Correlation of Rf Values and Distribution Coefficients in Amino Acid Paper Chromatography. (23692)

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Paper chromatography, as an analytical procedure, is particularly useful in analysis of biological materials in which the available samples are often extremely small. The method is quite popular for analysis of mixtures of amino acids, but the details of the procedures used by various investigators vary considerably(1-11). As part of a rather extensive program of investigation in regard to the various factors controlling the development of the chromatogram, in the analysis of amino acid mixtures, we have determined the relationship of solubility, of amino acids in phenol and water mixtures, to the corresponding Rf values obtained by paper chromatography(12), using phenol as the developing agent.

Methods. Redistilled phenol was emulsified with buffered water solutions of individual amino acids under controlled conditions of temperature. The layers were allowed to separate and the concentration of amino acid in each layer determined by the Kjeldahl Method. Distribution coefficients (Kd) were calculated by determining the ratio of Nitrogen concentration in the water phase to Nitrogen concentration in the phenol phase, at temperatures of 20, 25 and 30°C. A scatter diagram (Fig. 1) was prepared and the mathematical relationship between the 2 groups of data determined to be $Rf = 0.781 - .474 \log Kd - .433 (\log Kd)^2 + .259 (\log Kd)^3$. Theoretical Rf values were calculated and compared with published(12) Rf values (Table I). The Pearson Product Moment Coefficient of Correlation, r, was determined to be 0.965 (which is significant at the 1% level for 77 degrees of freedom).

Discussion. The data presented indicate that a relationship exists between distribution coefficients, of amino acids in the 2 phases of a phenol-water system, and Rf values, in amino acid paper chromatography, using the same phenol-water system as the developing agent. This would tend to support the hypothesis that, in paper chromatography, the paper acted as an inert mechanical support of the water or stationary phase and that the amino acid spot moved along the paper with the flow of developing agent by a process of continuous, or multiple, extraction and re-extraction. Several variables should be considered and standardized.

There probably is a "water in phenol" concentration gradient which would affect very greatly the reextraction process and the movement of the amino acid spot, and thus, the Rf value. This concentration gradient would be, in turn, affected by temperature, state of initial saturation of the phenol and the humidity of the air surrounding the strips. It would seem, therefore, that the relative amounts of water and phenol should be carefully standardized, that the humidity of the cabinet should be controlled and that the distance, on the paper strips to which the developing solution progresses, should be constant.

Summary. The distribution coefficients of amino acids between the 2 phases of a phenol-water mixture have been determined at various H^+ concentrations, and at temperatures of 20°, 25°, and 30°C. Rf values cal-

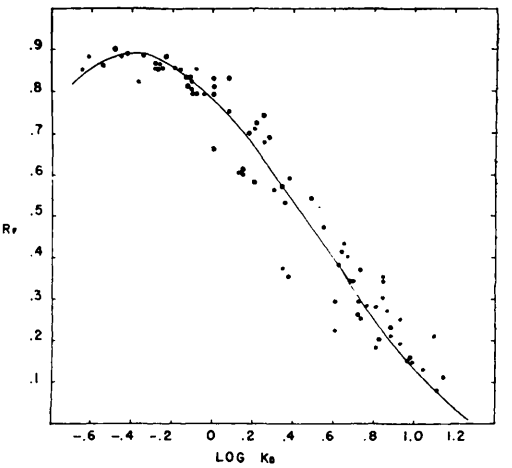

FIG. 1. Comparison of Rf (published) with log Kd (determined).

TABLE I. Comparison of Kd (Determined) with Rf (Calculated) and Rf (Published).

Amino acid	pH	Kd at °C				Rf calc. at °C (× 100)				Rf published (McFarren) (× 100)
		20	25	30	22	20	25	30	22	22
Alanine	2.0	4.5	4.4	4.3	4.5	36	37	38	36	43
	6.2	4.7	4.0	3.4	4.4	34	40	44	36	41
	10.0	3.4	3.8	3.4	3.6	44	41	44	43	47
	11.2	4.5	3.8	3.9	4.2	36	41	40	38	38
Arginine	2.0	7.4	6.8	5.7	7.2	21	24	28	22	35
	6.2	4.1	4.2	4.3	4.1	39	38	38	38	29
	10.0	.71	.74	.87	.72	84	84	81	84	87
	11.2	1.0	1.2	1.4	1	78	74	70	78	66
Aspartic acid	2.0	7.9	7.6	6.7	7.8	19	20	24	20	21
	6.2	9.9	9.3	8.8	9.7	14	15	16	14	15
	10.0	14.3	14.5	15.3	14.4	8	8	7	7	11
	11.2	14.9	10.9	13.2	13.3	5	12	7	7	8
Cystine	2.0	11.9	10.0	10.0	11.2	10	13	13	11	13
	6.2									
	10.0	5.3	5.5	5.5	4.1	31	29	29	30	29
	11.2	4.6	5.4	4.8	4.9	34	30	34	33	22
Glutamic acid	2.0	7.1	6.7	6.6	7.0	22	24	24	22	30
	6.2	6.4	7.0	7.0	6.6	25	23	23	24	28
	10.0	8.2	9.5	9.2	8.7	18	15	15	17	19
	11.2	9.1	10.5	10.5	9.6	16	13	13	15	16
Glycine	2.0	9.2	8.4	8.5	8.9	15	18	17	16	25
	6.2	5.2	5.8	5.8	5.4	32	28	28	30	26
	10.0	5.2	4.6	5.2	5.0	32	34	32	33	34
	11.2	5.2	6.0	5.2	5.5	32	27	32	30	25
Histidine	2.0	7.1	8.2	6.5	7.5	22	18	15	21	27
	6.2	2.4	2.3	2.4	2.4	55	56	55	55	35
	10.0	1.0	1.1	1.2	1.0	78	76	74	78	68
	11.2	1.1	1.2	1.2	1.1	76	74	74	76	68
Hydroxyproline	2.0	3.1	3.1	2.8	3.1	47	47	51	47	54
	6.2	2.4	2.2	2.4	2.3	55	58	55	56	53
	10.0	2.5	2.1	2.1	2.4	54	59	59	55	59
	11.2	2.1	2.4	2.4	2.2	59	55	55	58	57
Isoleucine	2.0	1.0	1.0	1.0	1	78	78	78	78	81
	6.2	.78	.83	.86	.79	83	82	81	83	82
	10.0	.74	.74	.74	.74	83	83	83	83	81
	11.2	.74	.80	.77	.76	83	82	82	82	83
Leucine	2.0	1.0	1.0	1.0	1.0	78	78	78	78	79
	6.2	.75	.82	.79	.78	83	82	82	83	85
	10.0	1.2	.75	.69	1.0	74	83	85	78	83
	11.2	.73	.71	.72	.73	85	84	84	84	83
Lysine	2.0	12.4	14.1	12.1	13.1	9	7	9	8	21
	6.2	10.5	8.7	8.5	9.8	12	17	17	14	15
	10.0	1.8	1.8	1.8	1.8	63	63	63	63	74
	11.2	2.1	2.5	3.9	2.2	58	54	40	57	37
Methionine	2.0	1.2	1.2	1.2	1.2	74	74	74	74	75
	6.2	.86	.97	1.0	.90	81	79	78	80	79
	10.0	.80	.84	.88	.82	82	81	80	82	79
	11.2	.79	.80	.85	.79	83	82	81	82	79
Phenylalanine	2.0	.50	.55	.53	.52	88	87	87	88	85
	6.2	.36	.39	.39	.37	88	89	89	89	89
	10.0	.34	.37	.41	.35	88	89	89	88	88
	11.2	.32	.34	.34	.33	88	88	88	88	90
Proline	2.0	1.0	.93	1.0	.97	78	79	78	78	80
	6.2	.69	.65	.81	.68	85	86	82	85	85
	10.0	.64	.74	.73	.68	85	83	83	85	84
	11.2	.62	.67	.75	.64	86	85	83	86	85

TABLE I (continued).

Amino acid	pH	Kd at °C				Rf calc. at °C (× 100)				Rf published (McFarren) (× 100)
		20	25	30	22	20	25	30	22	22
Serine	2.0	8.1	7.2	7.8	7.8	19	22	20	20	23
	6.2	6.5	7.2	7.0	6.8	25	22	22	23	20
	10.0	5.8	6.1	4.5	5.9	27	26	35	27	28
	11.2	6.6	6.5	6.2	6.6	24	24	26	24	18
Threonine	2.0	7.1	6.7	6.0	7.0	22	23	27	22	35
	6.2	5.6	5.0	5.0	5.4	29	33	33	30	37
	10.0	4.7	4.5	3.9	4.6	35	38	40	35	40
	11.2	5.0	4.8	4.6	4.9	33	34	35	33	34
Tyrosine	2.0	2.0	2.0	1.8	2.0	60	60	64	60	56
	6.2	1.4	1.4	1.5	1.4	70	70	68	70	60
	10.0	1.4	1.4	1.3	1.4	70	70	72	70	61
	11.2	1.4	1.4	1.4	1.4	70	70	70	70	60
Tryptophane	2.0	.41	.43	.44	.42	89	89	89	89	82
	6.2	.27	.29	.29	.28	86	87	87	86	85
	10.0	.22	.27	.29	.24	83	86	87	84	88
	11.2	.22	.23	.24	.22	83	84	84	83	85
Valine	2.0	2.0	1.7	1.8	1.9	60	65	63	62	69
	6.2	1.7	1.5	1.6	1.6	65	68	66	67	71
	10.0	1.5	1.5	1.5	1.5	68	68	68	68	70
	11.2	1.6	1.5	1.3	1.6	66	68	72	67	72

culated from each of these distribution coefficients have been correlated by statistical methods with corresponding Rf values as measured by paper chromatography and a significant relationship has been demonstrated.

1. Brimley, R. C., Barret, F. C., *Practical Chromatography,* Reinhold Pub. Co., New York 1953.

2. Cassidy, H. G., *Technique of Org. Chem.*, Vol. V (1951). *Absorption and Chromatography,* Interscience Pub., Inc., New York.

3. Clegg, D. L., *Anal. Chem.*, 1950, v22, 49.

4. Dent, C. E., *Biochem. J.*, 1948, v43, 169.

5. Landan, A. J., Fuevst, R., Awapara, J., *Anal. Chem.*, 1951, v23, 162.

6. Martin, A. J. P., *Ann. Revs. Biochem.*, 1950, v19, 517.

7. Moore, S., Stein, W. H., *ibid.*, 1952, v21, 521.

8. Strain, H. H., *Anal. Chem.*, 1950, v22, 41.

9. ———, *ibid.*, 1951, v23, 25.

10. Strain, H. H., Sato, T. R., *ibid.*, 1956, v28, 687.

11. Williams, T. I., *Introduction to Chromatography,* Chem. Pub. Co., Brooklyn, N. Y., 1947.

12. McFarren, E. F., *Anal. Chem.*, 1951, v23, 168.

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Host-Virus Relationships in Rous Sarcoma Tissues *in vitro*. I. Growth of Extraneous Viruses.* (23693)

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Considerable experimental work has been recorded on the growth of viruses in neoplastic tissues(1). For such investigations most workers have utilized transplantable tissues of tumors not known to be caused by viruses, while few studies have been made of the growth of extraneous viruses in the cells of virus-induced tumors. In investigating the biological properties of a tumor-inducing virus and the properties of the malignant tissue it produces, the ability of Rous sarcoma tissue

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