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Note

Separation of halogenated uracil derivatives from nucleobases and nucleosides by thin-layer chromatography on silica gel

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During studies on pyrimidine analogues in biological systems, a rapid and simple method was required for the detection of 5-fluorouracil, 5-chlorouracil, 5-bromouracil, 5-iodouracil, 5-fluorodeoxyuridine, 5-chlorodeoxyuridine, 5-bromodeoxyridine and 5-iododeoxyuridine in biological specimens and in mixtures of bases and nucleosides.

Nucleic acid constituents and their analogues have been extensively characterized by paper chromatography[1,2] and thin-layer chromatography[3,4]; the clear identification of the 5-halogenated uracil derivatives, however, remains a difficult problem.

In this paper, thin-layer chromatographic systems are described that allow the rapid separation of 5-halouracils and 5-halodeoxyuridines from the common nucleic acid constituents and the differentiation of these halogenated compounds.

EXPERIMENTAL

Analytical-reagent grade substances were purchased from Serva (Heidelberg, G.F.R.), Merck (Darmstadt, G.F.R.) or Calbiochem (San Diego, Calif., U.S.A.). Each substance (10–50 nmol) was applied to aluminium sheets (20 × 20 cm, not pre-treated) coated with a 0.2-mm layer of silica gel 60 with fluorescence indicator F_{254} obtained from Merck. The chromatograms were developed in standard chambers from Desaga (Heidelberg, G.F.R.) with the solvents given in Table I for 45–90 min at room temperature. When silica gel 60 HPTLC aluminium sheets (5.0 × 7.5 cm) were used, 2 nmol of each compound were sufficient and the time to develop the chromatograms was reduced to 10–15 min. Equilibration was not necessary. After air-drying of the developed chromatograms, the spots were detected under UV light (254 nm). When radioactive substances were employed, the spots were eluted or scraped off for scintillation counting.

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TABLE I

R_F VALUES FOR BASES, NUCLEOSIDES AND 5-HALOGENATED URACIL DERIVATIVES

Solvents: I, ethyl acetate–methanol (5:1); II, ethyl acetate–ethanol–water (32:4:1); III, diethyl ether–acetone–water (20:20:1); IV, methyl acetate; V, chloroform–ethanol–water (20:14:1); VI, chloroform–ethyl acetate–ethanol (5:24:6).

Compound	*Solvent*					
	I	*II*	*III*	*IV*	*V*	*VI*
Adenine	0.15	0.17	0.16	0.00	0.48	0.05
Guanine	0.05	0.06	0.09	0.00	0.26	0.00
Cytosine	0.00	0.03	0.02	0.00	0.20	0.00
Thymine	0.69	0.62	0.74	0.46	0.85	0.54
Uracil	0.57	0.54	0.62	0.29	0.75	0.39
5-Fluorouracil	0.82	0.72	0.87	0.71	0.85	0.63
5-Chlorouracil	0.86	0.78	0.90	0.78	0.89	0.72
5-Bromouracil	0.87	0.85	0.90	0.79	0.90	0.74
5-Iodouracil	0.89	0.85	0.91	0.82	0.93	0.77
Adenosine	0.16	0.18	0.17	0.00	0.51	0.08
Guanosine	0.00	0.02	0.02	0.00	0.27	0.00
Cytidine	0.00	0.02	0.03	0.00	0.18	0.00
Uridine	0.30	0.33	0.38	0.00	0.61	0.10
Deoxyadenosine	0.21	0.19	0.21	0.02	0.60	0.07
Deoxyguanosine	0.02	0.06	0.06	0.00	0.44	0.00
Deoxycytidine	0.04	0.05	0.05	0.00	0.35	0.00
Thymidine	0.59	0.48	0.65	0.20	0.82	0.41
5-Fluorodeoxyuridine	0.76	0.70	0.82	0.51	0.83	0.57
5-Chlorodeoxyuridine	0.80	0.68	0.85	0.58	0.90	0.60
5-Bromodeoxyuridine	0.79	0.75	0.86	0.61	0.88	0.63
5-Iododeoxyuridine	0.84	0.72	0.87	0.65	0.91	0.63

RESULTS

The R_F values of the compounds tested in six solvent systems are given in Table I; these are average values determined from 3–8 independent chromatographic runs. The results demonstrate that an efficient and rapid separation of the 5-halogenated uracil derivatives from the naturally occurring bases and nucleobases was achieved. This improvement over other solvent systems is due to the higher R_F values of the 5-halogenated uracil derivatives. To the best of the author's knowledge, these solvent systems provide much more rapid separations than comparable systems. Table I also shows that the different 5-halogenated base derivatives have distinct R_F values. In order to separate these closely related compounds completely, a combination of solvent systems is recommended.

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