[CONTRIBUTION FROM THE STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY]

Chromatographic Adsorption of Amino Acids on Organic Exchange-Resins. II

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This paper presents the results of a further investigation into the fundamentals of the behavior of amino acids toward organic exchange-resins. Preliminary investigations[3] into the adsorption of several representative amino acids by the exchange-resins Amberlite IR-100 and Amberlite IR-4, and frontal analyses of several mixtures, indicated that organic exchange-resins might be useful in the quantitative adsorption and separation of amino acids. Considerable progress has been made in recent years toward this end, as surveys of the literature show.[4]

In this paper we report a useful cascade method of studying the adsorption behavior of amino acids, and data and interpretations which clarify some of the difficulties met in using exchange-resins with amino acid solutions.

Methods and Materials

Preparation of Exchange-Adsorbents.—Since our earlier investigation,[3] many organic exchange materials have become available commercially. The synthetic organic cation-exchangers used during this work were: Amberlite IR-100,[5a] Ionac C-200,[5b] Duolite C-2,[5c] Dowex-50.[5d] The organic anion-exchange materials investigated were: Amberlite IR-4,[5a] Ionac A-300,[5b] Duolite A-3.[5c] Still other exchangers have lately become available. Commercial products were used because they were available and apparently quite uniform in properties.

Particle Size.—The moist resins, as received, were oven-dried at 80 to 85° for twenty-four hours. At the end of that time, they were placed in a ball mill and ground for several hours. This was followed by a mechanical sifting of the resin to produce dry resin of $-60 + 80$ mesh. This particle size was used throughout this work.

Activation.—The dry, meshed resin was placed in a beaker of water and allowed to swell for several minutes. The moist resin was then placed in a 55 × 5.7-cm. tube which was arranged to permit upward-flow cycling. The rate of upward flow was regulated by a water aspirator applied through a safety bottle attached to the top outlet of the tube. The activating solutions were gravity-fed to the bottom of the tube. The resin was activated by repeated treatment with aqueous solutions of the designated acids, bases or neutral salts in concentrations of 2 to 5%. The excess reagent of each half-cycle was washed from the column with distilled water before another half-cycle was commenced. Three complete cycles were made on each resin in all cases, and in the event that colored percolates were observed during the last cycle, the cycles were repeated until the resin "threw" no further color. After the last half-cycle of activation, the resin was washed with quantities of distilled water (15 to 20 l. per 100 g. of resin) to remove the excess activating reagent. However, if the previous treatment had been with acidic or basic solutions, the *p*H's of the final percolates were never exactly 7.0, presumably due to slow leaching and hydrolysis effects.

Drying.—After washing, the resin was extruded from the column, placed on paper towels, and allowed to dry exposed to air. This was prolonged (usually twenty-four hours) until the resin appeared dry, and did not lose weight during weighing procedures. The percentage of water of the air-dried resin was obtained by drying a known quantity of the resin in an oven at 80 to 85° for twenty-four hours. The difference in weight was calculated as water, although it was recognized that the acid-activated anion-exchangers may have evolved hydrogen chloride and acetic acid in addition to water during the drying procedure. Several resins were dried over calcium chloride in an evacuated desiccator for forty-eight hours. Where necessary during the experimental section, the dry weights of the resin have been used as a basis for comparing the results.

Analytical Procedures.—The manometric nitrous acid technique described by Van Slyke[6] was quite generally used throughout this work for the determination of total amino nitrogen. It was necessary at times to use the manometric ninhydrin technique described by Van Slyke, *et al.*,[7] since it was observed that the nitrous acid technique occasionally liberated more than theoretical amounts of nitrogen from solutions which had contacted the polyamine anion-exchange materials. This source of error was small and was practically eliminated by pre-treating the anion-exchange materials with 5% hydrochloric acid just before activation and use.[8] In one experiment, however (the adsorption of phenylalanine with anion-exchange materials) the extent of adsorption was slight and errors caused by the anion-exchange materials were large in comparison. It was essential to use the manometric ninhydrin technique in this case. When using the ninhydrin procedure, good results were obtained by utilizing the saturated sodium chloride solutions and calculation factors recommended by MacFadyen,[9] and the ground-glass stoppered reaction vessels recommended by Hamilton and Van Slyke.[10] Arginine, histidine and glycine were estimated colorimetrically by the techniques given in a previous paper.[3] Phenylalanine was estimated with an Evelyn colorimeter according to Block and Bolling's[11] adaptation of the Kapeller-Adler[12] method. Hydrogen ion concentrations were measured with a glass electrode. All experiments, unless otherwise reported, were carried out at room temperature with aqueous solutions. The amino acids used throughout this work were products ob-

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(3) C. S. Cleaver, R. A. Hardy, Jr., and H. G. Cassidy, THIS JOURNAL, **67** 1343 (1945). This report, and the present one, contain material taken from the dissertation submitted by Charles S. Cleaver to the faculty of the Graduate School, Yale University, 1948, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(4) R. K. Cannan, *Ann. N. Y. Acad. Sci.*, **47**, 135 (1946); R. Kunin, *Anal. Chem.*, **21**, 87 (1949); J. C. Winters and R. Kunin, *Ind. Eng. Chem.*, **41**, 460 (1949); R. J. Block and D. Bolling, "The Amino Acid Composition of Proteins and Foods," 2nd. ed., C. C. Thomas, Springfield, Ill., 1949; R. J. Block, in "Ion Exchange," F. C. Nachod, ed., Academic Press, Inc., New York, N. Y., 1949, p. 296 *et seq.*

(5) We wish to thank the following companies for making available to us generous supplies of these resins: (a) Resinous Products and Chemical Co., Philadelphia, Pa.; (b) American Cyanamid Co., New York, N. Y.; (c) Chemical Process Co., San Francisco, California; (d) Dow Chemical Co., Midland, Mich.

(6) D. D. Van Slyke, *J. Biol. Chem.*, **83**, 425 (1929).

(7) D. D. Van Slyke, R. T. Dillon, D. A. MacFadyen and P. B. Hamilton, *ibid.*, **141**, 627 (1941).

(8) R. K. Cannan, *ibid.*, **152**, 401 (1944).

(9) D. A. MacFadyen, *ibid.*, **145**, 387 (1942).

(10) P. B. Hamilton and D. D. Van Slyke, *ibid.*, **150**, 231 (1943).

(11) R. J. Block and D. Bolling, "The Amino Acid Composition of Proteins and Foods," C. C. Thomas, Springfield, Ill., 1945.

(12) R. Kapeller-Adler, *Biochem. Z.*, **252**, 185 (1932).

tained from laboratory supply houses.[13] Analyses by the techniques given above proved them to be sufficiently pure for all experiments reported here.

Section 1. Investigation of the Behavior of Anion-Exchangers in a Counter-Current (Cascade) Arrangement.—From our preliminary attempt[3] to separate an acidic (glutamic acid) a basic (arginine, as the hydrochloride) and a neutral (glycine) amino acid it became evident that whenever a mixture contained sodium chloride and glutamic acid the glutamic acid was incompletely adsorbed by Amberlite IR-4(*NaOH).[14] Competition occurred between chloride and glutamate for the resin surface. Also the adsorption of each chloride ion to the amine resin was accompanied by removal of a proton from the solution, thus making the solution eventually basic, and so further suppressing the adsorption of glutamate. The sodium chloride in the mixture with glutamic acid came from the cation exchanger [IR-100(*NaCl)], used to remove arginine cation from the mixture by exchange with Na^+. If the acid form of the cation exchanger could have been utilized instead of the sodium form this problem would not have arisen. However, it was found, in agreement with others[4] that the acid form of sulfonic acid resins adsorbs all amino acids to some extent (because of the high acidity of the surface), so that this strongly acid resin could not be used to remove arginine alone from the ternary mixture.

Before further attempts were made to separate the neutral and acidic amino acids by the use of amine resins it was felt that an investigation into the nature of the anion exchange-reactions in a counter-current technique was necessary, particularly since so little fundamental information was available concerning the behavior of these resins towards amino acids.

Experimental.—Three 500-ml., 3-neck flasks were fitted with mechanical stirrers; the first flask also contained calomel and glass electrodes. In making a run, equal volumes of the initial solution were placed in each flask. The solutions were stirred vigorously and equal amounts of exchange-resin added to each. Fifteen to twenty minutes was allowed for adsorption. This gave a sufficiently close approach to equilibrium. The acidity was then measured, and 5 ml. of solution removed from the first flask for amino acid analysis. The volume in this flask was corrected by adding 5 ml. of solution taken from the second flask, and this one corrected with 5 ml. from the third flask. Resin was again added to all flasks, equilibrium approached, and the procedure repeated. In this manner, the first three samples removed from the first flask were without errors of volume and concentration change, and all subsequent samples contained small errors which were "buffered" by the second and third flasks. The procedure approximated a counter-current technique in some respects. If the resin additions had been smaller and more numerous, and if the resin had been removed from the solution after each step, the analogy would be very good. The technique was utilized to obtain information regarding the counter-current behavior of the anion-exchangers, although the resin was not removed at each step. The conditions and data of these experiments are condensed in Figs. 1–3.

Discussion of Fig. 1 (IR-4(*NaOH)).—Curve A, Fig. 1, shows that IR-4(*NaOH) has a great affinity for glutamic acid if the solution is not contaminated with foreign anions. This curve is a yardstick for the others, since it represents the maximum adsorption of glutamic acid by IR-4 (*NaOH) under these conditions. The more nearly the other curves approximate to Curve A, the more nearly are the optimum conditions reached for the adsorption of glutamic acid in the presence of other anions, or at other acidities. The effect of Cl^- ions on the adsorption of glutamic acid is diagrammatically indicated by Curve D. An amount of Cl^- ion equivalent to glutamic acid decreased the adsorption of glutamic acid by nearly 70%, which shows that Cl^- ions are adsorbed to a greater extent than glutamate ions. The adsorption of the Cl^- ions (with H^+) raised the *p*H to a level where further adsorption of glutamic acid could not take place. A similar phenomenon has been observed with amine resins in other connections.[15] Curve B represents the effect of acidity on the adsorption of glutamic acid. The glutamic acid was 75% neutralized with sodium hydroxide for this run, and only the first few additions of resin adsorbed the amino acid. Subsequent additions of the resin were ineffective because the hydrogen ion concentration was too low to permit further adsorption of glutamic acid. This experiment revealed that glutamate anion concentration is not always the important factor in determining the extent of the adsorption of glutamic acid, since more glutamate ions were present (ionized) at the beginning of this experiment than in Experiment IA. A comparison of Curves D and B with Curve A showed that the adsorption of glutamic acid varies with hydrogen ion concentration as well as with foreign anion concentration. To adsorb glutamic acid in the presence of chloride ions, it seemed that an excess of protons must exist in solution after the removal of Cl^- ions. Acetic acid is an acid similar in strength to glutamic acid. It was thought that chloride ions, as hydrochloric acid, would be removed from the solutions containing acetic acid, glutamic acid and sodium chloride by the first additions of resin. Protons from the acetic acid would remain in solution after hydrochloric acid removal, and allow a simultaneous adsorption of glutamate and acetate ions until the proton supply was exhausted. This hypothesis found support in Curve F. The initial preferential adsorption of hydrochloric acid allowed only a slight removal of glutamic acid during the first few resin additions. After the Cl^- ion concentration had been decreased, glutamic and acetic acids were adsorbed in larger quantities, as evidenced by the slope of Curve F during the later few additions of resin. When the reservoir of protons was obtained from hydrochloric acid, (Curve G) the adsorption of glutamic acid commenced later and was smaller in degree than that observed in Curve F. This occurred in spite of lower initial acidities than those obtained with acetic acid as the proton reservoir. Presumably, the great excess of preferentially adsorbable Cl^- ions accounted for these results. Curve H was

(13) We are indebted to the George Sheffield Fund for the purchase of some of the amino acids for this work, and to the Nutrition Foundation, Inc., for others.

(14) Amberlite IR-4 (*NaOH) is the acid adsorbing resin activated with sodium hydroxide. This converts the resin to the free amine form.

(15) M. C. Schwartz, W. R. Edwards, Jr., and G. Boudreaux, *Ind. Eng. Chem.*, **32**, 1462 (1940); J. A. Bishop, *J. Phys. Chem.*, **50**, 6 (1946); R. Kunin and R. J. Myers, THIS JOURNAL, **69**, 2874 (1947).

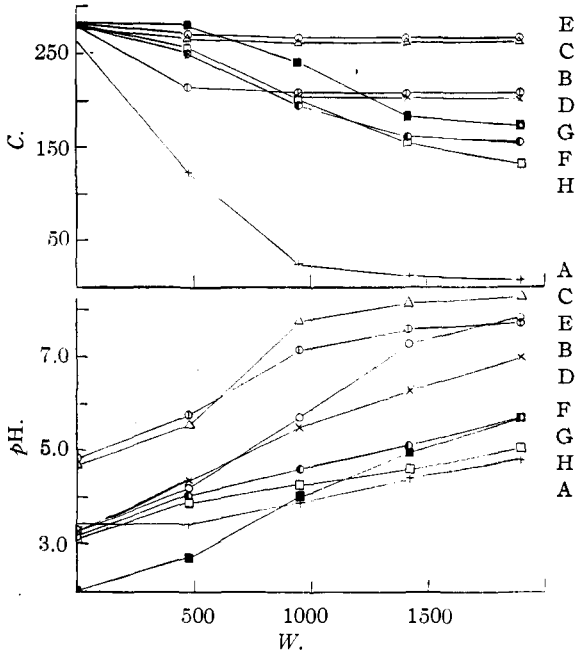

Fig. 1.—(a, upper) Adsorption of glutamic acid by Amberlite IR-4(*NaOH); (b, lower) concomitantly observed *p*H; *C* is concentration in γ of amino nitrogen per ml.; *W* is wt. in mg. of resin added to the solution, on dry basis. In each run 106 ml. of water per flask (see text), and a known weight of glutamic acid, close to 300 mg. (±3 mg.), were used: curve A, glutamic acid; B, glutamic acid + NaOH, 1:3/4 (molar ratio); C + NaOH + NaCl, 1:3/4:1; D + NaCl, 1:1; E + NaCl, 1:3; F + NaCl + CH_3COOH, 1:1:1; G + NaCl + HCl, 1:1:1; H + NaCl + CH_3COOH, 1:1:2.

similar to Curve F, although the "buffering" effect of acetic acid was much greater in this case. The slope of the tail of Curve H suggested that additional adsorption of glutamic acid would have occurred had more resin been added. Curve C approximated the counter-current effect of IR-4(*NaOH) on the IR-100(*NaCl) percolate of the previous group separation.[3] This curve showed why an incomplete separation had been obtained. Curve E showed the great undesirability of excessive Cl^- ion concentration. The ratio of glutamate to chloride anions in this experiment was 1:3, but the adsorption of glutamic acid was only one-tenth of that in Curve A.

Cannan[16] suggested that the acid salts of the anion-exchangers be used to produce anion-exchange reactions without large variations of *p*H. Experiments similar to those with IR-4-(*NaOH) were repeated with IR-4(*CH_3COOH). From these, it was possible to observe the effects of variations in foreign anions, and of *p*H on the adsorption of glutamic acid. The results are condensed in Fig. 2.

Discussion of Fig. 2 (IR-4(*CH_3COOH)).—Isotherm A, Fig. 2, is very similar to that ob-

(16) R. K. Cannan, see Ref. 4.

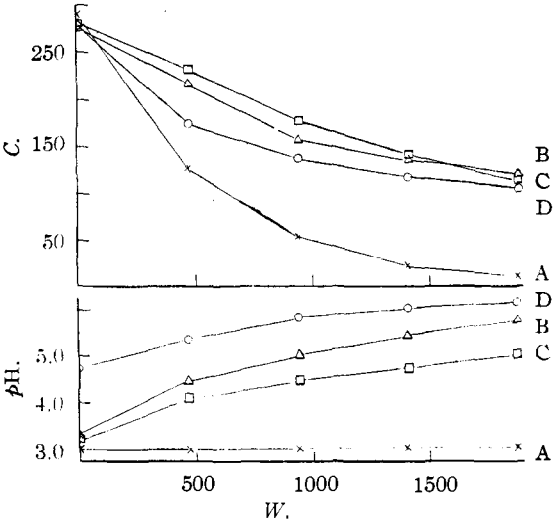

Fig. 2.—(a, upper) Adsorption of glutamic acid by Amberlite IR-4(*CH_3COOH); (b, lower) concomitantly observed *p*H; *C* and *W* as in Fig. 1: curve A glutamic acid; B glutamic acid + NaCl, 1:1 (molar ratio); C + NaCl + CH_3COOH, 1:1:1; D + NaOH, 1:3/4.

tained with glutamic acid and IR-4(*NaOH) (Fig. 1, A) and was used as the yardstick for the other curves. In the absence of other anions, glutamic acid is strongly adsorbed by IR-4-(*CH_3COOH). The *p*H during the exchange reaction did not vary. This effect was expected for acetate–glutamate exchange, and was in contrast to the results of the experiment with IR-4-(*NaOH). Curve D was obtained to test the theory that glutamate exchange depended to some extent on glutamate ion concentration. The weakly ionized glutamic acid was partially neutralized by sodium hydroxide to produce a greater number of glutamate ions. However, proton concentration apparently still played the major role in the adsorption of glutamic acid, since the removal of glutamic acid decreased when the initial *p*H was increased, and was much less than that described by Curve A. The adsorption was greater, however, than in the analogous experiment, Fig. 1, Curve B. This was due to the formation of a weaker base (sodium acetate) than in the previous case (sodium hydroxide) which buffered the solution, and restricted the *p*H increase. The effect of Cl^- ions on the adsorption of glutamic acid is observed in Fig. 2, Curve B. The initial slow fall in the curve was caused by the greater initial adsorption of Cl^- than glutamate ions. After the Cl^- ions had been removed by the first few additions of resin, more extensive glutamate exchange took place. The final adsorption of glutamic acid was greater than in Fig. 1, D because of a slower *p*H increase, which allowed more glutamic acid to be adsorbed. Curve C, Fig. 2, shows that buffering the solution with a source of protons increases the amount of glutamic acid adsorption after the chloride ions have been removed. The

tail of this curve has the greatest slope. This promised further adsorption of glutamic acid upon the addition of more resin. All of these curves showed slopes which suggested that further glutamic acid adsorption could take place in a chromatographic technique. Collectively, these results were more promising for a quantitative chromatographic adsorption of glutamic acid than those of Experiment 1. The acidities were also uniformly higher than those previously encountered with IR-4(*NaOH) due to the formation of sodium acetate rather than sodium hydroxide when the solutions were contaminated with salts.

IR-4(*CH_3COOH) showed a more neutral anion-exchange reaction than did IR-4(*NaOH). It was believed that IR-4(*HCl) would produce acidic solutions during the exchange, since some glutamate would exchange for chloride to produce the more highly ionized acid, hydrochloric acid, in solution. Investigations were made on this resin in the same manner as described for the experiments of Figs. 1 and 2. The results are condensed in Fig. 3.

Discussion of Fig. 3 (IR-4(*HCl)).—Isotherm A, Fig. 3, reveals the affinity of IR-4(*HCl) for pure glutamic acid. In comparison with OH^- or acetate ion, the chloride ion of the resin shows little tendency to exchange for glutamate ion. This appeared reasonable on the basis of the relative ionizations of glutamic acid and hydrochloric acid. That glutamate ion concentration can play a major role in these isotherms becomes evident in Curve C. The glutamic acid was first partially neutralized to form highly-ionized sodium glutamate. The adsorption of glutamate ion then increased with respect to Curve A. IR-4(*HCl) was the only resin which showed this dependence on glutamate ion concentration. The others could not display this relationship because of the more important superimposed *p*H effect. The effect of chloride ions is observed in Fig. 3B. As in the previous cases, the presence of Cl^- decreases the adsorption of glutamic acid. Curve D represents the counter-current behavior of an IR-100-(*NaCl) percolate with respect to IR-4(*HCl). The decrease of adsorption caused by chloride ions was opposed by the increase of adsorption caused by the sodium hydroxide. This made the over-all adsorption very similar to that of pure glutamic acid alone (Curve A). In all these experiments with IR-4(*HCl), the resin acted as the salt of a weak base and a strong acid. The hydrolysis of such a compound caused the high acidities encountered throughout these experiments. The high acidities ensured ultimate removal of glutamic acid, but the presence of Cl^- correspondingly decreased the relative adsorption, if compared to IR-4(*NaOH) and IR-4(*CH_3COOH). With respect to use in a counter-current technique, there seemed to be little choice between IR-4(*CH_3-COOH) and IR-4(*HCl). The neutral amino acids in the anion-exchanger percolate would be contaminated by inorganic salts in both cases.

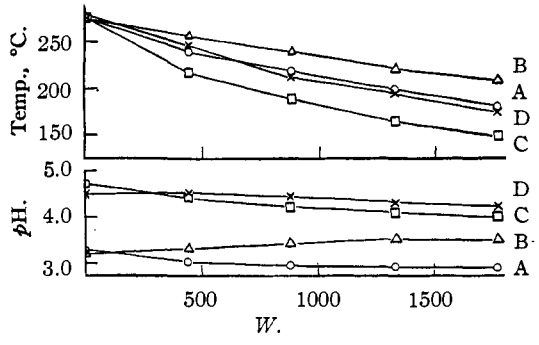

Fig. 3.—(a, upper) Adsorption of glutamic acid by Amberlite IR-4(*HCl); (b, lower) concomitantly observed *p*H; *C* and *W* as in Fig. 1: curve A glutamic acid; B glutamic acid + NaCl, 1:1 (molar ratio); C + NaOH, 1:3/4; D + NaCl + NaOH, 1:1:3/4.

Section 2. Mixed Adsorption of Anions by IR-4(*CH_3COOH).—The statement has been made in the previous section that the Cl^- ion was adsorbed "preferentially" to the glutamate anion. However, it was recognized that this may have been only a mass action effect, since at higher acidities, Cl^- ions were more numerous than the anions of the weakly ionized glutamic acid. To test this, adsorption isotherms were obtained for the two anions in the presence of each other at acidities such that both substances existed in a highly ionized state. A solution with the molar ratios glutamic acid:sodium chloride:sodium hydroxide = 1:1:3/4 was made up by diluting 2.1722 g. glutamic acid, 0.7986 g. sodium chloride, and 40 ml. 0.358 *N* sodium chloride to 250 ml. with distilled water. Five solutions of different concentrations but with the same ratio of components were made by appropriate dilution of portions of this initial solution. Twenty ml. of each of these five solutions were placed in contact with 500-mg. portions of air-dried IR-4(*CH_3COOH) and periodically shaken for eighteen hours. At the end of this time, the supernatant equilibrium solution was analyzed for glutamic acid by the Van Slyke manometric nitrous acid technique, and for halide by the usual gravimetric technique for silver chloride. Results are gathered in Fig. 4.

It is evident that chloride ion was adsorbed to a greater extent than glutamate ion at *p*H's which varied between 5.6 and 6.0. This difference in resin affinity for these ions showed that the presence of chloride ions would be particularly detrimental to the success of separations since in the presence of chloride, relatively few of the polar groups of the resin are utilized for the adsorption of acidic amino acid. This specific adsorption of chloride helps to explain some of the difficulties observed in attempts to separate amino acid mixtures quantitatively with exchange-resins.[4]

Section 3. Apparent Sorption of Amino Acids.—The data of Englis and Fiess[17] reveal

(17) D. T. Englis and H. A. Fiess, *Ind. Eng. Chem.*, **36**, 604 (1944).

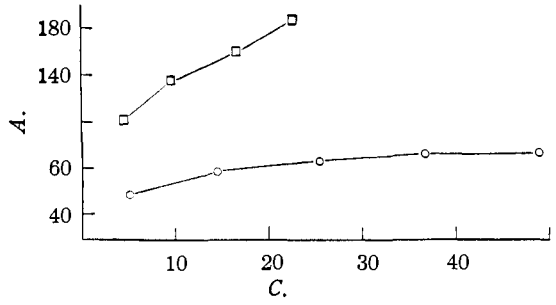

Fig. 4.—Co-adsorption of chloride and glutamate anions by Amberlite IR-4(*CH_3COOH): C = equilibrium concentration, millimoles/liter; A = specific adsorption, millimoles/g. resin on dry basis; □ = chloride; O, glutamate.

an interesting relation which has not previously been noted. The relative extents of adsorption of the neutral amino acids by the organic cation-exchange resins is paralleled by their relative distribution coefficients between organic solvents and water: tryptophan > phenylalanine > leucine > glycine.

With the acid form of the sulfonic acid cation-exchangers, this effect is apparently superimposed on the ionic adsorption of the neutral amino acids. It appeared that in addition to any ionic adsorption, the amino acids distributed themselves between the organic phase and the aqueous phase in the order of their distribution coefficients for organic solvent–water systems. Consden, Gordon and Martin[18] showed that in organic solvent–water systems, the neutral amino acids distributed themselves more and more into the organic phase as the hydrocarbon part of the molecule increased in size or aromaticity. Their data also indicated that the *absolute* distribution coefficients of the neutral amino acids varied with different organic solvents, but that the relative partition coefficients of the neutral amino acids between organic and aqueous phases tended to remain almost constant. These facts suggested that the neutral amino acids with aromatic or long alkyl chains were sorbed by organic ion-exchange substances to some extent by solution in the resin. If the liquid–liquid distribution hypothesis were correct as an explanation for this effect, it was expected that the sorption of the neutral amino acids by the salts of the organic exchange resins would increase in the same order as the distribution coefficients.

To test this idea, isotherms of a series of amino acids were obtained with various organic exchange resins at acidities where ionic exchange was improbable (nearly neutral solutions). Solutions of several amino acids were made up of different concentrations. Twenty ml. of each of these solutions were placed in contact with 1-g. quantities of air-dried organic ion-exchange material in oil sample bottles, and shaken periodically for twenty-four hours. (In the case of the anion-exchange resins, 10 ml. of solution and 500 mg. of resin were used.) At the end of this period, the supernatant equilibrium solutions were analyzed by the Van Slyke manometric procedures. All solutions were analyzed by the nitrous acid technique with the exception of the anion-exchange equilibrium solutions, which were analyzed by the ninhydrin manometric method. Both initial and equilibrium solutions were analyzed, and the difference calculated as sorption. For the results, see Figs. 5, 6, 7. (Unreported experiments also showed that tryptophan was sorbed by IR-100(*NaCl) to a larger extent than was phenylalanine.)

(18) R. Consden, A. H. Gordon and A. J. P. Martin, *Biochem. J.*, **38**, 224 (1944).

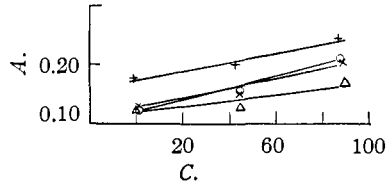

Fig. 5.—Sorption of *d,l*-alanine by exchange-resins: +, Duolite C-3(*NaCl); O, IR-100(*NaCl); △, Ionac C-200(*NaCl); ×, Dowex-50(*NaCl); C and A as in Fig. 4.

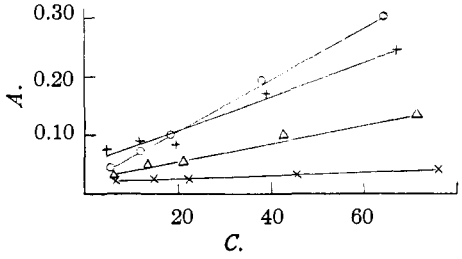

Fig. 6.—Sorption of *d,l*-norleucine by exchange-resins; symbols as in Fig. 5.

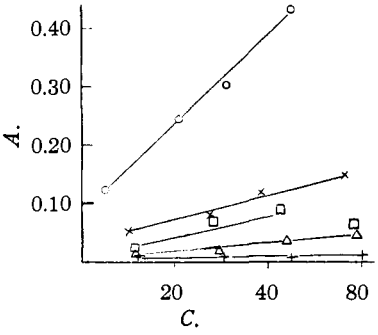

Fig. 7.—Sorption of *d,l*-phenylalanine by exchange-resins: O, IR-100(*NaCl); ×, Dowex-50(*NaCl); □, IR-4(*CH_3COOH); △, Duolite A-2(*CH_3COOH); +, Ionac A-300(*CH_3COOH); C and A as in Fig. 4.

The effect on sorption of increasing chain length of the neutral amino acids supported the resin–solution distribution hypothesis. Using the norleucine isotherms to compare the cation-exchange resins and the phenylalanine isotherms for the anion-exchange materials, it appeared that

Dowex-50 and Ionac A-300 exhibited the lowest sorption capacities for the neutral amino acids. In separation techniques, the advantages of more limited neutral amino acid sorption would depend also upon the quantities of each resin necessary to produce an exchange of the respective basic and acidic amino acids.

Tiselius, *et al.*,[19] have reported that the leucines were "retarded" by the carboxylic acid cation-exchanger, Wofatit-C. This could be caused by a similar solution effect, particularly since this cation-exchanger is surely less polar (see next section) than the sulfonic acid cation-exchangers. Wieland[20] did not test the separation of known mixtures of amino acids which contained amino acids higher than valine. It is felt that the long-chain basic amino acids, arginine and lysine, may be partially sorbed by a similar effect. This would account in part for the observed difficulty of elution of these amino acids from the cation-exchange resins.

The acid form of the cation-exchange materials behaves as a strong, insoluble acid. A titration of these macro-molecular acids with a strong base gives equivalent weights of the resins. The observed equivalent weight of a resin would be expected to depend on the previous treatment of the resin: drying, grinding, etc. The capacities of resins for *ionic* adsorption of amino acids would be expected to vary inversely with the equivalent weights of the resins. This would not be expected for non-ionic sorption.

Equivalent weights of four resins were determined by titration with sodium hydroxide. The values found were: Dowex-50(*HCl)[21] 227, Ionac C-200(*HCl) 324, Duolite C-3(*HCl) 390, IR-100(*HCl) 578.

The equivalent weights of the resins fell in the order: IR-100 > Duolite C-3 > Ionac C-200 > Dowex-50. This order is identical to that in Fig. 6 for decreasing norleucine sorption, with the organic cation-exchange materials. It seemed that the two were probably logically connected through the relative polar character of the resins. The resin of low equivalent weight contained more polar, hydrophilic $-SO_3^-$ groups per unit of mass than did the resin of high equivalent weight. These groups are probably randomly distributed throughout the body of the resin particle in each case. The higher the equivalent weight of the resin, therefore, the more non-polar area (or volume) available for sorption of the aromatic and aliphatic parts of the neutral amino acids.

The acid-adsorbent resins behaved as extremely weak bases, and equivalent weights of these materials were difficult to obtain. (Slow diffusion of acid and base resulted in no break at the end-point.) However, the exchange capacity of a resin is comparable to equivalent weight, and since glutamic acid was more extensively adsorbed by Ionac A-300 than by IR-4, it was concluded that Ionac A-300 had a lower equivalent weight, and hence was more polar than IR-4. This may have accounted for the difference in sorption of phenylalanine by the two acid-adsorbent resins. As a group, the acid-adsorbent resins showed much higher capacities for the acidic amino acids than did the cation-exchangers for the basic amino acids. It was inferred that the acid adsorbers have a higher density of polar groups in the surface, as a class, than the cation-exchangers. This would account for the collective small sorption capacities of the acid adsorbers for phenylalanine in comparison to the larger capacities of the cation-exchangers for this amino acid (Fig. 7). It was noticed that the equilibrium solutions of IR-100(*NaCl) with phenylalanine and tryptophan were discolored by minute resinous particles. Such was not the case with alanine solutions, and it is suggested that the former amino acids actually penetrated the resin and forced it to expand and crumble.

Acknowledgment.—The authors are indebted to the Nutrition Foundation for support of this work, and to Dr. Hubert B. Vickery for frequent counsel during its progress.

Summary

The counter-current response of a representative synthetic organic anion-exchange resin (IR-4) toward a typical acidic amino acid (glutamic acid) has been examined. The influence of the following factors upon the response have been investigated: type of resin, *p*H of solution and foreign anion contamination. Non-exchange distribution of neutral amino acids between organic exchange resins and aqueous phases has been postulated to explain observed effects.

NEW HAVEN, CONN. RECEIVED[22] AUGUST 23, 1949

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(22) Original manuscript received February 3, 1949.