Method for recovering sulfolipid from plant lipid extracts

WILLIAM E. KLOPFENSTEIN* and JAMES W. SHIGLEY

Department of Biochemistry, The Pennsylvania State University, University Park, Pennsylvania

SUMMARY Sulfoquinovosyl diglyceride from plant tissues is recovered in sizeable quantities, free from phospholipids and galactolipids.

KEY WORDS plant sulfolipid · 6-sulfo-α-D-quinovopyranosyl-(1 → 1′) diglyceride · alfalfa · chromatography · column · ion-exchange · thin-layer

SINCE THE DISCOVERY of the plant sulfolipid, 6-sulfo-α-D-quinovosyl-(1 → 1′) diglyceride, by Benson, Daniel, and Wiser (1), much interest has been focused upon it. This compound or similar compounds have been found in a variety of sources (2–8). Although Davies, Mercer, and Goodwin (6) have reported separating the sulfolipid by thin-layer chromatography, no method was available, until recently, for recovering the intact sulfolipid in moderate quantities. O'Brien and Benson (9) reported isolating the sulfolipid on Florisil and DEAE cellulose chromatography columns. A method is reported here for recovering the sulfolipid by means of ECTEOLA cellulose column chromatography followed by thin-layer chromatography.

Extraction. The lipids were extracted from 75 g of fresh alfalfa in Soxhlet extractors containing 175 ml of 95% ethanol. After addition of 100 ml of water to the resultant solution, it was extracted three times with a total of 125 ml of chloroform. The chloroform solutions of lipid thus obtained were concentrated to a volume of about 10 ml under a stream of nitrogen.

Column Chromatography. The column chromatographic procedure was a modification of that of Rouser, Baumann, Kritchevsky, Heller, and O'Brien (10). Seven grams of Whatman ECTEOLA cellulose powder, Et-11, was treated with 50 ml of glacial acetic acid and filtered. The remaining acid was removed by repeatedly washing with methanol and then with chloroform. The washed powder was suspended in about 100 ml of chloroform and poured into 15 × 500 mm glass columns onto a fiberglass plug. The concentrated lipid extract was introduced onto the column in about 15 ml of chloroform. The solvent mixtures used for elution were: (*a*) 50 ml of

Authorized for publication on 17 September 1965 as paper No. 3066 in the journal series of the Pennsylvania Agricultural Experiment Station.

* Present address: Department of Biochemistry, Kansas State University, Manhattan, Kansas.

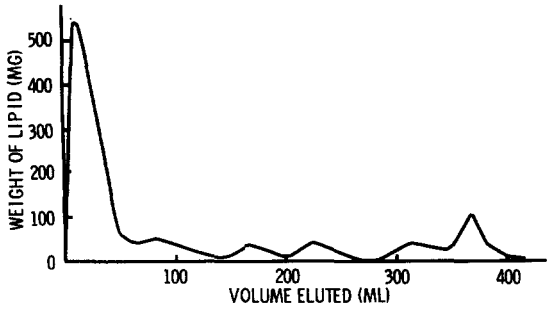

FIG. 1. Weight of alfalfa lipid eluted from ECTEOLA cellulose. The lipids from about 75 g (wet weight) of alfalfa were separated on an acetic acid-treated ECTEOLA cellulose column with the solvents listed in the text. The chlorophyll was essentially all in the first 50–60 ml of effluent. The final peak is largely sulfolipid; while phospholipids were eluted throughout the procedure. It is not necessarily the appearance of new components, but perhaps the appearance of a new solvent front, that causes the breaks in the curve.

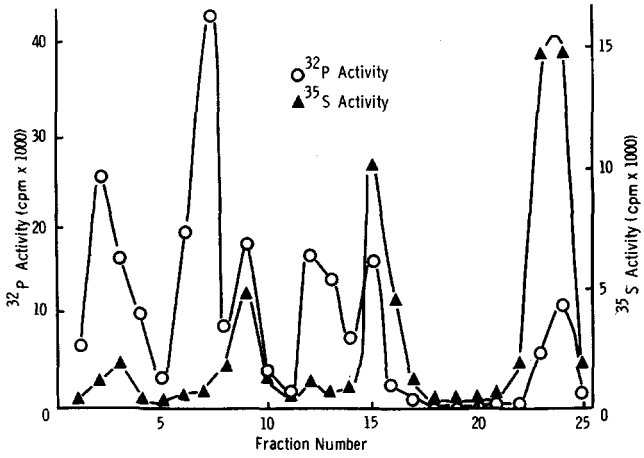

FIG. 2. Activity of ^{32}P- and ^{35}S-labeled alfalfa lipids eluted from ECTEOLA cellulose. Fractionation carried out as in Fig. 1; fractions, about 16 ml. The individual ^{32}P and ^{35}S activities were determined by using a Geiger-Mueller tube to find the total activity and then the activity was measured through an aluminum foil absorber sufficiently dense to eliminate all of the ^{35}S activity. From these values the separate activities were calculated.

chloroform, (*b*) 50 ml of chloroform–methanol 7:1, (*c*) 50 ml of chloroform–methanol 7:3, (*d*) 50 ml of chloroform–acetic acid 3:1, (*e*) 50 ml of acetic acid, (*f*) 50 ml of chloroform–methanol 4:1, (*g*) 75 ml chloroform–methanol–ammonium hydroxide 30:23:5. In mixture *f*, 1 ml of concd NH_4OH was added per 100 ml of solution. The elution pattern obtained is shown in Fig. 1. Acetic acid was added to those fractions which contained NH_4OH to prevent basic hydrolysis of the lipids.

When an alfalfa lipid extract containing ^{35}S-labeled sulfolipid and ^{32}P-labeled phospholipid was fractionated by this procedure, most of the ^{32}P activity was eluted by the chloroform and chloroform–methanol mixtures. The final eluent eluted the major portion of the ^{35}S activity with only a small amount of ^{32}P contamination (Fig. 2). The efficiency of the separation depended on the ratio (weight of lipid extract)/(weight of column packing), with less contamination of sulfolipid by phospholipid when large samples were used on small columns.

Thin-Layer Chromatography. The 4 × 10 inch plates were spread with a 0.35 mm thick layer of Silica Gel G. The plates were allowed to dry at room temperature and activated for 30 min at 105°C. After application of the sulfolipid-rich fraction from the ECTEOLA cellulose columns, the plates were dried for an additional 5 min at 105°C. The developing solvent was chloroform–methanol–acetic acid 65:25:10.

Components on the plates were located under UV light of short wavelength, exposure to iodine vapors, and spraying with Dittmer and Lester's phospholipid spray (11). Labeled lipids were located by radioautography. The sulfolipid had an R_f of 0.7 while the small amounts of phospholipid present exhibited R_f values between 0.3 and 0.6.

The combination of column chromatography and thin-layer chromatography is used in order to obtain a separation which is not always possible with thin-layer chromatography alone. The column chromatography removes the chlorophyll and galactolipids, which on thin-layer plates have R_f values of 0.8–1.0 and about 0.8, respectively, and might lead to contamination of the sulfolipid, R_f 0.7. When chlorophyll and galactolipids are not present in high concentration, the column chromatography might not be necessary.

This method provided sulfolipid free from all detectable impurities in quantities sufficient for analysis of component fatty acids by gas–liquid chromatography.

Manuscript received 30 November 1965; accepted 14 March 1966.

REFERENCES

1. Benson, A. A., H. Daniel, and R. Wiser. *Proc. Natl. Acad. Sci. U.S.* **45:** 1582, 1959.
2. Haines, T. H., and R. J. Block. *J. Protozool.* **9:** 33, 1962.
3. Zill, L. F., and E. A. Harmon. *Biochim. Biophys. Acta* **57:** 573, 1962.
4. Kates, M. *Biochim. Biophys. Acta* **41:** 315, 1960.
5. Middlebrook, G., C. M. Coleman, and W. B. Schaefer. *Proc. Natl. Acad. Sci. U.S.* **45:** 1801, 1959.
6. Davies, W. H., E. I. Mercer, and T. W. Goodwin. *Biochem. J.* **88:** 63p, 1963.
7. Rosenberg, A., and H. Pecker. *Biochemistry* **3:** 254, 1964.
8. Collier, R., and G. Y. Kennedy. *Marine Biol. Assoc. U. K.* **43:** 605, 1963 (*Chem. Abstr.* **60:** 9594c, 1964).
9. O'Brien, J. S., and A. A. Benson. *J. Lipid Res.* **5:** 432, 1964.
10. Rouser, G., A. J. Baumann, G. Kritchevsky, D. Heller, and J. S. O'Brien. *J. Am. Oil Chemists' Soc.* **38:** 544, 1961.
11. Dittmer, J. C., and R. L. Lester. *J. Lipid Res.* **5:** 126, 1964.