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THE CHROMATOGRAPHIC SPECTRA OF THE MONOMERIC COMPONENTS OF RIBONUCLEIC ACIDS

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The optimum solution of many problems of the separation and identification of natural compounds and their synthetic analogs can be achieved by using chromatographic spectra characterizing the comparative mobilities of the substances on paper chromatography in sets of different solvent systems.

The precursor of modern chromatographic spectra in the chemistry of the nucleic acids is the scheme for the separation of the spots of the nitrogenous bases present in the main natural nucleosides in eight solvent systems [1]. Chromatographic spectra are already widely used for the identification and classification of antibiotics [2, 3].

In order to facilitate the optimum choice of solvent systems for the separation and determination of the monomeric components of the nucleic acids, we have obtained the chromatographic spectra of four heterocyclic nitrogenous bases (adenine, guanine, uracil, and cytosine), and their nucleosides and nucleotides. Two sets of solvent systems were used. The first, standard, set we used to characterize anomalous nucleosides and other biologically active substances [4]. It consists of systems of organic solvents of simple composition as a rule (anhydrous, containing a definite percentage of water, or saturated with water). The first 22 systems in the standard set are arranged approximately in order of increasing polarity and the last five have a somewhat more complex composition. They are three-component systems and characterize the chromatographic mobility of various substances in the presence of acids and bases. The standard set of solvent systems is as follows: 1) benzene; 2) ethyl acetate; 3) butyl acetate; 4) petroleum ether; 5) carbon tetrachloride saturated with water; 6) benzene saturated with water; 7) chloroform saturated with water; 8) ethyl acetate saturated with water; 9) butyl acetate saturated with water; 10) acetone; 11) nitromethane saturated with water; 12) butanol saturated with water; 13) isopropanol + 10% of water; 14) acetone + 10% of water; 15) methanol; 16) methanol + 10% of water; 17) dioxane + 10% of water; 18) tetrahydrofuran + 10% of water; 19) water saturated with butanol; 20) dimethylformamide + 10% of water; 21) water; 22) water + 3% of ammonium chloride; 23) butanol saturated with water + 2% of piperidine; 24) butanol–pyridine–water (10 : 6 : 10); 25) butanol–acetic acid–water (5 : 2 : 3); 26) butanol–acetic acid–water (2 : 1 : 2); 27) isopropanol–ammonia–water (7 : 1 : 2).

The second set of solvent systems contains multicomponent systems that are frequently used in the chemistry of nucleic acids for separating nucleotides, nucleosides, and bases [1, 5–7]. We give the set of solvent systems for the components of the nucleic acids: 1') butanol–water–ammonia (86 : 14 : 1.33); 2') butanol–water–ammonia (81.3 : 10.9 : 7.8); 3') butanol–water–formic acid (22.5 : 2.5 : 2.5); 4') butanol–water–formic acid (77 : 13 : 10); 5') butanol saturated with 4% aqueous boric acid; 6') butanol saturated with 10% aqueous urea; 7') butanol–diethyleneglycol–water (20 : 5 : 5); 8') butanol–ethyleneglycol–0.1 N hydrochloric acid (4 : 1 : 1); 9') butanol–diethyleneglycol–morpholine–water (4.5 : 1 : 1.5 : 2); 10') isopropanol–ammonia–water (85 : 1.8 : 14.5); 11') isopropanol–hydrochloric acid–water (68 : 16.4 : 15.6); 12') isopropanol–hydrochloric acid–water (65 : 13 : 22); 13') acetone–trichloroacetic acid–water (75 : 6.25 : 25); 14') tert-butanol–hydrochloric acid–water (70 : 13 : 17); 15') isobutyric acid–0.5 N aqueous ammonia (60 : 36); 16') isobutyric acid–water–25% aqueous ammonia (60 : 31 : 0.06); 17') 5% solution of ammonium citrate saturated with isoamyl alcohol; 18') ethanol–M ammonium acetate buffer (7 : 3); 19') 5% solution of disodium phosphate saturated with isoamyl alcohol; 20') 5% solution of monopotassium phosphate saturated with isoamyl alcohol; 21') saturated solution of ammonium sulfate–isopropanol–water (79 : 2 : 19); 22') aqueous solution of ammonia (pH 10).

In the second set, the solvent systems are arranged in groups. The first nine systems form the "butan-1-ol group" and consist of n-butanol with various additives. Then comes the "isopropanol group," several groups of organic solvents with added acids and bases, and then the group of aqueous salt systems saturated with alcohols and, finally, aqueous ammonia with pH 10. Within the limits of each group, the systems are arranged approximately in order of increasing polarity.

Tables 1 and 2 give the R_f values of 12 components of the nucleic acids (nitrogenous bases, nucleosides, and nucleotides).

Typical examples of their chromatographic spectra are given in the figure.

It can be seen from the figure that in the standard set of solvent systems and for the nucleotides in some of the systems of the special set the chromatographic mobility of the components of RNA is equal to zero. In the majority of systems, the highest mobility on the paper chromatograms is possessed by uracil and its derivatives. As was to be expected, the least mobile, because of their low solubility, are guanine and its derivatives. In the majority of cases, cytosine and adenine and their derivatives occupy an intermediate position.

Table 1

Chromatographic Mobility of the Components of RNA in the Special Set of Solvent Systems

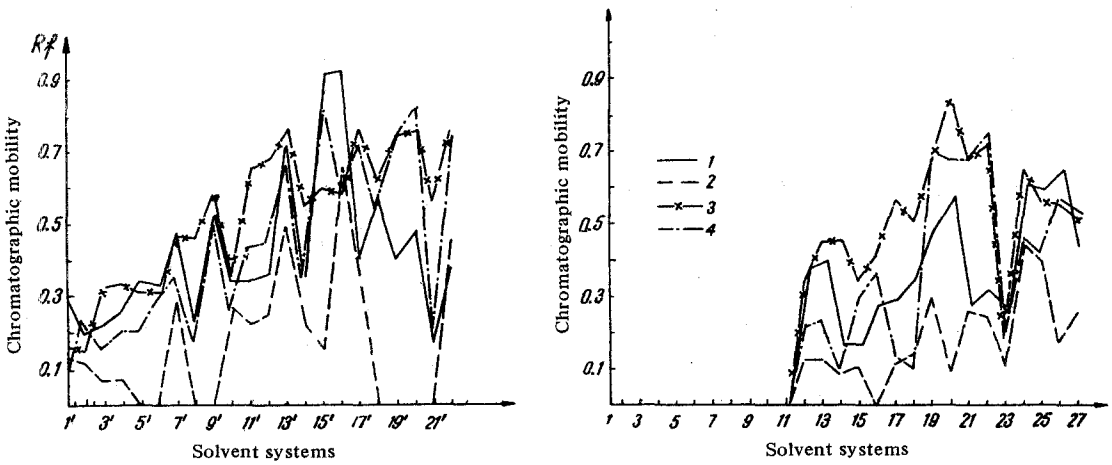
System no.	Adenine	Guanine	Uracil	Cytosine	Adenosine	Guanosine	Uridine	Cytidine	AMP	GMP	UMP	CMP
12	0.38	0.13	0.33	0.22	0.22	0.14	0.20	0.13	0	0	0	0
13	0.40	0.13	0.45	0.24	0.26	0.17	0.30	0.21	0	0	0	0
14	0.17	0.09	0.46	0.11	0.17	0.08	0.43	0.12	0	0	0	0
15	0.17	0.11	0.35	0.30	0.15	0.12	0.33	0.27	0.07	0.15	0	0
16	0.28	0	0.41	0.37	0.20	0.23	0.49	0.35	0.26	0.25	0	0
17	0.30	0.12	0.57	0.15	0.40	0.27	0.63	0.25	0.04	0	0.04	0
18	0.35	0.15	0.51	0.11	0.35	0.13	0.59	0.17	0	0	0	0
19	0.48	0.30	0.69	0.71	0.68	0.61	0.72	0.75	0.81	0.80	0.87	0.84
20	0.57	0.10	0.84	0.70	0.78	0.90	0.93	0.85	0.06	0.15	0	0
21	0.28	0.27	0.68	0.69	0.32	0.55	0.80	0.77	0.93	0.85	0.94	0.95
22	0.32	0.25	0.72	0.75	0.55	0.55	0.81	0.83	0.73	0.65	0.85	0.88
23	0.27	0.12	0.19	0.22	0.22	0.04	0.11	0.11	0	0	0	0
24	0.61	0.45	0.65	0.47	0.61	0.50	0.61	0.55	0.27	0.30	0.24	0.17
25	0.60	0.40	0.57	0.43	0.60	0.40	0.50	0.48	0.25	0.26	0.28	0.30
26	0.65	0.18	0.56	0.58	0.57	0.40	0.45	0.50	0.27	0.22	0.25	0.25
27	0.44	0.27	0.52	0.54	0.57	0.30	0.42	0.45	0.10	0.10	0.12	0.13

Note. In systems 1-11, the R_f values of all the components are equal to 0.

Table 2

Chromatographic Mobility of the Components of RNA in the Special Set of Solvent Systems

System no.	Adenine	Guanine	Uracil	Cytosine	Adenosine	Guanosine	Uridine	Cytidine	AMP	GMP	UMP	CMP
1′	0.29	0.13	0.16	0.09	0.18	0.03	0.10	0.05	0	0	0	0
2′	0.20	0.12	0.15	0.22	0.19	0.03	0.08	0.13	0	0	0	0
3′	0.22	0.07	0.32	0.15	0.15	0.08	0.15	0.10	0	0	0	0
4′	0.26	0.07	0.33	0.20	0.13	0.16	0.22	0.18	0	0	0	0
5′	0.34	0	0.31	0.20	0.10	0.08	0.15	0.08	0	0	0	0
6′	0.33	0	0.31	0.28	0.29	0.18	0.25	0.17	0	0	0	0
7′	0.47	0.28	0.46	0.35	0.27	0.22	0.37	0.29	0.06	0.08	0.10	0.07
8′	0.23	0	0.46	0.18	0.18	0.12	0.35	0.21	0.14	0.16	0.42	0.19
9′	0.50	0	0.58	0.50	0.55	0.43	0.53	0.58	0.17	0.13	0.17	0.23
10′	0.34	0.26	0.36	0.27	0.36	0.12	0.37	0.30	0	0	0	0
11′	0.34	0.22	0.65	0.43	0.32	0.30	0.64	0.42	0.47	0.45	0.83	0.55
12′	0.36	0.25	0.68	0.45	0.35	0.33	0.70	0.47	0.46	0.43	0.80	0.61
13′	0.70	0.48	0.75	0.67	0.66	0.63	0.53	0.70	0.54	0.33	0.56	0.46
14′	0.35	0.22	0.55	0.36	0.30	0.37	0.64	0.36	0.30	0.33	0.78	0.48
15′	0.91	0.15	0.59	0.80	0.93	0.51	0.54	0.68	0.55	0.35	0.36	0.56
16′	0.92	0.65	0.58	0.60	0.70	0.48	0.49	0.57	0.28	0.28	0.27	0.40
17′	0.40	0.35	0.75	0.72	0.54	0.59	0.84	0.82	0.67	0.69	0.87	0.97
18′	0.57	0	0.62	0.55	0.60	0.46	0.62	0.63	0.03	0.06	0.06	0.08
19′	0.40	0	0.74	0.73	0.53	0.63	0.83	0.79	0.77	0.84	0.91	0.93
20′	0.47	0	0.76	0.82	0.56	0.61	0.80	0.83	0.72	0.71	0.90	0.91
21′	0.17	0	0.56	0.21	0.13	0.35	0.60	0.71	0.32	0.50	0.65	0.71
22′	0.37	0.44	0.76	0.74	0.47	0.67	0.84	0.73	0.92	0.93	0.93	0.95



Chromatographic spectra of the RNA bases: 1) adenine; 2) guanine; 3) uracil; 4) cytidine.

The chromatographic spectra of the bases and nucleosides show that both sets of solvent systems can be used to separate the latter, while it is frequently possible to make do with simple systems from the first set. To separate the purine and pyrimidine bases the best systems are Nos. 12, 13, 15, 16, 18, 20, 3', 4', 11', 12', 15', 20', and to separate the nucleosides Nos. 13, 14, 20, 26, 2', 15', 21', 22'. The majority of the systems of the standard set are unsuitable for the separation of the nucleotides, since the R_f values of the nucleotides in these systems are fairly close to one another. Complete separation of the nucleotides can be achieved only in systems Nos. 22, 11', 12', and 21'. It is possible to use systems 15', 20', and others to separate the nucleotides using two-dimensional chromatography. The overlapping spectra of compounds of similar properties are of interest. The results of their comparison even when the R_f differences are small makes it possible to identify the substances unambiguously. By using the chromatographic spectra given in the figure it is always possible to select a system according to the complete problem both for the separation of many components and also for the isolation of one component from a mixture and for detecting impurities. Because of the close chromatographic mobilities of the components of the NA in many systems, to determine purity it is necessary to carry out control chromatography in several systems.

Experimental

The chromatographic mobilities of the components of RNA were determined with ascending chromatography on slow chromatographic paper of the Leningrad No. 2 paper mill.

For chromatography, the paper was cut into parallel strips 2.0 cm wide and 25.0 cm long. The starting line was drawn 2 cm from the lower edge and the line of the front 3 cm from the upper edge. The substances were deposited with glass capillaries in an amount of 2–3 drops of saturated aqueous solutions. After drying, the paper sheet was cut into individual strips.

The chromatographic systems were prepared from purified solvents immediately before the experiment. Glass cylinders with ground lids 25 cm high and 3–5 cm in diameter were used as the chromatographic chambers.

The spots of the substances were identified under a BUV-15 ultraviolet lamp with UFS-1 and UFS-2 filters 3–5 mm thick. The R_f values were determined by means of a uniformly stretched strip of rubber bearing a proportional decimal scale. To ensure reliability, many check experiments were carried out.

In the work, difficulties were found in the identification of guanine because of its very poor solubility in the majority of solvents. To obtain spots of guanine base with adequate absorption in the UV region, a solution of guanine hydrochloride or sulfate (about 10 μg) was deposited on the paper and then the paper was kept in ammonia vapors for 18 hr [1].

Conclusions

The chromatographic mobilities of the monomeric components of ribonucleic acids—adenine, guanine, uracil, and cytosine—and their nucleosides and nucleotides have been studied in two sets of solvent systems. The chromatographic spectra permitting the optimum choice of solvent systems in the solution of various problems on the separation and identification of the components of nucleic acids have been compared.

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