Vol. 98, No. 3, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 12, 1981 Pages 858-865

ISOLATION AND PRELIMINARY CHARACTERIZATION OF 1-O-OCTADEC-CIS-11-ENYL GLYCEROL FROM PARAMECIUM PHOSPHOLIPIDS

Dennis E. Rhoads, Kaniaulono B. Meyer and Edna S. Kaneshiro

Department of Biological Sciences
University of Cincinnati
Cincinnati, Ohio 45221

Received January 5,1981

SUMMARY

1-O-Octadec-cis-11-enyl glycerol, paramecyl alcohol, was isolated from Paramecium tetraurelia and P. caudatum phospholipids. This isomer of 1-O-octadec-9-enyl glycerol, selachyl alcohol, had not been previously reported as a component of naturally-occurring phospholipids. The preliminary characterization of the structure of this compound was determined by 1) gas chromatography of the parent compound as well as the products following hydrogenation, ozonolysis and oxidation, 2) mass spectrometry of the parent compound and an oxidation product, 3) infrared spectroscopy, and 4) proton magnetic resonance.

INTRODUCTION

The surface ciliary membrane of the protozoan, Tetrahymena, is known to be rich in the glyceryl ether, 1-O-hexadecyl glycerol (chimyl alcohol) (1). It has been postulated that the presence of surface membrane lipids containing resistant ether bonds serve to protect the organism against hydrolytic compounds in the aquatic environment (1). Recently, we reported that a related protozoan, Paramecium, is also rich in glyceryl ethers (2). Chimyl alcohol and another glyceryl ether, which has not yet been identified in phospholipids from other sources, were present in phospholipids of two species of Paramecium. This new compound, 1-O-octadec-cis-11-enyl glycerol, was isolated and characterized by GC[1], and MS, and IR and PMR spectroscopy. We propose that this compound be given the trivial name, paramecyl alcohol.

[1]Abbreviations used: GC - gas chromatography, IR - infrared, MS - mass spectrometry, M^+ - molecular ion, m/z - mass: charge ratio, PMR - proton magnetic resonance, TLC - thin-layer chromatography, TMS - trimethylsilane.

0006-291X/81/030858-08$01.00/0
Copyright © 1981 by Academic Press, Inc.
All rights of reproduction in any form reserved.

MATERIALS AND METHODS

LIPID EXTRACTION: Paramecium tetraurelia, strains 51s and d4 95, and P. caudatum were grown axenically at 25° in Fernbach flasks containing 500 ml of an enriched crude medium (3). Harvesting of cells and extraction of lipids were as previously described (2). 1-Alkyl glyceryl ethers were prepared from total lipids, total phospholipids or individual phospholipid classes by hydrogenolysis employing the "Vitride" method of Snyder et al. (2,4).

ISOLATION: Paramecyl alcohol was isolated and purified by two methods. 1. Preparative GC. The methaneboronate derivatives of glyceryl ethers were prepared (2) and the paramecyl alcohol derivative was collected from a Hewlett-Packard 5830A GC using a 6 ft 1/2 in o.d. stainless steel column packed with 10% Silar 10c on 100-200 mesh Gas Chrom Q. The column was attached to a 94% splitter. Column temperature was maintained at 200° with an injection temperature of 270° and detector temperature of 250°. The N_2 carrier gas flow was 25 ml/min. The preparation contained 10-15% chimyl methaneboronate, as estimated by GC of the fraction collected by this method. These samples were used for hydrogenation, oxidation, ozonolysis and GC-MS studies. 2. Argentation TLC and preparative GC. The isopropylidene derivatives of glyceryl ethers were prepared according to Hanahan et al. (5). The paramecyl alcohol derivative was isolated using TLC plates coated with 0.5 mm of 17% $AgNO_3$ on Silica Gel G. The plates were prerun in acetone and stored in the dark. After activation at 120° for 30 min, samples were applied and plates were developed in $CHCl_3$:ethanol (99:1, v/v) (6). The band containing the unsaturated compound was scraped off the plates and eluted with $CHCl_3$. This sample was further purified by preparative GC as described above. Samples isolated by this method were free of the chimyl alcohol derivative and were used for IR and PMR studies.

CHARACTERIZATION: The retention times of glyceryl ether derivatives were compared to that of the methaneboronate derivative of authentic batyl alcohol. Retention times of the TMS ether derivatives were also determined (Sil-Prep, Applied Sciences) before and after hydrogenation. However, use of methaneboronic acid resulted in more stable products, hence this reagent was employed in most of the studies. Unsaturation was detected by a change in retention times of the methaneboronate derivative of paramecyl alcohol in GC analyses following catalytic hydrogenation. Position of the unsaturated bond was determined by ozonolysis (7) or periodate-permanganate oxidation followed by GC analyses of fragments as described previously (8).

The methaneboronate derivatives of paramecyl alcohol and an authentic standard of the isomer, 1-O-octadec-9-enyl glycerol (selachyl alcohol) (Supelco), were analyzed on a Varian 1740 series GC equipped with a 3% OV-17 column and a Hitachi-Perkin Elmer RMU-7 MS. The GC was programmed to increase column temperature from 200° to 300° at 8°/min. Methyl esters of the dicarboxylic acid oxidation products of these glyceryl ethers, prepared by the sodium methoxide-BF_3 method (8), were analyzed on a LKB 9000 GC-MS interfaced with System 15 data system. The column was packed with 10% Silar 10c. Initial column temperature was maintained for 10 min at 200°, then it was increased to 250° at 3°/min.

The isopropylidene derivatives of paramecyl and selachyl alcohols were analyzed by IR as thin films between KBr plates employing a Perkin Elmer 599 IR spectrometer. The isopropylidene derivative of paramecyl alcohol was dissolved in $CHCl_3$ and analyzed by PMR on a 600 MHz spectrometer constructed at the Carnegie-Mellon University. The spectrometer was locked on the residual $CHCl_3$ resonance and the chemical shifts were converted to the TMS scale by adding 7.25 ppm to the observed chemical shift.

RESULTS AND DISCUSSION

Gas chromatography of methaneboronate or TMS derivatives of Paramecium glyceryl ethers indicated the presence of two major components, the major species

TABLE 1

RELATIVE RETENTION TIMES OF GLYCERYL ETHER METHANEBORONATES

GC Conditions			
Stationary phase	12% DEGS	10% EGSS-X	10% Silar 10c
Support	80-100 mesh Gas Chrom S	100-200 mesh Gas Chrom P	100-200 mesh Gas Chrom Q
Column length	6 ft glass	6 ft glass	6 ft glass
Column temperature (isothermal)	180°	180°	180°
N_2 flow (ml/min)	26	25	25
Glyceryl Ether Derivative			
1-O-hexadecyl glycerol (chimyl alcohol)	0.53	0.56	0.60
1-O-octadecyl glycerol (batyl alcohol)	1	1	1
1-O-octadec-9-enyl glycerol (selachyl alcohol)	1.07	1.18	1.19
1-O-octadec-11-enyl glycerol (paramecyl alcohol)	1.15	1.19	1.22

occurring in about twice the amount as the other. Derivatives of the more abundant ether cochromatographed with authentic chimyl alcohol derivatives (Table 1). Upon hydrogenation, derivatives of this ether did not change retention times. Mass spectra of chimyl alcohol derivatives from Paramecium were identical to those of authentic chimyl alcohol and agreed with published spectral data (9).

The second component had retention times close to those of authentic selachyl alcohol derivatives (Table 1). The product of hydrogenation of this component co-chromatographed with, and the mass spectra of this compound were identical to those of, authentic batyl alcohol derivatives. Hence, this suggested that this compound, paramecyl alcohol, was an unsaturated form of 1-O-octadecyl glycerol.

The end fragment obtained by ozonolysis of paramecyl alcohol was converted to its aldehyde derivative (8). The product cochromatographed with n-heptaldehyde when analyzed by GC on a Carbowax 20 M column. Also, paramecyl alcohol subjected to periodate-permanganate oxidation yielded a monocarboxylic end fragment which cochromatographed with n-heptanoic acid when analyzed by GC on a 12% DEGS column. These studies established the presence of an unsaturated bond at the Δ11 position of the alcohol.

The mass spectra of the methaneboronate derivatives of paramecyl and selachyl alcohols were qualitatively, and essentially quantitatively, identical, which

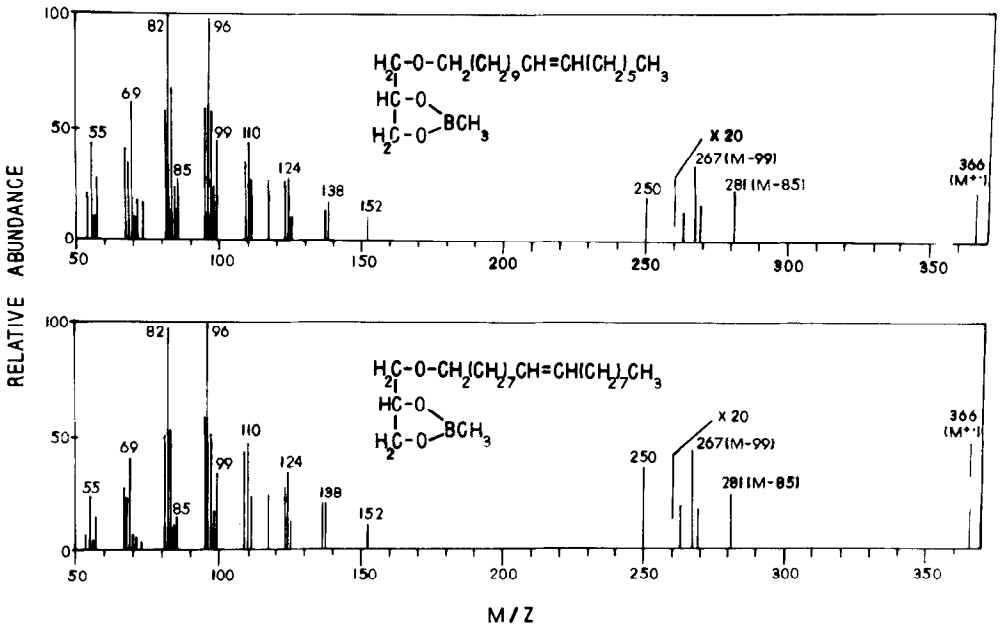


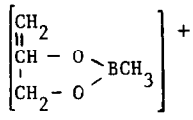
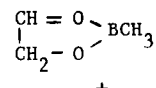
Figure 1. The 70 electron volt mass spectra of methaneboronate derivatives of 1-O-octadec-11-enyl glycerol (paramecyl alcohol, top) and 1-O-octadec-9-enyl glycerol (selachyl alcohol, bottom).

further indicated they were isomeric. Both showed the same molecular ion at m/z 366 (Fig. 1) and established the presence of one double bond in paramecyl alcohol The characteristic ions were those associated with fragmentation patterns typical of ether compounds (Fig. 1). Alpha cleavages produced two fragments at m/z 250 and m/z 99. The former is a hydrocarbon fragment, $C_{18}H_{34}$, indicating the length of the entire alkyl moiety in both compounds. The latter, along with the β cleavage product, m/z 85, represents products characteristic of glyceryl methaneboronate derivatives (Table 2). Below m/z 250, the spectra were similar to those of long chain hydrocarbons containing several series of fragments belonging to the classes: C_nH_{2n}, C_nH_{2n+1}, C_nH_{2n-1}, and C_nH_{2n-2}. The C_nH_{2n-2} class included the base ions, m/z 82 (C_6H_{10}) and m/z (C_7H_{12}), in addition to the m/z 250 fragment (Table 2).

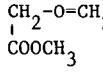
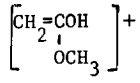
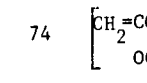
The positions of the double bond in the alkyl moiety of paramecyl and selachyl alcohols were confirmed by the mass spectra of methyl ester derivatives

TABLE 2

MAJOR FRAGMENTS OBTAINED FROM PARAMECYL AND SELACHYL ALCOHOLS[a,b]

m/z	Fragment
366	Molecular Ion[c]
250	$[C_{18}H_{34}]^+$
99	
96	$[C_7H_{12}]^+$
85	
82	$[C_6H_{10}]^+$

MAJOR FRAGMENTS OBTAINED FROM DICARBOXYLIC ACID PRODUCTS OF PARAMECYL AND SELACHYL ALCOHOLS FOLLOWING PERIODATE-PERMANGANATE OXIDATION[a,d]

Paramecyl Alcohol		Selachyl Alcohol	
m/z	Fragment	m/z	Fragment
288	Molecular Ion[e]	260	Molecular Ion[e]
256	$[M]^+-[CH_3OH]$	228	$[M]^+-[CH_3OH]$
215	$[HO{=}CH(CH_2)_9COOCH_3]^+$	187	$[HO{=}CH(CH_2)_7COOCH_3]^+$
183	$[215-CH_3OH]^+$	155	$[187-CH_3OH]^+$
103		103	
74		74	

a - Electron impact mass spectrometry.
b - Methaneboronate derivatives.
c - See fig. 1 for structures.
d - Methyl ester derivatives.
e - See fig. 2 for structures.

of their dicarboxylic acid oxidation products of periodate-permanganate oxidation (Fig. 2). The molecular ion of the paramecyl alcohol product was detected at m/z 288. The molecular ion of the selachyl alcohol product, expected at m/z 260, was not consistently observed. However, present in the spectra of both compounds were M-31 (CH_3O ion at m/z 229 for selachyl and 257 for paramecyl alcohol) and M-59 ($COOCH_3$ ion at m/z 201 for selachyl and 229 for paramecyl alcohol). These

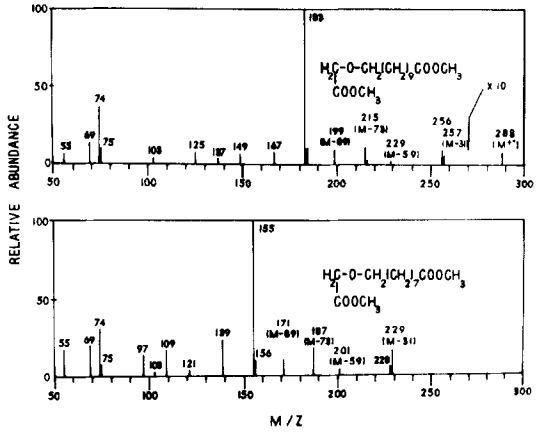


Figure 2. The 70 electron volt mass spectra of methylated dicarboxylic acid products following periodate-permanganate oxidation of 1-O-octadec-11-enyl glycerol (paramecyl alcohol, top) and 1-O-octadec-9-enyl glycerol (selachyl alcohol, bottom).

are characteristic ions in the spectra of compounds containing methyl esters. Their presence was consistent with the molecular weight assignments. Spectra of methyl esters of the ether oxidation products indicated that m/z 74 fragments arising by McLafferty rearrangement of methyl esters were also abundant. Characteristic ether fragmentation patterns were observed in these spectra as they were in the spectra of the methaneboronate derivatives of the parent compounds. The m/z 103 ion resulting from β cleavage was common to both compounds (Table 2). The α cleavage products, M-73 (m/z 187 and m/z 215) and M-89 (m/z 171 and m/z 199) differed by 28 mass units in the spectra of the two compounds, as did most of the major ions. Metastable ions at m/z 128.5 in the selachyl alcohol product and at m/z 155.8 in the paramecyl alcohol product indicated that in each case their base peaks, m/z 155 and m/z 183, respectively, resulted from further fragmentation (elimination of methanol) of their respective M-73 ions. This established the origin of the base peaks in both spectra. These mass spectral data indicated the presence of two additional methylene groups in the paramecyl alcohol product and confirmed the location of the double bond at carbon 11 in paramecyl alcohol.

The IR spectra of isopropylidene derivatives of paramecyl and selachyl alcohols were similar. The characteristic absorption band of the ether linkage at 1110 cm^{-1} in both spectra was consistent with the proposed structure of paramecyl alcohol. Other absorption bands detected in both spectra were at 840, 1050 (C-O), 1150, 1200, 1250, 1370 - 1380 (CH_3), 1440 - 1460 (CH_2, CH_3), 2850 (CH_2, CH_3) and 2900 cm^{-1}. An absorption band at 1667 - 1670 cm^{-1}, characteristic of double bonds, was not detected in either spectrum.

Consistent with the proposed structure was the PMR spectrum of the isopropylidene derivative of paramecyl alcohol. The presence of a double bond was indicated by the resonance at 5.3 - 5.4 ppm which showed coupling consistent with a cis configuration. The lack of a resonance at 2.1 - 2.2 ppm, which would be expected for a methylene group adjacent to an ester linkage, was consistent with the proposed ether structure. Resonances were also detected at 0.8 - 0.9 ($-CH_3$), 1.3 ($-CH_2-$), 1.8 and 2.2 (isopropylidene $-CH_3$), 2.0 - 2.1 ($-CH_2$ adjacent to the double bond), 3.3 - 3.8 (glycerol backbone protons) and 3.60 - 3.65 ($-O-CH_2$) ppm.

This paper described preliminary structural analyses of 1-O-octadec-cis-11-enyl glycerol which was isolated from Paramecium phospholipids. This glyceryl ether and chimyl alcohol were identified in all major glycerophospholipid classes of P. tetraurelia and in total phospholipids of P. caudatum.

ACKNOWLEDGEMENTS

Paramecium caudatum cultures were provided by Dr. A. Fok and Dr. R.D. Allen, University of Hawaii and P. tetraurelia cultures were from Dr. C. Kung, Univeristy of Wisconsin and Dr. J. Preer, Indiana University. Dr. R. Day and Dr. K. Jayasimhulu assisted in MS studies, Dr. M. Wilson and Mr. D. Moats assisted in IR studies and Dr. G. Kreishman and Dr. C. Ho (Carnegie-Mellon University) assisted in PMR studies. The GC-MS was purchased by the Department of Chemistry, University of Cincinnati, by a grant from the N.S.F. (GP-8490). This study was supported by grants from the N.S.F. (PCM77-19088) and the U.S.P.H.S. (GM20910).

REFERENCES

1. Thompson, G.A., Jr. (1972) J. Protozool. 19, 231-236.
2. Rhoads, D.E. and Kaneshiro, E.S. (1979) J. Protozool. 26, 329-338.
3. Soldo, A.T., Godoy, G.C. and VanWagtendonk, W.J. (1966) J. Protozool. 13, 492-497.

4. Snyder, F., Blank, M.L. and Wykle, R.L. (1971) J. Biol. Chem. 246, 3639-3645.
5. Hanahan, D.J., Ekholm, J. and Jackson, C.M. (1963) Biochemistry 2, 630-641.
6. Wood, R. and Snyder, F. (1966) J. Am. Oil Chem. Soc. 43, 53-54.
7. Beroza, M. and Bierl, B.A. (1969) Mikrochim. Acta 4, 720-723.
8. Kaneshiro, E.S., Beischel, L.S., Merkel, S.J. and Rhoads, D.E. (1979) J. Protozool. 26, 147-158.
9. Gaskell, S.J., Edmonds, C.G. and Brooks, C.J.W. (1976) Anal. Letters 9, 325-340