QUANTITATIVE CHROMATOGRAPHIC SEPARATIONS OF SYNTHETIC PEPTIDES

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THE following chromatographic separations of simple peptides have been reported in the last few years: fractionation of arginyl peptides on acid earths[1], partition chromatography of acetyl peptides on columns of silica gel[2], partition of free peptides on columns of starch[3], partition on two-dimensional paper chromatograms of a partial hydrolysate of gramicidin *S* [4] and of peptides of urine[5], and separations of synthetic peptides on columns of carbon by displacement analysis[6].

We now wish to report some simple chromatographic methods capable of separating quantitatively and specifically a complex mixture of peptides and amino-acids into different groups; the separation of each group into its constituents can be realized by paper chromatography or otherwise.

Adsorption of Neutral Peptides on Acid Alumina in 10 per cent Formaldehyde[7,12]

The following table contains the measurements of Dunn and Loshakoff[8] of the apparent acid dissociation constant (pKa') of neutral amino-acids and peptides in water and 9 per cent formaldehyde.

TABLE 1

Amino-acid	pKa'		Peptide	pKa'	
	Water	9% Formaldehyde		Water	9% Formaldehyde
Alanine	9·68	6·96	Glycyl-glycine	8·13	4·27
Valine	9·64	7·47	Di-glycyl-glycine	8·0	4·24
Norleucine	9·77	7·10	Glycyl-leucine	—	4·40
Phenylalanine	9·12	6·80	Alanyl-glycine	7·75	5·52
Glycine	9·60	5·92	Leucyl-glycine	—	5·57
Serine	9·15	5·63	Leucyl-tyrosine	—	5·07

Schramm and Primosigh[9] utilized the more acid pKa' of glycine and serine in 10 per cent formaldehyde to separate them from the other neutral amino-acids; glycine and serine are quantitatively adsorbed on acid alumina (Wieland[10]) and were eluted with alkali. Later they showed that threonine and cysteine are also adsorbed on alumina in 10 per cent formaldehyde[11].

We have applied the same principle to neutral peptides and found that they are all quantitatively adsorbed on acid alumina; they may be eluted quantitatively by hot water which dissociates the peptide-formaldehyde complex. β-Alanine, which has an $-NH_2$ group on a primary carbon like glycine, is also adsorbed quantitatively on acid alumina and may so be separated from α-alanine. Table 2 shows the results of some separations of binary mixtures of amino-acids and peptides.

TABLE 2. SEPARATIONS OF BINARY MIXTURES OF AMINO-ACIDS AND PEPTIDES IN 10 PER CENT FORMALDEHYDE

Non-adsorbed amino-acids	% in filtrate (i)	(ii)	Adsorbed substances	% in eluate (i)	(ii)
Alanine	99·4		Alanyl-phenylalanine	101·3	100
Alanine	99		Leucyl-glycine	100·5	
Alanine	100	96·4	β-Alanine	99·2	
Leucine	100	101·6	Glycyl-leucine	99	
Leucine	100·7		Leucyl-tyrosine	101·2	
Tyrosine	99·2	99	Alanyl-glycine	101·5	
Tryptophane	98		Alanyl-glycine	100·5	

(i), Nitrogen determinations by micro-Kjeldahl; (ii), determinations by known specific methods.

10 gm. of acid alumina[10] are washed in a column of 10 mm. diameter with 300 ml. of water; the *p*H of the last drop of effluent must be about 4·8–4·9 or else separations are not quantitative. After filtering on the column the solution of about 10 mgm. of the mixture of substances in 5 ml. of 10 per cent formaldehyde (*p*H 8·5), one washes the amino-acids into the filtrate with 50 ml. of 10 per cent formaldehyde (*p*H 8·5). Elution of the peptides is obtained by washing the column with 100 ml. of hot water (~90°).

Adsorption of Glycyl-peptides on Acid Alumina in 1 per cent Formaldehyde[12]

Table 1 shows that glycyl-peptides ($H_2N-CH_2-CONH-CHR-COOH$) have a more acid pKa' in 9 per cent formaldehyde than other (alanyl-, leucyl-, etc.) peptides. We have found that in 1 per cent formaldehyde glycyl-peptides are already sufficiently acid to be adsorbed quantitatively on acid alumina, whereas other peptides pass through the column, not being adsorbed. Elution of the glycyl-peptides is easily obtained by washing the column with hot water. The amino-acids glycine, serine, threonine, cysteine and β-alanine, which are adsorbed from 10 per cent formaldehyde solution, are not adsorbed from 1 per cent formaldehyde solution. Table 3 shows some separations of binary mixtures.

TABLE 3. SEPARATIONS OF BINARY MIXTURES OF AMINO-ACIDS AND PEPTIDES IN 1 PER CENT FORMALDEHYDE

Non-adsorbed substance	% in filtrate (i)	(ii)	Adsorbed peptide	% in eluate (i)	(ii)
Alanyl-leucine	102		Glycyl-leucine	98·3	99·2
Alanyl-leucine	100		Glycyl-tryptophane	98	100
Leucyl-glycine	100·7		Glycyl-leucine	98·6	
Leucyl-glycine	100		Glycyl-tryptophane	100·5	
Glycocolle	99·6	103	Glycyl-glycine	101·3	
Serine	97·6		Glycyl-tyrosine	100	93

(i), Nitrogen determinations by micro-Kjeldahl; (ii), specific determinations by known methods.

The optimum *p*H for separations in 1 per cent formaldehyde is 3·5 for effluent wash-water; acid alumina prepared according to Wieland[10] can be utilized as it is; the mixture of peptides (10–20 mgm.) dissolved in 5 ml. 1 per cent formaldehyde (*p*H 3·5) is poured on a column of 5 gm. acid alumina (10 mm. diameter); by washing with 50 ml. of 1 per cent formaldehyde, *p*H 3·5, the non-adsorbed substances are washed into the filtrate. Elution of adsorbed glycyl-peptides is obtained with 100 ml. hot water (~90°).

We suppose that seryl-, threonyl-, cysteyl- and β-alanyl-peptides will behave like the glycyl-peptides.

Adsorption of Aromatic Neutral Peptides on Carbon

Wachtel and Cassidy[13], Tiselius[6], Schramm and Primosigh[9] have studied adsorption of amino-acids on carbon columns, and have observed that the aromatic amino-acids (phenylalanine, tyrosine, tryptophane) are firmly bound. Elution of the adsorbed amino-acids is often difficult and seldom exceeds 85 per cent.

We have found that carbon columns retain quantitatively neutral peptides containing aromatic amino-acids; other neutral peptides containing only aliphatic amino-acids are not adsorbed. Elution is possible with water saturated with ethyl acetate[6,13]; yields attain 95 per cent if the carbon ('Activit *5OXP*' [14]) is first purified by boiling in 20 per cent

acetic acid and then treated with ephedrin[6]. 1 gm. of 'Activit' can adsorb about 30–50 mgm. of aromatic dipeptides. Table 4 shows some typical separations.

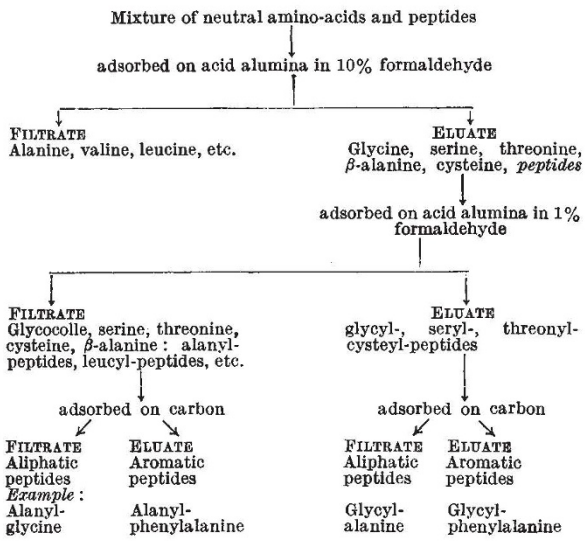
TABLE 4. SEPARATIONS OF BINARY MIXTURES OF ALIPHATIC AND AROMATIC PEPTIDES ON CARBON

Non-adsorbed aliphatic peptides	% in filtrate (i)	Adsorbed aromatic peptides	% in eluate (i)	(ii)
Leucyl-glycine	100·8	Leucyl-tyrosine	96·8	100
Glycyl-leucine	98·3	Glycyl-tyrosine	99·8	100
Alanyl-glycine	99·8	Alanyl-phenylalanine	100·4	99·1

(i), Nitrogen determinations by micro-Kjeldahl; (ii), specific determinations by known colorimetric methods.

0·5 gm. of 'Activit *50XP*' is boiled for 5 min. with 4 ml. of 20 per cent acetic acid, filtered, washed with 15ml. hot water, then treated in a beaker with 10 ml. water containing 2 mgm. of ephedrine; after shaking for 10 min. the carbon is poured into the column (10 mm. diameter) and washed with 15 ml. water saturated with hydrogen sulphide. The mixture to be separated (20–30 mgm.) in 5 ml. of 5 per cent acetic acid saturated with hydrogen sulphide is filtered on the column, which is then washed with 25 ml. of 5 per cent acetic acid saturated with hydrogen sulphide. Non-adsorbed substances are thus washed into the filtrate. The aromatic peptides are eluted with 125 ml. of water saturated with ethyl acetate and hydrogen sulphide.

By the methods described above a complex mixture of neutral amino-acids and peptides, such as may result from partial hydrolysis of a protein, can be separated into different groups as shown in the following scheme:



Adsorption of Acid Peptides on Acid Alumina

Preliminary experiments with glutathione and glycyl-glutamic acid show that acid peptides are quantitatively adsorbed on acid alumina, like glutamic and aspartic acid; elution can be obtained either by acid or, better, by alkali.

All these experiments were done with synthetic di- and tri-peptides; there are reasons to believe that the same methods will be applicable to higher peptides.

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THE TEACHING OF TAXONOMY

A JOINT meeting of the Linnean Society and the Systematics Association was held in the rooms of the former at Burlington House on February 6, when "The Teaching of Taxonomy" was the subject discussed. Sir Clive Forster-Cooper opened the discussion by noting the wide points of view and urging the importance of modern taxonomy. At one time taxonomy was the foremost branch of biology. It then suffered a decline relative to newer aspects and for a long time was considered a blind alley; but a revival of interest is now occurring. There are dangers in speaking too much of the 'professional' taxonomist, but the museum taxonomist is certainly overburdened in the answering of questions for others and has very little time left for research. It becomes a question whether all the wealth of facts and ideas now tending to be incorporated in taxonomy can be brought together in a unified syllabus for teaching in schools and universities. Sir Clive made the suggestion that the Systematics Association should appoint a committee to draw up some sort of guiding rules, of a sufficiently elastic nature, for the benefit of teachers, and to encourage them to teach students taxonomy along desirable lines.

Prof. James Gray considered it would be a pity if taxonomists should look upon their subject as a Cinderella, for though taxonomy is not the sole basis of biology it can play its part like all other branches in the development of the subject. Taxonomy must be seen as one of many points of view. Not more than 10 per cent of students of biology become taxonomists, and the problem for the teacher is how he can convey the taxonomic point of view and the discipline of the subject to the other 90 per cent so that they can carry away something which is to them a matter of intellectual pride. The systematist is to a large degree born such, and works much by general impressions. On the whole, the botanist seems to be more fortunate than the zoologist in having workable keys provided for him, and there is a great need in zoology for simple keys that can be used by students and teachers.

Dr. W. B. Turrill urged that plant taxonomy should be taught because it is indispensable to the proper study of every other branch of botany,