17091. Paper Chromatographic Identification of Some N-substituted Amino Acids.*

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Ever since Consden, Gordon and Martin[1] introduced the use of paper chromatography it has been successfully applied to the resolution of a great many biological mixtures. In the work presented here, paper chromatography was applied to the separation of a group of amino acid analogs studied for their tumor growth inhibitory effect on the transplanted sarcoma 37 in strain "A" mice.

The ascending capillary technic of Williams and Kirby[2] was adopted for the study of the separation of N-substituted amino acids in quantities of 25 γ. The paper strips were suspended from paper clips carried by a toothed non-corroding nickel alloy support which was firmly attached to the heavy glass of the jar. This toothed support permitted the simultaneous employment of 6 strips of Whatman No. 1 filter paper (40 × 12 cm). The solvent mixtures employed were phenol-water and collidine-water. After the solvents reached a 20 cm liquid front (L.F.) value which was within 20 hours, the strips were removed and dried in air with the aid of an electric fan. In developing the ninhydrin reaction the papers were kept at 90° for 10 minutes. The dried strips were then examined under an ultraviolet lamp (General Electric bulb BH4). The free amino acids showed up as faint blue spots, whereas the N-substituted amino acids were recognized by the presence of absorbing, non-emitting dark spots on the paper. After the ultraviolet analysis, the paper strips were sprayed with a 0.15% ninhydrin solution in saturated butanol-water.

Both the free amino acids and substituted analogs responded with spot formation on the positions outlined by previous ultraviolet analysis. The N-substituted analogs, however, gave spots far below the intensity of those of their corresponding amino acids. It was found that both color intensity and R_f values could be correlated with the rate of hydrolysis of the substituent and with the character of the steric arrangement. Thus, the size of the isopropyl chain is somewhat smaller than that of the propyl, which expresses itself in the somewhat greater R_f value of the latter. The phenyl N-substituted amino acids, however, disappeared from the paper either by this method of drying or by heat drying. The phenyl derivatives showed definite melting points between 110 and 180° and were quite soluble in organic solvents. Thus, it is very likely that the small amounts used are removed along with the solvent vapors from the paper strips during drying before any spot development can be observed, for, if control paper strips, untreated with solvent mixtures, carrying the phenyl derivatives were treated with ninhydrin solution, spots characteristic of traces of unsubstituted amino acid showed up, just as in the case of the other N-substituted amino acid-analogs.

Table I represents the tabulated R_f values of certain of the N-substituted and of the corresponding free amino acids. It must be emphasized that the spots developed by ninhydrin can not be attributed to the presence of traces of amino acids as impurities, as synthesis of the analogs did not employ amino acids at any stage of the work. Furthermore, the microanalytical values of the compounds agreed closely with the calculated ones. As the table shows, R_f values vary greatly according to the concentration of water in the solvent mixtures. In 65% phenol the N-substituted amino acids move almost as fast as the liquid front. Separation is not sharp and use of this solvent mixture becomes practical only in two dimensional work provided the chromatogram is developed first with 71%

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

2 Williams, R. J., and Kirby, H., *Science*, 1948, **107**, 481.

TABLE I.
R_f Values.

Compounds	Phenol		Collidin	
	65%	80%	71%	63%
DL-valine	0.73	0.69	0.25	0.43
Ethyl-N-DL-valine	0.94		0.41	
Isopropyl-N-DL-valine	0.97		0.43	
Propyl-N-DL-valine	0.99		0.44	
Phenyl-N-DL-valine*				
DL-leucine	0.81	0.74	0.37	0.55
Ethyl-N-DL-leucine	0.92		0.46	
Isopropyl-N-DL-leucine	0.96		0.56	
Propyl-N-DL-leucine	0.99		0.58	
Phenyl-N-DL-leucine*				
DL-phenylalanine	0.83	0.76	0.39	0.58
Ethyl-N-DL-phenylalanine	0.94		0.53	
Isopropyl-N-DL-phenylalanine	0.97		0.59	
Propyl-N-DL-phenylalanine	0.99		0.61	
Phenyl-N-DL-phenylalanine*				

* The phenyl derivatives evaporated from the paper upon drying (see text).

collidine and with phenol afterwards. It is important, as other authors have also suggested, that one run a control chromatogram of a known amino acid, to establish the necessary correction for the R_f values of the solvent mixtures used.

Of further interest is the observed degree of ninhydrin color sensitivity of the N-substituted amino acids. Recently Dent[3] reported that sarcosine reacts with an intense color formation. Plattner and Nager[4] have observed positive ninhydrin reactions with methyl-N-DL-valine and methyl-N-L-leucine. It was found that these compounds boiled with ninhydrin did not produce any aldehydes or ketones, because they do not have two hydrogen atoms on the nitrogen. The appearance of the ninhydrin reaction was positive irrespective of whether the paper was exposed to ultraviolet light or not. The positive ninhydrin color may be interpreted as being the result of a certain degree of hydrolysis that takes place under the effects of the solvents and heating that are used to develop the chromatograms. The intensity of the reaction decreases with increase of chain length in the homologous series of alkyl substitutions. The R_f values, on the other hand, increase with the length of the substituents, in as much as the n-alkyl group exhibits a somewhat faster rate of movement than the corresponding branched chain isomer.

Summary. N-substituted amino acid can be separated by paper chromatographic method. Also, N-substitution leads to the extinction of fluorescence of amino acids.

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3 Dent, C. E., *Biochem. J.*, 1948, **43**, 169.

4 Plattner, P. A., and Nager, U., *Helv. Chim. Acta*, 1948, **31**, 2203.