CHROM. 3505

An improved chromatography of organic iodine compounds on Tris-maleate buffer treated paper*

Conventional paper chromatography of iodide, iodotyrosines and iodothyronines requires 24 to 48 h for development and yields variable resolution[1]. Altering the structure of the supporting medium improves chromatography of many compounds. Resolution of α-keto acids improves in paper strips saturated with Veronal sodium, hydrochloric acid buffer, pH 8.6[2]. We find improved resolution of iodine containing compounds using chromatography paper saturated with alkaline TRIS (trishydroxymethylaminomethane) buffer.

Method

Whatman No. 3 or 3 MM strips, air dried after dipping in 0.25 *M* Tris–maleate buffer, pH 8.6 (Tris: 96.8 g, NaOH: 44.0 g, maleic anhydride: 78.4 g, water to 11 l) were used for chromatography with either tertiary amyl alcohol–ammonia–water (TA) (alcohol saturated with 2 *N* ammonium hydroxide) or butanol–acetic acid–water (BA) (78:10:12, v/v). ^{131}I-Labeled compounds were produced by enzymatic alteration of thyroxine (T_4)[3]. Sixty microliters of a carrier solution were added at the

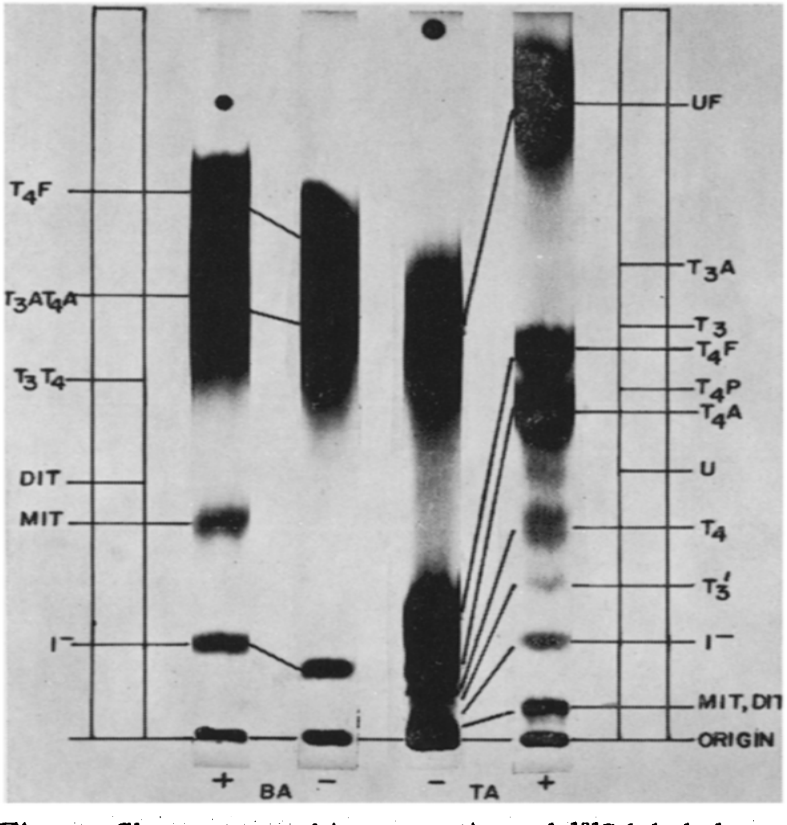

Fig. 1. Chromatographic separation of ^{131}I-labeled compounds from different experiments in butanol–acetic acid with and without Tris buffer and tertiary amyl alcohol–ammonia without and with buffer treatment (from left to right). UF and U are unknowns.

* Supported by USPHS grant AM-05825 and a grant from the United Health Foundation.

origin (T_4, triiodothyronine (T_3), tetraiodothyroacetic acid (T_4A), tetraiodothyropropionic acid (T_4P), triiodothyroacetic acid (T_3A), each 2 mg/ml; diiodotyrosine (DIT) and monoiodotyrosine (MIT), each 1 mg/ml; potassium iodide (I^-), 0.5 mg/ml; propylthiouracil, 0.8 mg/ml dissolved in 2 *N* ammonium hydroxide in methanol (1:7, v/v). After ascending (BA solvent) or descending (TA solvent) chromatography for 14–22 h at 22°, strips were dried, autoradiographed and later stained with Pauly's reagent (equal volumes of chilled, freshly mixed, 4.5 g/100 ml sodium nitrite and 0.8 g/100 ml sulfanilic acid in 10 % concentrated hydrochloric acid), and palladium chloride (0.1 g/100 ml) dissolved in 10 % concentrated hydrochloric acid). Stained strips were fixed with 10 g/100 ml potassium carbonate.

TABLE I

R_F VALUES FOR IODINE CONTAINING COMPOUNDS CHROMATOGRAPHED ON BUFFER TREATED PAPER[a]

Compound	*Solvent system*	
	BA	*TA*
I^-	0.17 ± 0.01	0.13 ± 0.01
MIT	0.37 ± 0.02	0.032 ± 0.02
DIT	0.49 ± 0.01	0.032 ± 0.02
T_3'	—	0.17 ± 0.03
T_4	0.67 ± 0.03	0.25 ± 0.02
T_4A	0.92 ± 0.02	0.41 ± 0.03
T_3	0.67 ± 0.03	0.52 ± 0.02
T_4F	—	0.46 ± 0.02
T_3A	0.92 ± 0.02	0.69 ± 0.02

[a] Values are mean and S.D. T_4F = Tetraiodothyroformic acid; T_3' = "reversed" T_3 (3',5',3-triiodothyroxine). See text for further abbreviations.

Results

Some dozen compounds readily separate in the TA system. The resolution of several compounds is compared on untreated and treated paper in Fig. 1. The mean R_F values for these compounds in the two solvent systems are shown in Table I. These are much more reproducible on paper treated with buffer than on untreated paper.

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Received March 13th, 1968