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The Reaction between Mercury(II) and Organic Compounds. IV.*[1] A Photometric Method for the Determination of Basic Amino Acids Precipitated with a Mercury(II) Perchlorate Precipitant and Some Properties of Their Products

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The particular reactivity of basic amino acids in presence of mercury(II) perchlorate gives white amorphous products (by X-ray powder diffraction). The structures of the products may be considered to be $[Arg\text{-}Hg]ClO_4 \cdot Hg(ClO_4)_2$, $[His\text{-}Hg]ClO_4 \cdot Hg(ClO_4)_2$ and $[Lys\text{-}Hg]ClO_4 \cdot Hg(ClO_4)_2$ on the bases of elemental analytical data and of infrared patterns showing the absorption bands of $\nu_{as}(Hg\text{–}O)$, $\nu(Hg\text{–}N)$ and perchlorate ions. The applicability of this peculiar reactivity to the separation and photometric determination of basic amino acids has been studied. The above mentioned products were obtained selectively as a mixture from a mixture of about twenty kinds of amino acids. The isolation of the mixture into each product could be performed satisfactorily by utilizing the different solubilities of the products in 10 N acetic acid containing sodium or potassium chloride (3.3 W/V %), and in an aqueous sodium hydroxide solution. Although the products showed characteristic ultraviolet absorption curves differing from those of the components, there were no absorption maxima in any case. The calibration curves, which followed Beer's law, could be obtained at any proposed wavelength. By using the calibration curves thus obtained, a very small amount of each basic amino acid could be determined spectrophotometrically in the 220—250 mμ region, within detection limits of about 10^{-5}—10^{-4} M. Constant absorbancies could be attained in the pH range of 1.5—10.7 of the sample solutions.

Numerous works of analyses of basic amino acids (Arg, His, and Lys) have hitherto been undertaken by many workers. The analyses, after the preparation of the derivatives of amino acids such as flavianate,[1] 3,4-dichlorobenzenesulfonate,[2] picrate,[3] and benzenesulfonate[4] are known as long-established techniques. Ion exchange resins,[5] electrophoresis,[6] various chromatographic techniques,[7] spectral reflectance,[8] and conductivity measurements[9] have also been used in recent years. Phosphotungstic acid[10] which is known specific for the simultaneous and preferential precipitant of basic amino acids, silver(I),[11] copper(II)[12] and mercury(II)[13] salts have been used as the coprecipitants of basic amino acids. Especially, mercury(II) chloride, acetate and sulfate had been used for the isolation of Asp, Glu, Cys, Met and Try besidese basic amino acids. However, in the separation of basic amino acids using mercury(II) salts by Warburg *et al.*,[13] His was almost exclusively obtained, while Arg and Lys were isolated by using silver salts.

*1 Presented in part at the 20th Annual Meeting of the Chemical Society of Japan, Tokyo, March, 1967.

1) A. Kossel and R. E. Gross, *Z. Physiol. Chem.*, **135**, 167 (1942).
2) H. B. Vickery, *J. Biol. Chem.*, **143**, 77 (1942).
3) E. E. Rice, *Biochem. Prep.*, **1**, 63 (1949).
4) E. V. McCollum and A. A. Rider, *J. Biol. Chem.*, **190**, 451 (1951).
5) F. Turba, *Ber.*, **74B**, 1829 (1941); J. W. Dubnoff, *J. Biol. Chem.*, **141**, 711 (1941); R. J. Block, *Proc. Soc. Exp. Biol. Med.*, **51**, 252 (1942); D. T. Englis and H. A. Fiess, *Ind. Eng. Chem.*, **36**, 604 (1944); C. S. Cleaver, R. A. Hardy and H. G. Cassidy, *J. Am. Chem. Soc.*, **67**, 1343 (1945); J. C. Winters and R. Kunin, *Ind. Eng. Chem.*, **41**, 460 (1949); J. E. Eastoe, *Biochem. J.*, **74**, 8 (1960); S. Jacobs, *ibid.*, **87**, 30 (1963).
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8) M. M. Frodyma and R. W. Frei, *ibid.*, **17**, 131 (1965); R. W. Frei, *Chem. Abstr.*, **64**, 10073 (1966).
9) A. Nieman, *Z. Anal. Chem.*, **205**, 403 (1964).
10) V. Slyke, *J. Biol. Chem.*, **22**, 281 (1915).
11) H. B. Vickery and R. J. Block, *ibid.*, **93**, 105 (1931); G. R. Tristram, *Biochem. J.*, **33**, 1271 (1939).
12) L. G. Brazier, *ibid.*, **24**, 1188 (1930); S. Town, *ibid.*, **22**, 1083 (1928).
13) G. L. Foster and D. Shemin, *Org. Syn.*, **18**, 43 (1938); O. Warburg and W. Christian, *Biochem. Z.*, **310**, 304 (1942).

In the previous paper[14] of this series,[14-16] the present author has shown that mercury(II) chloride as well as phosphotungstic acid reacted selectively and simultaneously with basic amino acids in various amino acids. He also suggested that instead of phosphotungstic acid, mercury(II) perchlorate, as well as chloride, could be utilized as selective precipitant.

The present investigation has been undertaken in order to establish the methods for the separation and determination of basic amino acids by mercury(II) perchlorate, and to discuss some properties of the resulting products.

The proposed methods are sufficiently rapid, precise, and accurate to be used for the selective and simultaneous separation and determination of a small amount of basic amino acids. Furthermore, there is no procedure, except that using phosphotungstic acid and that by mercury(II) chloride reported in the preceding paper,[14] for the selective and simultaneous separation of basic amino acids.

Experimental

Materials. All the amino acids were obtained from the Nippon Rikagaku Yakuhin Co., Ltd., while reagent-grade mercury(II) chloride was obtained commercially. Mercury(II) perchlorate was prepared from the chloride by a procedure similar to that reported previously.[15] All the other chemicals used were of an analytical or of a special grade; they were used without further purification. Solutions for the measurement of the absorption spectra were prepared using redistilled water.

Apparatus. The pH was measured with a glass electrode (Yokogawa type KPH-51A and Hitachi-Horiba type M-5). The ultraviolet absorption spectra and the absorbancies were recorded with a Hitachi recording spectrophotometer (EPS-2 type) and a Hitachi photoelectric spectrophotometer (EPU-2 type) with 10 mm cells respectively. The infrared spectra in the 4000—650 cm^{-1} and 700—200 cm^{-1} region were obtained using a Hitachi-Perkin Elmer (type 125) and a Hitachi EPI-L type spectrophotometer respectively. All the samples were examined as Nujol-mull.

The mercury contents in the products were determined by chelatometric titration or by colorimetric determination.[17] The pH was adjusted with dilute aqueous perchloric acid and sodium hydroxide solutions as occasion demands.

Procedure

The Selective Separation of Basic Amino Acids in a Mixture of Various Amino Acids. An aqueous solution of mercury(II) perchlorate below 10^{-2} M*2 was added to an acid sample solution (with $HClO_4$) composed of a mixture of about twenty kinds of amino acids. A dilute sodium hydroxide solution was then added, drop by drop, into the solution until the pH of the supernatant reached about 8.5. Thereby, the only basic amino acids were co-precipitated with mercury(II) perchlorate as $[Arg\text{-}Hg]ClO_4\cdot Hg(ClO_4)_2$, $[His\text{-}Hg]ClO_4\cdot Hg(ClO_4)_2$ and $[Lys\text{-}Hg]ClO_4\cdot Hg(ClO_4)_2$. In order to certify the complete precipitation, a small aliquot of the supernatant was taken out and the same procedure was carried out again after the addition of a slight excess of mercury(II) perchlorate.

The Isolation of Each Basic Amino Acid from the Mixture. The isolation could be carried out by utilizing different solubilities of the precipitates under various experimental conditions.

His. Acetic acid (10 N) containing sodium chloride (3.3 W/V %) was added to a wet mixture of Arg, His and Lys as mercury salts. By shaking the mixture for a while at room temperature, only $[His\text{-}Hg]ClO_4\cdot Hg(ClO_4)_2$ remained as it is, while $[Arg\text{-}Hg]ClO_4\cdot Hg(ClO_4)_2$ and $[Lys\text{-}Hg]ClO_4\cdot Hg(ClO_4)_2$ were dissolved completely.

Arg and Lys. By adjusting the pH of the mother liquor separated from His to about 8, a mixture of $[Arg\text{-}Hg]ClO_4\cdot Hg(ClO_4)_2$ and $[Lys\text{-}Hg]ClO_4\cdot Hg(ClO_4)_2$ was reprecipitated. It was suspended in water, and then aqueous sodium hydroxide was added to the suspension until the pH reached to 12.1—12.3 at room temperature. When this suspension was heated to boil, only $[Lys\text{-}Hg]ClO_4\cdot Hg(ClO_4)_2$ was dissolved, while $[Arg\text{-}Hg]ClO_4\cdot Hg(ClO_4)_2$ remained at this pH; filtration was then carried out keeping the suspension hot, and the residue was washed with hot water at the pH 12.1. When the filtrate reached to room temperature, $[Lys\text{-}Hg]ClO_4\cdot Hg(ClO_4)_2$ was reprecipitated.

Results and Discussion

The Structures and Some Properties of the Products. *Elemental Analysis.* The products were analyzed as follows:

Arg. Found: C, 8.01; H, 1.52; N, 6.99; Hg, 43.94; Cl, 13.51%. Calcd for $C_6H_{13}N_4O_{14}\cdot Hg_2Cl_3$: C, 8.26; H, 1.49; N, 6.42; Hg, 45.98; Cl, 12.19%. Mp 206—208°C (decomp.).

His. Found: C, 9.06; H, 0.89; N, 4.56; Hg, 46.57; Cl, 13.45%. Calcd for $C_6H_8N_3O_{14}\cdot Hg_2Cl_3$: C, 8.44; H, 0.94; N, 4.92; Hg, 47.0; Cl, 12.46%. Mp 215—218°C (decomp.).

Lys. Found: C, 6.10; H, 0.98; N, 3.20; Hg, 45.99; Cl, 14.78%. Calcd for $C_4H_9N_2O_{14}\cdot$

14) F. Kai, This Bulletin **40**, 2297 (1967).
15) F. Kai, *ibid.*, **40**, 1136 (1967).
16) F. Kai, *Nippon Kagaku Zasshi (J. Chem. Soc. Japan, Pure Chem. Sect.)*, **86**, 1040 (1965).
17) K. Ueno, "Chelate Titration" (in Japanese), Nankodo, Tokyo (1964), p. 268; E. B. Sandell, "Colorimetric Determination of Traces of Metals," Interscience Publ., New York (1959), p. 621; F. D. Snell and C. T. Snell, "Colorimetric Method of Analysis," C. Van Nostrand Co., Philadelphia (1959), p. 63.

*2 At a higher concentration, the added mercury(II) perchlorate was apt to decompose to mercury oxide.

Hg_2Cl_3: C, 5.90; H, 1.11; N, 3.19; Hg, 49.24; Cl, 13.10%. Mp 188—190°C (decomp').

These analytical results indicate the ratio of 2 : 1 for mercury(II) perchlorate to basic amino acid in all the products. All of the products were amorphous (by X-ray powder diffraction).

Infrared Spectra of the Products and Relative Substances. Figures 1—3 give the infrared spectra of the precipitates (A—C) and related substances. In the spectra of the precipitates, the absorption bands

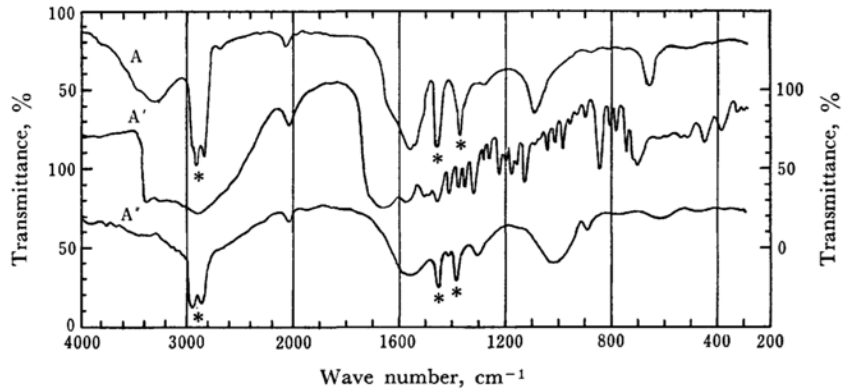


Fig. 1. Infrared spectra of Arg-mercury salt and relative compounds (Nujol).
A: $[Arg\text{-}Hg]ClO_4 \cdot Hg(ClO_4)_2$ A': Free Arg-hydrochloride
A'': Mixture of Arg and $Hg(ClO_4)_2$ (1 : 2) *, Absorption of Nujol

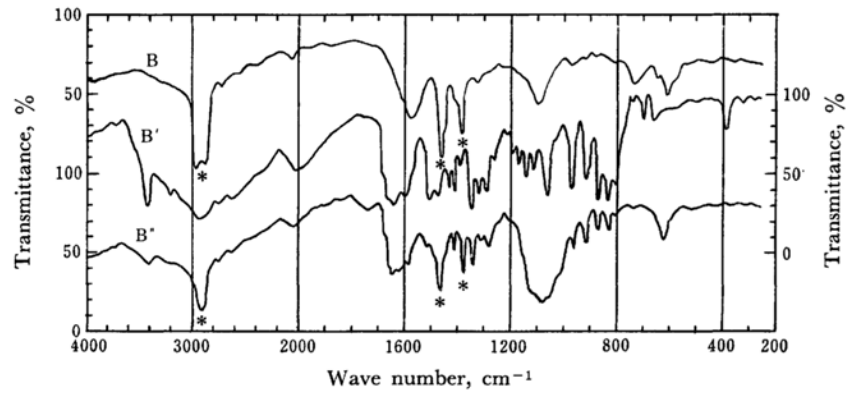


Fig. 2. Infrared spectra of His-mercury salt and relative compounds (Nujol).
B: $[His\text{-}Hg]ClO_4 \cdot Hg(ClO_4)_2$ B': Free His-hydrochloride
B'': Mixture of His and $Hg(ClO_4)_2$ (1 : 2) *, Absorption of Nujol

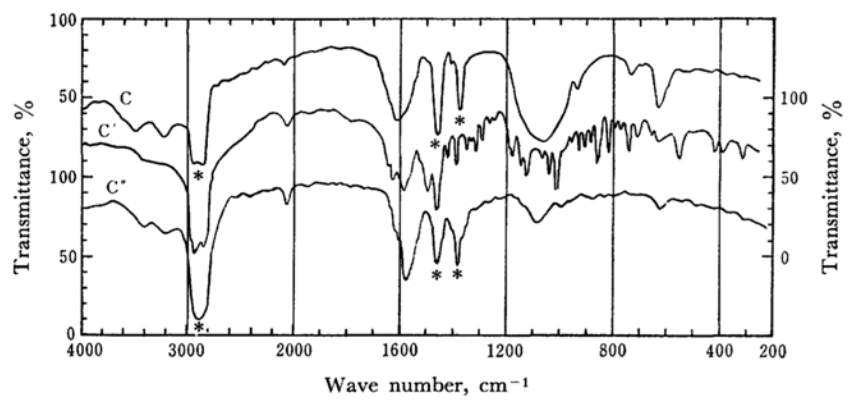


Fig. 3. Infrared spectra of Lys-mercry salt and relative compounds (Nujol).
C: $[Lys\text{-}Hg]ClO_4 \cdot Hg(ClO_4)_2$ C': Free Lys-hydrochloride
C'': Mixture of Lys and $Hg(ClO_4)_2$ (1 : 2) *, Absorption of Nujol

considered to be due to the ν_{as} (Hg–O)*3 shifted from the free carboxylic acid (A′—C′) are shown in the 1565—1570 cm^{-1} region. The bands considered to be ν(Hg–N)[18] are seen at 624 cm^{-1} for A and B, and at 622 cm^{-1} for C. Strong absorptions due to the perchlorate ions[19] are seen at 1090 cm^{-1} for A, at 1100 cm^{-1} for B and at 1085 cm^{-1} for C. Although free amino acids (A′—C′) as the components of the precipitates show many absorptions in the finger-print region, the precipitates give scarcely any absorptions in that region. Therefore, there is a possibility of the decomposition of the amino acids. However, since the stoichiometrical mixtures (A″—C″) of mercury(II) perchlorate and each basic amino acid (2 : 1) showed nearly the same absorption curves as those of A—C, the many absorptions in the finger-print region of A—C were considered to be hidden by the mercury(II) perchloarte which was predominant in quantity in the precipitates. As a result, the following structures of the precipitates are reasonable:

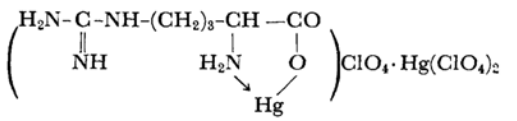
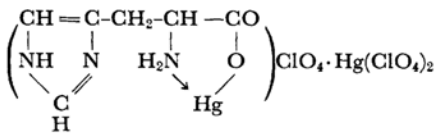
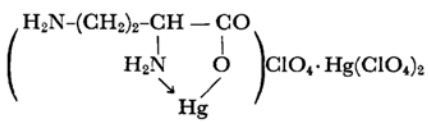


Abb. as [Arg-Hg]$ClO_4 \cdot Hg(ClO_4)_2$ (I)

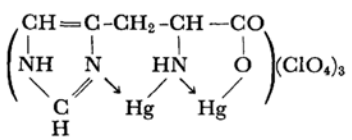


[His-Hg]$ClO_4 \cdot Hg(ClO_4)_2$ (II)

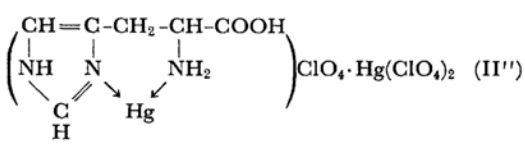


[Lys-Hg]$ClO_4 \cdot Hg(ClO_4)_2$ (III)

With respect to the II structure, the other structures shown below were considered possible:



(II′)



(II″)

However, II′ was considered to be unstable and II″*4 was unreasonable because the carboxylic group was expected to be fully utilized by the bonding with mercury(II).

The Solubilities of the Precipitates. The precipitates were insoluble in water and in organic solvents (even in DMF), but were soluble in aqueous mineral acid and alkaline solutions. Therefore, the amphoteric properties of amino acids remained. The I, II and III are soluble in hot alkaline solutions at the pH values of 12.8—13.0, 13< and 12.1—12.3 respectively. The II structure is, however, apt to decompose into mercury oxide under these conditions. Accordingly, isolation using different solubilities in alkali was not employed. In an acetic acid containing chloride ions (KCl, NaCl), it was found that I and III were dissolved completely, while II remained insoluble. Concentrations up to 10 N of acetic acid and up to 2% (W/V) of sodium or potassium chloride in acetic acid were required for a thorough dissolution.

When sodium or potassium acetate was used in place of chloride, I and III were insoluble. Accordingly, the presence of chloride ions seems to be indispensable for the dissolution of I and III. The isolation of I and III could be carried out by treating a mixture of them, obtained again in the way described hitherto, by utilizing their different solubilities in a sodium hydroxide solution.

Application to the Quantitative Determination of Basic Amino Acids. *The Stoichiometric Study of the Components.* The yields of the products varied with the ratios of the components. The results are illustrated in Fig. 4. The concentrations of both mercury(II) perchlorate and a basic amino acid in the original standard aqueous solutions were 5×10^{-3} M; the volume of each amino acid solution used was kept at 10 m*l*, corresponding to 5×10^{-5} mol. Into this solution of mixtures, dilute aqueous sodium hydroxide was added drop by drop while stirring. The yields of the products at a given ratio of two components were determined by the spectrophotometric method, by analyzing the nitrogen content, and by observing a ninhidryne reaction in a small aliquot of the supernatant. As can be seen in this figure, at the ratio of 1.0 the yields of Arg, His and Lys were 35, 42 and 45% respectively. Furthermore, at the ratio of 2.0, which was considered to be the theoretical value for a complete precipitate formation, the yields were no more than 65—77%. On increasing the ratio,

*3 In a homologous metal such as zinc, it is said that the band of ν(Zn–O) appears at 1656 cm^{-1}; Y. Sano and H. Tanabe, *J. Inorg. Nucl. Chem.*, **8**, 119 (1958).

18) S. Mizushima, I. Nakagawa and D. M. Seeny, *J. Chem. Phys.*, **25**, 1006 (1956); K. Broderson and H. J. Becher, *Chem. Ber.*, **89**, 87 (1956); I. Nakagawa, R. B. Penland, S. Mizushima, T. J. Lane and J. V. Quagliano, *Spectrochim. Acta*, **9**, 199 (1957); E. P. Bertin, *J. Am. Chem. Soc.*, **80**, 525 (1958).

19) B. J. Hathaway and A. E. Underhill, *J. Chem. Soc.*, **1961**, 3091.

*4 The possibility of the homologous formula of II″ was pointed out in Ref. 15.

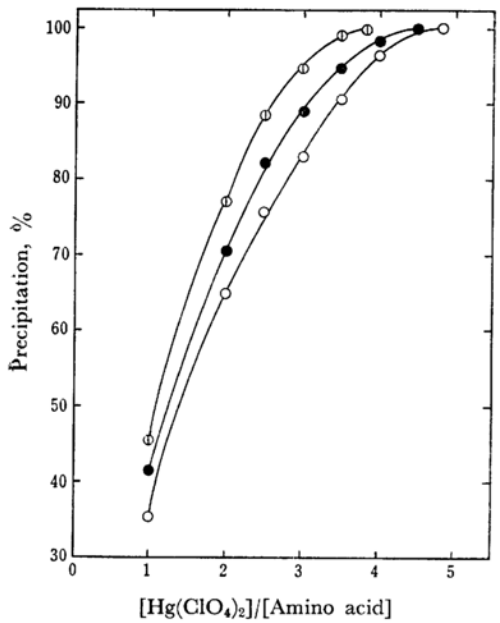


Fig. 4. Effect of varying ratio of component on precipitate formation.
-○- [Arg-Hg]$ClO_4 \cdot Hg(ClO_4)_2$
-●- [His-Hg]$ClO_4 \cdot Hg(ClO_4)_2$
-ⓘ- [Lys-Hg]$ClO_4 \cdot Hg(ClO_4)_2$
Original concn. of each component std. soln. was 5×10^{-3} M.

sufficient yields were obtained at the ratios of 3.8, 4.5 and 4.8 for Lys, His and Arg respectively. Therefore, more than five times as much mercury(II) perchlorate as each basic amino acid was required at least. The influence of excess mercury(II) perchlorate (about ten times as much mercury as each basic amino acid) on the precipitate formation was also studied. The formation, however, was not affected by the addition of excess mercury(II) perchlorate, and nor was any production of mercury oxide observed under these experimental conditions.

The Effect of pH on the Precipitate Formation. The yields of the products were affected by the pH of the sample solution. A dilute aqueous sodium hydroxide solution was added, drop by drop, to mixtures consisting of 2×10^{-4} M of each amino acid and 1.4×10^{-3} M of mercury(II) perchlorate. The results, shown in Fig. 5, suggest that the pH values of the solutions of mixtures which gave substantial yields were 7.8—7.9, 6.2—6.3 and 7.2—7.3 for Arg, His and Lys respectively. Therefore, in order to obtain substantially a mixture of the three kinds of precipitates, the pH of the solution was required to be at least 8.0. In order to obtain precipitates from a solution of more dilute amino acids, the effect of several organic solvents (methanol, ethanol, *n*- and isopropanol, acetone, dioxane, *etc.*), which are soluble in water, on the

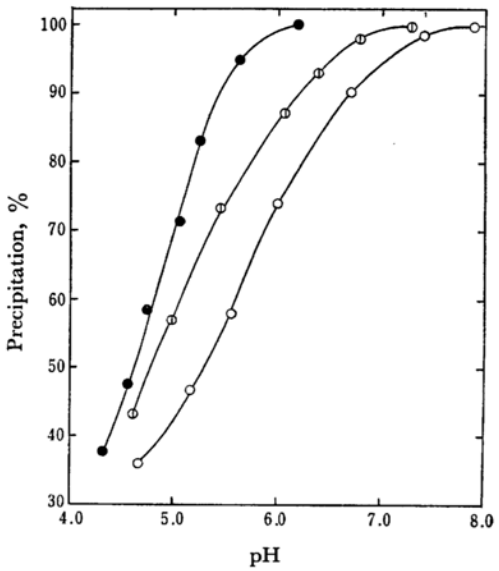


Fig. 5. Effect of pH on precipitate formation.
-○- [Arg-Hg]$ClO_4 \cdot Hg(ClO_4)_2$
-●- [His-Hg]$ClO_4 \cdot Hg(ClO_4)_2$
-ⓘ- [Lys-Hg]$ClO_4 \cdot Hg(ClO_4)_2$
Concn. of original std. soln.:
Amino acid $=2\times10^{-4}$ M
$Hg(ClO_4)_2=1.4\times10^{-3}$ M

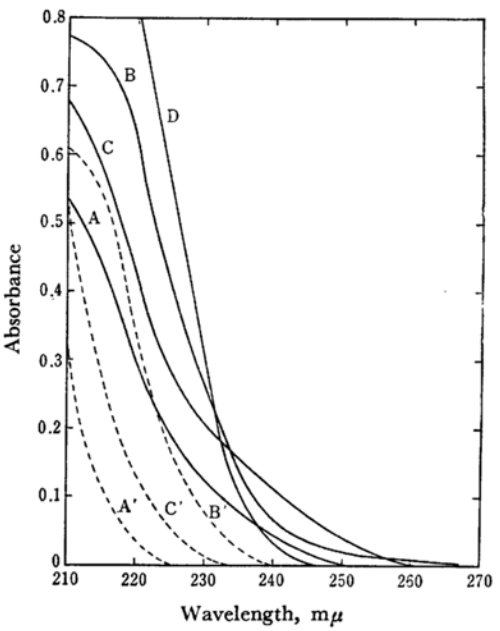


Fig. 6. Absorption spectra of the precpitates and their components in acidic aqueous solutions. (pH=2.1 with $HClO_4$)
A: [Arg-Hg]$ClO_4 \cdot Hg(ClO_4)_2$ (1×10^{-4} M)
B: [His-Hg]$ClO_4 \cdot Hg(ClO_4)_2$ (5×10^{-5} M)
C: [Lys-Hg]$ClO_4 \cdot Hg(ClO_4)_2$ (1×10^{-4} M)
A': Free Arg (1×10^{-3} M)
B': Free His (1×10^{-4} M)
C': Free Lys (1×10^{-2} M)
D: $Hg(ClO_4)_2$ (2×10^{-4} M)

formation of precipitates was investigated. The results made it clear that the formation of precipitates was facilitated by ethanol (ethanol: sample solution=1 : 1); thereby a small amount of basic amino acids could be precipitated from about a 10^{-5} M solution. This effect may presumably be explained by a reduction in the polarity of an aqueous sample solution.

Spectrophotometric Analyses. The ultraviolet absorption curves for the products and for their components are illustrated in Fig. 6. The spectra of the acidic solutions of I (curve A), II (curve B) and III (curve C) showed characteristic curves differing from those of the components, Arg (curve A'), His (curve B'), Lys (curve C') and mercury(II) perchlorate (curve D). As can also be seen from this figure, since the corresponding free amino acid content in these samples was as small as 1.9×10^{-5} M, 9.0×10^{-6} M and 1.4×10^{-5} M for Arg, His and Lys respectively, the proposed quantitative determination by the spectrophotometric method can be employed for a micro amount of basic amino acids.

Calibration Curves. The optimum wavelength for the quantitative determination must be ascertained, because there is no maximum in any of the absorption curves of the products. After many trials, the calibration curves in a good linear relationship at the proposed wavelength were obtained, as is shown below. The concentrations scaled in Figs. 7—9 show the calculated amounts of free amino acids in the corresponding mercury salts of basic amino acids.

Arg. The calibration curves on Arg in an acidic aqueous solution ($HClO_4$, pH 2.5), shown in Fig. 7, were obtained at a wavelength fixed arbitrarily in

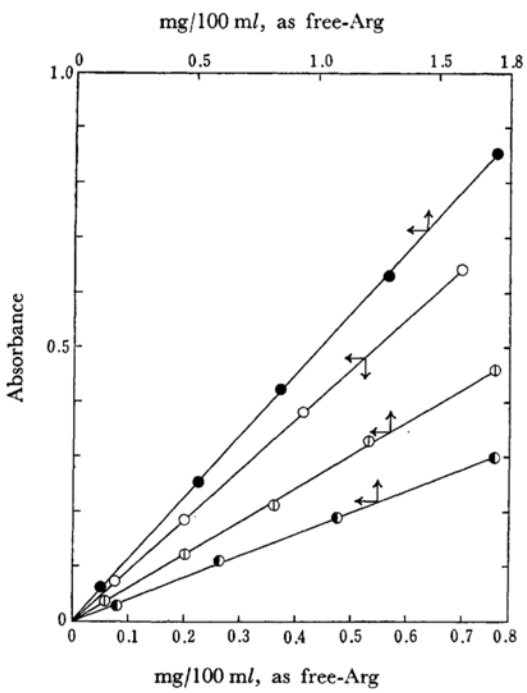


Fig. 7. Calibration curves on Arg, pH=2.5.
-○- 220 mμ -●- 230 mμ
-◑- 240 mμ -◐- 245 mμ

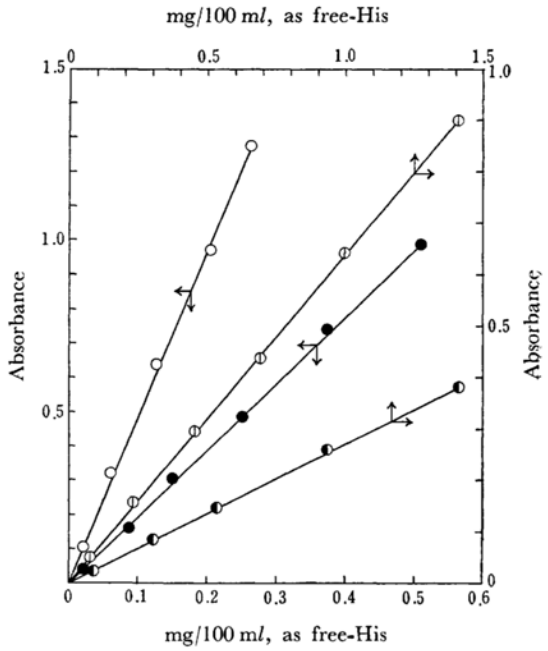


Fig. 8. Calibration curves on His, pH=2.0.
-○- 220 mμ -●- 230 mμ
-◑- 240 mμ -◐- 250 mμ

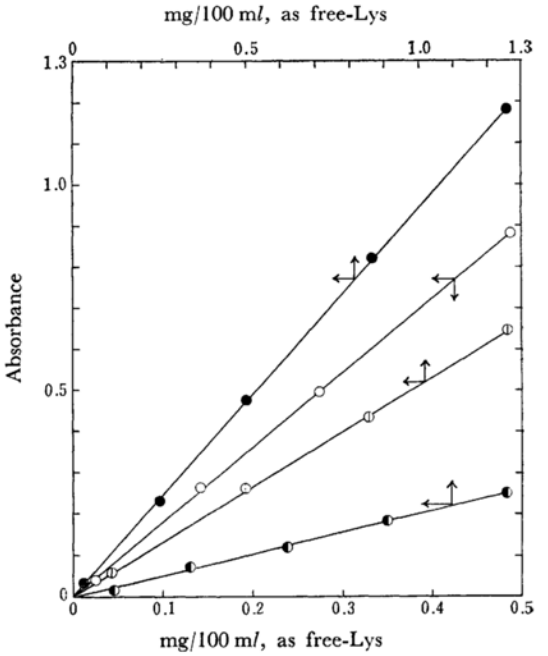


Fig. 9. Calibration curves on Lys, pH=2.3.
-○- 220 mμ -●- 230 mμ
-◑- 240 mμ -◐- 250 mμ

the region between 220—245 mμ. About 4×10^{-5} M of free Arg can be determined near 220 mμ, while about 1×10^{-4} M of free Arg can be determined in the 230—245 mμ region.

His. Figure 8 shows the curves of His (pH 2.0) in the range of 220—250 mμ. His up to 3.6×10^{-5} M in the 220—230 mμ and up to about 9.1×10^{-5} M in the 240—250 mμ region can be determined.

Lys. The curves for free Lys (pH 2.3) up to 3.5×10^{-5} M near 220 mμ and to 8.7×10^{-5} M in the 230—250 mμ region, shown in Fig. 9, have a fair linearity.

From these results, it was found that the quantitative determination of smaller amounts of basic amino acids may be carried out by the present calibration curves than those determined by using mercury(II) chloride[14] because the free basic amino acids contents in the products in this case of this perchlorate precipitant are lower than in those of chloride.

The Effect of the pH Value on the Absorbance of Sample Solutions. In order to study the effect of the pH on absorbancies, the pH of the sample solutions was adjusted to the required value with a dilute perchloric acid or a dilute sodium hydroxide solution.

Nevertheless these samples had been obtained at the pH values of 6—8 as described above (Fig. 5), at concentrations below 10^{-4} M of the sample solutions, there were, unexpectedly, neither any turbidity nor any conversion of the solution. The results are shown in Fig. 10. It was found that there are constant absorbancies extending over a wide range of pH values. His in the pH range of 1.5—8.5, Lys, in that of 2.5—10.7, and Arg, in that of 2.0—10.0 were determined at the respective constant absorbancies. Therefore, a precise set of pH values determined by a buffer solution was not necessary.

Recoveries by the Spectrophotometric Determination. The recoveries of basic amino acids by the present method were examined; the data obtained are listed in Tables 1 and 2. Table 1 shows the results obtained when each aqueous testing solution was composed of a mixture of mercury(II) perchlorate and an individual basic amino acid, while Table 2 shows those obtained from an aqoeus solution of a mixture of mercury(II) perchlorate and all the basic amino acids. Sufficient recoveries were shown in all cases.

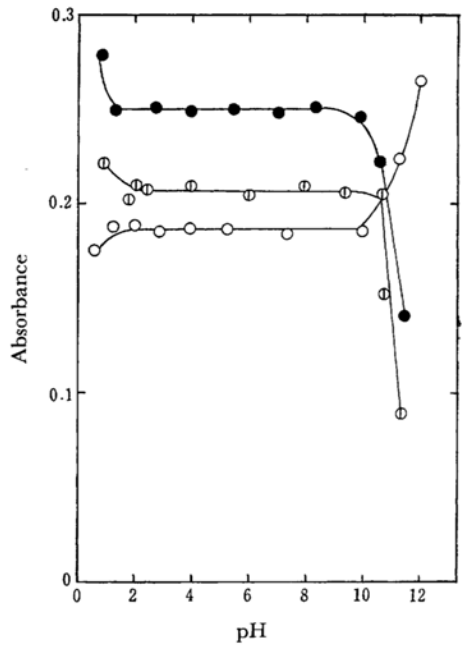


Fig. 10. Effect of pH on absorbancies.
-○- $[Arg\text{-}Hg]ClO_4\cdot Hg(ClO_4)_2$, 1×10^{-4} M at 225 mμ
-●- $[His\text{-}Hg]ClO_4\cdot Hg(ClO_4)_2$, 5×10^{-5} M, at 230 mμ
-○- $[Lys\text{-}Hg]ClO_4\cdot Hg(ClO_4)_2$, 1×10^{-4} M, at 230 mμ

Further confirmation of the usefulness of the proposed method was obtained by measuring the

Table 1. Recoveries of a basic amino acid from an each aqueous solution by the spectrophotometric determination

Arg			His			Lys		
Added mg	Found mg	Recoveries %	Added mg	Found mg	Recoveries %	Added mg	Found mg	Recoveries %
0.5	0.4	80	0.5	0.6	120	0.5	0.5	100
1.0	1.1	100	1.0	1.0	100	1.0	1.0	100
1.5	1.3	87	1.5	1.2	81	1.5	1.7	113
2.0	1.9	95	2.0	1.9	95	2.0	2.2	110
2.5	2.6	104	2.5	2.7	108	2.5	2.4	96
3.0	3.2	107	3.0	2.9	97	3.0	3.1	103
3.5	3.7	106	3.5	3.5	100	3.5	3.3	94
4.0	4.1	102	4.0	4.2	105	4.0	3.9	98

Concentration of each original aqueous standard solution was 10^{-3} M.

TABLE 2. RECOVERIES OF A BASIC AMINO ACID FROM AN AQUEOUS SOLUTION CONTAINING MIXTURE OF BASIC AMINO ACIDS AND VARIOUS AMINO ACIDS

a									b								
Added mg			Found mg			Recoveries %			Added mg			Found mg			Recoveries %		
Arg	His	Lys	Arg	His	Lys	Arg	His	Lys	Arg	His	Lys	Arg	His	Lys	Arg	His	Lys
1.0	1.0	1.0	1.1	0.9	1.0	110	90	100	2.0	2.0	2.0	2.1	2.0	1.7	105	100	85
3.0	3.0	3.0	2.9	3.1	3.1	97	103	103	4.0	4.0	4.0	4.3	4.1	3.8	107	102	95
5.0	5.0	5.0	5.2	4.8	4.7	104	96	94	6.0	6.0	6.0	5.7	6.1	6.0	95	101	100
7.0	7.0	7.0	7.0	7.1	6.8	100	101	97	8.0	8.0	8.0	8.4	7.8	8.3	105	98	104

a: Obtained from a mixture of three kinds of basic amino acids.
b: Obtained from a mixture of various amino acids.
Concentration of each original aqueous standard solution was 10^{-3} M.

basic amino acids in an ovalbumin. By comparing the present results with the data reported by Folin *et al.*[20] and Wieland,[21] it was confirmed that the proposed method is sufficiently rapid, precise and accurate to be used for the preferential and simultaneous separation and determination of a small amount of basic amino acids.

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