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CHLOROPHYTA

CHLOROPHYCEAE

GLYCOLIPIDS PRESENT IN EIGHT GENERA OF THE CHLOROPHYCEAE

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Monogalactosyl diglyceride (MGD) (2,3-di-*O*-acyl-1-*O*-β-D-galactopyranosyl-(D-glycerol),[1] digalactosyl diglyceride (DGD) (2,3-di-*O*-acyl-1-*O*-β-[6-*O*-α-D-galactopyranosyl-D-galactopyranosyl]-D-glycerol[2] and the corresponding trigalactosyl diglyceride (TGD)[3,4] have been found in a variety of plant tissues including the green alga, *Chlorella*.[5,6] 6-Sulpho-6-deoxy-α-D-glucopyranosyl diglyceride[7] has also been found in higher plants and algae. Fatty acid components of the galactolipids including those of *Chlorella* have been identified.[8]

In the present investigation the carbohydrate and fatty acid composition of the MGD, DGD and TGD of *Ulva lactuca*, *Enteromorpha intestinalis*, *Cladophora rupestris*, *Urospora*, *Codium fragile*, *Monostroma gravellei*, *Mougeotia* and *Acetabularia crenulata* all members of the Chlorophyceae have been investigated.

RESULTS AND DISCUSSION

The algae, apart from *Acetabularia* which came from Jamaica, were collected from various parts of the British Isles between October 1968 and June 1971. Although not deposited in an herbarium, samples of each of the algae have been kept and can be had on application to E. Percival.

Analysis of the 'non-acidic' glycolipid fractions on thin layer plates revealed three major bands with R_f values corresponding to those of MGD, DGD and TGD. After elution and hydrolysis only galactose and glycerol could be detected in the water-soluble products [PC, solvents (a), (f) and (g)]. This was confirmed by GLC of the trimethylsilyl (TMS) derivatives both as the sugar and the derived alcohol. After deacylation the products from each glycolipid had the published R_f values[3,6,9,10] for the corresponding galactosyl–glycerol derivatives from other sources in solvents (a) to (e).

Upon periodate oxidation deacylated MGD, DGD and TGD, from *Ulva lactuca*, reduced about 3, 5 and 7 moles of periodate respectively, the expected amount for 1,6-linked galactose units linked to C-1 of glycerol.

[1] H. E. CARTER, K. OHNO, S. NOJIMA, C. L. TIPTON and N. Z. STANACEV, *J. Lipid Res.* **2**, 215 (1961).
[2] H. E. CARTER, R. A. HENDRY and N. Z. STANACEV, *J. Lipid Res.* **2**, 223 (1961).
[3] T. GALLIARD, *Biochem. J.* **115**, 335 (1969).
[4] D. E. WEBSTER and S. B. CHANG, *Plant Physiol.* **44**, 1523 (1969).
[5] A. A. BENSON, R. WISER, F. A. FERRARI and J. A. MILLER, *J. Am. Chem. Soc.* **80**, 4740 (1958).
[6] R. A. FERRARI and A. A. BENSON, *Arch. Biochem. Biophys.* **93**, 185 (1961).
[7] H. DANIEL, M. MIYANO, R. O. MUMMA, T. YAGI, M. LEPAGE, I. SHIBUYA and A. A. BENSON, *J. Am. Chem. Soc.* **83**, 1765 (1961).
[8] B. W. NICHOLS and R. MOORHOUSE, *Lipids* **4**, 311 (1969).
[9] H. E. CARTER, R. H. MCCLUER and E. D. SLIFER, *J. Am. Chem. Soc.* **78**, 3735 (1956).
[10] B. URBAS, *Can. J. Chem.* **46**, 49 (1968).

The deacylated galactolipids from *Ulva* were incubated with a purified α-galactosidase and with a crystalline β-galactosidase and the release of galactose was followed by PC. The results (Table 1) indicated that the galactose unit of MGD is β-linked, whereas the terminal galactose unit of TGD is α-linked. The results obtained with DGD were contradictory, galactose being released from the deacylation product by both enzymes. The glycosidic linkage of the terminal galactose unit has been shown to be in the α-configuration for DGD from wheat flour,[1,2] runner bean leaves[11] and potato tubers.[3] Neufeld and Hall,[12] however,

TABLE 1. RELEASE OF GALACTOSE BY THE ACTION OF GALACTOSIDASES ON DEACYLATED GLYCOLIPIDS OF *Ulva* AND REFERENCE COMPOUNDS

Substrate	α-Enzyme	β-Enzyme
Deacylated MGD	−	+
Deacylated DGD	+	+
Deacylated TGD	+	−
Melibiose	+	−
Lactose	−	+
Raffinose	+	−

found that in the deacylation product of ^{14}C-labelled DGD formed in spinach chloroplasts by incubation with UDP-[^{14}C]galactose, 83% of the terminal galactose was released by β-D-galactoside galactohydrolase.

The major component of the 'acidic' glycolipid fraction had the same R_f values on TLC as the sulpholipid (SL) isolated by earlier workers.[13,14] After deacylation the derived material had the same R_f values on PC as authentic ^{35}S-glyceryl sulphoquinovose run both in parallel and as a mixture.

Quantitative estimation of the MGD, DGD and SL from 100 g dried weed gave respectively from *Ulva* 41, 62, and 43 mg, and from *Enteromorpha* 40, 90 and 20 mg. These results are in agreement with previous findings that the concentration of DGD is greater than that of MGD in algae, whereas the reverse is true in higher plants (see Radunz[15]).

Fatty acids. The pattern of FAME peaks obtained by GLC was very similar for MGD, DGD and TGD from any one weed. All the samples showed relatively small peaks corresponding to 11:0, 12:0, 13:0, 14:0 and 15:0 and larger peaks corresponding to 16:2 and 16:4 + 18:2. The major components in each of the glycolipids in all samples corresponded to 16:0 or 16:1, 18:3 + 20:0, 18:0 + 16:3 and 18:1. *Cladophora, Acetabularia* and *Codium* differed from the other weeds in having a 20:4 peak that was equal to or greater than any of the other components. All except *Codium* and *Cladophora* had a peak at the 20:1 position, and a small peak at the 20:2 position was given by the glycolipids from *Acetabularia* and *Cladophora* only.

EXPERIMENTAL

Extraction of glycolipids. All the samples were ground to a powder in liquid N_2 and the powder extracted with MeOH–$CHCl_3$ (7:3).[13] The antioxidant, butylated hydroxytoluene (2,6-di-*t*-butyl-*p*-cresol) was added to all extracting solvents.[16]

[11] P. S. SASTRY and M. KATES, *Biochem.* **3**, 1271 (1964).
[12] E. F. NEUFELD and C. W. HALL, *Biochem. Biophys. Res. Commun.* **14**, 503 (1964).
[13] P. G. ROUGHAN and R. D. BATT, *Anal. Biochem.* **22**, 74 (1968).
[14] A. RADUNZ, *Hoppe-Seyler's Z. Physiol. Chem.* **350**, 411 (1969).
[15] A. RADUNZ, *Hoppe-Seyler's Z. Physiol. Chem.* **349**, 1091 (1968).
[16] G. ROUSER, G. KRITCHEVSKY and A. YAMANOTO, in *Lipid Chromatographic Analysis* (edited by G. V. MARINETTI), Vol. 1, p. 99, Arnold, London (1967).

General methods. All evaporations were carried out under reduced pressure below 40°. The methods of Davies *et al.*[17] were used for deacylation and acid hydrolysis. The micro-method of Galliard[3] was used in the periodate oxidation of the deacylated glycolipids, the extent of oxidation being followed spectrophotometrically.[18] Quantitative analysis of the MGD, DGD, and SL, from *Ulva* and *Enteromorpha*, was carried out by the method of Roughen and Batt.[13]

Analytical procedures. The crude extract from each alga, after concentration, was separated into a 'non-acidic' and an 'acidic' fraction on a DEAE-cellulose column.[13] TLC was on glass plates coated with Kieselgel G (Merk) and developed with (i) $CHCl_3$–MeOH–HOAc–H_2O (85:15:10:3),[19] or (ii) $CHCl_3$–MeOH–EtOAc–2% NH_4OH (50:25:25:1·5).[14] Substances were located by spraying the edges of the plates with 1% iodine in MeOH. The respective glycolipids were extracted from the Kiesel gel with $CHCl_3$–MeOH–H_2O (115:115:25). Paper chromatograms were run on Whatman No. 1 paper by descent using systems (a) EtOAc–pyridine–H_2O (5:2:5, upper layer);[20] (b) *n*-BuOH–$EtCO_2H$–H_2O (151:75:100);[3] (c) *n*-BuOH–pyridine–H_2O (10:3:3);[10] (d) *n*-BuOH–pyridine–H_2O (4:6:3);[9] (e) H_2O satd. collidine;[9] (f) *n*-BuOH–EtOH–H_2O (40:11:19);[21] (g) EtOAc–HOAc–HCO_2H–H_2O (18:3:1:4);[22] (h) *n*-BuOH–HOAc–H_2O (5:3:1);[23] and sprayed with one of the reagents (i), (ii) or (iv) used by Bourne *et al.*[24] GLC was by the method of Percival.[25] The trimethylsilyl (TMS) ethers of galactose, and its derived alditol were run on column (4) (at 162°) and glycerol (at 155°) and on column (2) at 155°. The fatty acids as their methyl esters (FAME)[8] were chromatographed at 180° on Pye Model No. 104 gas chromatograph with glass columns (152 cm × 4 mm) packed with 15% diethylene glycol succinate on Gas Chrom W 80–100 mesh HMDS treated. They were identified by comparison with standard mixtures and from the retention times given by Chuecas and Riley.[26]

Enzymic hydrolysis. (a) *α-Galactosidase.* A sample of the deacylated glycolipid containing *ca.* 0·1–0·2 μmoles of sugar, as determined by the phenol–sulphuric acid method of Dubois *et al.*,[27] was incubated at 30° for 2 hr with 20 μl of enzyme solution in 0·1 M-acetate buffer, pH 5·6, in a total vol. of 0·5 ml.[28] The solution was then boiled for 15 min to stop the reaction, cooled, and centrifuged. Aliquots of the supernatant where chromatographed on paper. Control samples containing galactose, lactose, melibiose, raffinose and appropriate blanks were carried out at the same time. (b) *β-Galactosidase.* A similar sample of deacylated glycolipid was incubated at 30° for 2 hr with a mixture containing 0·1 M-potassium phosphate buffer, pH 7·0, 5 mM KCl and 0·1 mg enzyme in a total vol. of 0·2 ml.[3] The rest of the procedure was as described above.

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[17] W. H. DAVIES, E. I. MERCER and T. W. GOODWIN, *Phytochem.* **4**, 741 (1965).
[18] G. O. ASPINALL and R. J. FERRIER, *Chem. & Ind.* 1916 (1957).
[19] B. W. NICHOLS, in *New Biochemical Separations* (edited by A. T. JAMES and L. J. MORRIS), p. 321, Van Nostrand, London (1964).
[20] P. S. SASTRY and M. KATES, *Biochem.* **3**, 1280 (1964).
[21] L. HOUGH, J. K. N. JONES and W. H. WADMAN, *J. Chem. Soc.* 1802 (1950).
[22] E. L. HIRST and J. K. N. JONES, in *Modern Methods of Plant Analysis* (edited by K. PAECH and M. V. TRACEY), Springer-Verlag, Berlin (1955).
[23] M. KATES, *Biochim. Biophys. Acta* **41**, 315 (1960).
[24] E. J. BOURNE, P. G. JOHNSON and E. PERCIVAL, *J. Chem. Soc.* 1561 (1970).
[25] E. PERCIVAL, *Carbohy. Res.* **7**, 272 (1968).
[26] L. CHUECAS and J. P. RILEY, *J. Mar. Biol. Assoc. U.K.* **49**, 97 (1969).
[27] M. DUBOIS, A. GILLES, J. K. HAMILTON, P. A. REBERS and F. SMITH, *Analyt. Chem.* **28**, 350 (1956).
[28] P. M. DEY, Personal communication.

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