PN 1229

Lipid components of leaves
III. Isolation and characterization of mono- and digalactosyl diglycerides and lecithin*

CARTER and co-workers[1-3] isolated two galactolipids from wheat flour and established their chemical structures as 2,3-di-*O*-acyl-1-*O*-(β-D-galactopyranosyl)-D-glycerol (monogalactosyl diglyceride) and 2,3-di-*O*-acyl-1-*O*-(6-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-D-glycerol (digalactosyl diglyceride). These two galactolipids were also found in other plant sources[2], and BENSON *et al.* and WINTERMANS established their occurrence in algae[4,5], and in chloroplasts[6,7], but did not isolate the intact lipids. WEENINK[8] isolated a galactolipid fraction from the acetone-soluble lipids of red clover and showed that it contained mono- and digalactosyl glycerides. KATES AND EBERHARDT[9] found that glucose and arabinose, in addition to galactose, were present in a glycolipid-rich fraction of runner-bean leaves. Subsequently, the presence of at least four distinct glycolipids in these leaves was demonstrated (glycolipids a, b, c, and d, in increasing mobility)[10] by chromatography on silicic acid-impregnated paper. Recently, ZILL AND HARMON[11] also found glycolipids containing glucose and arabinose, as well as galactose, in spinach leaves and chloroplasts.

We now wish to report the isolation and characterization of the two major glycolipids in runner-bean leaves, namely mono- and digalactosyl diglycerides (corresponding to glycolipids d and a, respectively[10]), and of the major phosphatide, lecithin.

Total lipids (+ pigments) extracted from runner-bean leaves were chromatographed on silicic acid–Celite (4:1, w/w), as described previously[9], to obtain the glycolipid-rich fraction (Fraction I) and the lecithin-rich fraction (Fraction IV). Rechromatography of Fraction I on silicic acid yielded the monogalactosyl diglyceride in the second to third column volumes of 2 % methanol in chloroform (together with small amounts of acidic phosphatides); the digalactosyl diglyceride appeared in the third column volume of 5 % methanol in chloroform and in the first column volume of 10 % methanol in chloroform (together with the two minor glycolipids b and c, phosphatidyl glycerol and phosphatidyl ethanolamine).

Purification of glycolipids. The eluates containing the monogalactosyl lipid were concentrated *in vacuo* to a small volume and the phosphatide contaminants were removed by repeated precipitation with acetone, followed by passage through a column of Amberlite MB-3 in 95 % methanol. The effluent contained only one glycolipid component [R_F 0.71 in diisobutyl ketone–acetic acid–water (40:25:5) on silicic acid-impregnated paper[12]], and was free of phosphatides, but was still contaminated with brown pigments. The analytical data agreed with those expected for a monogalactosyl diglyceride (Table I), and alkaline hydrolysis yielded 1-*O*-β-D-galactopyranosyl-D-glycerol [m.p. 138–138.5°; reported m.p. (ref. 1) 139–140°; R_{Gal} 0.80, identical with that of authentic sample[1], in pyridine–ethyl acetate–water (1:2.5:2.5, v/v); infrared spectrum (KBr) identical with that reported for synthetic substance[13]].

The digalactosyl diglyceride was purified as follows: The eluates containing this glycolipid were concentrated *in vacuo* to a small volume and most of the phosphatides were removed by precipitation with acetone. The remaining traces of phosphatides

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TABLE I

ANALYTICAL DATA FOR GALACTOSYL DIGLYCERIDES FROM RUNNER-BEAN LEAVES*

Constituent	Monogalactosyl diglyceride		Digalactosyl diglyceride	
	Unhydrogenated	Hydrogenated	Unhydrogenated	Hydrogenated
C, %	—	68.84 (68.6)	—	65.10 (64.7)
H, %	—	11.16 (11.0)	—	9.93 (10.2)
Fatty acid methyl esters**, %	—	80.6 (76.0)	—	63.7 (63.0)
Mole ratio, Galactose[15]: glycerol[16]:ester[17]	1.00:1.00:1.97	1.00:1.08:2.12 (1:1:2)	2.00:1.11:2.04	2.00:1.05:2.11 (2:1:2)

* Values in brackets are data calculated for the distearoyl derivatives.
** By weighing methyl esters obtained after hydrolysis with methanolic HCl.

and the minor glycolipid c were removed by dissolving the acetone-soluble material in methanol at 0° and centrifuging the small amount of precipitate. The methanol-soluble material now contained mostly digalactosyl diglyceride and the minor glycolipid b. These could be separated by repeated chromatography on silicic acid using 5–10 % methanol in ethyl ether to elute the minor glycolipid, and 50 % methanol–ether to elute the digalactosyl diglyceride. The latter eluate now contained only one glycolipid component (R_F 0.45 on silicic acid-impregnated paper[12]) but was still contaminated with brown pigments. The analytical data were close to those expected for a digalactosyl diglyceride (Table I), and alkaline hydrolysis yielded 1-*O*-(6-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-D-glycerol [m.p. 188–189°; reported m.p. (ref. 1) 182–184°; R_{Gal} 0.20, identical with that of authentic sample[1], in pyridine–ethyl acetate–water; infrared spectrum (KBr) identical with that reported for authentic substance[13]].

Gas liquid chromatographic analysis on butanediol succinate polyester (180°) of the fatty acids from the purified mono- and digalactosyl lipids showed that both contained almost exclusively linolenic acid (96 and 93 %, respectively), the remaining acids being palmitic and stearic acids. An identical fatty acid composition was reported for the galactolipid fraction of red clover[8], but the wheat-flour galactolipids had more complex fatty acid compositions[2].

Hydrogenation of the mono- and digalactosyl dilinolenins in methanol with platinum as catalyst yielded the corresponding crystalline mono- and digalactosyl distearins which were freed from contaminating pigments by recrystallization from chloroform–methanol. These derivatives contained stearic acid almost exclusively (96–98 %) and had the expected analytical values (Table I).

Purification of lecithin. The crude lecithin fraction [Fraction IV (ref. 9), 3.66 % P; N/P, 1.1; choline-N/P, 0.96; fatty acid/P, 2.17] still contained about 10 % of nitrogenous lipids not containing choline plus traces of green pigments. The material was applied in chloroform to a column of alumina, and the lecithin fraction was eluted with 15 column volumes of 10 % methanol in chloroform. This fraction was now free from non-choline nitrogenous lipids but was contaminated with about 10 % of lysolecithin produced by hydrolysis on the alumina column. Final purification was achieved by chromatography on a column of silicic acid with 30 % methanol in chloroform, followed by acetone precipitation at 0°. The lecithin thus obtained was an

almost colourless oil which gave only one spot on silicic acid-impregnated paper[12] (R_F 0.50), and had the analytical data given in Table II. The fatty acids consisted of palmitic (27 %), stearic (8 %), oleic (5 %), linoleic (36 %) and linolenic (24 %) acids, and all of the saturated acids were found to be on the α'-position, as determined by

TABLE II

ANALYSIS OF PURIFIED LECITHIN FROM RUNNER-BEAN LEAVES

Constituent	*Lecithin*	*Hydrogenated lecithin*	*Theory**
P (%)	3.78	3.77	3.90
N/P (atomic ratio)	0.99	1.01	1.00
Choline-N/P (atomic ratio)	0.99	1.02	1.00
Fatty acids (%)	66.7	68.4	69.6
Neutral equivalent	278	275	277
Fatty acid/P (mole ratio)	1.97	2.04	2.00

* Calculated for 1:1 molar mixture of distearoyl and palmitoyl–stearoyl lecithins.

hydrolysis with phospholipase A (EC 3.1.1.4)[14]. Hydrogenation of the natural lecithin in methanol with platinum catalyst yielded a crystalline "hydrolecithin", containing only stearic and palmitic acids in the mole ratio of 3:1, and having analytical values close to those of a 1:1 molar mixture of distearoyl and palmitoyl–stearoyl lecithin (Table II).

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