid, α,ε-diaminopimelic acid, and

TABLE I

Compound	Column (cm)	Effluent ml to peak	Effluent ml of peak from buffer "front"	Remarks
L-Canavanine[a]	50	370		Eluted at 30°, beyond 3-methyl-histidine.
L-2,4-Diaminobutyric acid[a]	50	105		
S-Aminoethyl-cysteine[b]	50	153		
L-Cystine disulfoxide[c]	150	122		Three peaks resulted. Possibly all represent oxidation products of cystine.
		155		
		190		
allo-Hydroxy-D-proline[a]	150	149		Maximum at 440 mμ. Hydroxy-L-proline at 118 ml.
L-2-Thiolhistidine[a]	150	168		Skewed peak.
DL-Homocysteic acid[d]	150	40		Not resolved from L-cysteic acid; eluted essentially at column volume.
S-Methyl-cysteine[d]	150	247		Contained cystine also.
S-Ethyl-cysteine[d]	150	306		Small amount cystine present.
S-*n*-Butyl-L-cysteine[a]	150	492	54	
S-Carbamyl-L-cysteine[d]	150	192		
L-Ethionine[a]	150	483	45	
D-Penicillamine[a]	150	440		With pH 4.25 buffer front on 2 determinations. Small peak also at 247 ml.
L-Djenkolic acid[a]	150	452	14	Partially with cystathionine
α,ε-Diaminopimelic acid[a]	150	447	9	
Argininosuccinic acid[a]	150	551	13	
meso-Cystine[a]	150	412		Peak immediately prior to DL-cystine. Specimen apparently contained DL-cystine.
L-Homocystine[e]	150	629	19	Just preceding β-aminoisobutyric acid[3]. Not well separated from tyrosine, phenylalanine, and β-aminoisobutyric acid on 50 cm column.
	50	83		
DL + *meso*-Homocystine[f]	150	629	19	Peak not different from L-homocystine.
Asymmetrical disulfide of:				
(a) L-cysteine and L-homocysteine[g]	150	491	53	These optical isomers were not separable by the method. The peaks given by (b) and (c) were not wider than the peak of (a). Please see text.
(b) L-cysteine and DL-homocysteine[h]	150	491	53	
(c) D-cysteine and L-homocysteine[i]	150	491	53	

[a] Obtained from California Corporation for Biochemical Research, Los Angeles 63, California.
[b] Prepared by the method of CAVALLINI *et al.*[6].
[c] Prepared by the method of TOENNIES AND LAVINE[7]. Preparation performed 3 times with similar results.
[d] Obtained from Nutritional Biochemicals Corp., Cleveland 28, Ohio.
[e] Prepared by the aeration of L-cysteine at pH 7.5.
[f] Prepared by aeration of DL-cysteine at pH 7.5.
[g] Prepared by isolation from reaction mixture of oxidation of L-cysteine and L-homocysteine[2].
[h] Prepared by isolation from reaction mixture of oxidation of L-cysteine and DL-homocysteine[2].
[i] Prepared by isolation from reaction mixture of oxidation of D-cysteine and L-homocysteine[2].

the asymmetrical disulfide of cysteine and homocysteine. Arginino-succinic acid, D-penicillamine, L-ethionine, and S-*n*-butyl-L-cysteine, in addition to methionine, leucine, and isoleucine are also eluted in this general area. A published chromatogram of human urine reveals in this area several small peaks, presumably containing

unidentified amino compounds[8]. Moreover, the sensitivity of cystine to slight changes in buffer pH[4,9] also applies to the other diamino-dicarboxylic acids mentioned. Accordingly, workers interested in definitive separation of compounds from this area of the ion-exchange chromatogram would do well to consider a form of gradient elution[2].

The separation of *meso-* from DL-cystine has been noted[10,4]. However, it was of interest that *meso-* and DL-homocystine are not separated. It was interesting that the addition of one methylene group to just one side of the —S—S— group as in the asymmetrical disulfide of cysteine and homocysteine also prevents resolution of the optical isomers.

This study was aided by a grant from the National Institutes of Health, U.S. Public Health Service, and by a grant from the Kidney Disease Foundation of New York.

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Received July 14th, 1961

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A rapid paper chromatographic separation of estrogens*

Although there are many methods for the separation of estrogens[1], most procedures have limited applicability because effective separation of the three common estrogens is difficult in a single system or because of the prolonged chromatographic time required to move estriol from the origin. By modifying the well-tested chromatographic systems of ZAFFARONI[2], it is possible to obtain good separation of the estrogens in a short period of time.

The stationary phase is modified by the addition of formic acid to increase its acidity. Whatman No. 1 filter paper was used; Skellysolve B was substituted for hexane. All solvents were purified by standard techniques and redistilled prior to use. The results are given in Table I.

* Supported by Grant P-157 from the American Cancer Society.