Monoglucosyloxyoctadecenoic Acid—a Glycolipid from *Aspergillus niger*†

Roger A. Laine, Patricia F. S. Griffin, Charles C. Sweeley, and Patrick J. Brennan*

ABSTRACT: Details are given of the purification and structure of an apparently novel type of fungal glycolipid. It was obtained in a relatively pure form by fractionation of the acetone-soluble lipids of *Aspergillus niger* by Florosil column chromatography and finally purified by preparative thin-layer chromatography. On the basis of the evidence obtained from mass spectroscopy, infrared spectroscopy, chromatographic procedures, and the alkaline stability of the glycolipid, we propose that it is a monoglucoside of a *trans*-2-hydroxyoctadecenoic acid. The acidic nature of the glycolipid, combined with its relative prominence (about 6% of the total lipid), when phospholipid was depleted (less than 2% of the total lipid), indicated that it had an important role in the structure and function of the fungal membranes.

Glycolipids, other than sphingoglycolipids, have been fully identified in only a few fungi. They can be divided into two structural classes. One group is of the acylated polyol type and known examples are the 4-*O*-(2,3,4,6-tetra-*O*-acyl-β-D-mannopyranosyl)-D-erythritol of *Ustilago maydis* (Fluharty and O'Brien, 1969); the acylated mannitols and arabitols of *Rhodotorula* sp. (Tulloch and Spencer, 1964); acylated glucose of *Agaricus bisporus* and *Saccharomyces cerevisiae* (Brennan *et al.*, 1970); and the acylated trehaloses, the apparent endogenous reserve lipid in *Pullularia pullulans* (Merdinger *et al.*, 1968), and *Claviceps purpurea* (Cooke and Mitchell, 1969). The other type of fungal glycolipids have hydroxylated fatty acids attached glycosidically to the glucopyranose of a disaccharide. The known examples of these are the ustilagic acids, which are partially acylated cellobiosyl residues glycosidically linked in the β form to the hydroxyl groups of long-chain fatty acids (Bhattacharjee and Haskins, 1970), and the sophorosides. This latter group have a 2′-*O*-β-D-glucopyranosyl-D-glucopyranose (sophorose) attached β-glycosidically to hydroxy fatty acids, which may be a 17-L-hydroxy C_{18} acid, as in *Torulopsis apicola* (Gorin *et al.*, 1961), or a 13-L-hydroxydocosanoic acid, as in *Candida bogoriensis* (Tulloch *et al.*, 1968). These are extracellular components formed during fermentation, often in large amounts (2–5 g/l. of medium).

In this paper we report evidence for a new member of the second group of glycolipids, an intracellular component of *Aspergillus niger*. A structure whereby a single glucose is attached glycosidically to an α-hydroxy, monounsaturated C_{18} fatty acid, is proposed.

Materials and Methods

Materials. D-Glucose was obtained from Mallinckrodt Inc. Bistrimethylsilyltrifluoroacetamide was supplied by Regis Chemical Co. Glc supplies were obtained from Supelco Inc., Bellfonte, Pennsylvania. Thin-layer chromatography (tlc) supplies were purchased from Brinkmann Inc. or from Merck, Darmstadt, Germany. Florosil was purchased from Fisher Scientific Co.

Growth of Aspergillus niger. Spores of *A. niger* were collected from a stock culture maintained on 2% malt agar slopes. These were transferred to 100 ml of liquid medium, shaken for 24 hr, and transferred to fermentor vessels (New Brunswick Inc.). Growth proceeded for 5 days at 27° with vigorous agitation and aeration in a medium composed of: sucrose, 50 g; asparagine, 2 g; K_2HPO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $FeSO_4$, 0.01 g; in a total volume of 1 l.

Extraction of Lipids. The mycelium was harvested by filtration and washed with saline. It was then ground lightly with acid-washed sand and extracted four times with acetone by shaking at room temperature. Phospholipids were almost exclusively contained in chloroform–methanol extracts of the acetone-insoluble residue. Acetone-soluble lipids were concentrated, freeze-dried, and washed (Folch *et al.*, 1957). From 30 l. of medium, 1 kg, wet weight, of mycelium was obtained and this yielded 10 g of acetone-soluble lipid. Examination of the growth medium showed that it was devoid of glycolipid.

Purification of the Glycolipid. Acetone-soluble lipid was applied in 2-g lots to columns (35 × 4 cm) containing 250 g of Florosil, previously washed and activated (Radin, 1969). One-half of the applied lipid was eluted with chloroform (2 l.). Over 40% was eluted with a similar volume of chloroform–methanol (10:1, v/v) and about 7% was eluted with chloroform–methanol (2:1, v/v). The glycolipid under examination was present solely in the chloroform–methanol (2:1) eluate. Final purification of it was achieved by preparative tlc on plates (20 × 20 cm and 20 × 40 cm) of silica gel H. Lipid bands were located by spraying plates lightly with water, or by exposing them to iodine vapour. Lipids were eluted from the gel with chloroform–methanol (2:1 and 1:2, v/v) and washed (Folch *et al.*, 1957).

Acid-Catalyzed Methanolysis and Trimethylsilylation. The unknown glycolipid was subjected to acid-catalyzed methanolysis in 1 N methanolic HCl (Gaver and Sweeley, 1965). After extraction with hexane, the methanol fraction was neutralized with Ag_2CO_3 and taken to dryness under N_2. Pyridine (50 μl) and bistrimethylsilyltrifluoroacetamide (50 μl) were added and the mixture was allowed to react at 80° for 15 min.

† Joint contribution from Department of Biochemistry, Michigan State University, East Lansing, Michigan 48823 (R. A. L. and C. C. S.), and Departments of Biochemistry and Botany, Trinity College, Dublin 2, Ireland (P. F. S. G.), and Department of Biochemistry, University College, Dublin 4, Ireland (P. J. B.). *Received January 28, 1972.* This investigation was supported by Grant No. RR 00480 from the United States Public Health Service and by a grant from the National Science Council (Ireland).

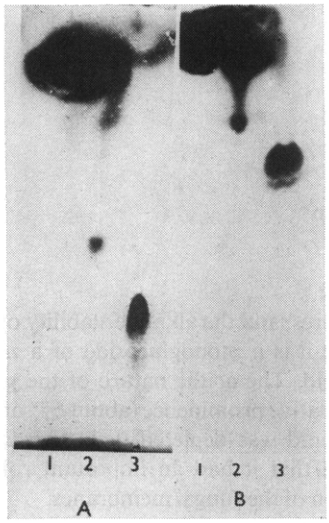


FIGURE 1: Thin-layer chromatographic patterns of the glycolipid from *Aspergillus niger*. A. Tlc in chloroform–methanol (9:1, v/v) (S2). B. Tlc in chloroform–methanol–water (65:25:4, v/v) (S3). 1. Chloroform eluate from Florosil column (composed largely of acylglycerols and sterol esters). 2. Chloroform–methanol (10:1, v/v) eluate from the column (containing principally free fatty acids). 3. Chloroform–methanol (2:1, v/v) eluate from the column (the unknown glycolipid was the principal component). Spots were detected with 0.2% anthrone in H_2SO_4 by heating at 110° for 10 min.

An aliquot of this mixture was injected into a 3% SE-30 glc column at 160°.

The hexane fraction was chromatographed on 3% SE-30 at 210°. A portion of this fraction was converted to the Me_3Si derivative with the acetamide and submitted to the same glc analysis.

For quantitative estimation of the molar ratios of constituents, trimethylsilylmannitol and methyl heptadecanoate were added as internal standards.

Mild Alkali-Catalyzed Methanolysis. Mild alkali-catalyzed methanolysis of the unknown glycolipid was carried out in 0.6 N methanolic NaOH for 1 hr at room temperature. The solution was neutralized with 0.6 N methanolic HCl and the mixture was partitioned between chloroform and water (Vance and Sweeley, 1967).

Analytical Procedures. Paper Chromatography. Whatman No. 1 paper was used and ethyl acetate–acetic acid–formic acid–water (18:3:1:4, v/v) and ethyl acetate–pyridine–water (10:4:3) were the solvents.

Thin-Layer Chromatography (Tlc). Tlc was performed on 250- or 500-μ plates of silica gel H and silica gel HR. Solvent systems were as follows: S1, petroleum ether–diethyl ether–acetic acid (85:15:2, v/v); S2, chloroform–methanol (9:1, v/v); S3, chloroform–methanol–water (65:25:4, v/v); S4, chloroform–methanol–water (100:42:6, v/v).

Gas–Liquid Chromatography (Glc). An F & M Model 402 was used employing a 2 m × 3 mm glass column packed with 3% SE-30 on 100–120 mesh Gas-Chrom Q. Isothermal temperatures used were 160, 210, and 240°. Hydrogen flame ionization detector and flash heater temperatures were 250°.

Mass Spectrometry, Mass spectra were recorded on an LKB 9000 combined glc–mass spectrometer. Instrumental conditions were as follows: glc column temperature, 210°; flash heater, 250°; molecular separator, 250°; ion source temperature, 290°; accelerating voltage, 3.5 kV; ionizing electron energy, 70 eV. A computerized data system was used for acquisition of mass spectra and preparation of tabular and bar graph data (Sweeley *et al.*, 1970).

Infrared Spectrophotometry (Ir). Ir spectra were recorded on a Perkin-Elmer Model 621 spectrophotometer using a KBr pellet or a solution of 1 mg of glycolipid in 0.2 ml of $CHCl_3$.

Other analytical procedures have been described previously (Brennan and Ballou, 1967).

Results and Discussion

Final Purification and Properties of the Glycolipid. Tlc in S2 and S3 of the three lipid fractions from a Florosil column is shown in Figure 1. In S1 the chloroform eluate was shown to contain mostly tri-, di-, and monoglycerides and sterol esters. The chloroform–methanol (10:1) eluate contained mostly free fatty acids and some polar unidentified lipids. The two-band lipid with R_F 0.64 in S3 was the only apparent component in the chloroform–methanol (2:1) eluate. When the plates were sprayed with 0.2% anthrone in H_2SO_4 and heated for a few minutes at 110° this lipid produced a distinct bluish purple colour, indicative of a glycolipid. Hydrolysis of this fraction, paper chromatography and visualization of chromatograms with aniline–phthalate or silver nitrate–sodium hydroxide (Brennan and Ballou, 1967) showed only glucose, with some slower moving material at the origin. The chloroform–methanol (2:1) fraction was subjected to preparative tlc in S3 or S4 to separate the major lipid from the minor slower moving band. The eluted lipid appeared homogeneous in the three chloroform–methanol-containing solvents.

When a thin-layer chromatogram of the unknown lipid was reacted with the Molybdenum Blue reagent (Dittmer and Lester, 1964) a negative result was obtained, indicating the absence of phosphorus. The lipid on thin-layer plates developed a pinkish purple colour with the periodate–Schiff reagent after 10 min, indicating unsubstituted hydroxyl groups (Shaw, 1968). When the lipid was subjected to acid-catalyzed methanolysis and extracted with hexane, and the trimethylsilyl (Me_3Si) derivatives of the components of the methanol residue analyzed by glc (SE-30 column at 240°), there were no significant extraneous peaks, save those of glucose (see below), and there were no peaks in the retention time region of sphingosine. Thus the possibility that the unknown was a sphingoglycolipid was excluded. An ir spectrum of the unknown glycolipid was compared with that of a glucosylceramide recently isolated from *Hansenula ciferri* by Kaufman *et al.* (1971). There was little similarity. The unknown had a very strong absorption at 1700 cm^{-1}, indicative of a free carboxylic group, whereas the glucosylceramide had no peak in this area or in the 1750-cm^{-1} region. Unlike the sphingoglycolipid the unknown glycolipid exhibited only minor absorption in the amide group area. Both lipids showed typical absorption bands for hydroxy groups and unsaturation. The ir evidence for the presence of a nitrogen-containing contaminant in the glycolipid preparation was confirmed when an acid-catalyzed methanolysate of the lipid showed small amounts of ninhydrin-positive material. When large amounts of a diethyl ether extract of this methanolysate were subjected to tlc, according to Sambasiverao and McCluer (1963), there were traces of three ninhydrin-positive components, corresponding to erythrosphingosine, dihydrosphingosine, and phytosphingosine.

Structure of the Glycolipid. Figure 2 (upper tracing) shows the glc pattern of the Me_3Si derivative of the neutralized methanol fraction obtained from acid-catalyzed methanolysis of the unknown glycolipid. Authentic D-glucose was also converted to its methyl glycosides and analysed as the Me_3Si de-

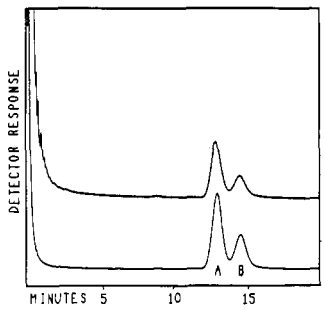


FIGURE 2: Gas–liquid chromatogram of tetra-*O*-trimethylsilyl-α- and -β-*O*-methyl glucosides prepared from authentic D-glucose (lower tracing, peaks A and B, respectively). The upper tracing represents the trimethylsilyl derivative of the sugar moiety from the *Aspergillus* glycolipid after acid-catalyzed methanolysis (3% SE-30, 160°).

rivative (lower tracing, Figure 2). The peaks shown represent α-methyl tetra-*O*-trimethylsilylglucoside (A) and its β anomer (B) (Sweeley *et al.*, 1963). This fraction contained no other volatile Me_3Si components when the temperature was increased to 240°, indicating that glucose was the only sugar constituent of the unknown lipid.

Tlc in benzene of the methylated fatty acids from the hexane fraction of the acid-catalyzed methanolysate showed that the bulk of them had an R_F of 0.15, indicative of hydroxylated fatty acids (Morrison and Smith, 1964). Traces of straight-chain fatty acids (R_F 0.60) were also obvious. The glc record of the aglycone moiety from the hexane fraction is shown in Figure 3 (lower tracing, component C). This peak demonstrated extensive tailing, indicative of a polar group on the molecule. The Me_3Si derivative of C gave a more symmetrical peak (D) when chromatographed under the same conditions, which suggested replacement of at least one hydrogen by an Me_3Si group. Component D had a similar retention time to methyl eicosanoate. Further analyses of C and D were carried out by mass spectrometry and ir spectrophotometry.

Molar ratios of the carbohydrate and aglycone components were estimated by integration of glc peaks of internal standards (trimethylsilylmannitol and methyl heptadecanoate) and comparison with the areas of component peaks. The ratio of glucose to aglycone was 1.09:1. After mild alkaline-catalyzed methanolysis was performed on the intact lipid (Vance and Sweeley, 1967), there was no difference in tlc behavior, which suggested a glycosidic linkage. The same conclusion was reached from the results of paper chromatography. It was found that when equal amounts (1 mg) of the glycolipid were subjected to mild alkaline hydrolysis (Brennan and Ballou, 1967) and to acid hydrolysis (2 N HCl at 100° for 3 hr) and chromatographed, there was a considerable amount of glucose in the acid hydrolysate but none in the aqueous fraction of the alkaline hydrolysate and the glycolipid was recovered from the organic phase with unaltered tlc mobility. These results also indicated that there were no *O*-acyl groups in the lipid.

The Me_3Si derivative of the intact glycolipid had two components with retention times of 4 and 5 min (a partially resolved doublet) at 270°, on a 0.3-m column of 0.05% OV-101 on etched glass beads. We have as yet no explanation for this phenomenon. An identical pattern for the Me_3Si derivative was observed after mild alkaline-catalyzed methanolysis.

Mass spectra of the lipid aglycone component (C) and its Me_3Si derivative (D) are shown in Figure 4, spectra I and III, respectively. The Me_3Si derivative (spectrum III) exhibited an ion of low intensity at *m/e* 384. Since Me_3Si derivatives have

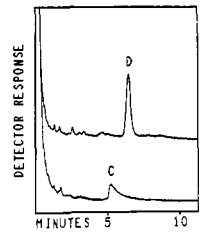
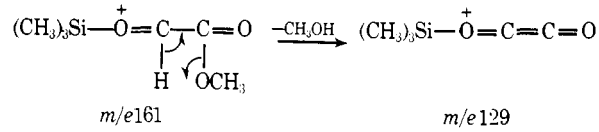


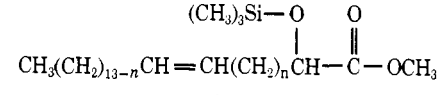
FIGURE 3: Gas–liquid chromatogram of the aglycone moiety from the unknown glycolipid, isolated by hexane extraction after methanolysis, peak C; and its trimethylsilyl derivative, peak D (3% SE-30, 210°).

been shown to lose $CH_3\cdot$ easily, an M − 15 ion is usually present in spectra of these compounds. If the ion at *m/e* 369 were the M − 15 fragment and *m/e* 384 the molecular ion, the strong ion at *m/e* 325 can be accounted for as an M − 59 fragment ion. This ion has been shown by Eglinton *et al.* (1968) to be characteristic for 2-hydroxy fatty acid methyl esters. The postulated fragmentation is shown in the structure drawn within the spectrum. Another ion which supports this proposed structure was observed at *m/e* 129. This may arise from homolytic cleavage between carbons 4 and 5 after loss of 59 (CO_2CH_3) (Eglinton *et al.*, 1968) or fission of the bond between carbons 2 and 3 with charge retention on the oxygen-containing fragment and subsequent loss of methanol:



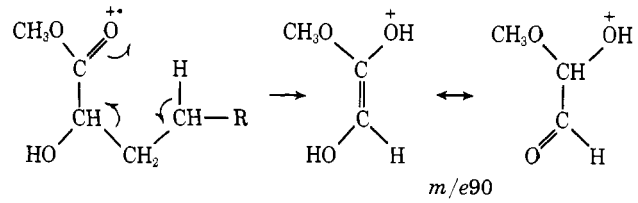
m/e 161 *m/e* 129

The molecular weight of a trimethylsilyl methyl 2-hydroxyoctadecenoate would be 384, as shown in the proposed structure:



mol wt 384

The molecular weight of methyl 2-hydroxyoctadecenoate is 312. Loss of 59 (CO_2CH_3) would give a fragment ion at *m/e* 253, as shown in mass spectrum I. Ions in the lower region of this mass spectrum are characteristic for an aliphatic carbon chain. The expected McLafferty rearrangement ion at *m/e* 90 was of low intensity in this spectrum.



m/e 90

Spectra of saturated 2-hydroxy fatty acid derivatives have been published by Eglinton *et al.* (1968). Catalytic hydrogenation was, therefore, carried out on the aglycone and mass spectra were recorded on the hydroxy ester and Me_3Si derivative. These mass spectra (II and IV) are shown in Figure 4. The expected molecular ion at *m/e* 314 was observed with the hy-

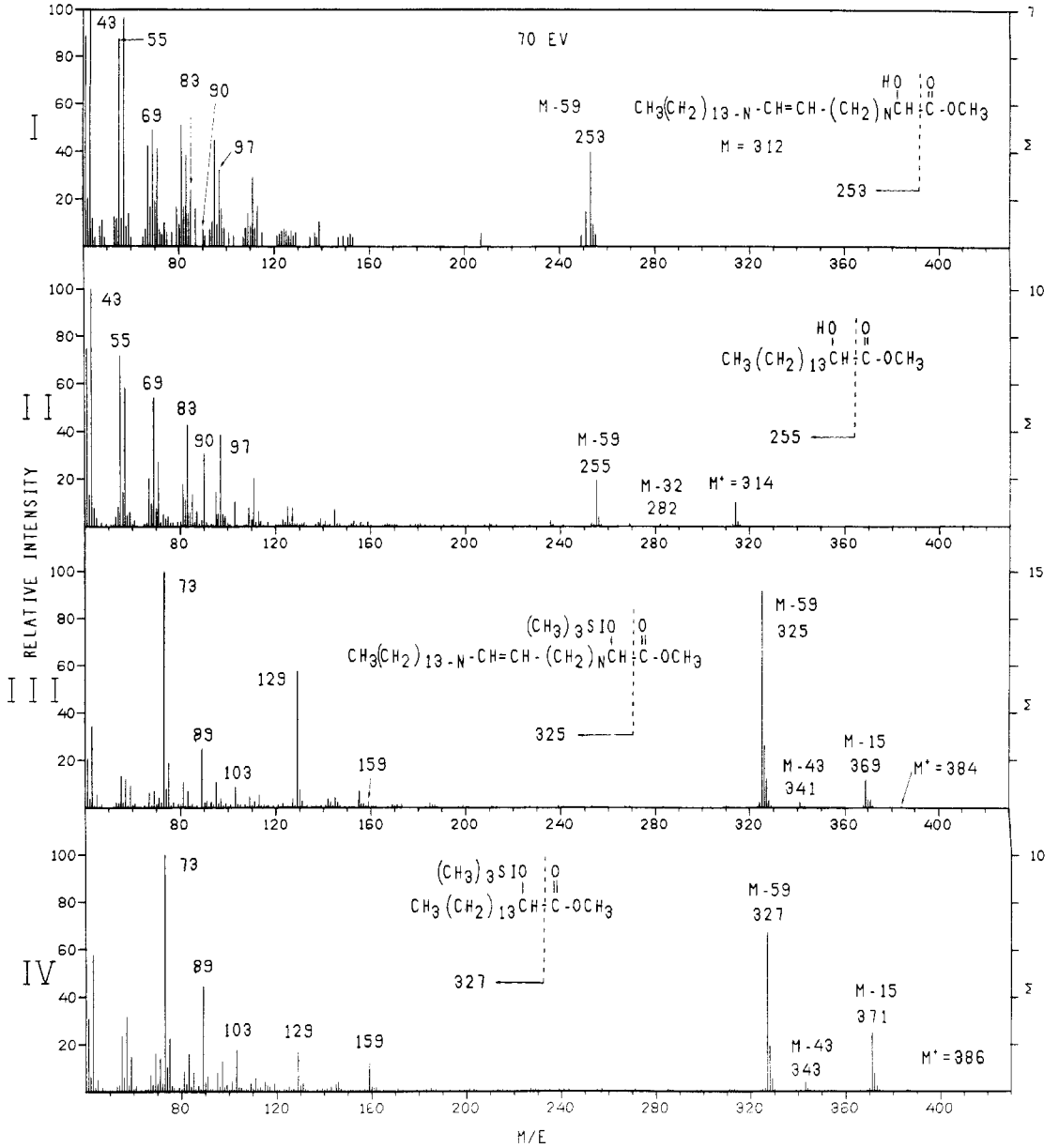


FIGURE 4: Mass spectra of the aglycone (I), its hydrogenation product (II), the trimethylsilyl derivatives of aglycone (III), and of reduced aglycone (IV). Recorded on an LKB 9000 at 70 eV.

droxy ester (II). The M − 59 ion has shifted 2 amu to *m/e* 255, as compared with the unsaturated compound. The ions were analogous to, and the fragment ions in the lower mass range were identical with, ions in mass spectra of methyl 2-hydroxyhexadecanoate published by Eglinton *et al.* (1968). The mass spectrum (IV) of the reduced Me_3Si derivative has a molecular ion at *m/e* 386, an M − 59 ion at *m/e* 327 and M − 15 at *m/e* 371, all of which were 2 amu higher than the corresponding ions before hydrogenation.

An infrared spectrum of the isolated aglycone methyl ester showed strong absorbance at 965 cm^{-1} which was of the same relative intensity as that observed for elaidic acid methyl ester, and characteristic for trans olefins (Silverstein and Bassler, 1967).

We propose on the basis of this evidence that the unknown glycolipid from *Aspergillus niger* is a glucoside of a *trans*-2-hydroxyoctadecenoic acid. Studies are in progress to determine the location of the double bond, the stereochemistry of the aglycone and the configuration of the glycosidic linkage.

Possible Functions for the Glycolipid. Under the growth conditions described here, the monoglucosyloxyoctadecenoic acid accounted for about 6% of the total lipid of *A. niger*, whereas phospholipids were barely evident, amounting to less than 2% of the lipid. This surprising observation has a precedent in bacterial lipids. Wilkinson (1968) reported extremely low contents of phospholipids in the cell walls of *Pseudomonas diminuta and P. rubescens* but high levels (about 15% of the total lipid) of a 1-*O*-hexuronosylglycerol. From this evidence it was concluded that acidic glycolipids could replace phospholipids as structural components in bacterial membranes and according to Minnikin *et al.* (1971) such acidic polar lipids should be accompanied by neutral polar lipids. Such considerations should also apply to fungal membranes. The fact that the monoglucosyloxyoctadecenoic acid is solely intracellular in location, unlike many other fungal glycolipids, may support the contention that it is membranous. However, we have as yet no evidence for the expected associated neutral polar lipid.

References

Bhattacharjee, S. S., and Haskins, R. H. (1970), *Carbohyd. Res. 13*, 235.
Brennan, P. J., and Ballou, C. E. (1967), *J. Biol. Chem. 242*, 3046.
Brennan, P. J., Flynn, M. P., and Griffin, P. F. S. (1970), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 8*, 322.
Cooke, R. C., and Mitchell, D. T. (1969), *Trans. Brit. Mycol. Soc. 52*, 365.
Dittmer, J. C., and Lester, R. L. (1964), *J. Lipid Res. 5*, 126.
Eglinton, G., Hunneman, D. H., and McCormick, A. (1968), *Org. Mass Spectrom. 1*, 593.
Fluharty, A. L., and O'Brien, J. S. (1969), *Biochemistry 8*, 2627.
Folch, J., Lees, M., and Sloane-Stanley, G. H. (1957), *J. Biol. Chem. 226*, 497.
Gaver, R. C., and Sweeley, C. C. (1965), *J. Amer. Oil Chemist's Soc. 42*, 294.
Gorin, P. A. J., Spencer, J. F. T., and Tulloch, A. P. (1961), *Can. J. Chem. 39*, 846.
Kaufman, B., Basu, S., and Roseman, S. (1971), *J. Biol. Chem. 246*, 4266.
Merdinger, E., Kohn, P., and McClain, R. C. (1968), *Can. J. Microbiol. 14*, 1021.
Minnikin, D. E., Ardolrahimzadeh, H., and Baddiley, J. (1971), *Biochem. J. 124*, 447.
Morrison, W. R., and Smith, L. M. (1964), *J. Lipid Res. 5*, 600.
Radin, N. S. (1969), *Methods Enzymol. 14*, 268.
Sambasivarao, K., and McCluer, R. M. (1963), *J. Lipid Res. 4*, 106.
Shaw, N. (1968), *Biochim. Biophys. Acta 164*, 435.
Silverstein, R. M., and Bassler, G. C. (1967), Spectrometric Identification of Organic Compounds, New York, N. Y., Wiley, p 73.
Sweeley, C. C., Bentley, R., Makita, M., and Wells, W. W. (1963), *J. Amer. Chem. Soc. 85*, 2497.
Sweeley, C. C., Ray, B. D., Wood, W. I., Holland, J. F., and Krichevsky, M. I. (1970), *Anal. Chem. 42*, 1505.
Tulloch, A. P., and Spencer, J. F. T. (1964), *Can. J. Chem. 42*, 830.
Tulloch, A. P., Spencer, J. F. T., and Deinema, M. H. (1968), *Can. J. Chem. 46*, 345.
Vance, D. E., and Sweeley, C. C. (1967), *J. Lipid Res. 8*, 621.
Wilkinson, S. G. (1968), *Biochim. Biophys. Acta 152*, 227.

Biosynthesis and Chemistry of 9α,15(*S*)-Dihydroxy-11-oxo-13-*trans*-prostenoic Acid†

P. S. Foss,‡ Charles J. Sih,* C. Takeguchi, and Heinrich Schnoes

ABSTRACT: Serotonin, when used as a cofactor in the biosynthesis of prostaglandins from *all-cis*-8,11,14-eicosatrienoic acid by bovine seminal vesicle microsomes, was found to enhance the overall synthesis of all the postaglandin and especially 9α,15(*S*)-dihydroxy-11-oxo-13-*trans*-prostenoic acid. This prostaglandin, which is coproduced with PGE and PGF$_{1\alpha}$, was purified and found to possess the structure 9α,15(*S*)-dihydroxy-11-oxo-13-*trans*-prostenoic acid. The chemistry of PGD$_1$ and related products as well as the absolute configuration were investigated.

Nugteren *et al.* (1966) first found that a variety of products were formed from *all-cis*-8,11,14-eicosatrienoic acid by an enzyme fraction of sheep vesicle glands when reduced glutathione was omitted from the incubation mixture. One of these products, when treated with methanolic KOH, resulted in rapid decomposition and a small increase in ultraviolet absorption at approximately 245 mμ was observed. Subsequently, Granstrom *et al.* (1968) reported the isolation of a small quantity of a similar compound, which had a weak absorption at 235 mμ after alkali treatment. This product was assumed to be 9α,15-dihydroxy-11-oxo-13-*trans*-prostenoic acid by both groups because a more polar compound with the same chromatographic behavior as PGF$_{1\alpha}$[1] was formed after treatment with $NaBH_4$.

In view of the finding that a small quantity of 8-iso-PGE$_1$ is produced in the incubation mixture and 8-iso-PGF$_{1\alpha}$ methyl ester is virtually indistinguishable from PGF$_{1\alpha}$ methyl ester with respect to chromatographic behavior, infrared, nuclear magnetic resonance (nmr), and mass spectra (Pike *et al.*, 1969) a more rigorous examination of the chemistry of this prostaglandin appears mandatory. Furthermore, the conditions governing the formation of this prostaglandin were unclear (Granstrom *et al.*, 1968). In this paper, we record the

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‡ Present address: School of Research Chemistry, Australian National University, Canberra, Australia.

* To whom correspondence should be addressed.

[1] Abbreviations used are: PGE$_1$, 11α,15α-dihydroxy-9-oxo-13-*trans*-prostenoic acid; PGE$_2$, 11α,15α-dihydroxy-9-oxo-5-*cis*,13-*trans*-prostadienoic acid; PF$_{1\alpha}$, 9α,11α,15α-trihydroxy-13-*trans*-prostenoic acid; PGF$_{2\alpha}$, 9α,11α,15α-trihydroxy-5-*cis*,13-*trans*-prostadienoic acid; 11-epi-PGF$_{1\beta}$, 9α,11β,15α-trihydroxy-13-*trans*-prostenoic acid; PGA$_1$, 15α-hydroxy-9-oxo-10,13-*trans*-prostadienoic acid; PGB$_1$, 15α-hydroxy-9-oxo-8(12),13-*trans*-prostadienoic acid; iso-PGD$_1$, 9α,15α-dihydroxy-11-oxo-12(13)-*trans*-prostenoic acid; PGD$_1$, 9α,15α-dihydroxy-11-oxo-13-*trans*-prostenoic acid; dihydro-PGD$_1$, 9α,15α-dihydroxy-11-oxoprostanoic acid.