

Some evidence of this peculiar phenomenon had previously been noted for RNA nucleosides⁵. In the present study, the best separations were obtained with agarose gel on glass. For solution analysis, cover slip glass which could be cut easily with a diamond point cutter was used as the carrier for the gel. The nucleotides resolved well and were eluted easily into dilute alkali. For densitometric analysis, the use of quartz glass was mandatory.

*Departments of Microbiology and Pathology,
Wayne State University School of Medicine,
and the Department of Pathology, Sinai Hospital,
Detroit, Mich. (U.S.A.)*

B. ZAK
L. M. WEINER
E. BAGINSKI

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Thin-layer partition chromatography of benzenecarboxylic and hydroxybenzenecarboxylic acids

Destructive oxidation of peat, lignites, bituminous coal and humic acids leads to the formation of complicated mixtures of low molecular weight polycarboxylic acids. Various chromatographic methods have been proposed for the separation of these acids¹⁻⁴.

We have shown^{5,6} that large quantities of water soluble organic acids are formed during the natural weathering of coals at temperature below zero (in the eternally frigid zone of the arctic area).

These water soluble acids contained many hydroxy compounds and could not be separated by previously mentioned methods. Satisfactory results were obtained by using partition chromatography on a thin non-adhering layer of silica gel.

The present report describes the separation conditions for thirteen synthetic polycarboxylic acids; eleven of them were detected in water soluble fractions of weathering coals.

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Experimental

Silica gel, 270 mesh, was washed with HNO_3 (sp.gr. 1.4), then with water, dried and activated for 2 h at 200° . To 100 g silica gel were added 50 ml 1 *N* acetic acid (stationary phase). Approximately 8.5 g silica gel were spread on a glass plate (18 × 24 cm) and a layer, 20 cm wide, was smoothed by means of a glass roller on a special support. The depth of the adsorption layer was 0.35–0.37 mm.

The acids used in the present investigation were synthesized in our laboratory (except *o*-phthalic acid). Data on these twelve acids are given in Table I.

TABLE I

No.	Acids	M.p. (°C)	%C	%H	Equiv. wt.
1	<i>o</i> -Phthalic	Sublimes	57.80	3.60	83.1
2	Benzene-1,3,5-tricarboxylic (trimesic)	320	51.50	2.92	70.4
3	Benzene-1,2,4-tricarboxylic (trimellitic)	232	51.60	2.87	70.6
4	Benzene-1,2,3-tricarboxylic (hemimellitic)	200	51.52	2.77	70.6
5	Benzene-1,2,3,5-tetracarboxylic (mellophanic)	252	47.41	2.50	63.6
6	Benzene-1,2,4,5-tetracarboxylic (pyromellitic)	272	47.32	2.48	63.6
7	Benzene-1,2,3,4-tetracarboxylic (prehnitic)	242	47.40	2.30	63.2
8	Mellitic	—	42.14	1.88	57.0
9	4-Hydroxy-5-methylbenzene-1,3-dicarboxylic	296	54.92	4.14	97.7
10	4-Hydroxybenzene-1,3-dicarboxylic	310	52.80	3.26	90.9
11	5-Hydroxybenzene-1,2,4-tricarboxylic	240	47.78	2.74	75.5
12	2-Hydroxybenzene-1,3,5-tricarboxylic	307	47.88	2.79	75.6
13	5-Hydroxybenzene-1,2,3,4-tetracarboxylic	243	44.56	2.20	67.7

All acids were dissolved in dioxan (concn. 0.5 %) and 5 μg of each acid applied at a distance of 20 mm from the wider edge of the prepared glass plate. The plates were developed in a horizontal position until the solvent reached the other side of the glass plate (45–90 min). Eight different solvent systems were chosen as mobile phases. They are reported in Table II together with the corresponding R_F values for the acids. All

TABLE II

Solvent systems used*	$R_F \times 100$												
	1	2	3	4	5	6	7	8	9	10	11	12	13**
A Benzene-acetone (2:3)	65	65	48	40	32	25	26	07	74	72	51	21	15
B Chloroform-acetone (2:3)	58	56	41	30	18	12	11	00	76	71	39	12	04
C Benzene-dioxan (2:3)	61	55	45	40	26	20	22	06	75	69	40	04	07
D Chloroform-dioxan (2:3)	57	49	39	33	21	15	14	00	71	66	35	05	05
E Benzene-methyl ethyl ketone (1:2)	60	—	51	35	30	26	19	04	70	68	50	13	10
F Chloroform-methyl ethyl ketone (1:2)	55	—	44	27	20	16	09	00	70	67	42	14	05
G Benzene- <i>n</i> -butanol (2:1)	66	74	57	37	27	21	14	04	83	78	51	16	07
H Chloroform- <i>n</i> -butanol (2:1)	62	68	49	33	24	11	07	00	82	78	32	07	03
I Chloroform-methyl ethyl ketone- <i>n</i> -butanol (1:1:2)	—	—	—	—	67	55	47	00	—	—	—	36	25

* All systems saturated with 50 % aqueous acetic acid.

** For key to numbers see Table I.

the solvent systems were saturated with 50% aqueous acetic acid. The acids were revealed by the following techniques:

1. Irradiation with U.V. light. The hydroxyacids appear as blue spots.
2. Spraying with 0.01% methyl orange or dimethylaminoazobenzene (for systems G, H, I, which contain butanol). After drying the acids appear as rose spots on a pale yellow background.
3. Spraying with a 1:1 v/v mixture 1% KMnO_4 and 5% Na_2CO_3 . All acids appear as yellow spots on a purple background.
4. Spraying with 2% FeCl_3 . Hydroxyacids Nos. 9 and 10 appear as violet spots, Nos. 11 and 13 as purple spots and No. 12 as a brown spot. (For the numbers of the compounds see Table I.)

The lower limit of detection is 0.5–2 μg .

It is difficult to separate the following pairs of acids: 4-hydroxyisophthalic and 4-hydroxy-5-methylisophthalic acid; and trimellitic and 5-hydroxytrimellitic acid. For the first pair the best result was obtained with the system C with benzene–dioxan (2:1). The second pair could be satisfactorily separated in system H but the proportion of butanol had to be increased.

For 2-hydroxybenzene-1,3,5-tricarboxylic acid and all the tetracarboxylic acids the resolving power of the systems A–H was poor. Satisfactory separations were obtained on using system I. The working conditions described may also be used for chromatography of the polycarboxylic acids on a preparative scale. The application of the gradient elution technique on columns of silica gel demonstrated the advantage of each system more clearly.

Institute of Biologically Active Substances,
Siberian Department of the Academy of Science of the U.S.S.R.,
Vladivostock (U.S.S.R.)

O. B. MAXIMOV
L. S. PANTIUKHINA

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