CHROMATOGRAPHY OF COMPOUNDS OF BIOLOGICAL INTEREST ON GLASS FIBER, PARAFFIN-COATED, AND UNTREATED CELLULOSE PAPER*

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The development of a rapid, sensitive, chromatographic method employing glass fiber paper by DIECKERT and coworkers[1] has made possible the analysis of a variety of compounds, including glycerides[2], phospholipids[3–5], sugars[6], polybromostearates[7], saponins[8], tung oil[9], bile acids[10], steroids[11,12], sterols[2,13], triterpenoids[14], and phosphatides[15]. The separation of cholesterol and its esters on paraffin-dipped paper has been described by SMITH[16]. ASHLEY AND WESTPHAL[17] have used paraffin oil-treated paper in the chromatography of aliphatic acids. Cellulose paper, untreated, has been employed for the separation of numerous compounds.

Because reports in the literature of studies employing glass fiber, paraffin-coated, and standard filter paper did not extend adequately to the biological compounds of interest in our studies with the rat, we have explored the use of several new solvent systems for the development of chromatograms prepared on these papers. In the present work, the migration characteristics of selected fatty acids, methyl esters of fatty acids, and other naturally occurring compounds have been studied on glass fiber and paraffin-coated paper. In addition to customary heating of the sulfuric acid-sprayed paper, iodine vapor has been used to detect compounds on glass fiber paper. The migration characteristics of fifty-five selected compounds, including amino acids, vitamins, purines and other biologically important substances, have been determined on untreated cellulose paper in one to six solvent systems.

PROCEDURES

Glass fiber paper***, 9 × 25 cm, was heated in a muffle furnace at 600° for 30 min. The paper was impregnated with a 0.4% solution of sodium silicate according to the procedure of SWARTWOUT *et al.*[13].

Large filter paper sheets† especially selected for chromatography were used to obtain values listed for compounds in Table III. Paraffin-coated paper was prepared

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*** No. 934 AH, Reeve Angel and Company, Clifton, New Jersey.

† No. 5-714, Whatman No. 1, 18 × 22 in., from Fisher Scientific Company, Silver Spring, Maryland.

by dipping sheets of this filter paper in a 5 % solution of paraffin in petroleum ether and allowing them to dry.

The compounds used as reference standards were secured from commercial sources. They were dissolved in suitable solvents and made up to contain 500 μg/ml. A line 5 cm from the bottom of the sheets was made with a lead pencil, and the positions of compounds were marked approximately 3 cm apart. Samples were applied to these reference positions with micropipets and allowed to dry. The paper was suspended in a covered rectangular glass jar with the upper 3 cm being held in the solvent by means of glass rods. With the treated glass paper, approximately 7 min were required for the solvent front to migrate to within 2 cm of the bottom edge of the paper. The chromatogram was removed and allowed to dry on a rack in a fume hood until the solvent odor could no longer be detected.

To locate the compounds after chromatography, the glass fiber papers were sprayed with concentrated sulfuric acid from an atomizer, placed on a glass rod support and heated in an oven at 110° for 4 min to produce charred spots. Some of the compounds were detected by reaction with iodine vapor to produce brown spots.

Approximately 15 to 24 h were required for descent of the solvents on the plain filter paper or paraffin-coated paper. The papers were supported on glass rods by stainless steel clamps and allowed to dry in a fume hood. Compounds on untreated or paraffin-coated cellulose paper were detected by reaction with iodine vapor, spraying with a 0.5 % solution of ninhydrin in 95 % ethanol, fluorescence under ultra-violet light, or spraying with bromocresol green or antimony trichloride (24 % solution in chloroform).

RESULTS AND DISCUSSION

The migration characteristics on glass fiber paper of a selected group of fat-soluble compounds in various solvents are shown in Table I.

Two spots were obtained for some of the compounds in the solvents studied. As these compounds were commercial samples which were not further purified, the appearance of two spots represents the presence of impurities therein. 7-Dehydrocholesterol may be separated from cholesterol by use of either isooctane or ethanol–ether–water (1:1:1 or 2:1:2). Ergosterol can be separated from these two compounds in isooctane. Greater separation between linolenic and linoleic acid, or cholesterol and related substances can be obtained by employing longer strips of paper. Limited success was achieved in separating the methyl esters of the fatty acids, hexane–isooctane–chloroform (2:4:1) giving greatest separation.

The R_F values of several compounds on paraffin-coated paper, with butanol–propionic acid–water* and chloroform–acetic acid–liquid paraffin (50:130:10) as solvents are presented in Table II. Slightly greater separation of cholesterol from 7-dehydrocholesterol and ergosterol is achieved in these solvents on paraffin-coated paper than on glass fiber paper.

Cholesterol may be detected on these chromatograms by spraying with Liebermann-Burchard reagent or a solution of ferric chloride in 87 % phosphoric acid (Zlatkis reagent).

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* Fresh solvent was prepared from equal volumes of two solutions: (A) 1246 ml of 1-butanol and 84 ml water, and (B) 620 ml of propionic acid and 790 ml of water.

TABLE I

R_F VALUES OF SELECTED COMPOUNDS ON GLASS FIBER PAPER

Compound	Quantity		R_F values[a]												
	μg	μl	1	2	3	4	5	6	7	8	9	10	11	12	13
Cholesterol	20	—	0.34	1.00	0.97	0.98	0.82	0.58	0.00	0.79	0.00	—	0.93	—	—
Choline	25	—	—	—	—	—	—	0.00	0.96	0.92	0.95	—	0.95	—	0.00
7-Dehydrocholesterol	50	—	0.03	—	0.98	0.96	0.24	0.13	0.96	0.78	0.38	0.79	—	0.74	0.15
	—	—	0.22[b]	—	—	—	0.80[b]	0.63[b]	—	0.82[b]	—	—	—	—	0.62[b]
Dihydrocholesterol	50	—	0.26	1.00	—	—	—	—	—	—	—	0.90	—	0.87	0.63
Ergosterol	50	—	0.01	—	0.99	0.95	—	—	—	0.86	—	0.15	0.93	0.85	—
	—	—	0.15[b]	—	—	—	—	—	—	—	—	0.71[b]	—	—	—
Fumaric acid	100	—	—	—	—	—	—	—	—	0.88	—	—	—	—	—
Linoleic acid	—	2	0.16	0.18	0.83	0.91	0.15	0.12	0.04	0.33	0.12	—	—	—	—
	—	—	—	—	—	—	—	—	0.80[b]	—	0.82[b]	—	—	—	—
Linolenic acid	—	2	0.12	0.20	0.91	0.92	0.23	0.17	0.06	0.39	0.84	—	—	—	—
	—	—	—	—	—	—	—	—	0.84[b]	—	—	—	—	—	—
Oleic acid	—	0.5	0.11	0.14	—	—	—	—	—	—	—	—	—	—	
Methyl arachidonate	—	—	1.00	—	—	—	—	—	—	0.91	—	0.96	0.90	0.96	0.16
Methyl laurate	—	—	—	—	—	—	—	—	—	0.05	—	0.00	—	—	—
Methyl linoleate	—	—	1.00	—	—	—	—	—	—	—	—	0.91	0.96	0.97	—
Methyl linolenate	—	—	1.00	—	—	—	—	—	—	0.87	—	0.94	0.96	0.96	0.63
Methyl myristate	—	—	—	—	—	—	—	—	—	0.89	—	0.92	0.96	—	—
Methyl oleate	—	—	1.00	—	—	—	—	—	—	0.90	—	—	0.97	—	0.97
Methyl stearate	—	—	0.94	—	—	—	—	—	—	—	—	0.90	—	—	—
Methyl palmitate	—	—	—	—	—	—	—	—	—	0.87	—	—	—	—	—

[a] Solvents: 1 = Isooctane
2 = Benzene–ethanol (100:9)
3 = Benzene
4 = Chloroform
5 = Carbon tetrachloride
6 = *n*-Hexane
7 = Ethanol–ether–water (2:1:2)
8 = Isooctane–chloroform (1:1)
9 = Ethanol–ether–water (1:1:1)
10 = Hexane–isooctane–chloroform (2:1:1)
11 = Hexane–chloroform–ethanol–ether (1:1:2:1)
12 = Hexane–isooctane–chloroform (4:1:1)
13 = Hexane–isooctane–chloroform (2:4:1)

[b] Two spots were obtained.

TABLE II

R_F VALUES OF SELECTED COMPOUNDS ON PARAFFIN-COATED PAPER

Compound	Quantity		Detection[a]	R_F value[b]	
	μg	μl		1	2
Adrenaline	50		Brown with I_2	0.29	—
Acetic acid		10	Yellow with BCG	0.20	—
Acetylcholine	50		Brown with I_2	0.40	—
Betaine	50		Brown with I_2	0.98	—
Cholesterol	100		Blue with $SbCl_3$	0.73	0.71
Choline	50		Brown with I_2	0.23, 0.46[c]	—
Cystathionine	20		Purple with Nin	0.02	—
7-Dehydrocholesterol	100		Fluorescent with U.V.	0.84, 0.94[c]	0.86
Ergosterol	100		Fluorescent with U.V.	0.84, 0.92[c]	0.80, 0.89[c]
Ethanolamine		4	Purple with Nin	0.55	—
Fumaric acid		10	Absorbs U.V.	0.54	—
α-Keto-γ-methiol-butyric acid	50		Purple with Nin	0.56	—
Lactic acid		2	Fluorescent with U.V.	0.75	—
Lecithin	100		Brown with I_2	0.85	0.88
Linoleic acid		5	Brown with I_2	0.84	0.76, 0.91[c]
Linolenic acid		5	Brown with I_2	0.84	0.73, 0.89[c]
N-Methylnicotinamide	50		Brown with I_2	0.87	—
Oleic acid		2	Fluorescent with U.V.	0.81	—
S-Adenosylmethionine	50		Purple with Nin	0.09	—
Serine	20		Purple with Nin	0.13	—

[a] I_2 = iodine vapor; BCG = bromocresol green; $SbCl_3$ = antimony trichloride; Nin = ninhydrin; U.V. = ultra-violet light.

[b] Solvents: 1 = butanol–propionic acid–water; 2 = chloroform–acetic acid–liquid paraffin (50:130:10).

[c] Separated into two spots.

In Table III are presented the R_F values of fifty-three compounds of biological interest in six solvent systems. Combinations of lutidine and methanol offer promise as solvents for the two-dimensional chromatography of compounds of biological interest, as excellent separation is achieved in them for a variety of compounds.

TABLE III

R_F VALUES OF SELECTED COMPOUNDS ON UNTREATED CELLULOSE PAPER

Compound	R_F values × 100[a]					
	Lutidine	Methanol	Butanol–acetic acid	Ethanol–acetic acid	Phenol	Butanol–propionic acid–water
Acetylcholine chloride	—	—	—	—	92, 91[b]	75, 64[b]
Adenine	70	—	—	—	—	—
Adenosine triphosphate	—	—	—	—	44, 17, 6[c]	18, 8, 4[c]
Adenylic acid	—	—	—	—	56	21
DL-α-Alanine	—	—	30	—	—	—
L-Alanine	22	70	31	—	—	—
Allantoin	—	50	—	—	—	—
p-Aminobenzoic acid	58	—	81	—	—	—
γ-Aminobutyric acid	—	—	—	44	—	—
DL-α-Amino-n-butyric acid	36	55	—	—	—	—

(continued on p. 353)

TABLE III (*continued*)

Compound	R_F values × 100[a]					
	Lutidine	*Methanol*	*Butanol–acetic acid*	*Ethanol–acetic acid*	*Phenol*	*Butanol–propionic acid–water*
4-Amino-5-imidazole-carboxamide	—	48	—	25	—	—
α-Aminoisobutyric acid	—	66	—	—	—	—
α-Aminopimelic acid	54, 15[b]	80, 73[b]	57, 31[b]	46, 26[b]	—	—
L-Aspartic acid	13	67	—	—	—	—
Adrenaline	—	70	—	—	—	—
S-Adenosylmethionine	—	4	—	—	—	—
Choline	36	73	38	39	—	—
Creatine	—	58	—	—	—	—
Creatinine	50	56	42	—	—	—
Cytidilic acid	—	—	—	—	41	14
Cytidine	—	43	—	—	76	42
Ethionine	51, 23[b]	74	52	—	—	—
L-Glutamic acid	13	—	—	—	—	—
Glycine	17	64	—	—	—	—
Guanylic acid	—	31	—	—	73, 38[b]	46, 16[b]
DL-Homocysteine	45, 17[b]	72, 55[b]	—	27	—	—
DL-Homocystine	16	55	—	—	—	—
Homocysteic acid	—	—	—	—	11	9
Homoserine	23	68	22	—	—	—
Isoleucine	47	63	59	—	—	—
α-Keto-γ-methiolbutyric acid	—	59	—	—	—	—
Leucine	50	—	61	—	—	—
Lysine	—	21	—	—	—	—
L-Methionine	45, 24[b]	53, 31[b]	—	—	—	—
Methionine sulfoxide	—	—	—	11	—	—
Methoxinine	—	—	—	—	85	50
N^2-Methylnicotinamide	82	—	81	—	100	77
Methylserine	—	51	—	—	—	—
Nicotinic acid amide	77	—	70	—	—	—
Pantothenic acid	—	71	—	—	—	—
Phenylalanine	47	51	—	—	—	—
Riboflavin	—	31	—	—	—	—
Serine	19	65	—	—	—	—
Taurine	29	—	—	—	—	—
Thiamine	—	—	37	—	—	—
Threonine	23	69	24	—	—	—
Tryptophan	—	35	—	—	—	—
Tyrosine	47	69	—	—	—	—
Urea	49	—	—	—	—	—
Uridylic acid	—	—	—	—	32	15
Valine	38	60	—	—	—	—
Vitamin B_{12}	—	30	—	—	—	—
Xanthine	—	28	—	—	—	—

[a] Solvents: Lutidine = 2,6-lutidine–water (65:35 v/v).
Methanol = absolute methanol–water (95:5 v/v).
Butanol–acetic acid = *n*-butanol–glacial acetic acid–water (80:20:20 v/v/v).
Ethanol–acetic acid = 95% ethanol–glacial acetic acid (95:5 v/v).
Phenol = Mallincrodt, USP "Gilt Label" phenol saturated with a solution of 6.3% sodium citrate and 3.7% potassium dihydrogen phosphate.
Butanol–propionic acid–water = equal volumes of two solutions: (A) 1246 ml *n*-butanol and 84 ml water, and (B) 620 ml propionic acid and 790 ml water.

[b] Two spots were obtained.

[c] Three spots were obtained.

SUMMARY

Selected organic compounds commonly occurring in plant and animal tissues have been chromatographed on glass fiber, paraffin-coated and untreated cellulose paper in a number of different solvent systems. The R_F values of these compounds represent new additions to the literature in the solvent systems studied.

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