[CONTRIBUTION FROM STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY]

Chromatographic Adsorption of Amino Acids on Organic Exchange-Resins

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This paper presents the results of an investigation of the behaviors of several amino acids toward two organic exchange-resins. Preliminary work was started early in 1941[3] as part of a project to investigate adsorption as a means of separating amino acids from their mixtures.[4] This early (unpublished) exploratory work indicated that exchange-resins would be useful as adsorbents for amino acids, and this paper reports later investigations of these resins. This work is presented now because it may be of use to others in the field. It is not entirely completed but present conditions make it uncertain when further work on this problem will be possible.

The purpose of the investigation reported here was to determine the response of the exchange-resins to different amino acids, to changes in relative concentration of amino acid and resin, to time of contact with solutions, etc. It was hoped that this would throw some light on the exchange process itself. It was intended, if it seemed feasible, to utilize the information so obtained for the separation of amino acid mixtures.

It was taken as a working hypothesis that it might be possible by the use of exchange-resins, particularly the newer, organic ones, to sort amino acids into three groups, the acidic amino acids, held perhaps by an acid-binding resin, the basic amino acids, which should be retained by a cation-exchanger, and the essentially neutral amino acids which might reasonably be expected not to be held by the resins. A number of workers appear to have thought of this idea at about the same time, and several papers have appeared in which work of this kind is reported. Block[5] has described a method for the separation of the basic amino acids from protein hydrolysates. He pretreated the hydrolysate to remove excess acid. The last step in this pretreatment involved the use of an organic acid-binding resin. The treated hydrolysate was then passed through a column of organic cation-exchange-resin which retained the basic amino acids as a group. These were recovered in good yield by treating the adsorbate with hydrochloric acid solution. Freudenberg, Walch and Molter[6] used organic cation-exchange and acid-binding resins to separate amino acids. The acid amino acids were adsorbed preferentially by an acid-binding resin. A cation-exchanger took up all amino acids, and the different groups of amino acids had then to be recovered from this resin by preferential elution. Platt and Glock[7] used organic ion-exchange-resins to purify biological solutions and extracts in the analysis for inositol. Turba, Richter and Kuchar[8] separated, in what they reported as a specific and highly quantitative manner, tryptophan from other amino acids by means of a specially treated organic exchange-resin. Cannan[9] has worked out a method for the adsorption of dicarboxylic amino acids from protein hydrolysates using an acid-binding resin. The amino acids were eluted with hydrochloric acid. The resulting solution "contains no more than traces of amino acids other than the dicarboxylic acids. From it, glutamic acid hydrochloride and copper aspartate may be crystallized directly in pure form and more readily than from Foreman's fraction."[9] A study of the behavior of a variety of amino acids on organic and other ion-exchange substances has been made by Englis and Fiess.[10] They investigated some of the factors which govern exchange. Their paper will be referred to below. It should be mentioned, to avoid possible confusion, that ion-exchangers of various inorganic kinds, such as permutits, pretreated aluminas and special clays, have been investigated in the separation of amino acids. A review of these does not properly belong here. A partial review to the middle of 1943 has been given by Wieland.[11]

The work referred to above makes it apparent that exchange adsorbents are promising tools which may be applied to the difficult problem of separating amino acids. All of the work referred to above, except that of Englis and Fiess, was designed to solve a given problem. Our approach, as that of Englis and Fiess, has been to examine wherever possible the fundamental problems of the exchange process so as to clarify the mode of operation of the organic exchange-resins in their application to the amino acids, and their general usefulness as tools. This paper is a contribution toward that end. The method used in this work is somewhat different from that of Englis and Fiess. Whatever of our work has confirmed theirs without contributing something additional has been not included.

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(4) A part of this work has been published: J. L. Wachtel and H. G. Cassidy, *Science*, **95**, 233 (1942); THIS JOURNAL, **65**, 665 (1943).

(5) R. J. Block, *Proc. Soc. Exptl. Biol. Med.*, **51**, 252 (1942).

(6) K. Freudenberg, H. Walch and H. Molter, *Naturwissenschaften*, **30**, 87 (1942).

(7) B. S. Platt and G. E. Glock, *Biochem. J.*, **36**, Proc., xviii (1942).

(8) F. Turba, M. Richter and F. Kuchar, *Naturwissenschaften*, **31**, 508 (1943).

(9) R. K. Cannan, *J. Biol. Chem.*, **152**, 401 (1944).

(10) D. T. Englis and H. A. Fiess, *Ind. Eng. Chem.*, **36**, 1054 (1944).

(11) Th. Wieland, *Die Chemie*, **56**, 213 (1943).

Materials

Two organic *exchange-adsorbents* were used in this work, a cation-exchanger Amberlite IR-100[12] and an acid-binding resin Amberlite IR-4.[12] These were obtained from the Resinous Products and Chemical Company, Philadelphia, Pa.[13] Amberlite IR-100 was conditioned by placing a quantity of resin in a vertical glass tube and passing suitable solutions through it. First distilled water was passed until the effluent liquid was colorless, then 5% hydrochloric acid until the effluent was strongly acid. The column was then washed with water until the effluent was nearly neutral to litmus and gave no halide test with silver nitrate. Either 5% sodium carbonate or ammonia solution as desired was then passed through the column until the effluent was strongly basic. The column was then washed with water until the effluent was neutral. The succession of treatments from acid through base and final washing, or base through acid and final washing, is a cycle, and the cycles were repeated, usually two to four as a total, until no colored material was given off by the resin anywhere in the last cycle. The cycle was stopped at the point which gave the desired resin: (H^+R^-), $(NH_4^+R^-)$, or (Na^+R^-), where the exchangeable cation is shown, and R^- is the resin body. Excess moisture was removed from the resin by pressing on filter paper. For some experiments oven-dried resin as noted was used. Amberlite IR-4 was conditioned in much the same way as the cation-exchanger. It was washed first with water, then 5% hydrochloric acid, then water, then 5% sodium carbonate or 2% sodium hydroxide, then water. Five or six cycles were usually needed. The last wash liquid was colorless, neutral to litmus and with no odor of formaldehyde. The resin was left at the point where it was activated by sodium carbonate or hydroxide, symbolized thus: IR-4 ($*Na_2CO_3$) and IR-4 (*NaOH). Excess moisture was removed from the resin by pressing on filter paper. The amino acids used were analyzed by the methods given below and were found to be sufficiently pure for the experiments reported here. Wherever possible analyses were made by the ninhydrin alpha-amino carboxyl method of Van Slyke, *et al.*,[14] in which the amino acids were decarboxylated with ninhydrin and the evolved carbon dioxide was determined. When very dilute solutions were encountered, and when mixtures of amino acids were involved it became necessary to use colorimetric methods, namely for histidine the modified diazo method developed by MacPherson,[15] for arginine the Sakaguchi method,[15,16] and for glycine the phthalaldehyde method.[17] The color densities were determined with an Evelyn colorimeter, using the appropriate filters and calibration curves. In the case of the diazo test for histidine it was observed that pure sulfanilamide when used in place of the usual sulfanilic acid gave an increase of about 10% in the photometric density for a given weight of histidine. The average density with sulfanilic acid was 90.3% of that with sulfanilamide. The histidine data given in this paper were obtained, unless otherwise stated, through the use of sulfanilamide. Hydrogen-ion concentration was measured with a glass electrode. All of the experiments were carried out at room temperature and with aqueous solutions.

Experimental

The conditions which would lead to the best operation of the resins were examined. Many conventional adsorption experiments were made in order to gain an insight into the behavior of the resins. These will not be reported in detail, due to lack of space, and a brief summary only will be given of the results. The cation-exchange-resins were studied first, and more thoroughly, than the acid-binding resins.

1. **Velocity of Adsorption.**—It was found, when 0.500 g. of resin was shaken with 20 ml. of solution containing 0.702 millimole of arginine hydrochloride, that equilibrium was reached by the hydrogen resin IR-100 (H^+R^-) in about one hour while with the sodium resin exchange continued for at least thirty-six hours (though the possibility of the destruction of amino acid, which would simulate adsorption, was not excluded). Behavior such as this has been observed with exchangers in other connections.[18]

2. **Type of Resin.**—Five-tenths of a gram of resin was shaken with 20 ml. of arginine hydrochloride solution, the *p*H of which had been adjusted with a few drops of hydrochloric acid or sodium hydroxide. It was found that the hydrogen resin (IR-100 (H^+R^-) dried at 110°) produced an equilibrium *p*H of from 1.9 to 2.9 even though the initial *p*H of the solution had been about 4.3, 6.6 or 8.5; the sodium resin (IR-100 (Na^+R^-), air-dried) had less effect on the *p*H; solutions of initial *p*H 2.4 to 2.8 showed at equilib-

(12) R. J. Myers, J. W. Eastes, and F. J. Myers, *Ind. Eng. Chem.*, **33**, 697 (1941); R. J. Myers, J. W. Eastes and D. Urquhart, *ibid.*, **33**, 1270 (1941). For a review of synthetic resin ion-exchangers see R. J. Myers, in E. O. Kraemer, "Advances in Colloid Science," Vol. I, New York, N. Y., 1942, p. 317.

(13) We wish to thank Dr. Frederick J. Myers of that company for making available to us generous supplies of these resins, and for giving us the information "that the exchange activity of Amberlite IR-100 is undoubtedly due to the presence of sulfonic acid groups and of IR-4 to amino groups. The former resin is a sulfonated phenol-formaldehyde resin composition and the latter an alkylene polyamine formaldehyde type condensate."

(14) D. D. Van Slyke, D. A. MacFadyen and P. Hamilton, *J. Biol. Chem.*, **141**, 671 (1941).

(15) H. T. MacPherson, *Biochem. J.*, **36**, 59 (1942).

(16) It was found that best results were obtained in the arginine analysis if the quantity of α-naphthol solution was reduced by half, to 1 ml., for this gave less color to the blank solution. A rigid time schedule was followed and the solution was vigorously stirred mechanically while adding the hypobromite solution. The color was developed in a known volume of 5 to 7 ml., diluted then to 25 ml., and read in the colorimeter. The development was followed until the color began to fade.

(17) A. R. Patton, *J. Biol. Chem.*, **108**, 267 (1935).

(18) See Myers, Eastes and Urquhart, ref. 12, and also R. H. Beaton and C. C. Furnas, *ibid.*, p. 1509.

rium 4.9 to 4.1 and those of *p*H 8.7 to 8.5 showed 9.2 and 8.6. At lower equilibrium concentrations the hydrogen resin adsorbed more amino acid than the sodium resin, and at higher equilibrium concentrations it adsorbed less amino acid than the sodium resin adsorbed.

3. The effect of the **weight of resin** relative to the volume of solution and amount of exchangeable ion was shown in the following experiment. Twenty-milliliter samples of histidine hydrochloride solutions were treated with weighed amounts of IR-100 (H^+R^-) which had been dried at 110–115°. (The resin developed an odor of SO_2 but appeared otherwise unaffected by this drying.) The resin was weighed to the nearest milligram. The ninhydrin amino carboxyl analytical method was used for most of the solutions. For the very dilute ones, the colorimetric method using sulfanilic acid was employed. The data are recorded in Table I. Column A gives adsorption in millimols per gram of resin (by difference); E gives equilibrium concentration in millimoles per liter.

TABLE I

EFFECT OF RATIO OF RESIN ON ADSORPTION OF HISTIDINE

Initial *p*H	A	E	Equilibrium *p*H	Apparent increase in H^+ ion concn., in millimole	Amino acid adsorbed, in millimole
0.5 g. resin; 20 ml. solution					
4.37	0.230	0.21	2.10	0.158	0.115
4.20	.433	1.10	1.92	.239	.216
4.03	.600	2.91	1.83	.294	.300
4.05	.663	7.28	1.84	.287	.331
4.01	.694	18.42	1.93	.233	.347
3.91	.709	30.0	2.02	.188	.355
0.25 g. resin; 20 ml. solution					
4.37	.411	0.82	2.20	.125	.103
4.20	.651	3.77	2.10	.158	.163
4.03	.692	9.23	2.13	.146	.173
4.05	.715	14.88	2.18	.130	.179
4.01	.747	26.42	2.29	.100	.187
3.91	.811	37.58	2.33	.091	.203
0.10 g. resin; 20 ml. solution					
4.37	.685	2.53	2.50	.062	.069
4.20	.752	8.16	2.50	.062	.075
4.03	.751	14.15	2.55	.054	.075
4.05	.811	19.78	2.60	.048	.081
4.01	.850	31.55	2.68	.040	.085
3.91	.864	43.35	2.75	.033	.086

The data in Table I are calculated to adsorption for 1 g. of resin. In the case of the 0.1-g. samples this involved multiplying any errors in analysis by 10; however, the differences observed are believed to be beyond experimental error. It may be seen that at equal equilibrium concentrations the **specific adsorption,** A, increased with decrease in the amount of resin. This should not be the case if simple, uncomplicated adsorption were occurring and it appears to be associated with the exchange. It would seem that the specific adsorption is greater with relatively less resin because at the same time the lesser amount of available hydrogen ion fails to lower the *p*H of the equilibrium solution enough to decrease the adsorption significantly. Analogous behavior was shown by arginine on IR-100 (H^+R^-), and is not tabulated here.

4. The resin (H^+R^-) appeared to be behaving like a strong, but very insoluble, acid, strong in the sense that much hydrogen ion was freely available if suitably evoked. A test of this behavior was made by titrating the resin with standardized alkali and acid, the *p*H change being followed with a glass electrode. The data obtained are too space-consuming to include here. It was found from the titration curves that the resin (H^+R^-) behaves like a strong acid and the resin (Na^+R^-) like the salt of a strong but insoluble acid.

The information reported above has shown that the degree of adsorption of a diamino-monocarboxylic acid was influenced by concentration of amino acid, relative concentration of resin when it was the hydrogen resin, concentration of hydrogen ion, and type of exchangeable group on the resin, and that the (H^+R^-) resin behaved like a strong acid. These resins were to be used in chromatographic adsorption columns, and it therefore seemed necessary to examine their behavior under conditions of flow. The chromatograms were to be of the type known as "liquid chromatograms." The remainder of the experiments will be concerned with the behavior of the resins when amino acid solutions percolate downward through columns of the resins in glass tubes. The liquid which flows from the bottoms of such columns will be called the **percolate.** The capacity and behavior of the resin under different conditions was followed by collecting the percolate in successive fractions and analyzing it. From the analyses a curve showing the break-through[12] point of the resin could be constructed for easy visualization of the behavior of the resin (Fig. 1). However the data will be given in the form of tables for the sake of greater definiteness. In these experiments two or more amino acids were used one of which was not adsorbed and one of which was adsorbed. The one which was not adsorbed showed by its dilution in the first fraction of percolate approximately the amount of liquid initially in the column. The one which was adsorbed indicated the exchange capacity of the resin. The two together showed whether the resin would be suitable for separating the mixture.

5. **Effect of Particle Size of Resin.**—The resin as received (largely −20 +48 mesh) was conditioned by putting it through cycles. One gram of IR-100 ($NH_4^+R^-$) was dried five hours at 75° and it then weighed 0.6220 g. Two grams of the undried resin was placed in a tube to form a column about 3.8 cm. high when wetted with water. A solution containing histidine hydro-

chloride and glutamic acid was passed through this column at a flow rate of 2 ml. per minute. The analytical data are given in Table II and are plotted in Fig. 1, curve 5.

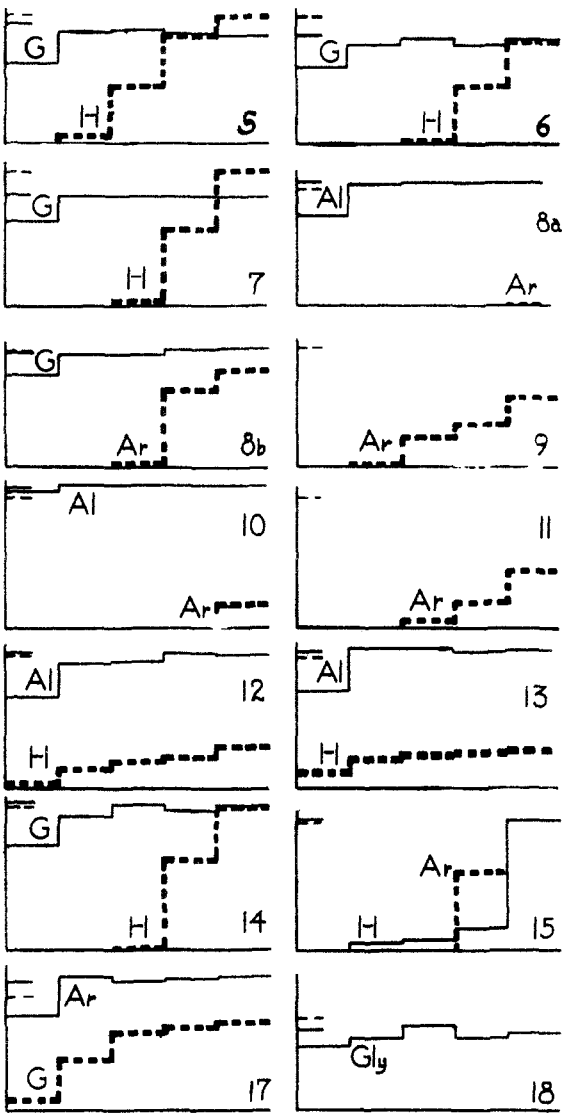

Figure 1 is designed for easy visualization of the results, and not to give quantitative information. On the abscissas are plotted the fractions of percolate collected (five) and on the ordinates the relative concentrations of these percolates. The numeral refers to the *experiment*, the data of which are plotted in the curve. The solid line is the amino acid which is not adsorbed and the broken line that which is adsorbed (except in 15, where both amino acids are adsorbed). The short lines near the tops of the ordinates show the initial concentrations of the amino acids. The letter indicates the amino acid, the curve of which is plotted, thus: Al, alanine; Ar, arginine; G, glutamic acid; Gly, glycine; H, histidine.

It is seen from Table II that histidine was retained during the first 10 ml. but broke through

TABLE II

BREAK-THROUGH DATA ON COARSE RESIN

Vol. collected	Histidine, millimoles/ml.	Glutamic acid, millimoles/ml.	*p*H
1st 10 ml.	2.05×10^{-4}	30.7×10^{-3}[a]	6.0
2nd 10 ml.	3.22×10^{-3}	42.0×10^{-3}	4.6
3rd 10 ml.	21.4×10^{-3}	42.8×10^{-3}	4.1
4th 10 ml.	40.6×10^{-3}	41.7×10^{-3}	3.8
5th 10 ml.	47.7×10^{-3}	40.5×10^{-3}	3.7
Initial soln.	52.8×10^{-3}	44.46×10^{-3}	3.2

[a] This solution was low in glutamic acid due to dilution with the water retained in the wet column, see preceding section. The effect is observed in all cases. There may also have been some adsorption.

afterward. Glutamic acid was not appreciably adsorbed. In all these experiments the concentration of glutamic acid or other amino acid for which no colorimetric method is available was determined by difference from the total amino acid and the value of the other amino acid determined colorimetrically. For this reason great accuracy cannot be claimed for the amino acid values, only adequate accuracy.

6. **Effect of Particle Size, Continued.**—The resin as received was conditioned, dried, ground and sieved, and then put through three cycles to activate the new surfaces. A column was prepared from $-60 + 80$ mesh IR-100 ($NH_4^+R^-$) resin. (One gram of the resin weighed after drying at 75°, 0.6280 g.) Two grams of resin was placed in a tube to give a column about 3.8 cm. high. A solution of histidine hydrochloride and glutamic acid was passed through the column at a rate of about 2 ml./min. The results are given in Table III and plotted in Fig. 1, curve 6.

TABLE III

BREAK-THROUGH DATA ON FINE RESIN

Vol. collected	Histidine, millimoles/ml.	Glutamic acid, millimoles/ml.	*p*H
1st 10 ml.	5×10^{-5}	28.4×10^{-3}	7.8
2nd 10 ml.	5×10^{-5}	37.2×10^{-3}	7.5
3rd 10 ml.	1.3×10^{-3}	39.4×10^{-3}	5.0
4th 10 ml.	21.9×10^{-3}	37.4×10^{-3}	4.0
5th 10 ml.	38.2×10^{-3}	39.4×10^{-3}	3.6
Initial soln.	47.66×10^{-3}	40.5×10^{-3}	3.2

It appears from this experiment that reducing the size of the particles of resin raised its exchange capacity for histidine (at this rate of flow) about three-fold. The break-through was delayed, and sharper. The particle size of the resin could have been made smaller with possible increase in capacity at this flow rate, but this size of particle was convenient to use: the rate of flow with it was satisfactory, and the break-through point occurred after a convenient volume, one which was great enough so that changes in either direction could readily be observed but not so great as to use up excessive amounts of amino acid solution. *For these reasons the remainder of the work reported here was done with $-60 + 80$ mesh resin.*

7. It next seemed desirable to find out the effect on the exchange of **height of column**. Two grams of the resin IR-100 ($NH_4^+R^-$) was placed in a narrower tube to give a column about 7.6 cm. in height. A solution of histidine and glutamic acid was passed through this column at a rate of about 2 ml. per minute. The data are given in Table IV and in Fig. 1, curve 7.

TABLE IV

EFFECT OF HEIGHT OF COLUMN ON EXCHANGE CAPACITY

Vol. collected	Histidine, millimoles/ml.	Glutamic acid, millimoles/ml.
1st 10 ml.	5×10^{-5}	31.5×10^{-3}
2nd 10 ml.	5×10^{-5}	41.0×10^{-3}
3rd 10 ml.	1.8×10^{-3}	40.8×10^{-3}
4th 10 ml.	28.6×10^{-3}	40.8×10^{-3}
5th 10 ml.	50.0×10^{-3}	40.8×10^{-3}
6th 10 ml.	52.3×10^{-3}	40.8×10^{-3}
7th 10 ml.	53.7×10^{-3}	36.4×10^{-3}
8th 10 ml.	51.7×10^{-3}	40.2×10^{-3}
9th 10 ml.	50.0×10^{-3}	39.3×10^{-3}
10th 10 ml.	52.3×10^{-3}	37.8×10^{-3}
Initial soln.	49.95×10^{-3}	41.60×10^{-3}

Increasing the height of the column apparently had no beneficial effect on the capacity at this rate of flow. This must mean that at this rate of flow the resin is already operating efficiently. The variations in concentration among the fractions from fifth to tenth for the histidine and from second to tenth for the glutamic acid illustrate the analytical difficulties encountered, and mentioned above in connection with experiment 5. Data beyond the fifth portion of percolate are not plotted in the figure.

8a. It seemed desirable to investigate the effects of **flow rate** on the break-through point of the resin. For this purpose mixtures of arginine and alanine were used. Arginine was somewhat better held than histidine. A solution containing arginine hydrochloride and alanine was passed through a 7.6 cm. column containing 2 g. IR-100 ($NH_4^+R^-$) at the rate of 2 ml. per min. The data are given in Table Va, and in Fig. 1, curve 8a.

TABLE Va

EFFECT OF FLOW RATE

Vol. collected	Arginine, millimoles/ml.	Alanine, millimoles/ml.	*p*H
1st 10 ml.	1.9×10^{-5}	33.7×10^{-3}	7.8
2nd 10 ml.	0.0	45.6×10^{-3}	7.8
3rd 10 ml.	4.8×10^{-5}	46.3×10^{-3}	7.8
4th 10 ml.	9.5×10^{-5}	46.3×10^{-3}	7.7
5th 7 ml.	7.9×10^{-4}	46.3×10^{-3}	7.6
Initial soln.	43.6×10^{-3}	46.7×10^{-3}	5.1

In the nearly neutral solution arginine is largely in the form of a cation and is well held. Arginine begins to break through at the fifth fraction. It may be noted that the resin shows high capacity at this *p*H. This behavior was supported by the findings of experiment 2. The *p*H appeared to begin to fall as the arginine began to break through. This indicated a break-through of hydrogen ion.

8b. There is included here, for purposes of later discussion, an experiment exactly like that reported in Table Va except that the solution used contained arginine and glutamic acid. The data are in Table Vb and Fig. 1, curve 8b.

TABLE Vb

EFFECTS OF FLOW RATE AND ACIDITY

Vol. collected	Arginine, millimoles/ml.	Glutamic acid, millimoles/ml.	*p*H
1st 10 ml.	4×10^{-5}	34.2×10^{-3}	7.6
2nd 10 ml.	4×10^{-5}	41.7×10^{-3}	7.0
3rd 10 ml.	1.5×10^{-3}	41.7×10^{-3}	4.6
4th 10 ml.	28.5×10^{-3}	43.9×10^{-3}	3.8
5th 10 ml.	36.1×10^{-3}	44.3×10^{-3}	3.5
Initial soln.	42.8×10^{-3}	42.3×10^{-3}	3.2

Here also the break-through is observed to be quite sharp.

9. Effect of Flow Rate, Continued.—An experiment similar to 8a, above, was made but which differed in that the rate of flow was increased ten-fold: from 2 ml./min. to 20 ml./min. The initial concentrations of amino acid were slightly different, and only the arginine was analyzed for since increase in flow rate could not in any likelihood appreciably change the behavior of the alanine on the column. The data are given in Table VI, and in Fig. 1, curve 9.

TABLE VI

EFFECT OF FLOW RATE, CONTINUED

The initial solution contained arginine hydrochloride, 44.6×10^{-3}, and alanine, 50.0×10^{-3}, millimoles per ml. In the five consecutive 10-ml. portions of percolate the arginine concentrations, in millimoles per ml., were: 4.8×10^{-5}; and 1.1, 11.4, 16.1 and 26.6, all $\times 10^{-3}$.

The effect of ten-fold increase in flow rate was to cause a much earlier break-through of arginine; however, adsorption from the first 10 or 20 ml. of solution was still fairly good.

10. The effect of **concentration of the solution** was next studied. The conditions were those of experiment 8a, above, except that the concentration of the solution in terms of both amino acids was reduced to a tenth while keeping the ratio of the two amino acids about the same. The solution was passed through 2 g. of the resin at the rate of 2 ml./min. and collected in 100 ml. portions. The data are given in Table VII, and Fig. 1, curve 10.

TABLE VII

EFFECT OF DILUTION

Vol. collected	Arginine, millimoles/10 ml.	Alanine, millimoles/10 ml.	*p*H
1st 100 ml.	4.8×10^{-5}	51.0×10^{-3}	8.3
2nd 100 ml.	4.8×10^{-5}	53×10^{-3}	7.9
3rd 100 ml.	4.8×10^{-5}	53×10^{-3}	7.6
4th 100 ml.	4.8×10^{-3}	53×10^{-3}	7.5
5th 100 ml.	8.6×10^{-3}	53×10^{-3}	7.2
Initial soln.	48.4×10^{-3}	52.1×10^{-3}	6.5

These data are very similar to those obtained with a solution almost ten times as concentrated (experiment 8a). This shows that the resin operates successfully over a ten-fold concentration change within this range. The concentration of alanine in the first 100 ml. of percolate should be noted especially. It is so nearly that of the original solution that it lends support to the conclusion of previous experiments that the lowering of the concentration of this amino acid in the first portion of percolate is a dilution effect due to the water already present in the resin. Here the amount of water on the resin is small compared with the volume of solution collected; in the previous experiments it was relatively large. It should be noted also that the *p*H values in both experiments show the same trend.

11. The two experiments above were then combined in order to determine the mutual effects of dilution and increased flow rate upon the behavior of the resin. The dilute amino acid solution was passed through a column containing 2 g. of IR-100 ($NH_4^+R^-$) at a rate of 20 ml. per min. The data are in Table VIII, and Fig. 1, curve 11. Alanine was not analyzed for.

TABLE VIII

EFFECTS OF DILUTION AND FLOW RATE

The initial solution contained arginine hydrochloride, 48.6×10^{-3}, and alanine, 50.5×10^{-3}, millimoles per ml. In the five consecutive 100-ml. portions of percolate the arginine concentrations, in millimoles per ml., were: 4.8×10^{-5}; 4.8×10^{-4}; and 2.5, 9.3 and 21.8, all $\times 10^{-3}$.

The results of these experiments indicate that the resin is sensitive to *rate* change within the range investigated, but not to *concentration* changes. It may be stated that experiments with the sodium resin IR-100 (Na^+R^-) gave substantially the same results as those with the ammonium resin, and hence they are not recorded here.

12. It is necessary in developing this report to show the behavior of histidine on the ammonium resin. Experiments given above showed that histidine was well adsorbed on the hydrogen resin. A solution of histidine hydrochloride and alanine was passed through 2 g. of IR-100 ($NH_4^+R^-$). The results are recorded in Table IX, and Fig. 1, curve 12.

TABLE IX

AN EFFECT OF *p*H ON ADSORPTION

Vol. collected	Histidine, millimoles/ml.	Alanine, millimoles/ml.	*p*H
1st 10 ml.	2×10^{-3}	33.8×10^{-3}	8.0
2nd 10 ml.	7.5×10^{-3}	47.2×10^{-3}	8.1
3rd 10 ml.	9.9×10^{-3}	47.7×10^{-3}	8.1
4th 10 ml.	12.0×10^{-3}	50.4×10^{-3}	7.8
5th 10 ml.	15.7×10^{-3}	50.2×10^{-3}	6.8
Initial soln.	49.23×10^{-3}	51.21×10^{-3}	4.0

It is evident that the histidine was not quantitatively retained even from the first 10 ml. This result agrees with predictions from conventional adsorption experiments (not given here) and may be explained on the following basis. The ammonium resin tends to yield a nearly neutral solution when it acts as an exchanger. Under this relatively low hydrogen ion concentration the histidine is not maintained wholly in the cationic form, and hence it is not in a state to be completely adsorbed by the ammonium resin. In addition there is competition with the hydrogen ion from the amino acid hydrochloride.

The explanation based on the results of this experiment agrees well with the results of experiments 5 and 6. Here histidine *in the presence of glutamic acid* was well held on the resin. Here the *p*H of the solution was consistently lower than in experiment 12. Results in agreement with those for the ammonium resin were obtained with the sodium resin in that since the sodium resin maintained a higher *p*H than the ammonium resin the histidine was even less well held by it. This is shown in the following experiments.

13. A solution of histidine hydrochloride and alanine was passed through 2 g. of IR-100 (Na^+R^-) at a rate of about 2 ml. per min. Column height was, as usual, about 7.6 cm. Data are given in Table X and Fig. 1, curve 13.

TABLE X

ADSORPTION OF HISTIDINE BY THE SODIUM RESIN

Vol. collected	Histidine, millimoles/ml.	Alanine, millimoles/ml.	*p*H
1st 10 ml.	6.3×10^{-3}	36.0×10^{-3}	8.6
2nd 10 ml.	11.7×10^{-3}	52.7×10^{-3}	8.6
3rd 10 ml.	13.4×10^{-3}	52.7×10^{-3}	8.6
4th 10 ml.	14.2×10^{-3}	51.7×10^{-3}	8.3
5th 10 ml.	15.1×10^{-3}	52.2×10^{-3}	8.1
Initial soln.	49.1×10^{-3}	51.4×10^{-3}	4.0

14. A solution of histidine hydrochloride and glutamic acid was passed through 2 g. of sodium resin at about 2 ml./min. The results are shown in Table XI, and Fig. 1, curve 14.

TABLE XI

ADSORPTION OF HISTIDINE BY THE SODIUM RESIN, CONTINUED

Vol. collected	Histidine, millimoles/ml.	Glutamic acid, millimoles/ml.	*p*H
1st 10 ml.	9.5×10^{-5}	38.8×10^{-3}	7.8
2nd 10 ml.	4.8×10^{-5}	49.4×10^{-3}	7.8
3rd 10 ml.	3.8×10^{-4}	54.1×10^{-3}	5.6
4th 10 ml.	33.4×10^{-3}	51.9×10^{-3}	4.2
5th 7 ml.	52.5×10^{-3}	53.4×10^{-3}	3.8
Initial soln.	52.5×10^{-3}	54.5×10^{-3}	3.2

These experiments show that the capacity of the sodium resin for histidine in the presence of glutamic acid is the same as that of the ammonium resin. An explanation is suggested under experiment 12. In the presence of alanine the histidine leaks through the column. The experiments suggested that perhaps histidine would be better held in the presence of alanine if the solution were made sufficiently acid to bring the histidine into

the cationic form. A preliminary experiment made with coarse resin with a histidine–alanine solution adjusted with hydrochloric acid to a *p*H of 3.1 suggests that this would not be the case.

15. It seemed desirable next to investigate a **ternary mixture** of amino acids in order to discover whether **displacement effects** occur. A solution containing arginine hydrochloride, histidine hydrochloride, and glutamic acid was passed through a column of 4 g. IR-100 ($NH_4^+R^-$) at the rate of 2 ml. per min. The percolate was collected in 25-ml. fractions and analyzed for arginine and histidine. The data are given in Table XII and in Fig. 1, curve 15 (compare curves 6 and 8b).

TABLE XII

BEHAVIOR OF A TERNARY MIXTURE

Vol. collected	Arginine, millimoles/2.5 ml.	Histidine, millimoles/2.5 ml.	Glutamic acid, millimoles/2.5 ml.
1st 25 ml.	4×10^{-5}	2×10^{-4}	
2nd 25 ml.	8×10^{-5}	3.2×10^{-3}	Not taken
3rd 25 ml.	4×10^{-4}	3.9×10^{-3}	
4th 25 ml.	29.0×10^{-3}	8.1×10^{-3}	
5th 25 ml.	Not taken	48.5×10^{-3}	
Initial soln. Initial *p*H 3.4	47.49×10^{-3}	48.52×10^{-3}	50.71×10^{-3}

These results, taken with those above, show that in the presence of glutamic acid, histidine and arginine together are well adsorbed. The histidine "leaked through" slightly but both amino acids broke through at about the same point. There appeared to be little or no preferential adsorption in the presence of glutamic acid.

Attention was also given to the acid-binding resin IR-4. When this resin, IR-4 (*Na_2CO_3), −60 +80 mesh, was placed dry in a tube, then wetted with water, and glutamic acid solution passed in, it swelled so as virtually to stop the flow of solution before glutamic acid could break through. At the same time bubbles of gas were given off which hindered the flow of the liquid, and a lightening in color of the resin appeared at the place where glutamic acid was being adsorbed.[19] To avoid the stoppage of flow, and the bubble-formation, the resin was conditioned with 2% sodium hydroxide instead of 5% sodium carbonate, and the column was formed by filling the tube with water and sifting the resin into the tube so that it was wetted as it settled into place. No flow difficulties were then encountered, neither was banding observed.

16. The **capacity of the resin** for glutamic acid was determined in a rough way as follows. A solution of glutamic acid was passed through a column containing 0.5 g. IR-4 (*NaOH), −60 +80 mesh, 5 cm. high, at the rate of 2 ml. per min. The percolate was examined by means of the ninhydrin color test for alpha amino acids. The results are given in Table XIII.

(19) Banding of columns treated with mixtures of hydrochloric and sulfuric acids was reported by Myers, Eastes and Myers, and Myers, Eastes and Urquhart.[13]

TABLE XIII

BEHAVIOR OF ACID-BINDING RESIN

Vol. collected	Ninhydrin color test	*p*H
1st 10 ml.	No color test	4.8
2nd 10 ml.	No color test	4.8
3rd 10 ml.	No color test	4.7
4th 10 ml.	Faint color test	3.9
5th 10 ml.	Strong color test	3.2

Initial soln. contained 0.5065 g. of glutamic acid in 100 ml., *p*H 3.1

It appears that the capacity of this resin is very high: about 0.2 to 0.25 g. glutamic acid for 0.5 g. resin.[20] It seems that the break-through behavior of the amino acid could be followed by measurements of *p*H.

17. Break-through experiments were then carried out. A solution of arginine hydrochloride and glutamic acid, *p*H 3.5, was passed through 0.75 g. of IR-4 (*NaOH) at the rate of 2 ml. per min. The analyses are in Table XIV, and Fig. 1, curve 17.

TABLE XIV

BREAK-THROUGH BEHAVIOR OF IR-4 (*NaOH)

Vol. collected	Arginine, millimoles/ml.	Glutamic acid, millimoles/ml.	*p*H
1st 10 ml.	35.6×10^{-3}	3.4×10^{-3}	6.2
2nd 10 ml.	50×10^{-3}[a]	19×10^{-3}	6.7
3rd 10 ml.	48×10^{-3}[a]	29×10^{-3}	6.7
4th 10 ml.	49×10^{-3}[a]	31×10^{-3}	6.5
5th 7 ml.	50×10^{-3}[a]	33×10^{-3}	6.5

[a] An analysis of the original solution gave a concentration of 48×10^{-3} millimole per ml., whereas the arginine hydrochloride weighed out was equivalent to 45.9×10^{-3} millimole/ml. This throws some doubt upon these analyses, but probably does not alter the general picture. The glutamic acid concentration in the original solution was 42.4×10^{-3} millimole/ml., and the *p*H 3.5.

It is seen that the glutamic acid here broke through early, and that the *p*H was higher than in the previous experiment. The glutamic acid should not, according to the previous experiment, have broken through until the fourth or fifth 10 ml. of percolate. It may be concluded tentatively that the resin shows preferential adsorption of the HCl from the arginine hydrochloride, adsorbing it to a greater extent than the glutamic acid, thus helping to raise the *p*H of the solution.

18. Adsorption behavior of glutamic acid in the presence of glycine was examined by passing a solution of the two amino acids through 0.75 g. of IR-4 (*NaOH) at the rate of 2 ml./min. Total amino acid was determined by the Van Slyke amino carboxyl method, and glycine by the *o*-phthalaldehyde method. Analysis of the initial solution by this method showed 29.8×10^{-3} millimole of glycine per ml., 30.3×10^{-3} had been weighed in. The data for this experiment are in Table XV, and Fig. 1, curve 18.

These results show that glycine was not adsorbed while glutamic acid was. Hence the two

(20) Myers, Eastes and Myers[12] showed that the capacity of this resin for sulfuric and hydrochloric acids was quite high.

TABLE XV

BREAK-THROUGH BEHAVIOR OF IR-4 (*NaOH), CONTINUED

Vol. collected	Glutamic acid, millimole/ml.	Glycine, millimoles/ml.
1st 10 ml.	0	24.6×10^{-3}
2nd 10 ml.	0	27.5×10^{-3}
3rd 10 ml.	0	32.0×10^{-3}
4th 10 ml.	0	27.5×10^{-3}
5th 7 ml.	0	29.3×10^{-3}
Initial soln.	34.5×10^{-3}	30.3×10^{-3}

amino acids could be quantitatively separated by this method. It would be interesting to examine by this method the separation of glutamic acid from a monoamino monocarboxylic acid hydrochloride and from free arginine.

19. It now appeared that this work would have to be interrupted. Time was, however, left, for a preliminary attempt at the separation of a mixture of basic, acidic and neutral amino acids, one of each. Accordingly a solution was prepared containing 2.4657 g. of arginine hydrochloride, 1.5107 g. glutamic acid, and 0.8620 g. glycine in 500 ml. Two hundred ml. of this solution was passed through 10 g. of IR-100 (Na^+R^-) at the rate of 2 ml./min. The column was then washed with three 10-ml. portions of water. The entire percolate was collected, the *p*H adjusted to 3, and the solution transferred to a 250-ml. volumetric flask and made up to volume. Two hundred ml. of this liquid was then passed through 6 g. of IR-4 (*NaOH) at the rate of 2 ml./min. The column was washed with three 10-ml. portions of water and the total percolate made up to 250 ml. The percolates were analyzed for glycine, arginine and total amino acids with the results given in Table XVI.

TABLE XVI

ATTEMPT AT A SEPARATION

Amino acid	Concn. by wt. in original soln., mg./ml.	Found by anal. in percolates. Cor. for dilution and removal of samples for analysis; mg./ml. Percolate from IR-100 (Na^+R^-)	Percolate from IR-4 (*NaOH)
Arginine	4.08 (as arginine)	Less than 0.01	Less than 0.006
Glutamic acid	3.02	3.0 (by diff.)	1.2 (by diff.)
Glycine	1.72	1.8	1.8

A 200% excess of resin was used in each column, the use of an excess being deemed necessary particularly in the case of IR-4, for HCl was used to adjust the *p*H of the solution. Better results would have been obtained if the *p*H had not been adjusted as it was but had been brought to about 6, as has been shown by Cannan.[9] It would appear that the IR-100 retained virtually all the arginine (99.8%) and held back little or none of the other amino acids. The second column retained 60% of the glutamic acid and no glycine. The separation by this second column can probably be made quantitative by following the observations of Cannan.[9] It was found that *o*-phthalaldehyde cannot be used to determine glycine in the presence of arginine hydrochloride.

No elution experiments are reported in this paper because that problem has not been sufficiently well investigated. The reversibility of the adsorption, which bears a relation to the elution, has not been sufficiently investigated.

An attempt to separate glutamic and aspartic acids on IR-4 was not successful though the problem seemed worthy of further study. The possibility was suggested by the findings of Myers, Eastes and Urquhart[12,19] that a mixture of hydrochloric and sulfuric acids could be separated to some extent on IR-4 resin.

It would seem likely from the results reported in this paper that arginine and histidine can be separated chromatographically on Amberlite IR-100. Adsorption isotherms for arginine, histidine and lysine (not published) seem to confirm this since they have different slopes and are well separated. It is planned to investigate this.

An experiment with arginine hydrochloride and IR-100 (H^+R^-) showed that chloride ion took little or no part in the actual exchange. The concentrations of chloride in solution remained unchanged when this resin was shaken with solutions of arginine hydrochloride.

Discussion

In a solution of *p*H 7 containing amino acids, those which are monoamino and monocarboxylic (such as alanine and glycine) will be present largely as dipolar ions with a net charge of zero; any dicarboxylic amino acids will be present largely as ions with a net negative charge; and of the diamino acids arginine and lysine will be present largely as ions with a net positive charge, and histidine will be present only partially in the form of the mono-cation. Thus if no factor were present to change the hydrogen ion concentration of the solution, and if no other kinds of adsorption were to occur, it would be expected that the neutral amino acid molecules would not be exchanged, the dicarboxylic amino acids would be held by acid-binding resins and that the basic amino acid cations would be adsorbed by cation-exchangers. The problem is usually less simple than this because hydrochloric or other strong acid is usually present to compete in the exchange process, or if this has been neutralized salt ions are present, and in addition the exchangeable ion of the resin may affect the *p*H of the solution. Also adsorptions other than those of exchange or of acid-binding may occur.

The cation-exchange-resin (H^+R^-) behaves as an insoluble acid with freely available hydrogen ions, experiments 2, 3, 4, which can also form insoluble salts with all cations. If an amino acid is passed through a column of such a resin it is likely to be adsorbed to some extent from its solution, no matter what the original *p*H. This comes about either because the acidifying action

at the surface of the resin brings all amino acids at least partially into the cation form, and thus makes them "exchangeable," or else "-onium" formation occurs at the surface of the resin, converting the strongly acid resin, strongly acid due to sulfonic acid groups, into a less strongly acid adsorbate-resin, acid now due to carboxylic acid groups. The finding is actually that all amino acids which have been studied are to some extent adsorbed by the acid resin.[10] We have confirmed this in preliminary experiments (not published) with IR-100 (H^+R^-): when solutions of arginine hydrochloride and glutamic acid were passed through a column of the coarse resin both amino acids were held to some extent. This also applied to arginine hydrochloride and alanine, and histidine hydrochloride and alanine.

The adsorption is a process which may proceed to equilibrium and which is reversed in the presence of excess hydrogen ion, thus although an amino acid such as glycine is adsorbed by the hydrogen resin it is less well adsorbed at *p*H 2 than at *p*H 5.[10] This is because at *p*H 2 the mass action effect of hydrogen ion in reversing the exchange is much greater than at *p*H 5 while the increase in glycine cations in going from *p*H 5 to *p*H 2 is small. The basic amino acids will be in the cationic form when the solution is acid, and also, except for histidine, largely so when it is neutral. They will be adsorbed from neutral or acid solution. But from excessively acid solution they will not be well adsorbed for the same reason that glycine was not well adsorbed from very acid solutions. The data in Table II are explainable on this basis. This argument applies only in the case of the hydrogen resin.

The case of the resins IR-100 ($NH_4^+R^-$) and (Na^+R^-) is somewhat different. These resins do not adsorb neutral amino acids (in agreement with Englis and Fiess[10]) and they do not adsorb glutamic acid. This is explainable on the basis that they do not contribute a common ion (H^+) to the solution. These resins adsorb arginine well and histidine less well.

The properties of the resins will be exaggerated by the (counter-current) chromatographic method for this imposes a stress upon the system. That is, the downward flow at first removes exchanged ions from their source before they can set up a condition of equilibrium; it destroys the effectiveness of the reverse components of the processes. This lasts until the column finally comes into equilibrium with the flowing liquid at its original concentration.[21]

The problem of correlating the number of amino acid molecules adsorbed with, for example, the number of hydrogen ions which appear in the solution (Table I) is complicated, as is the whole problem of the adsorption, not only by the fact that the amino acid molecules are amphoteric, but also by the probability that the acid (in this example) groups of the resin are not all of the same kind. The dissociations of some of these groups may be markedly affected by *p*H change.

When the question turns to the use of these resins in *separations* it would seem that the following tentative remarks may be made by way of guidance. The use of the resin (H^+R^-) is inadvisable because of its non-selective nature: it tends to adsorb all amino acids, some of them incompletely. The resins ($NH_4^+R^-$) and (Na^+R^-) are quite selective and tend to remove the cationic amino acids: the arginine (and probably lysine) quantitatively, and the histidine less well unless acid is present. The sodium resin contributes a difficultly removable ion to the solution and hence of the two, the ammonium resin must at present be the one of choice.

Less work has been done with the acid-binding resin. Cannan[9] found that Amberlite IR-4 adsorbed only "traces of amino acids other than the dicarboxylic acids," and this was confirmed for the two amino acids studied by Englis and Fiess.[10] Our results are in agreement with these findings (expts. 17, 18, 19). The resin appears to bind whole molecules of acid and has a very large capacity.

Much work remains to be done before these resins can be used with complete confidence in the analytical separation of amino acids, and it may be worth while to support this with a brief statement of some of the factors which need to be investigated. The cation-exchange and the acid-binding resins appear not to owe their effectiveness in each case to single types of functional groups. In the former the functional groups are sulfonic acid groups, phenolic groups and possibly carboxylic acid groups also; in the latter there may be primary, secondary or tertiary amino groups as well as some phenolic groups (see Cannan,[9] p. 402, ref. 2). It would be desirable to investigate somewhat more homogeneous preparations or even pure substances partaking of the properties of exchange-resins. One can visualize exchangers in which a functional group is made progressively more acid, say by the influence of appropriately placed electronegative substituents, so that a whole series of exchange activities is produced. It would then become possible to make rate studies of the behaviors of such resins, useful for theoretical as well as for practical purposes. It might be useful to know whether different adsorbabilities were accompanied by different rates of adsorption. Part of the necessary mathematical equipment for such rate studies has already been developed.[22]

The question of the existence on these resins of adsorptions which are other than exchange or acid-binding in nature needs to be investigated. This would involve widening the range of amino acids studied. The question of diffusion into the resin also needs further study. Some of our

(21) H. G. Cassidy and S. E. Wood, THIS JOURNAL, **63**, 2638 (1941).

(22) H. C. Thomas, *ibid.*, **66**, 1664 (1944).

(unpublished) results indicate that the amino acid must not be allowed to remain too long upon the resin else it becomes difficult to remove it completely. This suggests the very important problem of elution. Work with the resins seems to indicate that the exchange or acid-binding is quite reversible in the absence of side-reactions. It may be mentioned that these resins are prepared with formaldehyde and if there remains in the resin any residual formaldehyde or if any residual reactive groups are present then chemical reaction with amino acids might be expected. This point is made here not because we have any definite evidence bearing upon it but because we feel that it should be borne in mind. There also exist the possibilities of using the resins for the chromatographic separation of amino acid esters, or acyl derivatives; or of influencing the ionization of the amino acids by the use of alcohol or other solvent mixtures[23] or by modifying the amino acid by means of formol,[24] so that differences between individual amino acids may be accentuated for the purpose of separating them.

(23) Th. Wieland, *Naturwissenchaften*, **30**, 374 (1942); also ref. 8.

(24) G. Schramm and J. Primosigh, *Ber.*, **76**, 373 (1943). See also Englis and Fiess[10] who made some studies of this kind but who used probably too dilute a formaldehyde solution and too low a *p*H.

Acknowledgment.—We wish to record our indebtedness to Professor Hubert B. Vickery for encouragement and advice in this work.

Summary

The responses toward several amino acids of a cation-exchange and an acid-binding synthetic organic resin have been examined. The influence of the following factors upon the responses have been investigated: type of resin, particle size, length of adsorption column, rate of flow, concentration of amino acid in solution, hydrogen ion concentration of the solution. The investigation has been extended to several binary and two ternary mixtures of amino acids, and evidence regarding their separability has been obtained. An attempt has been made to explain the effects observed. The problems involved in separating amino acids on exchange-resins have been considered as well as the factors needing further investigation.

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[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY OF PRINCETON UNIVERSITY]

Preparation of (+)2-Methylbutanal-1

BY ELMER J. BADIN AND EUGENE PACSU

It was necessary in connection with other investigations to know the properties of the optically active aldehyde, (+)2-methylbutanal-1, and its derivatives; the optical rotations for the aldehyde obtained by Ehrlich[1] and by Levene and Kuna[2] were at variance with one another. The aldehyde was prepared by oxidizing amyl alcohol fractions containing various percentages of active 2-methylbutanol-1 and inactive 3-methylbutanol-1 obtained by fractionation of fusel oil. In this work the procedure of Ehrlich, involving oxidation with sodium dichromate in sulfuric acid solution, was modified by reducing the oxidation time and by fractionating the oxidation products to isolate the aldehyde instead of first preparing the bisulfite addition compound and hydrolyzing it with sodium carbonate. The latter step was avoided to prevent racemization of the aldehyde which presumably occurred during the hydrolysis by others of the bisulfite addition compound. By these modifications both the yield and purity of the product were greatly improved.

Experimental

Preparation of Active Amyl Alcohol.—The optically active amyl alcohol was prepared by fractionation of fusel oil with $[\alpha]_D$ −1.40° through a twelve theoretical plate glass helix packed column. In one series of distillations there was obtained an alcohol with $[\alpha]_D$ −5.42°, b. p. 128–129°, d^{20}_4 0.8186. This was accomplished by carrying out eight complete distillations and by taking from three to five cuts per distillation. Based on an optical rotation of $[\alpha]_D$ −6.0°[3] for pure (−)2-methylbutanol-1, this alcohol contained 90.3% (−)2-methylbutanol-1 and 9.7% 3-methylbutanol-1. Other fractions obtained had 29.8% of the active alcohol with $[\alpha]_D$ −1.79° and 59.2% of the active alcohol with $[\alpha]_D$ −3.55°.

Oxidation of Active Amyl Alcohol.—Sixty grams (0.682 mole) of the active amyl alcohol, $[\alpha]_D$ −5.42°, was placed in a one-liter flask equipped with a dropping funnel, electric mercury seal stirrer, and a modified Claisen distillation head packed with saddles and fitted to a downward condenser. A sulfuric acid solution of 68 g. (0.227 mole) of sodium dichromate dihydrate, 55 ml. of concentrated sulfuric acid, and 400 ml. of water was prepared. The alcohol was heated to incipient boiling and the sodium dichromate solution added at a constant rate to the vigorously stirred alcohol over a period of twenty-two minutes. Volatile products were allowed to distil out as formed; the bath surrounding the reaction flask was maintained at 95° throughout the oxidation. After addition of all the dichromate solution the bath was heated to 140° over a period of fifteen minutes to remove the remainder of the products.

The organic layer of the distillate was dried twice with anhydrous sodium sulfate and magnesium sulfate and then fractionated. The cut boiling below 95° was collected and dried with calcium chloride and sodium sulfate and redistilled; 23 g. of aldehyde with b. p. 90–92°, d^{25}_4 0.8055, $[\alpha]_D$ +28.50° (homogeneous, 1-dm. tube) was obtained. The yield of the aldehyde was 52% of the theoretical based on the amount of the alcohol oxidized.

(1) Ehrlich, *Ber.*, **40**, 2556 (1907).

(2) Levene and Kuna, *J. Biol. Chem.*, **110**, 323–328 (1935).

(3) Whitmore and Olewine, THIS JOURNAL, **60**, 2569–2570 (1938).