CCXXXVI. EXPERIMENTS ON AMINO-ACIDS

I. THE PARTITION OF ACETAMINO-ACIDS BETWEEN IMMISCIBLE SOLVENTS

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THE work described in this series of papers was undertaken with a view to extending the methods available for the isolation of amino-acids from protein hydrolysates. At this stage of the work it seems desirable to publish what has already been done, so that others may make use of the observations.

Neuberger [1938, 1] observed that after acetylation by ketene of an enzymic digest of egg albumin, followed by acidification, a proportion of the total N was rendered extractable from aqueous solution into chloroform. From this, it seemed possible that acetamino-acids might have widely differing partition coefficients between chloroform and water, and that this might afford the basis of a general method for the isolation of amino-acids from protein hydrolysates. With Dr Neuberger's agreement a preliminary investigation of the partition coefficients of a number of acetamino-acids was therefore undertaken. The results of this are summarized in the present paper.

The partition coefficients of the *N*-acetyl derivatives of the naturally occurring amino-acids between chloroform and water were found to cover a very wide range. Some ether-water and ethyl acetate-water systems were also investigated. The figures obtained with ether did not differ very strikingly from those obtained with chloroform. A selection of the ethyl acetate figures is given below.

The experimental technique used for determining the partition coefficients was to shake together in a separating funnel known volumes of chloroform and an aqueous solution of the solute under investigation, and then to estimate the amount of solute in each phase either by titration against standard alkali or by N determination. In some cases, however, where the coefficient was too high for such direct determination, a rough estimate could be made by subjecting an aqueous solution of the compound to extraction by a known volume of chloroform in the continuous liquid-liquid extractor described by Neuberger [1938, 1]. The amount of chloroform passing through the aqueous layer in this apparatus can be readily determined with reasonable accuracy by measuring the temperature rise and rate of flow of the condenser water from time to time. The Neuberger extractor shows a complete absence of entrainment; a saturated aqueous solution of Na_2SO_4 was extracted in the apparatus with 50 vol. of chloroform, and on evaporating the chloroform extract to dryness it was impossible to detect $SO_4^=$ in the residue.

By using a substance of known partition between chloroform and water, and by determining its rate of disappearance from the aqueous layer in the apparatus after extraction by different volumes of chloroform, it was found that the "plate efficiency" of the Neuberger extractor (aqueous layer 500 ml., passing 1·0–1·5 l. of chloroform per hr.) was 70–80 %.

The preparation and properties of the compounds used are described in an appendix to this paper.

In the following tables:

$$P = \frac{\text{concentration of solute in aqueous phase}}{\text{concentration of solute in organic solvent phase}},$$

c = concentration of solute in aqueous phase, expressed as mg. per ml.

Effect of concentration on P

This is not very marked, and P tends to reach a constant value at high dilutions. In general P does not vary by more than a factor of 2 at concentrations below 10 mg. per ml. in the aqueous phase. Fig. 1 illustrates the variation

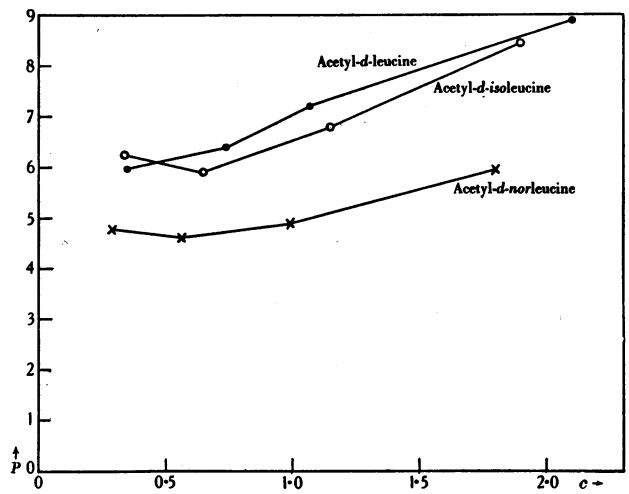

Fig. 1. Partition isotherms of acetyl-*d*-leucine, acetyl-*d*-*iso*leucine and acetyl-*d*-*nor*leucine between chloroform and water phases at 37°. (P and c have same significance as in text.)

of P against c for acetyl-*d*-*iso*leucine, acetyl-*d*-*nor*leucine, and acetyl-*d*-leucine at 37°. No attempt has been made to give a theoretical treatment of these isotherms in terms of ionic dissociation or of association in the non-polar solvent.

Effect of temperature on P

P has in every case a considerable negative temperature coefficient. This is illustrated by the figures for the partition of acetyl-*d*-leucine given in Table I.

Table I. *Effect of temperature on partition of acetyl*-d-*leucine between water and chloroform*

Temp. °C.	P	c
4	13·4	0·6
24	8·8	0·5
37	6·0	0·4

Effect of salt on P

Raising the concentration of neutral salt in the aqueous phase leads invariably to a lowering of P for any given value of c. This "salting-out" effect is particularly noticeable with *N*-acetyl-*l*-tyrosine in the presence of Na_2SO_4.

Table II. *Approximate partition coefficients of acetamino-acids between chloroform and water*

Compound	Temp. °C.	P	c	
ON-Diacetyl-*dl*-tyrosine	18	7	8	
Acetyl-*l*-phenylalanine	37	5	1	
Acetyl-*d*-*nor*leucine	37	5	1	
Acetyl-*d*-*iso*leucine	37	6	1	
Acetyl-*d*-leucine	37	7	1	
Acetyl-*l*-proline	37	15	2	
Acetyl-*l*-methionine	37	22	2	
Acetyl-*l*-valine	37	30	2	
Acetyl-*dl*-α-aminobutyric acid	37	40	2	
Acetyl-*dl*-alanine	37	160	2	
Acetylglycine	18	600	7	
N-Acetyl-*l*-tyrosine	37	600	4	
Diacetyl-*dl*-lysine	20	800	10	See Note 1
Acetyl-*l*-glutamic acid	20	>1000	6	
N-Acetyl-*dl*-serine	20	>1000	—	
N-Acetyl-*l*-hydroxyproline	20	>1000	—	
NN'-Diacetyl-*l*-cystine	20	>1000	10	See Note 2

Note 1. Diacetyl-*dl*-lysine could not be prepared crystalline. (Neuberger [1938, 2] also reports failure to obtain a crystalline product on treating *l*-lysine with ketene and NaOH in aqueous solution.) For this determination, therefore, synthetic *dl*-lysine dihydrochloride was acetylated in aqueous solution by the method of du Vigneaud & Meyer [1932], with acetic anhydride and NaOH. A Van Slyke amino-N determination on an aliquot of the resulting solution (30 min. shaking) showed that 88% of the original —NH_2 groups were acetylated, corresponding to the conversion of at least 76% of the original lysine residues into diacetyllysine. N corresponding to 2% of this diacetyllysine was extracted by the passage through the Neuberger apparatus of 17 vol. of chloroform after acidification of the aqueous phase. The figure $P=800$ given above is probably lower than the true figure for diacetyllysine in pure aqueous solution, owing to the considerable amount of salt present in the acetylation mixture after acidification.

Note 2. *NN'*-diacetyl-*l*-cystine does not crystallize [Hollander & du Vigneaud, 1931]. It was accordingly prepared in solution by acetylation of *l*-cystine, and P was determined in the same way as for diacetyllysine (see note 1). This value of P is probably low, as a result of "salting-out".

Table III. *Partition of acetamino-acids between ethyl acetate and water phases*

Compound	Temp. °C.	P	c
Acetylglycine	18	36	3
Acetyl-*l*-glutamic acid	37	23	10
Acetyl-*dl*-alanine	18	18	3
Acetyl-*dl*-α-aminobutyric acid	18	7	4
N-Acetyl-*l*-tyrosine	37	1·4	8

It will be seen by comparison of the above tables that the ethyl acetate figures cover a much less wide range than the chloroform figures, also that the positions of *N*-acetyltyrosine and acetylglutamic acid are entirely different in the two series.

α-*N*-Acetylarginine and α-acetylhistidine contain free basic groupings, and are therefore unlikely to be extractable from water into chloroform. Evidence is presented in Paper II of this series that for α-acetylarginine this is the case.

APPENDIX

The preparation and properties of acetamino-acids

In preparing acetamino-acids from the corresponding free amino-acids, the acetylation was carried out, unless otherwise stated, with acetic anhydride and NaOH in aqueous solution at 0° [cf. Bergmann & Zervas, 1928; Chattaway, 1931; du Vigneaud & Meyer, 1932]. This method has been shown by du Vigneaud & Meyer [1932] to cause very little racemization. The molecular proportions used by these authors were employed in each preparation, unless otherwise stated, and the yields obtained were uniformly good. After adding H_2SO_4 exactly equivalent to the NaOH employed in the preparation, the product was separated from the resulting Na_2SO_4 by extraction with hot alcohol. After evaporation of the alcohol *in vacuo*, the product was recrystallized from a suitable solvent, as described below. The acid equivalent weights of the compounds were determined by titration against a standard NaOH solution, using phenol red as indicator. Compounds described here for the first time are indicated with an asterisk.

Acetyl-dl-*alanine.* Prepared from *dl*-alanine (B.D.H.). Recrystallized from acetone. M.P. 136°. Fischer [1903] gives M.P. 137°.

**Acetyl*-dl-*α-aminobutyric acid.* Prepared from *dl*-α-aminobutyric acid (B.D.H.). Recrystallized from ethyl acetate. M.P. 129–131°. (Found: C, 48·2; H, 7·20; N (Dumas), 8·9%. $C_6H_{11}O_3N$ requires C, 49·6; H, 7·58; N, 9·6%. Acid. equiv. wt.: found 148; calc. 145.)

NN′-*Diacetyl*-l-*cystine.* See Note 2, Table II.

Acetyl-l-*glutamic acid.* Prepared from *l*(+)-glutamic acid [cf. Bergmann & Zervas, 1928; du Vigneaud & Meyer, 1932].

Acetylglycine. Prepared from glycine (B.D.H.) [cf. Chattaway, 1931].

**Acetyl*-l-*hydroxyproline.* The preparation and properties of this compound are described in Paper III of this series.

**Acetyl*-d-iso*leucine.* *dl-iso*Leucine (prepared from *sec*-butylmalonic ester) was resolved by the method of Locquin [1907]. The resulting *d*(−)-*iso*leucine had $[\alpha]_D^{21°}$ −11·5° (water, $l=2$, $c=2·7$). This on acetylation yielded a product which was recrystallized from water. M.P. 150–151°. $[\alpha]_D^{20°}$ −15·6° (alcohol, $l=2$, $c=2·3$). (Found: C, 55·5; H, 8·60; N (Kjeldahl), 8·4%. $C_8H_{15}O_3N$ requires C, 55·5; H, 8·67; N, 8·1%. Acid equiv. wt.: found 173; calc. 173.)

**Acetyl*-d-*leucine.* *dl*-Leucine (prepared from *iso*butylmalonic ester) was resolved for me by Mr S. Williamson, to whom I express my thanks. He used the method of Fischer & Warburg [1905]. The resulting *d*(+)-leucine was acetylated, and yielded a product which was recrystallized from water. M.P. 186–188°. $[\alpha]_D^{22°}$ +23·2° (alcohol, $l=2$, $c=3·7$). (Found: C, 55·8; H, 8·76; N (Kjeldahl), 7·8%. $C_8H_{15}O_3N$ requires C, 55·5; H, 8·67; N, 8·1%. Acid equiv. wt.: found 175; calc. 173.)

Cherbuliez *et al.* [1930] describe the isolation of acetyl-*l*-leucine from a protein by distillation of its ethyl ester. Their product had M.P. 181° and $[\alpha]_D^{20°}$ −16·99°. They remarked that their product had higher rotation and melting point than that of Karrer *et al.* [1921]. It will be noted that the present compound, of synthetic origin, has still higher M.P. and rotation than that of its optical antipode as isolated by Cherbuliez *et al.*

Diacetyl-dl-*lysine.* See Note 1, Table II.

Acetyl-l-*methionine.* Prepared from *l*-methionine isolated from an enzymic protein digest by Mr N. W. Pirie, to whom I express my thanks for the gift [cf. du Vigneaud & Meyer, 1932].

**Acetyl*-d-nor*leucine.* *dl-nor*Leucine (prepared from *n*-hexoic acid by bromination to α-bromo-*n*-hexoic acid, followed by amination) was resolved by the method of Marko [1908]. The resulting *d*(−)-*nor*leucine had $[\alpha]_D^{22°}$ −22·9° (18% HCl, $l=0·5$, $c=5$).

This on acetylation yielded a product which crystallized slowly from water. The compound is much more soluble in water than the corresponding derivatives of *iso*leucine and leucine, and might therefore be of use as an intermediate in the study of leucine fractions of protein origin.

The acetylated product had M.P. 112–114° and $[\alpha]_D^{28°}$ −0·2° (alcohol, $l=2$, $c=2·4$). This very low rotation is not due to racemization, since the compound on acid hydrolysis shows the correct rotation for *d*(−)-*nor*leucine in acid solution. (Found: C, 55·9; H, 8·81; N (Kjeldahl), 8·0%. $C_8H_{15}O_3N$ requires C, 55·5; H, 8·67; N, 8·1%. Acid equiv. wt.: found 172; calc. 173.)

Acetyl-l-*phenylalanine*. Prepared from *l*(−)phenylalanine (Hofmann-La Roche) [cf. du Vigneaud & Meyer, 1932]. The present product had M.P. 170–172°.

Acetyl-l-*proline*. Prepared from *l*-proline (isolated from a gelatin hydrolysate) [cf. Bergmann, 1935; du Vigneaud & Meyer, 1932].

*N-*Acetyl*-dl-*serine*. The preparation and properties of this compound are described in Paper III of this series.

N-*Acetyl*-l-*tyrosine*. Prepared from *l*-tyrosine [cf. du Vigneaud & Meyer, 1932].

ON-*Diacetyl*-dl-*tyrosine*. Prepared from *l*-tyrosine [cf. du Vigneaud & Meyer, 1932].

The *O*-acetyl group of this compound may be completely split off by keeping it in excess of 0·005 *N* NaOH at 20° for 10 hr. The *N*-acetyl group is unaffected by this treatment.

**Acetyl*-l-*valine*. Prepared from *l*(+)-valine (sold by Hofmann-La Roche as "*d*-valin"). The product was recrystallized from water. M.P. 157–158°. $[\alpha]_D^{20°}$ +5·8° (alcohol, $l=2$, $c=1·7$). (Found: C, 53·8; H, 8·29; N (Dumas), 8·5%. $C_7H_{13}O_3N$ requires C, 52·8; H, 8·17; N, 8·8%. Acid equiv. wt.: found 163; calc. 159.)

**Acetyl*-dl-*valine*. Prepared from synthetic *dl*-valine, the gift of Dr B. C. J. G. Knight, to whom I express my thanks. The product was recrystallized from water. M.P. 144–146°. (Found: C, 52·8; H, 8·08; N (Kjeldahl), 8·5%. $C_7H_{13}O_3N$ requires C, 52·8; H, 8·17; N, 8·8%. Acid equiv. wt.: found 159; calc. 159.)

SUMMARY

The partition of a number of acetamino-acids between immiscible solvents has been studied.

In an Appendix, the preparation of these compounds is described. Six compounds not previously described are characterized.

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