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Note

Paper chromatographic and electrophoretic separations of orotic acid, 5-fluoroorotic acid, 5-fluorouracil, and 5-fluorouridine 5'-monophosphate

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Numerous fluoropyrimidines have been found to have antitumor activity¹. For our enzymatic studies with the antitumor agent 5-fluoroorotic acid, we needed to separate orotic acid from 5-fluoroorotic acid and to separate 5-fluoroorotic acid, 5-fluorouracil, and 5-fluorouridine 5'-monophosphate. To our knowledge, these separations have not been reported. Separations of fluorouracil and its nucleosides and nucleotides, however, have been reported²⁻⁵ as well as separations of these compounds from orotic acid and its nucleoside and nucleotide⁶⁻⁸.

We report here our descending paper chromatographic and electrophoretic systems used to carry out these separations.

EXPERIMENTAL

5-Fluorouracil and orotic acid monohydrate were purchased from Sigma (St. Louis, Mo., U.S.A.), and 5-fluoroorotic acid was bought from P-L Biochemicals (Milwaukee, Wisc., U.S.A.). 5-Fluorouridine 5'-monophosphate was synthesized by the procedure of Robins *et al.*⁹. The synthesis of [carboxyl-¹⁴C]5-fluoroorotic acid will be reported elsewhere¹⁰. Whatman 3MM paper was used for all separations.

Chromatography

The compounds (0.05–0.1 μ mole each) were separated by descending chromatography using freshly prepared solvent systems and eluting at room temperature for 16 h: (A) isopropanol–conc. ammonia–water (7:1:2), or (B) 0.5 mM Na₃B₄O₇–isopropanol (1:2). The spots were visualized by UV light (254 nm). With [carboxyl-¹⁴C]5-fluoroorotic acid, radioactivity was determined by cutting out 1-cm strips from the origin to the solvent front and counting each strip in 10 ml Aquasol (New England Nuclear, Boston, Mass., U.S.A.) on a Packard Tri-Carb Model 3380 liquid scintillation counter.

Electrophoresis

Electrophoresis was carried out on a Savant flat-plate high-voltage instrument at 1,000 V for 60 min in 0.1 M HCl–KCl buffer (pH 1.8). Spots were visualized with UV light (254 nm).

RESULTS AND DISCUSSION

The R_F values for chromatography of the pyrimidines are listed in Table I.

TABLE I
 R_F VALUES FOR CHROMATOGRAPHY OF PYRIMIDINES

<i>Compound</i>	<i>Solvent system</i>	R_F
Orotic acid	A	0.34
5-Fluoroorotic acid	A	0.28
5-Fluoroorotic acid	B	0.39
5-Fluorouracil	B	0.64
5-Fluorouridine 5'-monophosphate	B	0.18

Each of the compounds separated in solvent system B was eluted from the paper with water and shown to have the same UV absorption spectrum of the single component under identical conditions.

When the separation of 5-fluoroorotic acid and orotic acid was performed with [carboxyl- ^{14}C]5-fluoroorotic acid, essentially none of the radioactivity was found in the region of orotic acid¹⁰.

Electrophoretic mobilities for orotic acid and 5-fluoroorotic acid are 4.1 cm and 5.9 cm from the origin towards the anode.

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