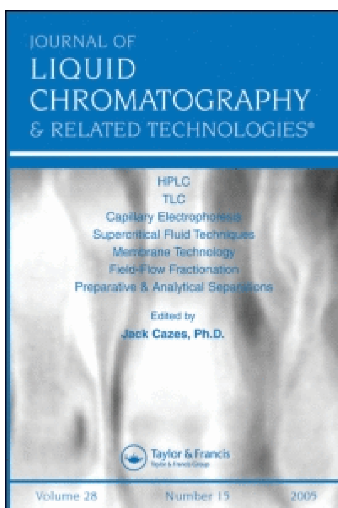
This article was downloaded by:
On: *25 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Chromatographic and Spectroscopic Analysis of Globotriaosyl Ceramide from Bovine Spermatozoa

Juan G. Alvarez[ab]; Bayard T. Storey[ac]; Mark L. Hemling[d]; Robert L. Grob[e]
[a] Obstetrics and Gynecology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania [b] Department of Obstetrics and Gynecology, Philadelphia, PA [c] Departments of Physiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania [d] SmithKline Beecham Laboratories, king of Prussia, Pennsylvania [e] Department of Chemistry, Villanova University, Villanova, Pennsylvania

To cite this Article Alvarez, Juan G. , Storey, Bayard T. , Hemling, Mark L. and Grob, Robert L.(1992) 'Chromatographic and Spectroscopic Analysis of Globotriaosyl Ceramide from Bovine Spermatozoa', Journal of Liquid Chromatography & Related Technologies, 15: 10, 1621 — 1638

To link to this Article: DOI: 10.1080/10826079208018314

URL: http://dx.doi.org/10.1080/10826079208018314

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 15(10), 1621-1638 (1992)

CHROMATOGRAPHIC AND SPECTROSCOPIC ANALYSIS OF GLOBOTRIAOSYL CERAMIDE FROM BOVINE SPERMATOZOA

JUAN G. ALVAREZ[2*], BAYARD T. STOREY[1,2],
MARK L. HEMLING[3], AND ROBERT L. GROB[4]
[1]Departments of Physiology and [2]Obstetrics and Gynecology
University of Pennsylvania
School of Medicine
Philadelphia, Pennsylvania
[3]SmithKline Beecham Laboratories
King of Prussia, Pennsylvania
[4]Department of Chemistry
Villanova University
Villanova, Pennsylvania

Downloaded At: 09:20 25 January 2011

ABSTRACT

The isolation and characterization of globotriaosyl ceramide from bovine spermatozoa is presented. Analysis of Gb_3 by high-resolution proton nuclear magnetic resonance (^{1}H-NMR), fast atom bombardment mass spectrometry (FAB-MS), mobility on HPTLC, and HPTLC-immunostaining; GLC/MS analysis of the partially methylated alditol acetates of the sugar units, and GLC analysis of the sphingoid bases and fatty acids present in the ceramide moiety, resulted in the characterization of this major glycosphingolipid as Gal(α1-4)Gal(β1-4)Glc(β1-1)Cer.

INTRODUCTION

Although the different phospholipid components of mammalian spermatozoa have been well characterized, the chemical characterization of the different glycolipid classes present

*To whom correspondence and reprint requests should be addressed: Dr. Juan G. Alvarez, Department of Obstetrics and Gynecology, 581 Dulles Building, 3400 Spruce Street, Hospital of the University of Pennsylvania, Philadelphia, PA 19104-6080.

Copyright © 1992 by Marcel Dekker, Inc.

in mammalian spermatozoa has not yet been established. In a recent report (2) we described the characterization of seminolipid from bovine spermatozoa as the major component of a particular glycolipid class almost exclusive of mammalian spermatozoa: glycoglycerolipid.

Another class of glycolipids present in biological membranes are the glycosphingolipids which result from the glycosylation of a molecule of ceramide to one or more sugar units. When the latter correspond to unsubstituted hexoses the resulting glycolipids are designated neutral glycosphingolipids. Globotriaosyl ceramide (Gb_3) has been isolated as a major glycosphingolipid from a variety of sources, including blood group P^k erythrocytes (3), Burkitt lymphoma cell line (4), embryonal carcinoma PA1 cell line (5), and human myometrium (6). It has also been partially characterized as the major component of human spermatozoa (7). In this report we present the characterization of globotriaosyl ceramide as the major neutral glycosphingolipid in bovine spermatozoa.

Downloaded At: 09:20 25 January 2011

MATERIALS AND METHODS

Reagents

The glycolipid standards from bovine brain including, galactosyl ceramide (CMH), lactosyl ceramide (CDH), Gb_4, GM_1, GD_{1a}, GD_{1b}, GT_{1a}; and the orcinol ferric chloride spray reagent, were purchased from Sigma Chemical Co (St. Louis, MO). The partially-O-methylated alditol acetates standards, 1,3-di-O-acetyl-2,3,6-O-tri-O-methylgalactitol, and 1,3-di-O-2,3,6-O-tri-O-methylglucitol, were purchased from BioCarb Chemicals (USA). Seminolipid (SML) from bovine spermatozoa was purified as described previously (2). Precoated silica gel HP-K high performance plates (10 x 10 cm, 250 μm thickness) were obtained from Whatman Inc. (Clifton, N.J.). Silica gel (60A pore, 35-75 μm particle size) for column chromatography, was purchased from Analtech Inc. (Newark, DE, U.S.A.).

DEAE-Sephadex A-25 was obtained from Pharmacia Fine Chemicals (New Jersey). Solvents were EM Science chromatographic grade. Inorganic salts were from J.T. Baker (Phillisburg, N.J.) and of the highest purity available.

Sample preparation

Thirteen ejaculates from seven different bulls were collected at the Dairy Breeding Center, University Park, Pa. The seminal plasma was separated by centrifugation at 800 *g* for 20 min and the resulting pellet resuspended in 50 mL of isotonic saline. The sperm suspension was centrifuged at 800 *g* for 20 min and the supernatant aspirated. The final pellet (40 mL of packed sperm with a total concentration of 7 x 10^{10} cells) was then stored at -20ºC until extraction was performed, as described previously (2)

Downloaded At: 09:20 25 January 2011

Glycosphingolipid extraction and purification

The frozen pellet was thawed in a water bath at 37ºC, resuspended in 50 mL of distilled water (W) and subjected to mechanical disruption with an Ultra-Turrax polytron homogenizer for 2 min.as described elsewhere (2). Then 134 mL of methanol (M) were added and the homogenate stirred for 1 hr at 4ºC, followed by addition of 67 mL of chloroform (C) and stirring for 30 min at 4ºC. (final C-M-W ratio was 4:8:3 v/v/v). The organic liquid phase was separated by centrifugation at 800 *g* for 5 min, and the resulting pellet extracted twice as indicated above. The combined supernatants were evaporated to dryness and then partitioned by addition of 50 mL of a mixture of C-M-W (4:2:1 v/v/v), followed by centrifugation at 800 *g* for 5 min. The aqueous upper phase (AUP) was aspirated and a volume of Folch's theoretical upper phase (8) (C-M-0.74% KCl, 1:10:10 v/v/v) equal to the AUP obtained, was added to the organic lower phase, Vortex mixed, and centrifuged at 800 *g* for 5 min. This procedure was repeated five times. The final organic lower phase was evaporated to dryness under rotatory evaporation, redissolved in

2 mL of C-M-W (30:60:8 v/v/v) (solvent 1), and then loaded onto a DEAE-Sephadex A-25 column (acetate form, 1 x 10 cm), prepared according to Ledeen *et al.* (9). The neutral fraction containing the neutral glycosphingolipids was eluted with 100 mL of solvent 1, evaporated to dryness, peracetylated with a mixture of pyridine-acetic anhydride (2:1, v/v), and the peracetylated glycolipids separated on a Florisil column according to the method of Saito and Hakomori (10). The purified peracetylated glycolipid fraction was evaporated to dryness and deacetylated by adding 1 mL of 0.1N $NaOCH_3$ at room temperature in the dark for 30 min (8), The hydrolysate was then loaded onto a Dowex (H^+ form) column (0.5 x 0.5 mL) packed in methanol. The deacetylated glycolipids were eluted with 4 mL of C-M (2:1, v/v), evaporated to dryness, dissolved in 1 mL of chloroform, and loaded onto a silica gel column (1 x 10 cm) packed in the same solvent. Then 50 mL of C, C:M 9:1, C:M 6:1, C:M 4:1, C:M 3:1, C:M 2:1, C:M 1:1, and M, were separately loaded onto the column and 1mL fractions collected at a flow rate of ca.1 mL/min. The eluted lipids were monitored by HPTLC. The fractions containing the pure Gb_3 were combined (total yield of 2 mg), evaporated to dryness, and stored at -20°C until further analysis

Downloaded At: 09:20 25 January 2011

Acid methanolysis

100 μg of the purified Gb_3 were methanolysed at 100°C for 14 hr in 1 mL of 1N anhydrous methanolic-HCl (10). The methanolysate was then neutralized, partitioned with 7 mL of C-M-W (4:2:1 v/v/v), centrifuged at 800 *g* for 5 min and the lower phase aspirated and evaporated to dryness. The methanolysate (N-methylated sphingoid base + fatty acid methyl ester) was then redissolved in 1 mL of methanol and the fatty acid methyl esters extracted and analyzed by GC on a WCOT capillary column (Supelco-Wax-10) as described previously (2). When analysis of hydroxylated fatty acids was sought, the hexane extracts were combined, evaporated to dryness and derivatized with a mixture of pyridine-hexamethyldisilazane-trimethylchlorosilane (5:1:1, v/v/v) at room temperature.

The O-trimethylsilyl (TMS) derivatives were evaporated to dryness and dissolved in 20 μL of n-hexane. The TMS methyl esters were analyzed by GC on a WCOT capillary column (Supelco-Wax-10) with initial and final temperatures in the column oven of 150°C and 250°C respectively, programmed to increase at a rate of 10°C/min. The analysis was performed on a Varian 3700 instrument equipped with a flame ionization detector operated at a temperature of 260°C. Fatty acid methyl ester peaks were identified by comparison of retention times of standard mixtures of hydroxylated fatty acids and quantified using a Hewlett-Packard 3392A integrator, using methyl heptadecanoate as the internal standard.

High performance thin layer chromatography

Downloaded At: 09:20 25 January 2011

Four μL aliquots of each, Gb_4, GM_1, GD_{1a}, GD_{1b}, GT_{1a}; the purified Gb_3 from bovine spermatozoa; LacCer (CDH); and seminolipid (SML), dissolved in C-M (1:1 v/v) at a concentration of 1mg/mL, were streaked on Whatman silica gel plates as a thin band 5 mm from the lower edge of the plate. Four μL aliquots of each , the ganglioside mixture including, GM_1, GD_{1a}, GD_{1b}, GT_{1a}; seminolipid (SML) from bovine spermatozoa purified as described previously (2); Gb_3 from bovine spermatozoa; LacCer (CDH); and GalCer (CMH), dissolved in the same solvent and at 1 mg/mL, were streaked on Analtech diphasic plates. The plates were then dried, predeveloped in C-M (1:1 v/v) to 10 mm from the lower edge of the plate, dried, and placed in 4 x 13 x 14 cm size tanks using C-M-W (65:30:8 v/v/v) (solvent 2) for the Whatman plates and C-M-0.6M $CaCl_2$-0.12M sodium acetate (55:45:4:6 v/v/v/v) (solvent 3) for the Analtech plates, as the mobile phases. Following development the plates were thoroughly dried, sprayed with the orcinol ferric chloride reagent, and placed in a Frigidaire microwave oven for 5 min at setting 9 (11). The stained chromatograms were then scanned with a Shimadzu CS-9000 spectrodensitometer at 550 nm in the transmission mode.

Carbohydrate composition and anomeric linkages

Two hundred µg of the purified Gb_3 from bovine spermatozoa and Gb_3 standard were permethylated according to the procedure of Hakomori (12). Then the permethylated glycolipids were hydrolyzed separatedly in 80% acetic acid containing 0.7N HCl at 70°C for 18 hr, reduced with $NaBH_4$, and acetylated with acetic anhydride (13). The resulting partially O-methylated alditol acetates were analyzed as described previously (2), and the retention times compared to those corresponding to the standards, 1,3-di-O-acetyl-2,3,6-O-tri-O-methylgalactitol, and 1,3-di-O-2,3,6-O-tri-O-methylglucitol and to the partially-O-methylated alditol acetates obtained following hydrolysis of Gb_3 standard under the same experimental conditions

Downloaded At: 09:20 25 January 2011

Sphingoid base analysis

One hundred µg of the purified Gb_3 from bovine spermatozoa were hydrolyzed with 1 mL of 1N HCl at 90C for 14 hr.and and the sphingoid bases analyzed as described previously (14).When indicated, after analysis in the fluorescence mode, the plates were sprayed with a 3% solution of $Cu(COOCH_3)_2$ in 8% H_2PO_4, dried at room temperature, heated in an oven at 180°C for 10 min, and the resulting bands scanned in the transmission mode at 400 nm This latter reaction has been shown to be specific for lipids containing unsaturated moieties thus allowing visualization of (4E)-sphingenine, (4E)-icosasphingenine and (4E)-phytosphingenine but not their saturated homologues (14).

Proton NMR spectroscopy

The purified Gb_3 was dried in vacuo in a dessicator over anhydrous silica gel for at least 24 hr prior to analysis, and the resulting residue dissolved in 0.5 mL of freshly prepared Me_2-SO-d_6 to yield 4 mM sample solutions. The samples were then transferred to 200 x 5 mm

NMR tubes (Wilmad Glass Co., Buena, N.J.). The spectra obtained in this solvent are referred as non-deuterated. And the spectra obtained in Me_2-SO-d_6-D_2O (98:2 v/v) as deuterated. Proton spectra were obtained on a Bruker AM-500 (500 MHz) spectrometer equipped with an Aspect 3000 computer, operating in the Fourier-transform mode with quadrature detection. Probe temperature was 298 + 2°K. Integrated, one-dimensional spectra were obtained over a spectral range of 5 KHz.(0-10 ppm) and 16,384 sampling points. Chemical shifts are expressed in ppm and referenced to internal dimethylsulfoxide (∂ 2.486).

HPTLC-immunostaining

Downloaded At: 09:20 25 January 2011

High performance thin-layer chromatography-immunostaining was carried out according to the method of Magnani *et al* (15) as modified by Dubois *et al.*. (16). Gb_3 was chromatographed on aluminum-backed high performance thin-layer chromatography plates (silica gel 60, E. Merck, Darmstadt, West Germany) in chloroform-methanol-water (65:35:8, v/v/v), as indicated previously. The dried chromatogram was soaked for 1 min in a 0.1 % solution of polyisobutylmethacrylate (Polysciences) in hexane. After drying in air, the chromatogram was sprayed with buffer A solution of the following composition: 0.005 M Tris, 0.15 M NaCl, pH 7.8, with 1% bovine serum albumin and 0.1 % sodium azide. Immediately, the plate was soaked in the same buffer until all the silica gel was wet. The plate was then removed and overlayed with a solution the monoclonal antibody 38-13 diluted 1:10 with the same buffer (60 $\mu L/cm^2$) and incubated for 1 hr at room temperature. The chromatogram was washed by dipping in 4 successive changes of cold phosphate-buffered saline (PBS) at 2 min intervals and overlayed with buffer A containing 2 x 10^6 cpm/mL of ^{125}I-labeled goat anti-mouse Ig M antibodies. After 1 hr at room temperature, the chromatogram was washed as before in PBS, dried and exposed to XAR-5-x-ray film (Eastman Kodak).

Negative ion fast atom bombardment mass spectrometry

The FAB mass spectrum of Gb_3 was obtained on a VG ZAB-HF spectrometer with a high field magnet and VG 11/250 data system. An Ion Tech saddle field atom source was operated at 9 kev and a discharge current of 1 mA. Samples were dissolved (10 µg/µL) in methylene chloride/methanol (ca. 1:1) and 2 µL was added to ca. 2 µL of triethylenetetramine as matrix on a stainless steel FAB target ribbon. The samples were introduced into the spectrometer through the FAB/FD probe inlet, in line with the flight axis (17).

Downloaded At: 09:20 25 January 2011

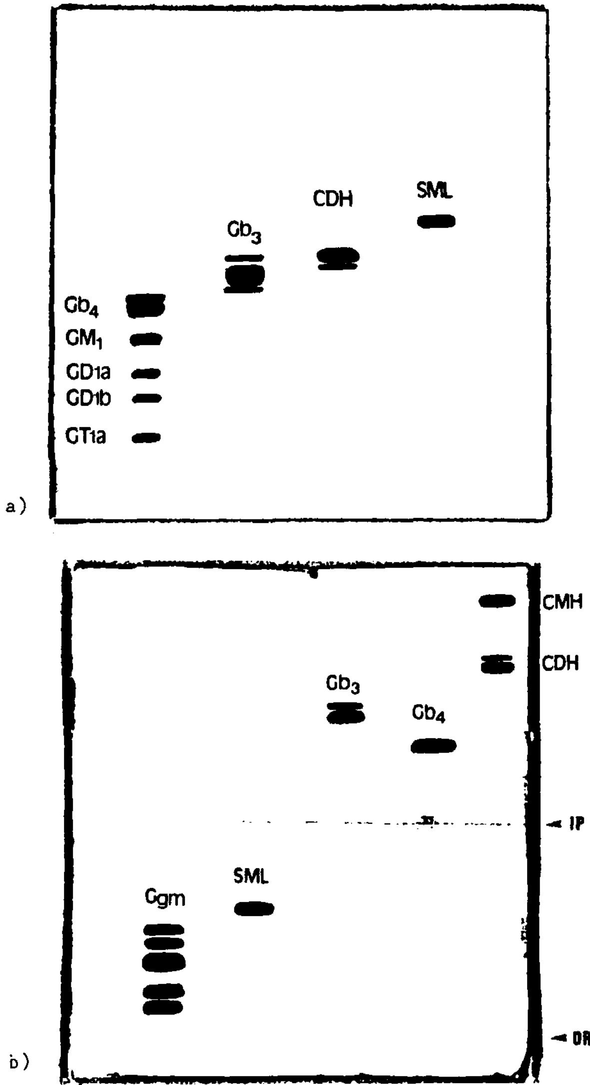
RESULTS AND DISCUSSION

High performance thin layer chromatography

The orcinol-sprayed chromatogram of Gb_4, GM_1, GD_{1a}, GD_{1b}, GT_{1a}; the purified Gb_3 from bovine spermatozoa; LacCer (CDH); and seminolipid (SML) from bovine spermatozoa, is shown in Fig.1a. The migration of Gb_3 from bovine spermatozoa is consistent with Gb_3 isolated from other cells. Glycolipid separation on diphasic silica gel has recently become available (2) (see Materials and Methods). This HPTLC modality provides additional information concerning not only the acidic character of the glycolipid but also its degree of purity. As shown in Fig. 1b, the acidic glycolipids including, the ganglioside mixture, GM_1, GD_{1a}, GD_{1b}, GT_{1a}; and seminolipid (SML) from bovine spermatozoa, were separated in the lower NH_2-hemiplate. Gb_3 from bovine spermatozoa; Gb_4; LacCer (CDH); and GalCer (CMH) GM2 migrated into the silica gel-hemiplate.. This indicates that Gb_3 is a neutral glycolipid.

Sugar analysis

GLC-MS analysis of the partially methylated alditol acetates of the purified Gb_3 and Gb_3 standard indicated the presence of 1,3-di-O-acetyl-2,3,6-O-tri-O-methylgalactitol, and 1,3-



Downloaded At: 09:20 25 January 2011

FIGURE 1: a) Orcinol-Sprayed Normal Phase HPTLC Chromatogram of Globotriaosyl Ceramide from Bovine Spermatozoa (see Materials and Methods for details).b) Orcinol-Sprayed Diphasic TLC Chromatogram of Globotriaosyl Ceramide from Bovine Spermatozoa.

TABLE 1: Partially Methylated Alditol acetates of Globotriaosyl Ceramide from Bovine Spermatozoa

	Peak ratio of components
	Globotriaosyl Ceramide
2,3,6-trimethyl-glucitol	1.00
2,3,6-trimethyl-galactitol	0.85
2,3,4,6-tetramethyl-galactitol	1.05

TABLE 2: Fatty Acid Methyl Esters of Globotriaosyl Ceramide from Bovine Spermatozoa

Fatty acid	%
14:0	2.20
15:0	1.80
16:0	49.80
18:0	22.05
18:1	2.80
20:0	7.35
20:1	0.46
22:0	1.20
22:1	0.54
24h:1	4.40
24h:0	7.35

Downloaded At: 09:20 25 January 2011

di-O-2,3,6-O-tri-O-methylglucitol with a molecular ratio of 2:1 respectively, consistent with the sugar composition of Gb_3 (Table 1).

GC analysis of fatty acid methyl esters

The GC chromatogram of the n-hexane extract of acid treated Gb_3 (see Materials and Methods) revealed the presence of palmitic and stearic acids as the major fatty acid components. Significant amounts of the hydroxylated fatty acids, C24:0h and C24:1h were also found (see Table 2 for more details).

Sphingoid base analysis

Downloaded At: 09:20 25 January 2011

HPTLC-fluorescence spectrodensitometric analysis of the sphingoid bases corresponding to Gb_3 from bovine spermatozoa revealed the presence of sphingenine as the major sphingoid base (98%) and trace amounts of sphinganine and icosasphinganine.

Proton NMR spectroscopy

Figures. 2a and 2b show the 1-D spectrum of the purified Gb_3 from bovine spermatozoa. Outside of the region between 3 and 4 ppm in which most of the sugar ring resonances reside, several classes of resonances can be identified. In the up-field alkyl region, a 6-proton alkyl methyl triplet can be seen at 0.83 ppm, which corresponds to the terminal methyl groups of sphingosine and fatty acid moieties (H14) (Fig. 2a). And a 50-proton multiplet is found at 1.24 ppm corresponding to the alkyl methylene chain (H10).

In the down-field region, proton resonances corresponding to the ceramide moiety are found (Fig.2b). A 1-proton doublet of doublets resonance at 4.09 ppm (H3') and a two-proton doublet of doublets at 4.0 ppm (H1') could be ascribed the hydroxyl geminal proton and to glycosidic methylene protons respectively.

Downloaded At: 09:20 25 January 2011

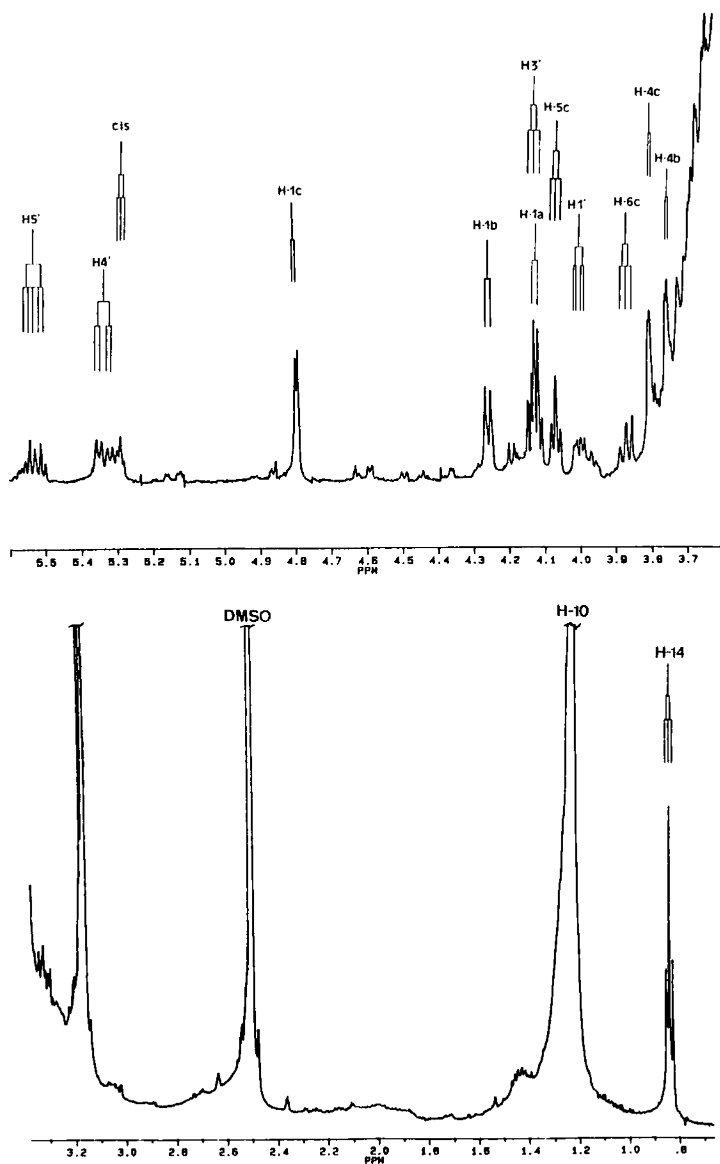


FIGURE 2: a) Integrated One-Dimensional 500 MHz Proton NMR Expanded Subspectrum of Deuterated Globotriaosyl Ceramide from Bovine Spermatozoa. This spectrum corresponds to the up-field region between 0.8 and 3.2 ppm.
b) Integrated One-Dimensional 500 MHz Proton NMR Spectrum of Globotriaosyl Ceramide from Bovine Spermatozoa. The limits of the spectrum ranged between 3.7 and 5.6 ppm.

In the down-field anomeric region three well resolved 1-proton doublets are detected at 4.8 ppm (H-1c), 4.25 ppm (H1b), and 4.12 ppm (H1a) (Fig. 2b). Consideration of the chemical shifts (Table 3) and coupling constants (Table 4) for these anomeric proton resonances allowed the identification and anomeric configuration of the sugar units: α-galactopyranosyl, β-galactopyranosyl and β-glucopyranosyl, respectively (18). And finally, two 1-proton multiplets at 5.45 ppm and 5.65 ppm were assigned to the olefinic protons (H4 and H5, respectively) (Fig 2b) corresponding to the *trans* double bond at the 4 position of the sphingoid base moiety. These results are in excellent agreement with the proton NMR characterization of Gb_3 by Hakomori *et al* (18) and Koike *et al* (19)

Downloaded At: 09:20 25 January 2011

HPTLC immunostaining

The results of the immunostaining of Gb_3 from bovine spermatozoa with monoclonal antibody 38-13 are shown in Fig 3. The binding of mAb 38-13 to the putative Gb_3 from bovine spermatozoa confirms its identity as globotriaosyl ceramide.

Fast atom bombardment mass spectrometry

The negative ion FAB mass spectrum of bovine spermatozoa, Gb_3 is shown in Fig. 4. Pseudomolecular ions $(M-H)^-$ are observed at m/z 1022, 1050, 1106, 1148, and 1150. These can be assigned as coming from Gb_3 with C16:0, C18:0, C22:0, hydroxy-C24:1, and hydroxy-C24:0 fatty acyl constituents. The peak at m/z 1132 may be attributed to H_2O loss from m/z 1150 or to native C24:1 component. Each of the ions in the molecular region has a corresponding peaks due to three succesive hexosyl losses. The fatty acyl portion of the ceramide is further defined in the low mass end of the spectrum by the w, x, y peaks. These peaks correspond to C16:0, C18:0, and C22:0 fatty acyl groups. No evidence of the hydroxy fatty acids in this region suggests that H_2O loss is relatively facile. These results are in good agreement with those obtained by GC analysis of the fatty acid methyl esters obtained following alkaline methanolysis.

TABLE 3: Chemical Shifts for Methylene and Methyne Protons of Globotriaosyl Ceramide from Bovine Spermatozoa.

Proton chemical shifts (ppm)								
H-1a	H-1b	H-1c	H-4b	H-4c	H-5c	H-6c	H-1'	H-3'
4.124	4.242	4.798	3.751	3.808	4.082	3.875	3.998	4.135
[a](4.186)	(4.246)	(4.815)	(3.776)	(3.820)	(4.065)	N.A.	(3.950)	(4.246)

[a]values in parenthesis correspond to those reported by Koike *et al* for synthetic globotriaosyl-E-ceramide

N.A: not available

TABLE 4: Apparent Coupling Constants for Globotriaosyl Ceramide Proton Resonances

Proton-Proton Coupling Constants (±0.05 Hz)								
$J_{1,2a}$	$J_{1,2b}$	$J_{1,2c}$	$J_{4,5b}$	$J_{4,5c}$	$J_{5,6c}$	$J_{6,7c}$	$J_{1,2'}$	$J_{3,4'}$
7.80	7.63	3.81	2.81	2.73	6.12	4.55	5.61(A) 10.3(B)	8.2
[a](7.80)	(7.60)	(3.9)	(2.8)	(2.70)	(6.10)	N.A.	5.60(A) 10.30(B)	8.1

[a]values in parenthesis correspond to those reported by Koike *et al.*

N.A.: not available

Downloaded At: 09:20 25 January 2011

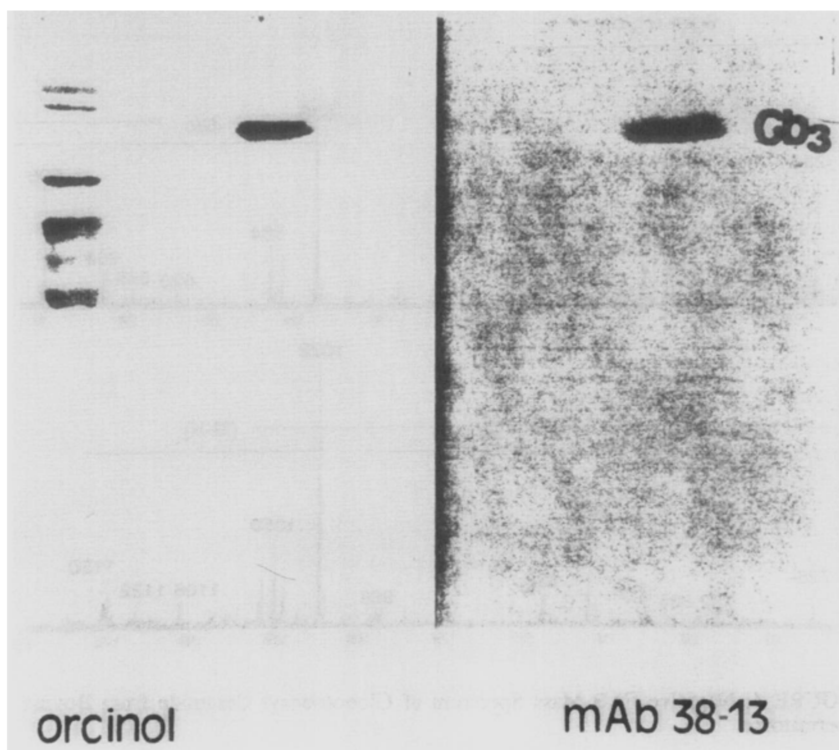


FIGURE 3: HPTLC-Immunostaining of Purified Globotriaosyl Ceramide from Bovine Spermatozoa. The left panel, left column, corresponds to the orcinol-sprayed chromatogram of a standard glycolipid mixture including, GlcCer, GalCer, LacCer, Gb3, GM1, GD1a, GD1b, and GT1b; and Gb3 from bovine spermatozoa in the right column. The right panel corresponds to an autoradiography of Gb3 following immunostaining with monoclonal antibody 38-13.

Downloaded At: 09:20 25 January 2011

The positive ion mass spectrum shows weak pseudomolecular ions as $(M+Na)^+$ species at m/z 1046, 1074, 1130, 1172, and 1174. The observed fragment ions due to loss of succesive hexosyl units appears to be more strongly derived from the C18:0 than C16:0 as expected although this series is very weak in general.

SUMMARY

The isolation and characterization of globotriaosyl ceramide from bovine spermatozoa is presented. Analysis of Gb_3 by high-resolution proton nuclear magnetic resonance (^{1}H-

Downloaded At: 09:20 25 January 2011

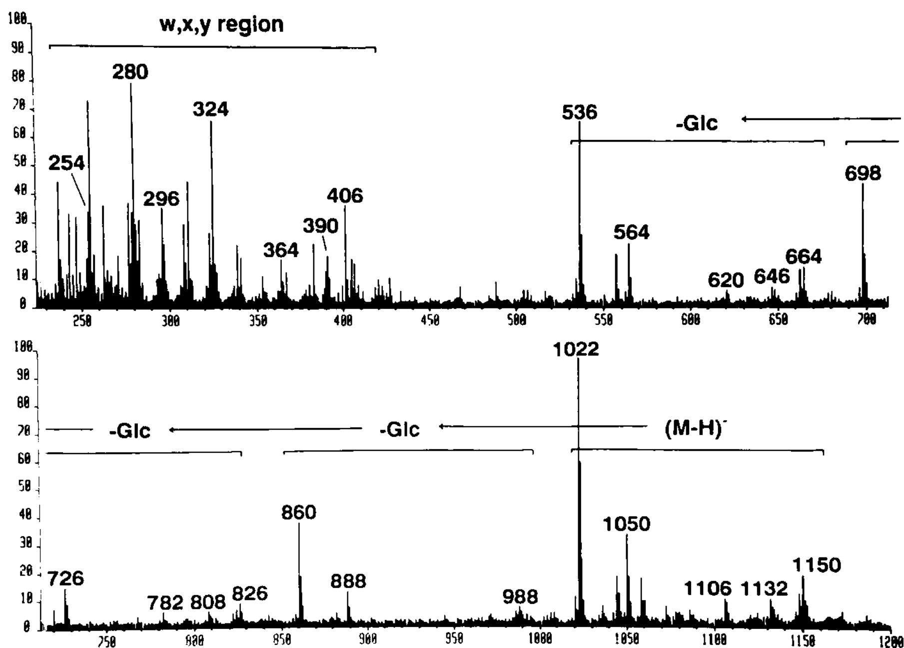


FIGURE 4: Negative FAB Mass Spectrum of Globotriaosyl Ceramide from Bovine Spermatozoa.

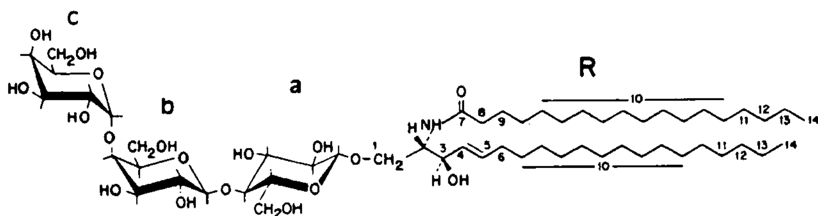


FIGURE 5: Structure of Globotriaosyl Ceramide from Bovine Spermatozoa. The sugar units, corresponding to β-glucose, β-galactose, and α-galactose are labeled a, b, and c, respectively. The ceramide moiety is labeled as R.

NMR), fast atom bombardment mass spectrometry (FAB-MS), mobility on HPTLC, and HPTLC-immunostaining; GLC/MS analysis of the partially methylated alditol acetates of the sugar units; HPTLC analysis of the sphingoid bases; and GC analysis of the fatty acids present in the ceramide moiety, resulted in the characterization of this major glycosphingolipid as Gal(α1-4)Gal(β1-4)Glc(β1-1)Cer (Fig. 5).

ACKNOWLEDGMENTS

The authors wish to thank Dr. Steven Spitalnik for the generous gift of monoclonal antibody 38-13 and for providing his expertise in the isolation and purification of glycosphingolipids. We also would like to thank Dr. Gary J. Killian of Dairy Breeding Research Center, Penn State University, for kindly providing the frozen specimens of bovine spermatozoa used in this study. This work was supported by NIH grants HD-15842 and HL-19737.

Downloaded At: 09:20 25 January 2011

REFERENCES

1. IUPAC-IUB Commission of Biochemical Nomenclature. . The Nomenclature of lipids (Recommendations 1976). J. Lipid Res. 19, 114, 1978

2. Alvarez,J. G., Storey, B. T., Hemling, M. L., and Grob, R. L. J. Lipid Res. 31, 1073, 1990

3. Marcus, D. M., Sundu, S. K., Suzuki, A.. Seminars in Hematol. 18, 63, 1981

4. Nudelman, E., Kannagi, R., Hakomori, S., Parsons, M., Lipinski, M., Wiels, J., Fellous, M., and Tursz, T.. Science 220, 509, 1983

5. Hakomori, S.. Ann. Rev. Immunol. 2, 103, 1984

6. Zhu, Z., Deng, H., Fenderson, B. A., Nudelman, E. D., and Tsui, Z.. J. Reprod. Fert. 88, 711, 1990

7. Ritter, G., Krause, W., Geyer, R., Stirm, S., and Wiegandt, H.. Arch. Biochem. Biophy. 257, 370, 1987

8. Folch, J., Lees, M., and Sloane Stanley, G. H.. J. Biol. Chem. 226, 497, 1957

9. Ledeen, R., W., Yu, R., K., and Eng, L., F.. J. Neurochem. 21, 829, 1973

10. Saito, T., and Hakomori, S-I.. J. Lipid Res. 12, 257, 1971 .

11. Alvarez, J., G., and Touchstone, J., C.. J. of Chromatogr. 436, 515, 1988.

12. Hakomori, S., I.. J. Biochem. (Tokyo). 55, 205, 1964.

13. Nakamura, K., Hashimoto, Y., Suzuki, M., Suzuki, J., and Yamakawa, T.. J. Biochem. 96, 949, 1984

14. Alvarez, J. G., Touchstone, J. C., Storey, B. T., and Grob, R. L.. J. Liquid Chromatogr. 12, 3115, 1989

15. Magnani, J. L., Nilsson, B., Brockhaus, M., Zopf, D., Steplewski, z., Koprowski, H., and Grinsburg, V. J. Biol. Chem. 257, 14365, 1982

16. Dubois, C., Magnani, J. L., Grunwald, G. B., Spitalnik, S. L., Trisler, G. D., Nirenberg, M., and Grinsburg, V. J. Biol. Chem. 261, 3826, 1986

17. Hemling, M., E., Yu, R., K., Sedgwick, D., and Rinehart, K., L., Jr.. Biochemistry 23, 5706, 1984

18. Hakomori, S., Siddiqui, Y., Li, T., and Hellerqvist, C. G. J. Biol. Chem. 246, 2271, 1971

19. Koike, K., Sugimoto, M., Sato, S., Ito, Y., Nakahara, Y., and Ogawa, T. Carbohydrate Res. 163, 189, 1987

Downloaded At: 09:20 25 January 2011