PAPER CHROMATOGRAPHY OF AMINO ACIDS AND OTHER ORGANIC COMPOUNDS IN SELECTED SOLVENTS

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The simplicity of apparatus and the ease with which many analyses can be performed have made paper chromatography an outstanding biochemical technique. However, additional information on the characteristic migration rates of compounds in different solvent systems and the influence of variations in temperature and other factors on the R_F values of these compounds is needed to permit ready application of the method in the identification of compounds encountered in routine laboratory work and research. The present paper compares the R_F values of amino acids and other organic compounds in water-saturated phenol, butanol–propionic acid–water, and other selected solvents under controlled and uncontrolled conditions of temperature and humidity. Several compounds for which R_F values are not given in the literature are reported.

The R_F values of several amino acids in phenol have been reported in the literature [1–4]. Though much work has been done with phenol, it is frequently necessary to use two-dimensional chromatography to obtain discrete separation of compounds for identification purposes. The objectional features of collidine and other solvents have been cited by others[5]. A few studies employing butanol–propionic acid–water have been reported[2,6]. However, much of this work has been conducted without adequate control over such conditions as temperature, etc. The use of phenol and butanol–propionic acid–water as solvents in two-dimensional paper chromatography provides a convenient and rapid technique for the separation of compounds in small quantities of biological fluids. The work described herein extends information available on these solvents.

EXPERIMENTAL PROCEDURES

The compounds listed in Tables I and II were applied individually to 18 in. × 22 in. filter paper sheets (Whatman No. 1, especially selected for chromatography) in the upper right corner. The solutions were usually prepared in 50% ethanol in concentrations of 0.5%. 4 λ of a 0.5% solution of Tropaolin 000 No. 1, a dye, was applied 15 mm above the point of application of the sample to facilitate identification when mixtures or solutions containing unknown compounds were studied. Water-saturated

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phenol, unbuffered, or buffered with 8-quinolinol[7], 25 mg in 500 ml water-saturated phenol, or a solution of 6.3% sodium citrate and 3.7% potassium di-hydrogen phosphate per 100 g phenol[5] was used for the first dimension. The water used to saturate the phenol must be free of all traces of metals[9], therefore glass-distilled water (triple distilled) was used in all instances. A mixture of butanol–propionic acid–water was used for the second dimension[2*].

The papers were run by the descending technique in plywood boxes, 30 in. in height, 19 in. in width, and 34 in. in length. The insides of the boxes were coated with paraffin prior to use to prevent impregnation of the wood by solvents. One chromatocab was always used with phenol, the other with butanol–propionic acid–water. The solvents were contained in pyrex cradles, 24 in. long with a semi-circular cross section 1½ in. in diameter, which rested in stainless steel troughs. Each box was fitted with a 12 in. × 25 in. glass plate on one end to facilitate viewing the papers as the solvent progressed, and was covered with a tightly fitting, felt-stripped lid. Where temperature control is indicated, the chromatocabs were housed in a special room maintained at a temperature of 24 ± 0.5° and at constant humidity. In other instances, the chromatocabs were used in a typical laboratory room where temperature and humidity fluctuated with the weather, though it ranged between 29–35° when the analyses were conducted.

A small quantity of the solvent was placed in a dish at the bottom of the box to bring the atmosphere to equilibrium more quickly with the solvent. 18 to 22 hours were required for migration of phenol down the papers whereas 15 to 16 hours were required for butanol–propionic acid–water. After the phenol run, the papers were dried in a fume hood overnight. They were turned at a 90° angle counterclockwise and butanol–propionic acid–water allowed to descend the papers. They were again dried overnight in a fume hood. Amino acids were located by spraying with ninhydrin (0.2% in ethanol). Color was developed by heating in an air oven at 90° for 5 min. Urea was located by spraying with phenol and sodium hypochlorite, according to the method of BERRY[8]; creatinine was detected with pircic acid[3]; purines were treated with 0.5% nitric acid and ammoniacal silver nitrate[3].

The R_F values (distance travelled by the compound/distance travelled by the solvent) were calculated in the various solvent systems. Following detection, the position occupied by the compounds was encircled with a lead pencil because colors faded on standing over a period of time.

In addition, some compounds were applied to filter paper strips, 1¼ in. wide. The strips were run in selected solvents in a small glass chromatocab, 24 in. high × 12 in. wide, using the descending technique. A tightly fitting glass plate served to cover the glass chromatocab. Ethanol–acetic acid (19:1), 95% ethanol, butanol–ethanol–water (4:1:1), and butanol–acetic acid–water (4:1:5) were used as solvents. The strips were allowed to dry in a fume hood prior to spraying.

* Fresh solvent was prepared from equal volumes of two solutions: A (1246 ml *n*-butanol and 84 ml water) and B (620 ml propionic acid and 790 ml water).

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RESULTS AND DISCUSSION

The R_F values for 47 amino acids are given in Table I. The values listed represent, in most cases, the average of two or more runs.

The presence of either 8-quinolinol or the sodium citrate–potassium phosphate buffer affected the migration rates in phenol–water of several of the compounds studied (cysteic acid, cysteine, cystine, glutamic acid, histidine, hydroxyproline, isoleucine, methionine sulfoxide, norvaline, phenylalanine, proline, serine, tryptophan, tyrosine, and valine). Variations in room temperature affected the migration of cysteic acid, glutamic acid, glycine, isoleucine, norleucine, serine, and valine.

Similarly, in butanol–propionic acid–water, the R_F values of arginine, aspartic

TABLE I

R_F VALUES OF AMINO ACIDS AND OTHER SELECTED COMPOUNDS IN PHENOL AND BUTANOL–PROPIONIC ACID–WATER UNDER VARIOUS CONDITIONS

Compound	R_F value × 100							Concentration µg
	Phenol–water				Butanol–propionic acid–water			
	Room temp.	25°	+ 8-Quinolinol 25°	+ Buffer 24 ± 0.5°	Room temp.	25°*	24 ± 0.5°**	
DL-α-Alanine	63	61	63	61	32	32	32	20
L-Alanine	—	—	—	61	—	—	36	20
DL-α-Amino-n-butyric acid	—	—	—	67	—	—	39	20
β-Amino-n-butyric acid	—	—	—	81	—	—	40	30
γ-Aminobutyric acid	—	—	—	80	—	—	41	30
α-Aminoisobutyric acid	—	—	—	74	—	—	38	20
β-Aminoisobutyric acid	—	—	—	79	—	—	41	30
α-Aminopimelic acid	—	—	—	91 42***	—	—	52 34***	30
Arginine	55	60	64	64	28	28	19	20
L-Aspartic acid	25	27	32	34	17	24	22	5
Cysteic acid	11	18	16	6	5	9	6	30
Cysteine	—	20	32	20	—	11	—	20
Cystine	20	17	28	14	5	10	8	20
Ethionine	—	—	—	80	—	—	50	20
Ethionine sulfoxide****	—	—	—	80	—	—	30	—
L-Glutamic acid	28	52	41	22	24	32	25	20
Glutathione	—	—	—	32	—	—	5	20
Glycine	37	44	44	40	57	31	20	20
Glycyl-DL-methionine	—	—	—	76	—	—	42	30
Histidine	—	52	76	82	—	24	15	20
DL-Homocysteine	—	—	—	81 39***	—	—	45 20***	20
DL-Homocystine	—	—	—	33	—	—	19	20
Homoserine	—	—	—	64	—	—	32	50
L-Hydroxyproline	65	68	78	64	25	28	21	20
Isoleucine	89	94	93	79	64	68	63	20
Leucine	—	—	—	79	—	—	67	20
Lysine	—	57	53	47	—	19	13	20
D-Methionine	—	—	—	77	—	—	45	30
DL-Methionine	78	81	*****	78	52	57	44	20
DL-Methionine sulfoxide****	—	87	85	76	—	25	24	—

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TABLE I (*continued*)

Compound	R_F value × 100							Concentration µg
	Phenol–water				Butanol–propionic acid–water			
	Room temp.	25°	+ 8-Quinolinol 25°	+ Buffer 24 ± 0.5°	Room temp.	25°*	24 ± 0.5°**	
L-Methionine	—	—	—	72	—	—	53	20
L-Methionine sulfoxide****	—	—	—	72	—	—	26	—
Methionine sulfone	—	67	67	67	—	25	30	20
Methionine sulfoxide	—	—	—	75	—	—	28	20
Methionine sulfoximine	—	—	—	71 67***	—	—	41 18***	20
Norleucine	95	89	93	88	54	76	71	20
DL-Norvaline	86	87	87	79	47	63	41	20
DL-Ornithine	—	—	—	40	—	—	13	20
Phenylalanine	90	94	91	78	63	67	50	20
Proline	91	95	96	85	37	42	32	20
DL-Sarcosine	—	—	—	72	—	—	27	20
L-Serine	30	42	37	27	18	25	16	20
Taurine	—	—	—	34	—	—	17	20
L-2-Thiolhistidine	—	—	—	25	—	—	15	30
Threonine	48	50	51	46	24	31	25	20
L-Tryptophan	73	75	82	74	54	49	40	20
L-Tyrosine	69	67	63	54	46	45	36	20
Valine	80	87	85	71	57	57	49	20

* Papers were run first in phenol + 8-quinolinol.
** Papers were run first in buffered phenol.
*** Two spots obtained, lower spot.
**** Formed in phenol by oxidation.
***** Completely oxidized to the sulfoxide.

TABLE II

R_F VALUES OF SELECTED COMPOUNDS IN PHENOL AND BUTANOL–PROPIONIC ACID–WATER

Compound	Quantity µg	R_F value × 100*			
		Buffered phenol–water		Butanol–propionic acid–water	
		Average	Range	Average	Range
Purines					
Adenine	50	87	85–89	57	51–64
Hypoxanthine	50	90	87–92	38	36–39
Uric acid	50	21	20–22	21	20–21
Xanthine	50	48	45–51	32	31–33
Miscellaneous					
Allantoin	50	55	54–56	27	26–28
4-Amino-5-imidazole carboxamide	30	89	—	39	—
Creatine	50	91	91–92	37	35–40
Creatinine	20	90	—	48	—
Cystathionine	50	26 18**	—	14 8**	13–15 7–9**
Dimethylaminoethanol	5	79	—	38	—
Ethanolamine	0.5	76	74–79	40	39–41
Glutaric acid	75	68	—	72	—
Urea	60	68	—	49	—

* At 24 ± 0.5°.
** Two spots obtained, lower spot.

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acid, glutamic acid, glycine, histidine, hydroxyproline, lysine, DL-methionine, norleucine, norvaline, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine were different when temperature was not controlled in contrast to being controlled.

The R_F values for L-methionine were higher than those for either D- or DL-methionine in butanol–propionic acid–water. R_F values for other selected compounds in phenol–water and butanol–propionic acid–water are given in Table II.

Chromatography of methionine with other compounds

Methionine was chromatographed with other selected amino acids because it was often necessary in our work to identify this amino acid in the presence of other compounds which migrated to approximately the same position. In such instances, a

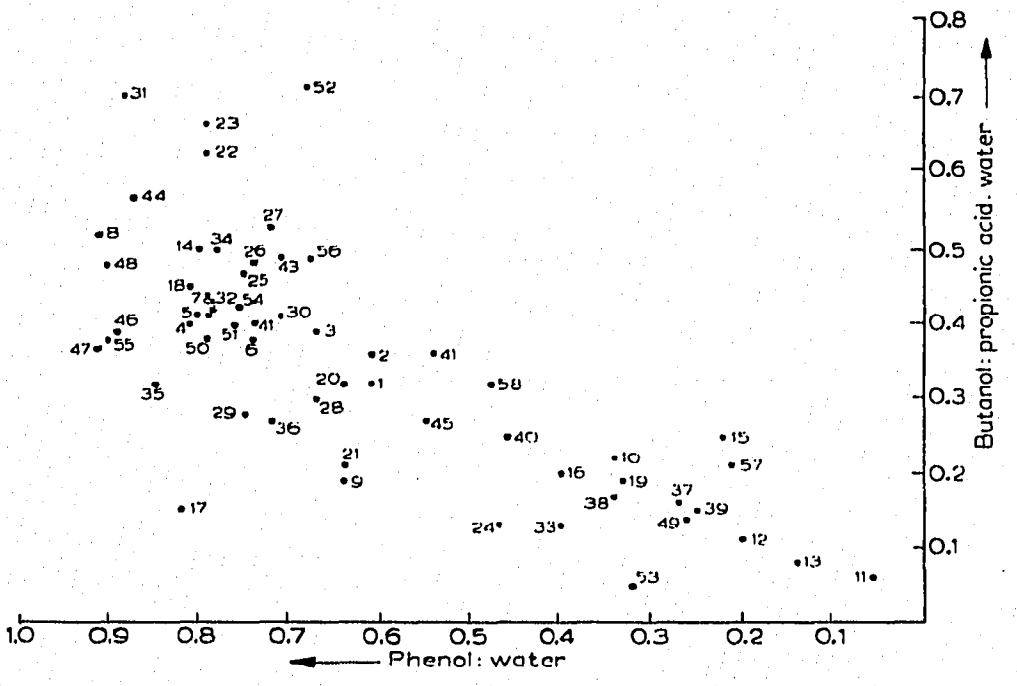

Fig. 1. Map of R_F values of amino acids and other selected organic compounds. 1. DL-α-Alanine; 2. L-Alanine; 3. DL-α-Amino-n-butyric acid; 4. β-Amino-n-butyric acid; 5. γ-Aminobutyric acid; 6. α-Aminoisobutyric acid; 7. β-Aminoisobutyric acid; 8. α-Aminopimelic acid; 9. Arginine; 10. L-Aspartic acid; 11. Cysteic acid; 12. Cysteine; 13. Cystine; 14. Ethionine; 15. L-Glutamic acid; 16. Glycine; 17. Histidine; 18. DL-Homocysteine; 19. DL-Homocystine; 20. Homoserine; 21. L-Hydroxyproline; 22. Isoleucine; 23. Leucine; 24. Lysine; 25. D-Methionine; 26. DL-Methionine; 27. L-Methionine; 28. Methionine sulfone; 29. Methionine sulfoxide; 30. Methionine sulfoximine; 31. Norleucine; 32. DL-Norvaline; 33. DL-Ornithine; 34. Phenylalanine; 35. Proline; 36. DL-Sarcosine; 37. L-Serine; 38. Taurine; 39. L-2-Thiolhistidine; 40. Threonine; 41. L-Tryptophan; 42. L-Tyrosine; 43. Valine; 44. Adenine; 45. Allantoin; 46. 4-Amino-5-imidiazole carboxamide; 47. Creatine; 48. Creatinine; 49. Cystathionine; 50. Dimethylaminoethanol; 51. Ethanolamine; 52. Glutaric acid; 53. Glutathione; 54. Glycyl-DL-methionine; 55. Hypoxanthine; 56. Urea; 57. Uric acid; 58. Xanthine.

solution of the compounds was prepared in 50% ethanol, and aliquots of this were applied to 18 in. × 22 in. filter paper sheets or 1 ¼ in. wide filter paper strips.

These data are shown in Table III.

A comparison of the data in Table III with those in Tables I and II reveals that

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TABLE III

EFFECT OF OTHER COMPOUNDS ON THE R_F VALUE OF METHIONINE

Compounds chromatographed with L-methionine	R_F values × 100*			
	Buffered phenol–water		Butanol–propionic acid–water	
	L-Methionine	Other compound	L-Methionine	Other compound
α-Aminoisobutyric acid	77	74	52	43
Allantoin	82	52	46	25
Arginine	81	64	48	—
Creatinine	74	90	51	56
Ethionine	75	78	59	68
Histidine	79	77	46	20
Leucine	75	82	47	57
Leucine and isoleucine	80	85 85	47	56 56
Methionine sulfone	77	66	41	22
Methionine sulfone and sulfoxide	79	66 78	41	22 22
Methionine sulfoxide	78	77	42	23
Norleucine	76	83	47	47
Norvaline and urea	81	79 80	53	49 50
Phenylalanine	77	81	55	63
Phenylalanine, urea, and creatinine	77	83 91 77	46	53 52 49
Proline	74	85	47	38
Tryptophan	75	75	48	50
Valine	77	78	48	48

* Run at 24 ± 0.5°.

the presence of other compounds in the applied solution did not affect R_F values in phenol–water as much as the presence of buffers in the solvent or variations in temperature. The presence of other compounds along with methionine, however, appeared to influence the R_F values of methionine in butanol–propionic acid–water (methionine sulfone, methionine sulfoxide, methionine sulfone and sulfoxide, tryptophan, arginine, valine, ethionine, leucine and isoleucine, histidine, norleucine, leucine, proline, phenylalanine, urea and creatinine, and allantoin).

Both the R_F values for methionine and the R_F values for methionine sulfoxide, tryptophan, arginine, ethionine, norleucine, leucine, and proline were affected when these compounds were chromatographed together in pairs or in groups of three in butanol–propionic acid–water. However, though the R_F value for methionine was influenced by creatinine and phenylalanine, R_F values of the latter compounds were not affected when they were run with methionine.

Effect of group chromatography on the R_F values of individual compounds

It was observed early in our work that R_F values of individual compounds may differ slightly when chromatographed in the presence of several other compounds. Accordingly, this influence was evaluated in the two solvent systems. Solutions of selected amino acids were applied to filter paper sheets in the manner described previously.

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TABLE IV

EFFECT OF GROUP CHROMATOGRAPHY ON THE R_F VALUES OF AMINO ACIDS AND OTHER COMPOUNDS

Compound	R_F value × 100*			
	Buffered phenol–water		Butanol–propionic acid–water	
	Average**	Range	Average	Range
DL-α-Alanine	57	54–60	30	—
L-Alanine	51	46–57	29	—
DL-α-Amino-n-butyric acid	64	54–69	31	—
α-Aminoisobutyric acid	71	67–75	33	—
Arginine	61	59–65	21	—
Cystine	18	—	10	—
Ethionine	80	76–89	51	45–57
L-Glutamic acid	29	23–35	22	—
Glutathione	30	25–32	6	5–6
Glycine	38	36–42	20	—
L-Hydroxyproline	64	58–69	21	20–22
Isoleucine	83	77–89	51	45–56
Leucine	82	79–85	51	45–56
Lysine	50	47–53	15	—
L-Methionine	79	77–83	47	41–51
L-Methionine sulfoxide	77	76–79	26	24–28
Methionine sulfone	60	—	22	—
Methionine sulfoxide	77	73–79	22	—
Norleucine	82	77–89	49	45–52
DL-Norvaline	76	72–79	50	48–54
DL-Ornithine	39	37–40	15	—
Phenylalanine	82	79–89	54	49–58
Proline	86	84–89	32	31–32
DL-Sarcosine	75	72–78	26	25–27
L-Serine	32	29–34	20	19–21
Taurine	39	33–45	15	—
Threonine	48	45–51	22	—
L-Tryptophan	68	67–69	43	37–49
L-Tyrosine	50	47–56	36	35–37
Valine	76	72–79	48	—
Allantoin	52	49–55	25	—
Creatinine	92	88–95	63	52–67
Urea	76	70–80	50	45–51

* Run at 24 ± 0.5°.
** Average of 2–7 values.

Solution A contained threonine, tyrosine, arginine, α-amino-n-butyric acid, lysine, glycine, glutamic acid, cysteine, aspartic acid, isoleucine, methionine, norleucine, methionine sulfone, ethionine, histidine, methionine sulfoxide, and α-aminoisobutyric acid. Solution B contained proline, hydroxyproline, alanine, taurine, cystine, homocystine, sarcosine, leucine, tryptophan, and phenylalanine. Solution C contained α-alanine, serine, valine, ornithine, norvaline, and glutathione. The values from these three analyses and others in which groups of compounds were chromatographed were averaged and are shown in Table IV.

When several compounds were present simultaneously in the applied solution, the effect on R_F values of individual components appeared to be great. In both phenol–water and butanol–propionic acid–water, R_F values of alanine, aspartic acid, glutamic acid, L-methionine, methionine sulfone, and norleucine were different when

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TABLE V

R_F VALUES OF COMPOUNDS IN VARIOUS SOLVENT SYSTEMS

Compounds	R_F values × 100*			
	Ethanol–acetic acid	Butanol–acetic acid–water	Butanol–ethanol–water	Ethanol
4-Amino-5-imidazole carboxamide	25	—	—	—
Creatinine	42	—	25	—
Ethionine	37	—	35	—
Glycyl-methionine	26	—	—	—
Hydroxyproline	13	—	—	—
Isoleucine	53	61	39	—
Leucine	50	62	37	—
Methionine	28	46	—	—
Phenylalanine	33	—	30	—
Proline	22	—	—	—
Tryptophan	22	56	19	—
Urea	42	70	37	—
Valine	47	48	24	—
Creatinine and phenylalanine	42 34***	— —	27 32***	— —
Isoleucine and leucine	52***	61***	35***	—
	52	61	35	—
Methionine and tryptophan	21**	47	26	—
	21	52	19	—
Methionine and urea	28	46	23	—
	39	70	32	—
Methionine and valine	26	47***	23***	—
	40	47	23	—
Phenylalanine and leucine	30	—	31	—
	50		39	—
Phenylalanine and ethionine	31***	—	31	—
	31		35	—
Tryptophan and valine	20		18	—
	40	—	25	—
Urea and tryptophan	37 19	— —	— —	— —
Urea and valine	34 35	— —	— —	— —
Methionine, creatinine and urea	— —	46 38 45	28 30 36	20 28 38
Valine, creatinine and urea	— —	41 34 41	30 31 38	28 28 38

* Room temperature.
** Incomplete separation of spots.
*** One spot.

chromatographed in the group in contrast to treatment as individual compounds.

The R_F values of DL-α-amino-*n*-butyric acid, α-amino-isobutyric acid, isoleucine, leucine, methionine sulfoxide, norvaline, and urea were affected only in butanol–propionic acid–water, whereas those for glutamic acid, L-methionine sulfoxide, and tryptophan were different only in phenol–water.

Comparative migration of compounds in different solvents

In several instances, the solvents commonly employed in our work did not cause discrete separation of amino acids and other compounds. Since it was necessary to

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identify certain substances in the presence of others, R_F values of selected compounds were determined in different solvent systems. Table V presents these data.

It will be noted that, by use of the appropriate solvents, all of the compounds listed in Table V, which migrate to the same positions in phenol–water and butanol–propionic acid–water, with the exception of isoleucine and leucine, can be separated.

Formation of methionine sulfoxide from methionine

It was noted early in our work that two spots appeared on methionine chromatograms when these were run in phenol–water (buffered and unbuffered) and butanol–propionic acid–water systems. From a series of side experiments in which methionine was chromatographed with suspected compounds, it was learned that the "lower spot" was methionine sulfoxide.

Chromatograms were prepared of methionine-2-^{14}C, non-radioactive methionine, methionine sulfoxide, other amino acids whose R_F values were close to the position of the lower spot and various combinations of these. The papers were run in buffered phenol–water and in butanol–propionic acid–water one-dimensionally; and two-dimensionally in both solvents. Autoradiograms were made of the chromatograms containing radioactive methionine.

Autoradiograms of methionine-2-^{14}C presented two areas of radioactivity, corresponding to R_F values of 0.72 and 0.53 in phenol–water and butanol–propionic acid–water, respectively, for methionine and in the position below methionine, 0.72 and 0.26. Subsequent studies were initiated to identify the lower spot.

Radioactive and non-radioactive methionine always presented two spots when run both in phenol–water and butanol–propionic acid–water two-dimensionally. Combinations of methionine and methionine sulfoxide gave only two spots, corresponding exactly to the two positions obtained when methionine was chromatographed alone. The radioactivity in the lower spot from methionine on autoradiograms always coincided with the ninhydrin-positive spot from methionine sulfoxide on chromatograms. This was not true for combinations of methionine with other amino acids. Co-chromatography tests in which the radioactive ninhydrin spots were excised, eluted, concentrated and reapplied with methionine revealed that the methionine lower spot always traveled on chromatograms with methionine sulfoxide.

In one-dimensional runs in phenol–water or in butanol–propionic acid–water, however, only one spot was observed from methionine. In addition, when the two-dimensional chromatograms were run first in butanol–propionic acid–water, and then in phenol–water, only one spot was obtained from methionine.

It was apparent from these findings, therefore, that methionine is oxidized by phenol to methionine sulfoxide, but because the R_F values of methionine and methionine sulfoxide are the same in phenol–water, these compounds appear as one spot on one-dimensional chromatograms. However, because the R_F values for methionine and methionine sulfoxide are different in butanol–propionic acid–water, papers containing methionine run previously in phenol–water show two spots, indicating separation of methionine from its sulfoxide. Similarly, when the solvents are reversed, that is, when

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TABLE VI

EFFECT ON R_F VALUES OF CHROMATOGRAPHING AMINO ACIDS IN BUTANOL–PROPIONIC ACID–WATER FIRST, THEN IN PHENOL

Compound	R_F values × 100*			
	Buffered phenol–water		Butanol–propionic acid–water	
	first	second	first	second
α-Aminoisobutyric acid	74	73	38	48
Cystine	14	20	10	11
Glutamine	58	33***	20	19***
	23**		23**	
Homocysteine	81	22***	45	16***
	39**		20**	
Homocystine	33	25	19	17
Isoleucine	79	83	63	72
Leucine	79	87	67	76
L-Methionine	72	78***	53	56***
	72**		26**	
Methionine sulfoximine	71	63***	41	19***
	67**		18**	
Phenylalanine	78	82	50	66
L-Serine	27	31	16	20
L-Tryptophan	74	75	49	57
L-Tyrosine	54	56	36	39
Valine	71	76	49	56
Creatinine	90	93	48	52
Urea	68	75	49	60

* Run at 24 ± 0.5°.
** Lower spot.
*** Only one spot was obtained with the reversed solvent systems.

papers are run in butanol–propionic acid–water first, and then in phenol, only one spot is present after two-dimensional runs because the sulfoxide is formed in the second run with phenol and does not separate because the R_F values are identical in phenol. This latter point was proved by permitting papers which had already been exposed to the two solvents in reversed order, to run again after a 90° turn in butanol–propionic acid–water. Two spots again appeared.

The development of two spots from methionine in the two-dimensional solvent systems proved extremely helpful in later work in which radioactive methionine was fed to rats and its metabolites were studied by chromatographic techniques.

R_F values of amino acids in reversed solvent runs

When amino acids were run first in butanol–propionic acid–water, then in buffered phenol–water the shapes and sizes of the spots revealed after spraying with ninhydrin were different from those run in the usual manner. It was of interest to determine whether the R_F values of the compounds were also changed appreciably. It will be noted from Table VI that values in buffered phenol–water for cystine, leucine, and urea were altered when butanol–propionic acid–water was the first solvent. R_F values in butanol–propionic acid–water for α-aminoisobutyric acid, isoleucine, leucine, phenylalanine, tryptophan, valine, and urea were higher also when this solvent system was used first.

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Though several compounds separated into two spots when run in phenol–water first, then in butanol–propionic acid–water, when the solvents were reversed, only one spot was obtained. This would indicate that the second spot was formed from the compound by oxidation with phenol, as in the case of methionine discussed earlier in this paper.

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SUMMARY

The influence of variations in temperature, presence and absence of buffers, and presence of other compounds in the applied solution on the R_F values of 70 organic compounds has been evaluated in phenol–water and butanol–propionic acid–water systems. R_F values of selected compounds in ethanol–acetic acid, butanol–ethanol–water, butanol–acetic acid–water, and ethanol are given. The production of methionine sulfoxide from methionine in phenol–water is discussed.

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