CHROMATOGRAPHIC STUDIES OF THE LIPID COMPONENTS OF LEAVES[1]

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Plant lipids have recently been shown to contain lecithin, phosphatidyl ethanolamine, phosphatidyl serine and phosphatidyl inositol (Kates and Eberhardt, 1957; Benson and Maruo, 1958); phosphatidyl glycerol (Benson and Maruo, 1958); three glycolipids and a sulfolipid (Benson et al, 1958); and two monoglycerides (Zill and Harmon, 1959). Attempts to separate plant lipids by silicic acid column chromatography (Kates and Eberhardt, 1957), by two-directional paper chromatography (Benson and Maruo, 1958), or by chromatography on silicic acid-impregnated paper (Ferrari, 1959) have not resulted in clear-cut separations of the components. In this laboratory, it was found that a separation of the lipids of runner bean leaves into at least ten components could be achieved by chromatography on silicic acid-impregnated paper using a slight modification of the procedure of Marinetti et al. (1957).

Total lipids were extracted from runner bean leaves which had been supplied with orthophosphate-P^{32}, sulfate-S^{35}, or $C^{14}O_2$, respectively, in the light for about 2 hrs. (Eberhardt and Kates, 1957). The lipids

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Table I

Characteristics of Chromatographically Separated Lipid Components

Spot No.[a]	R_f value	Rhodamine 6G Stain[b]	Ninhydrin Stain	Phospho-molybdate Stain[c]	Periodate-Schiff Stain	Radioisotope incorporation % of total[d]			Products of mild alkaline hydrolysis
						P^{32}	S^{35}	C^{14}	
1	0.25	purple	-	-	w	18	0	1	P^{32}-GPI
2	0.32	purple	-	-	m	0	100	<1	S^{35}-sulfoglycoside
3	0.41	yellow	-	s	s	0	0	1	C^{14}-glycoside a
4	0.44	pink	-	m	s	29	0	14	P^{32}-GPC + P^{32}-cyc-GP + C^{14}-glycoside b
5	0.50	purple	w	-	m	23	0	6	P^{32}-GPG
5'	0.60	-	-	-	-	4	0	<1	-
6	0.63	pink	s	w	trace	23	0	7	P^{32}-GPE
7	0.68	purple	-	-	m	0	0	13	C^{14}-glycoside c
8	0.75	yellow	-	s	s	0	0	14	C^{14}-glycoside d
9	0.82	purple	-	-	-	2	0	13	P^{32}-GP
10	0.90	violet	-	-	-	0	0	30	Chlorophyll hydrolysis products + C^{14} fatty acids.

Abbreviations: w = weak; m = moderate; s = strong; - = negative; GPI, glycerylphosphoryl inositol; GPC, glycerylphosphoryl choline; cycGP, cyclic glycerophosphate; GPG, glycerylphosphoryl glycerol; GPE, glycerylphosphoryl ethanolamine; GP, glycerophosphate.

[a] Most of the plant pigments were in spot 10, but traces were also present in spots 7 and 9.

[b] Chromatograms viewed under UV light (366 mμ).

[c] This reagent reacts with some non-choline containing lipids (Marinetti et al., 1957).

[d] Determined by scanning the autoradiograms with a Spinco recording densitometer.

were dissolved in isoamyl alcohol-benzene (1:1), applied as spots (4-5 μg.P/spot) to a strip of silicic acid-impregnated Whatman 3MM paper, and chromatographed in diisobutyl ketone-acetic acid-water (40:25:5) at 25° for 18-20 hrs. (Marinetti et al, 1957). The chromatogram was dried for 15-20 min. in the fume hood and immediately redeveloped in the same solvent for 20 hrs. Radioactive lipids were located by autoradiography and total lipids by staining the chromatogram with Rhodamine 6G (Marinetti et al, 1957). Amino-lipids were detected with ninhydrin, choline-containing lipids with phosphomolybdic acid-stannous chloride, and lipids containing vicinal hydroxyl groups with the periodate-Schiff reagent.

For further identification, the separated components were eluted from the chromatogram with chloroform-methanol-water (70:25:5) followed by methanol, and subjected to milk alkaline hydrolysis (Dawson, 1954; Benson and Maruo, 1958). The hydrolysis products were chromatographed one-dimensionally in saturated phenol-water (PW) and in butanol-acetic acid-water (BAW, 5:3:1). Phosphate esters were identified by their R_f values in these solvents compared to those of authentic compounds. Other products were eluted from the chromatograms with water, hydrolyzed with 3N HCl for 1-2 hrs. at 100°, and the hydrolyzates were chromatographed in pyridine-ethyl acetate-water (PEAW, 4:10:10, upper phase). Sugars, glycosides, and polyhydric alcohols were detected with silver nitrate and identified where possible by their R_g values (movements relative to glucose) compared to those of authentic compounds.

The characteristics, radioisotope data, and

hydrolysis products of the separated components are summarized in Table I.

On the basis of this data, the probable identity of the lipid spots is as follows: 1 - phosphatidyl inositol; 2 - sulphoglycolipid; 3 - glycolipid a; 4 - lecithin + glycolipid b; 5 - phosphatidyl glycerol (+ trace of phosphatidyl serine); 5' - unidentified phosphatide; 6 - phosphatidyl ethanolamine; 7 - glycolipid c (+ trace of pigments); 8 - glycolipid d; 9 - phosphatidic acid (+ pigments); 10 - pigments + glycerides.

Further information concerning the structure of the sulfolipid and of the four glycolipids was obtained from the nature of the products of acid hydrolysis of the corresponding mild alkaline hydrolysis products (glycosides), as given in Table II.

TABLE II

Products of Acid Hydrolysis of Glycosides

Glycoside	R_f value in		Products of acid hydrolysis (silver nitrate reducing)					
	PW	BAW	S^{35} compound[2]	Glucose	Galactose	Arabinose	Unknown sugar[3]	Glycerol
S^{35}-glycoside[1]	0.19 (0.23)	0.04	+	++	-	-	+	-
Glycoside a*	0.44	0.09	-	++	+*	-	+	trace
Glycoside b*	0.45	0.09	-	+	+++*	-	+	+
Glycoside c*	0.55	0.28	-	++	+*	trace	+	trace
Glycoside d*	0.58	0.30	-	++	+++*	+	+	+

[1] R_g in PEAW, 0.32. [2] R_g in PEAW, 0.29.

[3] R_g in PEAW, 1.36; as compared to xylose, 1.28, and ribose, 1.43.

* C^{14}-labeled compounds.

In addition to the compounds listed in Table II, each hydrolyzate also contained a compound (R_g in PEAW, 0.60) which did not reduce silver nitrate but appeared as a white spot in the chromatogram. Also, no inorganic sulfate was detected in the acid hydrolyzate of the sulfo-glycoside, suggesting that the sulfur is in a very stable form. The absence of glycerol and galactose and the presence of glucose and an unknown pentose in the hydrolyzate indicates that the chemical structure of the sulfolipid is more complex than that deduced by Wiser and Benson (1959) for the sulfolipid of Chlorella and other plants.

The hydrolysis data (Table II) suggest that each of the glycolipids either contains a complex oligosaccharide, or is a mixture of glycolipids containing relatively simple glycoside moieties. The fact that galactose was the only C^{14}-labeled sugar detected probably indicates that its turnover rate in the glycolipids is very much greater than that of the other sugars. In any case, the glycolipids obtained here appear to have more complex structures than those deduced by Benson et al. (1958) for the glycolipids of Chlorella.

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