CHROM. 5751

Detection of non-protein amino acids in the presence of protein amino acids. II.

In a previous publication[1], the detection of several non-protein amino acids in the presence of protein amino acids was described utilizing a Beckman 120 amino acid analyzer. These studies have been extended to the JEOL 5AH amino acid analyzer. This instrument allows programming of the chromatography process and this has resulted in larger average distances amongst the amino acid peaks and should be applicable to amino acid analysis where hitherto new compounds are to be tested. In addition to cysteic acid, taurine, methionine sulfone, hydroxyproline, sarcosine, α-amino-N-butyric acid, betaine, norvaline, norleucine, homocystine, and β-alanine previously shown we have now also run chromatograms with ornithine, N-amidino alanine, iminodiacetic acid, S-carboxymethyl cysteine, *allo*-threonine, lanthionine α-amino adipic acid, pipecolic acid, and isovaline. Urea was also run. We wish to present new data for N-amidino alanine, S-carboxymethyl cysteine, isovaline, lanthionine, *allo*-threonine, and *allo*-isoleucine.

Experimental

Materials. The JEOL Corporation 5AH amino acid analyzer was used in these studies. The following is a list of compounds and the companies from which they were purchased:

L-ornithine-$(HCl)_2$, D.L-norvaline, D.L-α-amino adipic acid, pipecolic acid, D,L-*allo*-isoleucine, D,L-norleucine, D,L-proline, D,L-lanthionine, D,L-isovaline, D,L-*allo*-threonine, D,L-methionine, and D,L-methionine sulfone from Mann Research Laboratories;

D,L-threonine, D,L-homocystine, L-lysine·HCl, D,L-valine, L-histindine·HCl, D,L-isoleucine, β-alanine, urea, D,L-leucine, α-amino-N-butyric acid, D,L-aspartic acid, glutamic acid, taurine, D,L-tyrosine, glycine, betaine·HCl, sarcosine·HCl, hydroxyproline, L-cystine, D,L-α-phenylalanine, D,L-tryptophan, aspartic acid, hydroxy-L-proline, and L-arginine·HCl from Nutritional Biochemicals Corporation;

L-cysteic acid·H_2O from Sigma Chemical Company; S-carboxymethyl cysteine from BDH Chemicals, Ltd.; and N-amidino alanine from Eastman Organic Chemicals.

Drs. F. WOELLER AND C. PONNAMPERUMA of the N.A.S.A. Ames Research Center supplied a sample of iminodiacetic acid. (This compound was recrystallized from dilute HCl with the use of acetone, prior to use.)

The buffers were purchased from the Pierce Chemical Company in concentrated form and were diluted prior to use.

The resin used is JLC-R2 cation-exchange resin.

Chromatography and programming. Solutions of the standard compounds were made so that 5–100 nmoles of each compound were applied to the appropriate 'basic' or 'neutral-acidic' columns. The pH of these solutions was pH 2 in HCl. Chromatograms of the compounds were run singly as well as in mixtures.

The JEOL punch-tape was used to program the amino acid analyzer so that the runs were made automatically. 'Basic' column length was 25 cm and buffer pH 5.28 and was carried out at 55° with a flow rate of 0.8 ml/min. 'Neutral-acidic'

column length was 55 cm. Change from the 'basic' to 'neutral-acidic' column was programmed to occur after 250 min. The temperature was programmed to change from 45° to 55° after 80 min operation of the 'long' column and the buffer was programmed to change from pH 3.25 to 4.30 after 140 min operation of the 'long' column. Initially the buffer change was programmed at 120 min; however, the later buffer change resulted in better separations in the α-amino-N-butyric acid to isoleucine retention time region. Buffer for the basic column was 0.35 *N* sodium citrate whereas both buffers for the 'neutral-acidic' column were 0.2 *N* sodium citrate and 5% in methanol (v/v) with pHs as above.

Results

The color constants for the amino acids and urea are indicated in Table I. Fig. 1 shows the elution profiles of these compounds.

TABLE I

The color constant is calculated with the use of the equation $Hw = CD$, where H is the height of the peak measured with the use of the appropriate JEOL ($-\log T$) scale (× 10, × 3, or × 1) w is the number of dots above the peak-half-height, D is the number of μmoles of amino acid applied to the column, and C is the color constant. The 570 nm data were used except for hydroxyproline, proline, and pipecolic acid for which the 440 nm data were used. The conditions for the chromatographic runs are described in the text and are programmed especially on the JEOL 5AH amino analyzer to maximize the average separations amongst the amino acid peaks. T means transmittance.

Amino acid	*Color constant*	*Amino acid*	*Color constant*
Tryptophan	17	Proline	4
Ornithine	22	α-Amino adipic acid	24
Lysine	25	Glycine	25
Histidine	21	α-Alanine	22
N-Amidino alanine	9	Pipecolic acid	0.1
Arginine	44	Lanthionine	9
Cysteic acid	22	α-Amino-N-butyric acid	70
Urea	4	Cystine	22
Taurine	5	Betaine	1
Iminodiacetic acid	22	Isovaline, valine, nor-valine	18
S-Carboxymethyl cysteine	35	Methionine	14
Aspartic acid	23	Isoleucine, *allo*-isoleucine	12
Threonine, *allo*-threonine	18	Leucine	21
Methionine sulfone	7	Norleucine	16
Serine	24	Tyrosine	21
Sarcosine	4	α-Phenylalanine	20
Hydroxyproline	4	Homocystine	42
Glutamic acid	60	β-Alanine	4

We may note the wide retention time separation from tryptophan to arginine, the separation of ornithine and lysine, the separations of iminodiacetic acid, S-carboxymethyl cysteine, and aspartic acid, the wide separation of α-amino-N-butyric acid and cystine, the separation of homocystine and β-alanine and the long retention

interval between α-amino-N-butyric acid and leucine. However, we should note that no separations were accomplished for threonine and *allo*-threonine, or isoleucine and *allo*-isoleucine, or valine, isovaline, and norvaline, or proline and lanthionine. (However, for proline 440 nm/570 nm > 1 whereas for lanthionine 570 nm/440 nm > 1 so that these two compounds are distinguishable when they are not present simultaneously although they have identical retention times.)

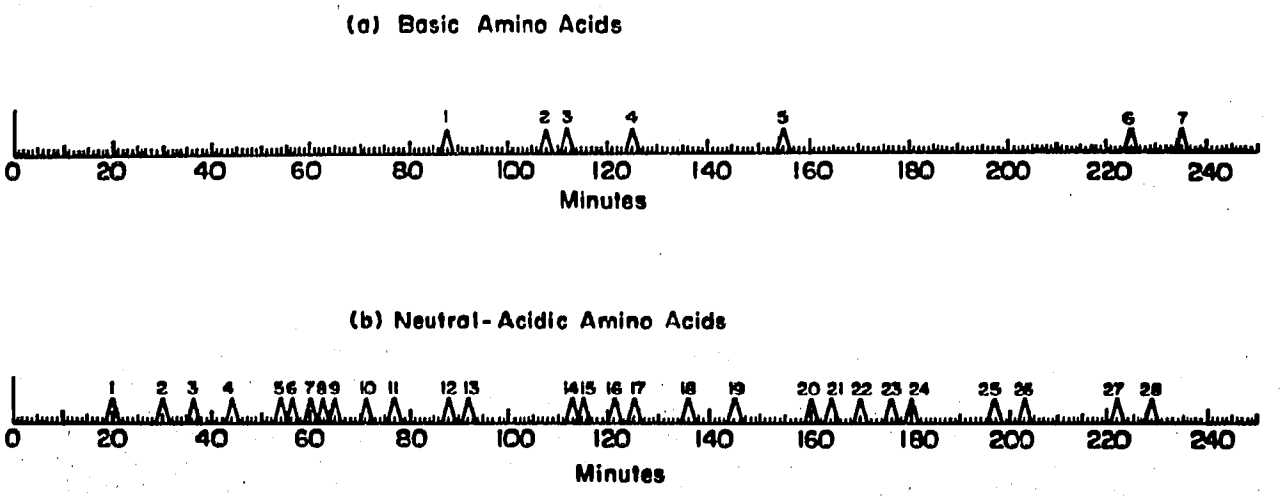

Fig. 1. Amino acid analyzer chromatograms of non-protein and protein amino acids. The distances between peaks are measured between apexes in the reduction of the data represented in the figure. Conditions are described in the text. (a) 1 = tryptophan, 2 = ornithine, 3 = lysine, 4 = histidine, 5 = ammonia, 6 = N-amidino alanine, 7 = arginine. (b) 1 = cysteic acid, 2 = urea, 3 = taurine, 4 = iminodiacetic acid, 5 = S-carboxymethyl cysteine, 6 = hydroxyproline, 7 = aspartic acid, 8 = methionine sulfone, 9 = threonine and *allo*-threonine, 10 = serine, 11 = sarcosine, 12 = glutamic acid, 13 = proline (440 nm) and lanthionine (570 nm), 14 = α-amino adipic acid, 15 = glycine, 16 = α-alanine, 17 = pipecolic acid, 18 = α-amino-N-butyric acid, 19 = cystine, 20 = isovaline, norvaline and valine, 21 = methionine, 22 = *allo*-isoleucine and isoleucine, 23 = leucine, 24 = norleucine, 25 = tyrosine, 26 = α-phenylalanine, 27 = homocystine, 28 = β-alanine.

Discussion

As previously mentioned[1] data showing the positions of 147 compounds in amino acid chromatograms have appeared in the literature[2]. In a previous publication[1] separations of fifteen non-protein amino acids in the presence of fourteen protein amino acids were presented. Here, we present data showing the separations of seventeen non-protein amino acids in the presence of eighteen protein amino acids. The color constants of the amino acids have been found to vary amongst different buffers. This has been found previously[3]. The time scale of the chromatography is much expanded using the amino acid analysis program described in this publication, the peak retention times are distributed more uniformly and are further apart as described in the previous section.

Data for several hitherto untested compounds are presented as well as coincidences of four sets of compounds at four retention times.

It is interesting to note the distinct separations amongst the following chemically similar compounds: ornithine and lysine, N-amidino alanine and arginine, and iminodiacetic acid and S-carboxymethyl cysteine and aspartic acid.

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