Phytochemistry, 1965, Vol. 4, pp. 741 to 749. Pergamon Press Ltd. Printed in England

THE OCCURRENCE AND INTRACELLULAR DISTRIBUTION OF THE PLANT SULPHOLIPID IN MAIZE, RUNNER BEANS, PLANT TISSUE CULTURES AND *EUGLENA GRACILIS*

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(*Received* 27 *February* 1965)

Abstract—The major sulpholipid in green and etiolated maize, two varieties of runner bean, tissue cultures of Paul's Scarlet Rose stem, and in green and etiolated *Euglena gracilis* strain Z is, in all cases, 6-sulphoquinovosyl diglyceride; no evidence could be obtained for a different sulpholipid in runner beans. In green tissues the major part, if not all, the sulpholipid is in the chloroplasts; it is, however, also present in etiolated maize and *Euglena*, and in tissue cultures which do not have chloroplasts.

INTRODUCTION

IN 1959 Benson and his colleagues[1] showed that ^{35}S was rapidly incorporated into the lipids of the green algae *Chlorella pyrenoidosa* and *Scenedesmus*, the photosynthetic bacterium, *Rhodospirillum rubrum* and in the leaf tissue of many higher plants, e.g. barley, clover, lucerne and New Zealand spinach. Eventually Daniel *et al.*[2] demonstrated that the structure of the major sulpholipid into which ^{35}S was incorporated into these organisms was 6-sulpho-6-deoxy-α-D-glucopyranosyl diglyceride (6-sulpho-α-D-quinovosyl diglyceride). The major fatty acid components are palmitic acid and linolenic acid.[3] 6-Sulphoquinovosyl glycerol and 6-sulphoquinovosyl have also been reported in *Chlorella*.[4]

The concentration of the sulpholipid in leaves equals or exceeds those of the well-known phospholipids[5] and its high concentration in green tissues led to the suggestion that it plays a functional role in chloroplast structure and function.[6] However, the generally prevalent view that the sulpholipid was located exclusively in the photosynthetic tissues was questioned by Wintermans[7] who found that in green and yellow leaves of *Sambucus nigra* the concentration of the sulpholipid does not vary with differing chlorophyll concentrations; he also noted its presence in both the chloroplasts and cytoplasm of leaves of *Beta vulgaris*.

According to Kates[8] the sulpholipid in the primary leaves of runner beans (Scarlet) is not the conventional one, but is composed of an S-compound, glucose, an unknown sugar, an unknown compound and fatty acids.

[1] A. A. BENSON, H. DANIEL and R. WISER, *Proc. Nat. Acad. Sci. U.S.* **45**, 1582 (1959).
[2] H. DANIEL, M. MIYANO, R. O. MUMMA, T. YAGI, M. LEPAGE, I. SHIBUYA and A. A. BENSON, *J. Am. Chem. Soc.* **83**, 1765 (1961).
[3] L. P. ZILL and E. A. HARMON, *Fed. Proc.* **18**, 359 (1959); B. W. NICHOLS and A. T. JAMES, *Fette, Seifen, Anstrichmittel* **66**, 1003 (1964); and C. F. ALLEN, P. GOOD, H. F. DAVIS and S. D. FOWLER, *Biochem. Biophys. Res. Communs.* **15**, 424 (1964).
[4] A. A. BENSON and I. SHIBUYA, In *Physiology and Biochemistry of Algae* (Edited by R. A. LEWIN), Academic Press, New York (1962).
[5] A. A. BENSON, *Proc. 5th Int. Congr.* Moscow (1961).
[6] A. A. BENSON and I. SHIBUYA, *Fed. Proc.* **20**, 79 (1961).
[7] J. F. G. M. WINTERMANS, *Biochim. Biophys. Acta* **44**, 69 (1960).
[8] M. KATES, *Biochim. Biophys. Acta* **41**, 315 (1960).

The present investigation was undertaken to examine the nature and intracellular distribution of the sulpholipid in green and etiolated maize seedlings and *Euglena* sp., tissues used in this Department for studying the biochemistry of chloroplast development,[9] to see if the sulpholipid is present in plant tissue cultures, and to check the claim that the sulpholipid present in runner beans is atypical.

A short report of part of this work has already appeared.[10]

RESULTS

Detection and Assay of Sulpholipid by Thin-Layer Chromatography

Although there have been several reports[3] of the isolation and identification of unlabelled sulpholipid, the administration of ^{35}S to the tissue prior to extraction of the sulpholipid has two important advantages. Firstly it enables the sulpholipid to be unequivocally distinguished from other leaf lipids with similar solubility and chromatographic properties, and secondly it enables much smaller quantities of sulpholipid to be detected.

TABLE 1. R_f VALUES FOR 6-SULPHOQUINOVOSYL DIGLYCERIDE AND ITS HYDROLYSIS PRODUCTS ON KIESELGEL-G IN THREE SOLVENTS

Solvent	R_f value		
	6-Sulphoquinovosyl diglyceride	6-Sulphoquinovosyl glycerol*	6-Sulphoquinovose†
Butanol: propionic acid: H_2O (6:3:4)	0·6	0·1	0·05
Phenol: saturated with H_2O (23°)	0·5	0·1	0·05
Diisobutylketone: acetic acid: H_2O (8:5:1)	0·4	—	—

* Product of alkaline hydrolysis.
† Product of acid hydrolysis.

Benson *et al.*[1] carried out two-dimensional chromatography on paper of the lipid extract (2 days) followed by autoradiography (about 14 days); a single experiment thus taking about 16 days. We have devised a single dimension thin-layer system in which the operation is complete within 24 hr. With Kieselgel G as adsorbent the R_f values of the plant sulpholipid and its deacylated derivative (6-sulphoquinovosyl glycerol) in three solvents are recorded in Table 1. The development of the chromatograms is complete within 60 min. Autoradiography is also much more sensitive and faster with thin layer than with paper chromatography.[11] A spot containing 1000 dis/min will produce a well-defined image after 24 hr exposure, whereas on paper even as much as 13,000 dis/min will only produce a faint image after 24 hr. Figure 1 illustrates this difference in sensitivity; it records a comparison of the autoradiogram when one-dimensional thin layer and paper chromatograms of the same amounts of an ethanolic extract of *Chlorella* grown in the presence of $^{35}SO_4^{2-}$, are developed with *n*-butanol–propionic acid–water, and exposed to X-ray films for different periods of time.

[9] T. W. GOODWIN, In *Biosynthetic Pathways* (Edited by J. D. PRIDHAM), Academic Press, London (1965) (In press).
[10] W. H. DAVIES, E. I. MERCER and T. W. GOODWIN, *Biochem. J.* **88**, 63P (1963).
[11] A. A. BENSON, J. R. COOK and T. YAGI, *Plant Physiol.* **37**, xliv (1962).

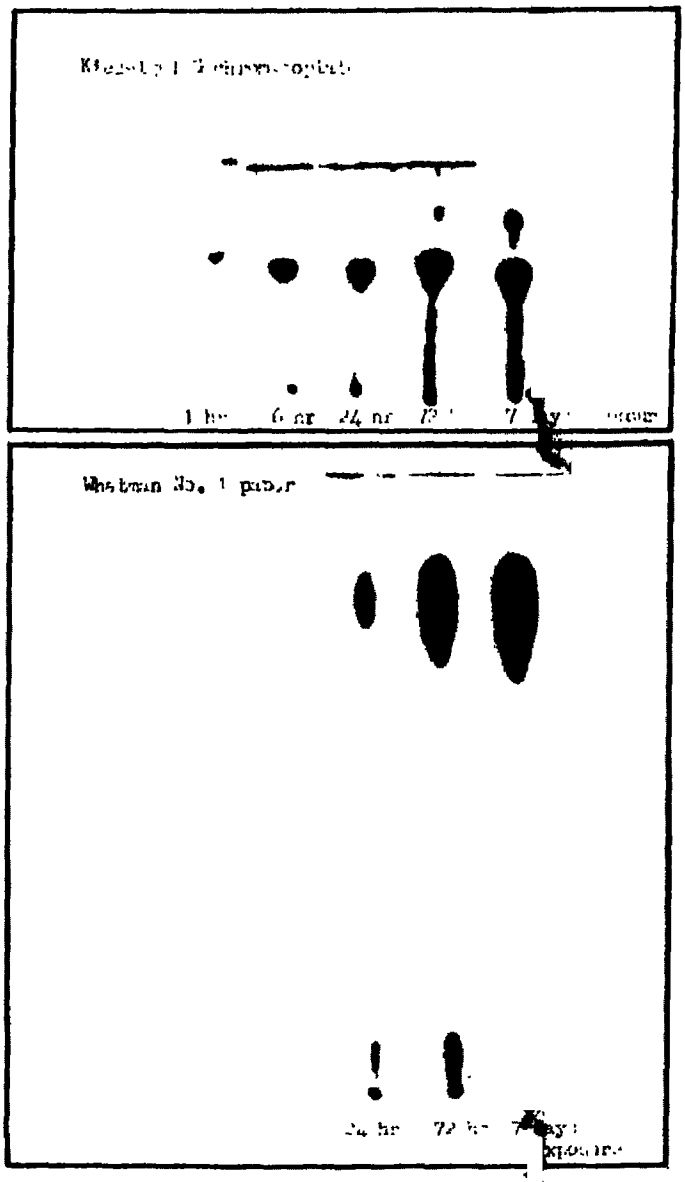

FIG. 1. A COMPARISON OF THE SENSITIVITY OF PAPER CHROMATOGRAMS AND THIN-LAYER CHROMATOGRAMS OF PLANT SULPHOLIPID AUTORADIOGRAPHY.

The same amount of sulpholipid extract exposed for 1 hr to 7 days. (A) on thin-layer chromatogram; (B) on paper chromatogram. Solvent in both cases *n*-butanol: propionic acid: H_2O (6:3:4).

[*facing page* 742]

An exposure of 1 hr is adequate for the detection of the sulpholipid on the thin-layer plate whilst a minimum of 24 hr is required with the paper chromatogram. Furthermore the greater sensitivity of the thin-layer method is seen on longer exposures; after 7 days up to eleven ^{35}S-containing compounds can be detected whilst after this time only three or four are visible on paper.

This method was used throughout the present work, but was occasionally supplemented by the original Benson technique for comparison purposes.

Maize

Maize seedlings were germinated for 6 days in the dark, excised from their roots and divided into two groups, each of which was placed in 25 ml tap water containing 1 mc of $^{35}S—SO_4^{2-}$. One group was illuminated for 24 hr whilst the other was returned to darkness for the same period. The extracted lipid from both groups contained 6-sulphoquinovosyl diglyceride as the major ^{35}S-component as determined by chromatography on Kieselgel G plates in three solvent systems (see Experimental section); in comparison with the authentic compound isolated from *Chlorella pyrenoidosa*[1] the deacylated product of this compound

TABLE 2. INTRACELLULAR DISTRIBUTION OF 6-SULPHOQUINOVOSE IN 6-DAY ETIOLATED MAIZE SEEDLINGS ILLUMINATED FOR 24 HR

Fraction	Activity in sulpholipid (dis/min × 10^{-3})	Percentage of total activity in sulpholipid	Chlorophyll content (mg)	Activity in sulpholipid per mg chlorophyll (dis/min × 10^{-5})
3000 *g*	409	54·2	0·48	8·44
20,000 *g*	267	35·5	0·316	8·48
105,000 *g*	71·1	9·4	0·078	9·18
Supernatant	6·83	0·9	0·0	—

was similarly shown to be 6-sulphoquinovosyl glycerol and the product of acid hydrolysis 6-sulphoquinovose.

The results of a typical experiment on the intracellular distribution of the sulpholipid in the greened seedlings (Table 2) indicate that the sulpholipid segregates with the chlorophyll. That is, the sulpholipid present in the 20,000 *g* and 105,000 *g* fractions can be accounted for as contamination by chloroplast fragments because the concentration per unit of chlorophyll is essentially the same in these fractions as in the chloroplast fraction. These findings are in agreement with the views of Benson *et al.*[11] that the sulpholipid is absent from mitochondrial preparations, but are somewhat at variance with the claim that it is present in the supernatant of homogenates of green leaves of *Beta vulgaris*,[7] for less than 1 per cent of the total is present in the supernatant fraction of our preparations.

The distribution of sulpholipid in homogenates of etiolated seedlings (Table 3) are somewhat different. In this case there is no chlorophyll to indicate the degree of unhomogeneity of the various fractions but there is clearly a greater percentage of sulpholipid in the 105,000 *g* fractions of the etiolated seedlings than the green seedlings. Two possible explanations for this exist: (*a*) the plastids are more fragile than chloroplasts and are thus more easily disrupted, or (*b*) the sulpholipid is associated in etiolated tissue with particles of the same size as microsomes. The implication of (*b*) is that during greening of etiolated seedlings the sulpholipid is mobilized from these particles into the developing chloroplasts.

TABLE 3. INTRACELLULAR DISTRIBUTION OF 6-SULPHOQUINOVOSYL DIGLYCERIDE IN 7-DAY ETIOLATED MAIZE SEEDLINGS

Fraction	Activity in sulpholipid (dis/min × 10^{-2})	Percentage of total sulpholipid recovered
3000 *g*	120	40·1
20,000 *g*	88	29·5
105,000 *g*	88	29·5
Supernatant	3·0	1·0

Runner Beans

In one set of experiments runner bean seeds (Prizewinner and Scarlet) were germinated in the dark for 5 days. The stems were excised just above the seed, and placed in water containing 1 mc $^{35}SO_4^{2-}$ and illuminated for 24 hr. Our standard methods of procedure (discussed in the previous section) revealed 6-sulphoquinovosyl diglyceride as the major sulpholipid in the primary leaves of both varieties. As these results were completely at variance to those reported by Kates[8] the experiments were repeated using his cultural conditions and extraction procedures.[8,11] Thin-layer chromatography in three solvent systems (Table 1), one-dimensional chromatography on paper (Whatman No. 1) in two solvent systems (phenol: water and butanol: propionic acid: water), and one-dimensional chromatography on silicic acid impregnated paper in a diisobutylketone-acetic acid: water system all revealed 6-sulphoquinovosyl diglyceride as the major sulphur-containing phospholipid; its deacylated product was also identified as 6-sulphoquinovosylglycerol. The presence of the complex lipid reported by Kates could not be demonstrated.

Plant Tissue Cultures

Williams and Goodwin[12], Threlfall and Goodwin[13] and D. R. Threlfall (unpublished observations) have shown that, with one exception, cultures of meristemic cells of Paul's Scarlet Rose contain only traces of terpenoids characteristic of functional chloroplasts (e.g. carotenoids, tocopherol, vitamin K) and no chlorophyll; the exception is plastoquinone.[14] The cultures do, however, contain considerable amounts of sterols which are present in plastids and do not require light for synthesis.[9] From the point of view of chloroplast development it was important to see if undifferentiated plant cells contained 6-sulphoquinovosyl diglyceride. Labelled sulphate was added to a freshly inoculated culture and the cells harvested 14 days later. Analysis revealed the presence of small amounts of labelled 6-sulphoquinovosyl diglyceride; gentle homogenization (see Experimental) of the cells followed by fractional centrifugation demonstrated that the bulk of the activity was located in the supernatant (Table 4). The same distribution was observed with sterols,[12] which are, however, entirely particulate in homogenates of etiolated cells. It would be reasonable to assume that such gentle homogenization as grinding with sand would not entirely disrupt the organelles present in the cells of plant tissue culture, but more work is required before this can be stated with complete confidence.

[12] M. KATES and F. M. EBERHARDT, *Can. J. Bot.* 35, 1907 (1957).
[13] B. L. WILLIAMS and T. W. GOODWIN, *Phytochem.* 4, 81 (1965).
[14] D. R. THRELFALL and T. W. GOODWIN, *Biochim. Biophys. Acta* 78, 532 (1963).

TABLE 4. THE INTRACELLULAR DISTRIBUTION OF 6-SULPHOQUINOVOSYL DIGLYCERIDE IN TISSUE CULTURES OF PAUL'S SCARLET ROSE MERISTEM

Fraction	Activity in sulpholipid (dis/min 10^{-2})	Percentage of sulpholipid present before extraction
3000 *g*	26	5·0
20,000 *g*	3·8	0·7
105,000 *g*	4·7	0·9
Supernatant	488	93·4

Euglena gracilis *Strain Z*

A well-known characteristic of *Euglena gracilis* is that when grown heterotrophically in the dark it is colourless and produces no chloroplasts; when such cells are washed free from medium, resuspended in buffer and illuminated they rapidly become green as they produce functional chloroplasts. Grown either heterotrophically or autotrophically in the light *E. gracilis* is green. The development of chloroplasts in greening *Euglena* has been studied from the viewpoint of the formation of chloroplast terpenoids;[15] the present study extends this investigation to the sulpholipid.

Three media (see Experimental) containing the same amount of $^{35}SO_4^{2-}$ were inoculated with the same culture of *Euglena gracilis* strain Z. After 6 days incubation the three sets of cells (heterotrophic-dark, heterotrophic-light and autotrophic light) were harvested, and examined for sulpholipid; in all cases the major S-containing lipid was 6-sulphoquinovosyl diglyceride as demonstrated by our standard procedures. The autoradiograms suggested that the green cultures contained far more sulpholipid than the etiolated cultures; as no quantitative assay for sulpholipid is available an attempt to compare the amounts in the two cultures was made by measuring the total radioactivity in the sulpholipid isolated from cultures grown autotrophically and heterotrophically under otherwise identical conditions, and comparing them with the total $^{35}SO_4^{2-}$ uptake in each case. Table 5 shows the results of such an experiment with 6-day cultures grown on the "heterotrophic medium" in light and darkness; it will be seen that although light does not greatly increase $^{35}SO_4^{2-}$ uptake (1·32:1), the relative amount of sulpholipid is some 4·6 times greater in the green than in the etiolated cultures.

TABLE 5. A COMPARISON OF THE UPTAKE OF $^{35}SO_4^{2-}$ AND ITS INCORPORATION INTO 6-SULPHOQUINOVOSYL DIGLYCERIDE BY ETIOLATED AND GREEN *Euglena gracilis**

Nature of cells	Total uptake of $^{35}SO_4^{2-}$ (dis/min $\times 10^{-8}$)	Activity in sulpholipid (dis/min $\times 10^{-5}$)	Ratio of uptake (light:dark)	Ratio of activity in sulpholipid (light:dark)
Green	5·36	28·4	1·32:1	4·61:1
Etiolated	4·06	6·16		

* Cultures of identical composition inoculated with the same mother culture and incubated at 18° for 6 days either in light or darkness.

[15] T. W. GOODWIN, *Experientia* **10**, 213 (1954).

The intracellular distribution of sulpholipid in green autotrophic and green heterotrophic *Euglena* cells is very similar (Table 6) and, as in the case of the green tissue of maize, the sulpholipid segregates with the chlorophyll, thus indicating that the major portion is located in

Table 6. Intracellular distribution of 6-sulphoquinovosyl diglyceride in green *Euglena gracilis* grown in the light on autotrophic and heterotrophic media

Fraction	Activity of sulpholipid (dis/min × 10^{-4})	Percentage of total sulpholipid	Chlorophyll (mg)	Activity of sulpholipid (dis/min × 10^{-6}/mg chlorophyll)
A. Cells grown autotrophically				
3000 *g*	617	62·3	3·62	1·71
20,000 *g*	260	26·4	1·38	1·89
105,000 *g*	112	11·3	0·52	2·16
Supernatant	0	0·0	0·00	0·00
B. Cells grown on heterotrophic medium in light				
3000 *g*	223	64·3	2·17	1·03
20,000 *g*	71·4	20·6	0·63	1·14
150,000 *g*	50·8	14·7	0·49	1·03
Supernatant	1·29	0·4	0·00	—

the chloroplasts. Again, similarly to the observations in maize, in etiolated *Euglena* the bulk of the sulpholipid is found in the 105,000 *g* fraction (Table 7), which once again suggests that in the development of chloroplasts the sulpholipid may be transferred from this particle to the plastid.

Table 7. Intracellular distribution of 6-sulphoquinovosyl diglyceride in "etiolated" *Euglena gracilis*

Fraction	Activity of sulpholipid present (dis/min × 10^{-5})	Percentage of total sulpholipid
3000 *g*	21·6	21·8
20,000 *g*	29·7	29·9
105,000 *g*	46·8	47·1
Supernatant	1·16	1·2

EXPERIMENTAL

Materials

The following were examined: Higher plants—maize (*Zea mais*, South African White Horse Tooth hybrid No. 159C/4221 obtained from Messrs. Gunsons (Seeds) Ltd., London); runner beans (*Phaseolus multiflorus* v. Scarlet and v. Prizewinner, obtained from Messrs Bees and Carter, respectively). Algae—*Chlorella pyrenoidosa* (wild type) and *Euglena gracilis* strain Z (1221–5Z), both from the collection of Algae and Protozoa, Botany School, Cambridge. Tissue culture—the cultures of cambial tissue of Paul's Scarlet Rose was originally obtained from Professor P. W. Brian, F.R.S., University of Glasgow; it has been maintained in this laboratory for some years.

Growth and culture conditions. The seeds of maize and runner beans were soaked in distilled water for 24 hr and then planted in a John Innes No. 1 soil mixture and germinated at 23° in complete darkness. When required etiolated seedlings were greened by illumination for 24 hr. The Scarlet runner beans were also grown under greenhouse conditions in the light at 18°. *Chlorella pyrenoidosa* was maintained on slopes consisting of Agar (1·5% w/v) and Difco bacteriological yeast extract (0·2% w/v) in distilled water. For experimental purposes it was grown in the light at 18° for 7 days in a liquid medium.[15] The cells were harvested by centrifuging for 3 min at 300 *g* (M.S.E. Minor bench centrifuge). *Euglena gracilis* strain Z was maintained on slopes under the same conditions as *C. pyrenoidosa*. It was grown in either a heterotrophic[16] medium in light or darkness or on an autotrophic medium[17] in the light at 18°. The cells were harvested in the same way as *C. pyrenoidosa*. Paul's Scarlet Rose tissue cultures were grown as previously described in detail.[12]

Administration of radioactive compounds. Carrier-free $^{35}SO_4^{2-}$ (Radiochemical Centre, Amersham, Bucks.) was used throughout. Etiolated seedlings at an appropriate stage of growth were excised from their roots and placed in distilled water containing the appropriate amounts of $^{35}SO_4^{2-}$. For the algal and plant tissue cultures the labelled sulphate was either added to the medium before sterilization and inoculation or added aseptically to an actively growing culture.

Cell Fractionation Procedures

(*a*) *Higher plants.* The greened or etiolated tissues were cut with a scissors into a glass container of a blender (M.S.E. Atomix) precooled to 1°. All subsequent operations were carried out at this temperature. Homogenization was carried out for 30 sec in a buffer[18] (0·35 M-NaCl, 0·01 M-K_2HPO_4 and 0·01 M-Versene) adjusted to pH 7·4, and the homogenate was filtered through eight layers of cheese cloth[19] and centrifuged at 140 *g* for 5 min (M.S.E. Minor centrifuge fitted with swing-out head). These two steps remove all the cell debris and fibrous tissue. The supernatant was then centrifuged in 50 ml polypropylene tubes at 5000 rev/min for 15 min in a Servall Superspeed RC-2 automatic refrigerated centrifuge fitted with a S5-34 head. This sedimented the 3000 *g* fraction. The resulting supernatant was recentrifuged in the same apparatus in stainless-steel cups at 15,000 rev/min for 15 min; this sedimented the 20,000 *g* fraction. A 105,000 *g* fraction was obtained by centrifuging the supernatant from the previous spin for 90 min in a Beckman Spinco Model L preparative centrifuge fitted with a No. 40 rotor head.

(*b*) *Euglena.* The cells, suspended in distilled water, were disintegrated ultrasonically at 0° with a Mullard Ultrasonic Disintegrator. The disintegrated cells were suspended in 10% sucrose (w/v) and differentially centrifuged in the same way as homogenates of higher plants (see above).

Plant tissue culture. The plant cells (10 g) were washed with the buffer used in homogenizing whole plant tissue (see above), placed together with 10 g acid-washed sand in a large precooled mortar. Sufficient chilled buffer was added to cover the cells which were well ground with a pestle. The resulting mixture was filtered through eight layers of cheese cloth and centrifuged at 140 *g* for 5 min. The supernatant was then fractionated in the same way as maize homogenates (see above).

[16] J. J. WOLKEN, *Euglena*, Quinn and Boden, London, (1961).
[17] G. BRAWERMANN and E. CHARGAFF, *Biochim. Biophys. Acta* **31**, 164 (1959).
[18] P. K. STUMPF and A. T. JAMES, *Biochim. Biophys. Acta* **70**, 20 (1963).
[19] G. V. MARINETTI and E. STOTZ, *Biochim. Biophys. Acta* **21**, 168 (1956).

Extraction of Lipid

(*a*) *Intact tissue of higher plants.* The tissue was cut into small fragments and placed in a 50 ml Vortex homogenizer beaker. Sufficient hot absolute ethanol was added to cover the tissue which was then homogenized for 60 sec in a M.S.E. homogenizer at full speed. The resultant mixture was filtered through a sintered glass funnel. The residue was re-extracted twice, once with the same solvent and once with hot ethanol containing 25% (v/v) chloroform. The combined extracts were then reduced to dryness at 35° in a rotary evaporator (Wright). The residue was dissolved in a small volume of absolute ethanol for chromatography. In some experiments the runner beans were extracted according to the method of Kates and Eberhardt.[11]

(*b*) *Algal and plant tissue culture cells, centrifuged pellets.* These preparations were washed into a 500 ml beaker with hot absolute ethanol and homogenized with an Ultra-Turrax homogenizer. The procedure from then on was the same as with plant extracts.

Supernatant from differential centrifugations. This material was extracted by adding 4 vol of ethanol and boiling the mixture for 5 min, this denatured soluble protein. The solution was extracted exhaustively with 4 vol of diethyl ether. The ether extract was washed twice with water and dried by standing over anhydrous Na_2SO_4 for 2 hr. The solution was filtered and the ether removed *in vacuo*. The residue was taken up in a small volume of absolute ethanol for chromatography.

Chromatography of Lipid Extracts

Paper. The two-dimensional system of Benson *et al.*[1] was used. Up to 40 hr were required for suitable development of the chromatogram. Ascending chromatography in one direction on silicic acid impregnated paper[19] was effective with diisobutylketone:acetic acid:H_2O (40:30:7) as solvent. The time for suitable development was 14 hr.

Thin layer. It was found that good separation of the sulpholipid was obtained by chromatography at 24° on Kieselgel G (Merck) with butanol:propionic acid:water (6:3:4), phenol saturated with water or diisobutylketone:acetic acid:water (8:5:1) as developer. The average time for satisfactory development was 60 min.

Detection and Assay of Sulpholipid

The sulpholipid is only detectable as radioactive spot, so it was located by autoradiography with "Kodirex" X-ray film. The thin-layer chromatograms are some twenty times more sensitive than the paper chromatograms[20] (see also Results section).

Quantitative determination of the radioactivity in the sulpholipid spot was carried out by eluting the spot with toluene, adding it to a liquid scintillator, also dissolved in toluene, and assaying it in a Packard Tri-Carb Scintillation Spectrometer series 314E.

Deacylation of 6-sulphoquinovosyl diglyceride. The eluted spot was dissolved in 1 ml toluene:methanol (1:1) and 1 ml of 0·2 N methanolic KOH added. The solution was warmed at 37° for 20 min when 1 ml of water was added and the mixture deionized by passing through a column of Dowex-50 (H^+ form). The eluate was evaporated to a small volume and the deacylated sulpholipid (6-sulphoquinovosyl diglycerol) detected by chromatography on paper and thin layers followed by autoradiography.

Acid hydrolysis of 6-sulphoquinovosyl diglyceride. To the sulpholipid dissolved in 1 ml 50% aqueous ethanol, 0·5 ml of 6 N HCl was added and the solution heated at 100° for 30 min.

[20] T. W. GOODWIN, *Lab. Practice*, April (1964).

After cooling, 2 ml of water were added and the solution extracted with diethyl ether (5 ml). The aqueous phase was taken to dryness *in vacuo* and the residue dissolved in 1 ml water. The resulting 6-sulphoquinovose was detected by chromatography on paper and thin layer followed by autoradiography.

Determination of Chlorophyll

Arnon's procedure[21] based on that originally proposed by Mackinney[22] was used.

Acknowledgement—We wish to thank the Agricultural Research Council for a grant to cover the cost of this investigation.

[21] D. I. Arnon, *Plant Physiol.* **24**, 1 (1949).
[22] G. Mackinney, *J. Biol. Chem.* **140**, 315 (1941).