66. THE ISOLATION OF AMINO-ACIDS FROM RUBBER LATEX

BY G. S. WHITBY AND H. GREENBERG

From the Department of Chemistry, McGill University, Montreal

(Received 21 May 1941)

AFTER an examination of the chemical composition of the 'resin' of rubber had been carried through [Whitby *et al.* 1926] there was initiated a chemical examination of the aqueous serum left on separating rubber from the latex of *Hevea brasiliensis*. This latter was carried as far as a study of the amino-acids present, and had then to be interrupted. As it has not been possible since to resume it, and as several papers on the amino-acid composition of the proteins of *Hevea* latex have recently appeared, it is now thought desirable to put on record the results which were obtained.

While several investigations, mentioned below, have been made of the amino-acids derived by hydrolysis from the proteins of rubber and latex serum, only one previously published investigation has been concerned with the amino-acids present as such in latex. McGavack & Rumbold [1934] isolated *d*-alanine from latex serum and secured some evidence of the presence of phenylalanine. In the present investigation the following seven amino-acids were isolated from the serum: tyrosine, *l*-leucine, *d*-isoleucine, *d*-valine, *d*-arginine, *l*-aspartic acid and *i*-proline. Indications were also obtained of the presence of phenylalanine.

It may be noted that one of these, *d*-valine, was previously isolated from the acetone extract of rubber (sheet form) [Whitby *et al.* 1926]. Clearly, it had been taken up by the coagulum from the serum and had escaped complete removal in the preparation of the rubber.

It is interesting to compare these findings as to the free amino-acids present in *Hevea* latex with the results of the examination by various investigators of the amino-acids obtained by the hydrolysis of protein from rubber and serum. Belgrave [1925, 1] obtained evidence of the presence of glycine and proline in proteins obtained (*a*) from latex serum by precipitation with alcohol, (*b*) from rubber by exhaustive extraction with benzene. Later work by Belgrave [1925, 2] on the former protein indicated the presence, in addition, of arginine (in high proportion), cystine, histidine and lysine. Midgley *et al.* [1937] obtained the following from protein isolated from rubber: glycine, proline, arginine, histidine, lysine, leucine, aspartic acid and a representative of the group comprising alanine, phenylalanine, hydroxyproline and serine. They state that the following were definitely absent: cystine, glutamic acid and tyrosine. Subsequent authors, however, working it is true with protein isolated from rubber or latex in different ways, have identified these three amino-acids in hydrolysates.

In protein obtained from latex serum by precipitation with ammonium sulphate, Altman [1935] identified and estimated proline, arginine, histidine, leucine, aspartic acid, alanine, dihydroxyphenylalanine, tyrosine, glutamic acid, hydroxyproline and valine. Tristram [1940] analysed protein obtained by extraction of dried, fresh latex for the following nine amino-acids, all of which were present and which in total constituted about 50% of the protein: arginine, histidine, leucine, aspartic acid, tyrosine, glutamic acid, cystine, methionine and tryptophan.

In the present work a cursory examination of the heat-coagulable protein of latex serum led to the isolation from its hydrolysate of glycine, proline, aspartic acid, glutamic acid and tyrosine, and gave evidence of the presence, in addition, of histidine, arginine and lysine.

Table 1 summarizes the results of investigations made to date on the identification, isolation and estimation of the amino-acids of rubber, latex and latex serum.

In isolating, in the present investigation, amino-acids from latex serum, the method of fractionation employed was essentially that of Vickery [1924; 1925] and of Vickery & Leavenworth [1925], in which use is made of the mercuric acetate-sodium carbonate reagent of Neuberg & Kerb [1912] to precipitate the amino-acids as their carbonates and the purines as mercury complexes. The chief modification introduced was in the separation of the monoamino-acids and proline from the bases and dicarboxylic amino-acids. For this separation use was made of the butyl alcohol extraction procedure of Dakin [1918; 1920] which has proved so successful when applied by that worker to acid hydrolysates of proteins. The results of applying this procedure to the present case, however, were in keeping with the experience of Hunter [1925], who found that a sharp separation could not be obtained with enzymic digests of proteins containing peptide material. It is indeed hardly surprising that the butyl alcohol extraction method did not follow the same course and was not as complete and clear cut with the present material as with a protein hydrolysate, because of the presence, in addition to amino-acids, of a host of other organic and inorganic compounds. There is, for example, little doubt that acid-hydrolysable peptides were present, as Vickery has shown to be the case with alfalfa juice. Nevertheless, in spite of these circumstances, the method was of value and made it possible to isolate and identify tyrosine, *l*-leucine, *d*-isoleucine, *d*-valine, *d*-arginine, *i*-proline. The general scheme of fractionation is outlined in Table 2.

Table 2. *Outline of fractionation procedure*

Every attempt to arrive at the composition of a complex organic mixture of biological origin such as a solution of chemical entities constituting a plant juice

requires in the first instance much preliminary pioneering work to determine what substances and groups of substances are actually present. In the initial stages of the present study an attempt was made to keep an audit of the nitrogen separation, but many of the fractions obtained proved on further investigation to be so intractable that this had soon to be abandoned.

EXPERIMENTAL

A sample of serum solids, supplied by the Central Rubber Station, Buitenzorg, Java, and prepared by evaporating serum obtained by the coagulation of fresh latex with acetic acid, was used in some preliminary experiments. It was found that, although originally completely water-soluble, an appreciable amount had become insoluble during evaporation and storage. This insoluble material was isolated by filtration, washed with hot water and dried. The resulting hard, dark brown material had no melting point and was found to be protein in nature with 2.15 % ash. Repeated solution in NaOH and precipitation with acid gave a fairly white product. It will thus be seen that originally this protein was a normal soluble constituent of rubber latex, and that, while it was not precipitated by acetic acid (in the cold) during the coagulation of latex, subsequent heating had denatured it.

According to the view of Belgrave [1925] and of Belgrave & Bishop [1923] the non-protein nitrogenous compounds in latex are destined for the synthesis of proteins or are their decomposition products. If this be true then an analysis of the protein for constituent amino-acids should give helpful information as to nitrogenous substances which might be expected to occur in the free state in latex serum. With this object in view a cursory examination of the protein was made.

The protein (100 g.) was hydrolysed with six times its weight of H_2SO_4 (38 %) for 24 hr. The mineral acid was removed by adding baryta to pH 3 and the solution of amino-acids was concentrated to 350 ml., when tyrosine began to crystallize out. The solution was then brought to pH 5.5 with KOH and concentrated to 200 ml. After 18 hr. at 0° the separated material was filtered off. On repeated crystallization from hot water, this gave 2.5 g. of pure tyrosine.

The concentrated solution of amino-acids was then subjected to a continuous butyl alcohol extraction [Dakin, 1918; 1920]. The fraction separating from the butyl alcohol (monoamino-monocarboxylic acids, aliphatic and aromatic) weighed 31 g. From this mixture glycine was isolated as the ethyl ester hydrochloride: m.p. 144°.

The fraction soluble in butyl alcohol (proline and hydroxyproline) weighed 5.14 g. Some difficulty was experienced in isolating proline as hydantoin but evidences of its presence were found. The fraction not extracted by butyl alcohol was, after removal of the solvent, passed through the Foreman [1914] procedure for the separation of the dicarboxylic acids. In this way 11.4 g. of copper aspartate and 1.75 g. of glutamic acid hydrochloride (m.p. 196–197°) were obtained. The base fraction obtained from the Foreman filtrate by precipitation with phosphotungstic acid was examined by the Kossel procedure as given by Plimmer [1917] and although, for various reasons, the analysis was not completed, evidence of the presence of arginine, histidine and lysine was obtained.

From the cursory examination of the protein described above it will be seen that it is quite similar to most plant proteins, and, on the hypothesis of Belgrave, gives hints as to the possible amino-acids present in the serum of *Hevea brasiliensis*.

The actual work on serum solids was done on a quantity of serum solution (kindly furnished by the Naugatuck Chemical Company) obtained from latex preserved with ammonia and containing sodium alginate, which had been used to cause separation of the rubber by creaming. An attempt was made to evaporate this serum solution *in vacuo*, but the presence of sodium alginate caused violent frothing and it was necessary to concentrate from 55 gallons to 10 l. at atmospheric pressure and 75° with the aid of constant stirring and an electric fan to carry off the vapour. The time taken was 74 hr., and during the concentration about 2 kg. of rubber separated in small flocks, which were removed, together with coagulated protein, by skimming. Towards the end of the operation magnesium oxide was added to remove ammonia. Finally, the cooled concentrate was diluted with alcohol to precipitate the residual rubber and protein, together with the major part of the alginic acid, which could then be filtered off. To the filtrate were added 1200 g. normal lead acetate to remove proteoses and inorganic acids having insoluble lead salts and also to help clarify the solution under investigation. Not much precipitation occurred, however, until the solution was diluted with water. The precipitate was filtered, washed and the filtrate and washings saturated with H_2S to remove lead. The clarified solution was then concentrated *in vacuo* at 30° in the presence of charcoal, filtered and made up to a volume of 10 l. Of this 3800 ml. were taken for further investigation, and the results of a preliminary analysis by standard methods are given in Table 3.

Table 3

	g. in 3800 ml.	% of total N
Total N	124.6	100
Ammonia-N	17.4	14
Amide-N	4.1	3.3
Amino-N	23.4	18.5
Other N	80.0	64.1
Solids	2180.0	—
Ash	286.0	—

Precipitation with Neuberg's reagent

The solution was mechanically stirred and 40% Na_2CO_3 added until the liquid was acid to phenolphthalein but alkaline to litmus. After standing 2 hr., 21 g. of inorganic solids were removed by filtration. To the filtrate were added in small alternate quantities 1900 g. Na_2CO_3 (in 10% solution) and 4750 g. mercuric acetate (in 25% solution) until red mercuric oxide began to separate. After a day at 4–5° the voluminous precipitate was filtered off and washed with 80% alcohol. The filtrate and washings were combined and will be referred to later as the 'Neuberg filtrate'.

The Neuberg precipitate was suspended in as small a quantity of water as possible and treated with H_2S . When the liberation of the nitrogenous material was complete, the solution was kept at 70° for a number of hours to granulate the mercuric sulphide. After filtering and washing with hot water, the filtrate and washings were concentrated *in vacuo* at 35° and the volume made up to 2 l. The analysis at this stage is given in Table 4.

On comparing Tables 3 and 4, it will be seen that the Neuberg precipitate has carried down 82% of the total N of the original solution, and that the amide-N has disappeared, probably by hydrolysis in the alkaline medium, thus augmenting the yield of ammonia-N.

Table 4

	g. in 2 l. ml.	% of total N
Total N	101.9	100
Ammonia-N	19.0	18.65
Amide-N	None	None
Amino-N	23.9	23.5
Other N	59.0	57.9
Solids	1094.2	—
Ash	211.8	—

H₂SO₄ was next added to the above-mentioned solution obtained from the Neuberg precipitate until the concentration of the acid was 5%; this was followed by 25% aqueous phosphotungstic acid containing 5% H₂SO₄, added in small quantities at a time with stirring, until a drop of the supernatant liquid gave a white precipitate with a drop of baryta. The reaction mixture was kept at 5° for 50 hr., filtered and the precipitate washed well with 5% H₂SO₄. The procedure up to this point has separated the original solution of serum solids into three fractions (cf. Table 1):

(a) 'Neuberg filtrate': a fraction containing nitrogenous substances not precipitated by mercuric acetate-sodium carbonate.

(b) 'Phosphotungstic acid precipitate': a fraction containing nitrogenous substances precipitated by mercuric acetate-sodium carbonate and by phosphotungstic acid, viz. purines, diamino-acids.

(c) 'Phosphotungstic acid filtrate': a fraction containing nitrogenous bodies precipitated by mercuric acetate-sodium carbonate but not by phosphotungstic acid, viz. monoamino-acids.

Neuberg filtrate

The Neuberg precipitate having been removed, the filtrate was diluted with an equal volume of 95% alcohol (18 l.) and kept for 24 hr., after which some mercuric acetate was removed as a co-ordination compound with mercuric oxide [Berthelot, 1883]. The filtrate was now acidified with acetic acid and H₂S passed in to remove traces of mercury. After filtration the combined filtrate and washings were concentrated *in vacuo* at 35° to 8 l. Since 1900 g. of Na₂CO₃ had been introduced into this solution during the Neuberg precipitation and since the sodium was now present in the form of both acetate and carbonate, the following method was used to remove the sodium ion as chloride and the acetate ion as acetic acid.

The solution was first acidified to pH 3 with HCl. This caused the separation of a large quantity of NaCl and liberated all the acetic acid. The salt was removed by filtration and the filtrate concentrated until sufficient NaCl had separated to make the vacuum distillation difficult. The salt was again removed and distillation continued. In this way practically all the acetic acid was removed and the volume of the solution reduced to 2.5 l. At this point 2.5 l. of 95% alcohol were added to the concentrate, whereby 500 g. of NaCl and KCl were removed. The clear filtrate was reduced *in vacuo* to 1 l. and an equal volume of alcohol added. This threw down much quebrachitol and some inorganic material. These were filtered off and washed with alcohol. The filtrate and washings were concentrated to remove all alcohol, and then submitted to the continuous butyl alcohol extraction of Dakin [1918; 1920] for 200 hr. to remove any proline that might have escaped precipitation by the Neuberg reagent. It was found that the butyl alcohol extracted a fair amount of material, including most of the pigment, from the aqueous solution, and that on removing the solvent the

resulting thick paste was very soluble in water. 5 g. of this paste were treated for proline by the method of Dakin [1920]. To the solution in 50 ml. of water 8 g. of potassium cyanate were added and the whole evaporated to dryness on the water bath. The mass was taken up with water and acidified with H_2SO_4 until acid to Congo red (pH 3). The solution was then extracted with ether for 7 hr. in a continuous extractor [Dakin, 1920] to remove monoamino-acids, if present. When the extraction was complete $1/5$ vol. of H_2SO_4 (50 %) was added and the solution heated on the water bath for 2 hr. to convert the proline into the hydantoin. The solution was again extracted with ether for 50 hr. when there were obtained 26 mg. of substance which melted at $141\text{--}144^\circ$. The melting-point of *i*-proline hydantoin is given by Dakin [1920] as 143° . (Found by Folin's colorimetric method [1925]: N, 21.1 %; $\text{C}_6\text{H}_8\text{O}_2\text{N}_2$ requires N, 20.0 %.) The small yield did not permit of further confirmatory evidence being obtained. Small-scale experiments using mercuric chloride gave no evidence for the presence of betaines in this fraction.

Phosphotungstic acid precipitate

The wet precipitate was suspended in 3 l. of water and decomposed by adding baryta until alkaline to litmus. The magma was kept warm for 3 hr., filtered, the barium phosphotungstate well washed with hot water and the filtrate and washing reduced *in vacuo* to 3 l. The last trace of barium was now removed in the usual way and the final volume adjusted to 2 l. This is the 'purine and diamino-acid fraction', which analysed as follows:

	g.
Total solids	203.6
Ash	98.4
Total N	18.6

The fraction was treated with AgNO_3 (120 g.), which threw down a very dense canary yellow precipitate of purine-silver nitrate complexes. This was filtered, washed with hot water and decomposed with H_2S . The filtrate on evaporation gave 91 g. of a mixture of yellow crystals and a dark-brown syrup having an ash content of 65.1 %, the residue being almost pure tungstic oxide. The mixture contained 2.8 g. N, but attempts to separate the purine bases from it were unsuccessful.

The histidine fraction. The above-mentioned filtrate, which might contain the three bases, was submitted to the usual Kossel procedure for their isolation. Baryta was added until a drop of supernatant liquid no longer gave a white precipitate with a reagent made by adding ammonia to a 10 % silver nitrate solution until the silver just dissolves. The precipitate was kept in the cold for several hours before filtering and washing with cold water. It was then decomposed with H_2S and the clarified filtrate taken to dryness *in vacuo*; 1.5 g. of a light lemon-yellow coloured solid were obtained, which gave none of the usual reactions for histidine. It is inferred, therefore, that the latex serum does not contain this base.

The arginine fraction. The base solution was next saturated with baryta at 40° . After several hours the arginine-silver complex was separated by filtration, decomposed in the usual way and concentrated under reduced pressure. The solid material thus obtained weighed 4 g., and the ninhydrin test showed that α -amino-N was present. Arginine was obtained from it as flavianate by the method of Vickery [1926] and for further characterization was converted into the carbonate by the method of Pratt [1926]. The yield of white crystalline

material was 1.42 g.: 0.2410 g. when heated at 105° for 4 hr. lost 0.104 g., showing the presence of 0.5 mol. of water of crystallization (no previous reference in the literature). For the air-dried material: C=37.6; H=6.66%. $C_7H_{14}O_4N_4$, 0.5 H_2O requires C, 36.95; H, 6.61%. For the anhydrous material: N=25.15%. $C_7H_{14}O_4N_4$ requires N, 25.7%. The silver salt of arginine was made from 0.2 g. by adding sufficient HNO_3 to form the mononitrate and then adding the equivalent of silver nitrate. On diluting with alcohol, then with ether and keeping the mixture in the dark, white needles separated slowly (m.p. 181–183° with decomposition; Riesser [1906] gives 183°). Found: Ag, 26.64%. $C_6H_{14}N_4O_2 \cdot HNO_3 \cdot AgNO_3$ requires Ag, 26.15%. The picrate melted at 203–205° (Mulliken [1916] 205–206°) and the anhydrous copper salt at 238–240° (Riesser [1906] 232–234°). A solution made by dissolving 1% of the carbonate in 5% HCl showed in a 1 dm. tube $[\alpha]_D^{20} + 9.1^\circ$.

From the above evidence it is certain that the compound isolated was *D*-arginine and that it therefore occurs as such in the original solution of serum solids.

The lysine fraction. Silver and barium were completely removed from the arginine filtrate and the clarified solution concentrated to 510 ml. The mercuric chloride-baryta treatment of Winterstein [1904] gave 5 g. of a dark-brown hygroscopic mass from which it was not found possible to isolate lysine as its picrate. Other attempts to obtain some evidence for the presence of this base also failed.

Phosphotungstic acid filtrate

The amino-acid fraction which was precipitated by Neuberg's reagent but not by phosphotungstic acid was submitted to continuous extraction with butyl alcohol to give:

- (a) A fraction soluble in butyl alcohol.
- (b) A monoamino-acid fraction insoluble in, but extracted by, butyl alcohol.
- (c) A monoaminodicarboxylic acid fraction not extracted by butyl alcohol.

It must be stated that this extraction was by no means as clear cut as in the case of hydrolysed proteins for which it was primarily designed, but even so a sufficient separation of material was obtained to lead to the isolation of a number of amino-acids.

The extraction was continued for 200 hr., the butyl alcohol being changed every 24 hr. At the end of each day the butyl alcohol was filtered to remove fraction (a) (see above) and the residue washed with fresh alcohol. The filtrates were collected, the alcohol removed *in vacuo* and the residue taken up in water (fraction (b)). The aqueous solution of unextracted material was evaporated *in vacuo* to remove butyl alcohol and some acetic acid, and there was thus obtained a thick golden-yellow paste (fraction (c)).

Fraction (a). The solution (500 ml.) on standing deposited 4.4 g. of nearly colourless crystals which gave a strong Millon reaction indicating the presence of tyrosine. The material was suspended in 150 ml. of 95% alcohol and boiled, water being added slowly until no more of the suspension went into solution. The residue was filtered off and washed with alcohol. The dried material (0.69 g.) was dissolved in hot water, treated with norit (0.1 g.), filtered and concentrated on the water bath to incipient crystallization. On cooling, characteristic crystals of tyrosine separated and were filtered off (0.41 g.). The mother liquor on further concentration yielded an additional 0.11 g. The Millon test was positive. (Found: C, 59.9; H, 6.0; N, 7.69%. $C_9H_{11}O_3N$ requires C, 59.7; H, 6.1; N, 7.73%.) On standing, the solution from which the crude tyrosine had separated deposited

thin lustrous crystals. These were filtered off and washed with cold alcohol (1.62 g.). Millon's test was negative, showing the absence of tyrosine. Their greasy feel, the fact that they were moistened by water only with difficulty, and especially their analysis, suggested that they were leucine (found: N, 10.68; amino-N, 10.94%; mol. wt. (freezing point of water) 126.7; $C_6H_{13}O_2N$ requires N, 10.7; amino-N, 10.7%; mol. wt. 131), but the specific rotation in water, $[\alpha]_D^{20} - 13.6^\circ$, was too low and in 20% HCl was too high $[\alpha]_D^{20} + 27.1^\circ$, the recorded values in the literature being -10.42° and $+15.53^\circ$ respectively. Further investigation showed that the product was a mixture, and *l*-leucine was identified as its phenylhydantoin, m.p. 119–120°, and *d*-isoleucine as its benzoyl derivative, m.p. 116°.

The mother liquor from the above separation was concentrated to a syrup and kept at 0° for 24 hr. A mass of crystalline material was deposited which was filtered off and washed with ethyl acetate. The product (6.73 g.) was recrystallized from hot water to give ill-defined colourless platelets (2 g.) which were subsequently shown to be a mixture of *l*-leucine and *d*-isoleucine. The final mother liquor was taken to dryness (473 g.) dissolved in the minimum quantity of water and treated with much alcohol. On standing, another crop of crystalline material (2.6 g.) was obtained. This was filtered off, dissolved in water and precipitated by adding a large volume of alcohol. The product was *d*-valine. (Found: C, 50.4; H, 9.45; N, 11.88%. $C_5H_{11}O_2N$ requires C, 51.3; H, 9.40; N, 11.96%.) The specific rotation in water was $[\alpha]_D^{20} + 6.3^\circ$ and in 20% HCl $[\alpha]_D^{20} + 28.6^\circ$, while the phenylcarbamido-acid melted at 154–153° and the phenylhydantoin at 129–130.5°, values that are in keeping with those recorded in the literature.

Fraction (b). This weighed 7.8 g. and contained 30% of ash. 2 g. of the material were dissolved in 10 ml. of *N* NaOH and to the cooled solution an equivalent of phenylisocyanate was added. The mixture was shaken, with cooling, until the odour of isocyanate had disappeared. After filtration the phenylcarbamido-acids in the filtrate were precipitated by adding 10 ml. of *N* HCl. On recrystallization 1 g. of the phenylcarbamido derivative of *isoleucine*, m.p. 119–120°, was obtained. The hydantoin obtained by dehydration with HCl melted at 80°.

Fraction (c). After standing for about two months this fraction showed no signs of crystallization; it was therefore saturated with gaseous HCl, which precipitated much sodium and potassium chlorides. These were removed by filtration, and the filtrate set aside at 4–5° for 5 weeks, but no glutamic acid separated. A similar result was obtained in attempting to isolate glycine ethyl ester. The material was therefore collected, water removed, the residue covered with 4 vol. of 95% alcohol and saturated with dry HCl to precipitate most of the inorganic material (150 g.) and to bring the remainder into solution (as ethyl ester). After filtration, the alcohol and water were then removed *in vacuo*, 4 vol. of absolute alcohol were added, and the mixture again saturated with gaseous HCl.

The esterified solution was evaporated almost to dryness under reduced pressure and the residue dissolved in the smallest quantity of water. The esters were liberated with NaOH and extracted with ether after saturation of the liberated ester solution with K_2CO_3 . When the greater part of the dry ether was evaporated, 6 vol. of distilled water were added and the solution refluxed for 6 hr. to hydrolyse the esters to the free amino-acids and ethyl alcohol. Ordinary distillation removed most of the alcohol and evaporation under reduced pressure left a solid residue weighing 38 g. and also gave a turbid distillate from which were obtained 2 ml. of a pungent-smelling oil. This oil was set aside to be

investigated at some future date. The solid residue was dissolved in as little hot water as possible and decolorized with animal charcoal. To the clear filtrate 500 ml. of acetone were added, which caused the separation in two crops of 16 g. of material, m.p. 259–263°. Appropriate tests showed that this contained no glutamic acid, but that the ninhydrin reaction was positive. Negative Millon and positive xanthoproteic tests indicated the presence of phenylalanine. It was found, after a great deal of experimentation, that this material was not completely soluble in cold glacial acetic acid; 4 g. were accordingly suspended in cold glacial acetic acid and filtered rapidly, the residue being washed repeatedly with small quantities of the acid. In this way 1.04 g. of a pure white crystalline substance were obtained. Analysis of the substance, and of its insoluble copper salt, showed that it was *L*-aspartic acid. (Found: C, 36.4; H, 5.27; N, 10.58%. $C_4H_7O_4N$ requires C, 36.1; H, 5.26; N, 10.52%.) The specific rotation in 1% aqueous solution was $[\alpha]_D^{20} -4.1^\circ$. The copper salt contained 23.0% Cu; $C_4H_5O_4NCu \cdot 4.5H_2O$ requires Cu 23.05%.

SUMMARY

The following amino-acids are shown to occur in the serum of the latex of *Hevea brasiliensis*, all except the last having been actually isolated from it: tyrosine, *L*-leucine, *D*-isoleucine, *D*-valine, *D*-arginine, *L*-aspartic acid, *t*-proline, phenylalanine. A cursory examination of the amino-acid composition of the heat-coagulable protein of latex has been made. A tabular summary is given of the results obtained by various workers to date on the amino-acid composition of latex and latex proteins.

The authors are deeply indebted to Prof. A. C. Chibnall for assistance in revising the manuscript of this paper.

REFERENCES

- Altman (1935). *Arch. Rubbercult. Ned.-Ind.* **23**, 239. (Reprinted *Rubb. Chem. and Technol.* **13**, 505 (1940).)
- Belgrave (1925, 1). *Malay. agric. J.* **13**, 154.
- (1925, 2). *Malay. agric. J.* **13**, 367.
- & Bishop (1923). *Malay. agric. J.* **11**, 371.
- Berthelot (1883). *Ann. Chim. (Phys.)*, **29**, 392.
- Dakin (1918). *Biochem. J.* **12**, 290.
- (1920). *J. biol. Chem.* **44**, 499.
- Folin (1925). *Laboratory Manual of Biological Chemistry*, p. 131.
- Foreman (1914). *Biochem. J.* **8**, 463.
- Hunter (1925). *Trans. roy. soc. Can.* **19**, (V), 1.
- McGavack & Rumbold (1934). *Industr. Engng Chem.* **26**, 789.
- Midgley, Henne & Renoll (1937). *J. Amer. chem. Soc.* **59**, 2501.
- Mulliken (1916). *Identification of Pure Organic Compounds*. London: Chapman & Hall.
- Neuberg & Kerb (1912). *Biochem. Z.* **40**, 498.
- Plimmer (1917). *The Chemical Constitution of the Proteins*, Part 1. London.
- Pratt (1926). *J. biol. Chem.* **87**, 351.
- Riesser (1906). *Hoppe-Seyl. Z.* **49**, 217.
- Tristram (1940). *Biochem. J.* **34**, 301; cf. Tristram (1941). *Biochem. J.* **35**, 413.
- Vickery (1924). *J. biol. Chem.* **60**, 647; **61**, 117.
- (1925). *J. biol. Chem.* **65**, 81, 91, 657.
- (1926). *J. biol. Chem.* **71**, 303.
- and Leavenworth (1925). *J. biol. Chem.* **63**, 579.
- Winterstein (1904). *Hoppe-Seyl. Z.* **45**, 77.
- Whitby, Dolid & Yorston (1926). *J. chem. Soc.* p. 1448.
- Zwicker (1914). *Pharm. Weekblad*, **51**, 21; *Chem. Abstr.* **8**, 841.