Journal of Chromatography, 272 (1983) 9—20
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1474

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC RESOLUTION OF *p*-NITROBENZYLOXYAMINE DERIVATIVES OF BRAIN GANGLIOSIDES

T. DENNIS TRAYLOR*

Oak Ridge Associated Universities, Comparative Animal Research Laboratory, P.O. Box 117, Oak Ridge, TN 37830 (U.S.A.)

and

DANIEL A. KOONTZ and EDWARD L. HOGAN

Department of Neurology, Medical University of South Carolina, Charleston, SC 29425 (U.S.A.)

(First received May 18th, 1982; revised manuscript received August 13th, 1982)

SUMMARY

A new quantitative procedure for the high-performance liquid chromatographic (HPLC) resolution of human brain gangliosides employing reversed-phase chromatography is described. To provide a derivative which can be determined by UV absorption techniques, *p*-nitrobenzyloxyamine was coupled to the gangliosides. Derivatization involves ozonation and cleavage of the ceramide double bond followed by oxime formation to the nascent aldehyde. Individual gangliosides, as they were resolved by HPLC, were collected. These fractions were then identified by thin-layer chromatography (TLC) and by gas chromatography of their monosaccharides. Quantitative results were obtained along with a marked increase in sensitivity over conventional resorcinol—hydrochloric acid quantitation of TLC-resolved gangliosides.

INTRODUCTION

The study of gangliosides is of wide interest among neurochemists since they appear to be actively involved in a number of plasma membrane functions that may include cell surface receptor mechanisms. Alterations in ganglioside content or distribution have often been associated with cell transformation [1] and hereditary gangliosidoses [2,3]. It is now known that the membrane receptor for cholera toxin is a specific ganglioside — GM1 [4]. Gangliosides are usually resolved by thin-layer chromatography (TLC) and determined by

0378-4347/83/0000—0000/$03.00

colorimetry or densitometry. TLC resolution is affected by plate-to-plate variations and by a lack of equilibrium conditions in the vapor phase. Quantitation following their resolution by scraping of silica gel and colorimetric determination of sialic acid is laborious and time consuming, while densitometry suffers considerable variability. These limitations render quantitation of a minor band difficult and further seriously limit studies where only small amounts of gangliosides are available, such as with some cell cultures, clinically relevant volumes of cerebrospinal fluid, micro-amounts of tissue, etc. Consequently, some investigations are simply reported as a photograph of a resorcinol-sprayed TLC plate or just as total lipid N-acetylneuraminic acid (NANA). Such reports suggest the problems inherent in TLC resolution and quantitation are compromising results and further suggest the need for improved methodology.

High-performance liquid chromatography (HPLC) offers an obvious solution to some of these difficulties since columns featuring a large number of theoretical plates are commercially available and since fast resolutions offered by HPLC should provide a separation of gangliosides that is more rapid and distinct than that by TLC. Because native gangliosides have little absorbancy in the ultraviolet and refractive index detectors do not offer sufficient sensitivity, we chose to derivatize the gangliosides with an adduct that absorbs in the ultraviolet and one that can be added rapidly and quantitatively. Our plan was to derivatize in the ceramide portion in order that subsequent structural characterization of the carbohydrate moiety would not be compromised. In this way it should be possible to study minor gangliosides and obtain adequate quantities to chemically characterize their structure.

MATERIALS AND METHODS

A Waters Assoc. (Milford, MA, U.S.A.) high-performance liquid chromatograph, composed of two Model 6000 pumps, Model 660 solvent programmer, Model U6K injector, and a Model 440 UV detector was used in this study. The reversed-phase column (Waters Assoc. μBondapak C_{18} 10 μm particle size, 30 cm × 2 mm) was run at ambient temperature with a flow-rate of 1 ml/min. The UV detector was operated at 254 nm. Solvent programming began with the initial solvent composition methanol—water (50 : 50) held constant for 1 min and then linearly adjusted over 15 min to methanol—water (70 : 30). Peak areas were quantitated by a Columbia Scientific Industries (Austin, TX, U.S.A.) Model CRS-208 integrator. Gangliosides were isolated from human brain by the method of Folch et al. [5], as modified by Suzuki [6]. Methanol and chloroform were purchased from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). *p*-Nitrobenzyloxyamine hydrochloride was obtained from Regis Chemical Company (Morton Grove, IL, U.S.A.), triphenylphosphine and N-acetylneuraminic acid were purchased from Supelco (Bellefonte, PA, U.S.A.), DEAE-Sephadex was obtained from Pharmacia Fine Chemicals (Piscataway, NJ, U.S.A.), and other chemicals used were purchased from Fisher Scientific (Pittsburgh, PA, U.S.A.).

Ozonolysis of gangliosides

Mixed human brain ganglioside was dissolved in methanol (1 mg/ml) and a 10-μl aliquot was retained for estimation of NANA by the method of Svennerholm [7] and Miettinen and Takki-Luukkainen [8]. Molar concentration of ganglioside was estimated from the pattern determined by TLC and total NANA. Ozonolysis at −70°C was performed with a Supelco Microozonizer at an oxygen flow-rate of 10 ml/min. Ozonation was promptly terminated when ozone began to evolve from the reaction tube and the tube was then flushed with nitrogen. Commonly, ozonation required about 2 min per mg of ganglioside. Triphenylphosphine was added to selectively cleave ozonide to aldehydes. Fuchsin test for aldehydes was routinely positive within 1 min (native ganglioside remained negative in the Fuchsin test after 1 h).

p-Nitrobenzyloxyamine derivatization of gangliosides

Extensive investigation of reaction conditions (see Results) led us to the adoption of standard conditions for derivatization of ganglioside-aldehyde without detectable by-product formation. We have routinely used a molar ratio of *p*-nitrobenzyloxyamine to the sum of ganglioside-aldehyde and long-chain fatty aldehyde of 1.3:1. The reaction mixture was incubated in methanol for 15 min at 40—45°C. Immediately following derivatization, chloroform was added to the reaction mixture, adjusting solvent composition to methanol—chloroform (7:2), and the mixture applied to a previously prepared DEAE-Sephadex column (25 cm × 2 cm) [9]. DEAE-Sephadex A-25 was washed four times with chloroform—methanol—0.8 *M* sodium acetate (30:60:8) and three times with chloroform—methanol—water (30:60:9). The column was prepared by suspension of the gel in methanol—chloroform (7:2) and then poured into a small glass column. The reaction mixture was applied to the column at a flow-rate of 1 ml/min and the column washed at that rate with 300 ml of methanol—chloroform (7:2). Labeled gangliosides were eluted with 200 ml of methanol—chloroform (7:2) that had been made 0.2 *M* in ammonium acetate. The column eluate was monitored at 254 nm by an Instrumentation Specialities (Lincoln, NE, U.S.A.) Model UA-5 absorbancy monitor equipped with a Type-6 optical unit. All peaks were examined for sialic acid by the resorcinol method of Svennerholm [7]. Following concentration, the sample was dialyzed to remove ammonium acetate and lyophilized.

Thin-layer chromatography of p-nitrobenzyloxyamine-labeled gangliosides

Individual peaks collected after resolution by HPLC were dried under a stream of nitrogen, dissolved in a small quantity of chloroform—methanol (1:1), and carefully applied to an E. Merck (Darmstadt, G.F.R.) precoated silica gel 60 HPTLC plate (Cat. No. 5641). The TLC plates were chromatographed twice in chloroform—methanol—water (60:35:8) containing 20 mg calcium chloride. Plates were then sprayed with resorcinol—hydrochloric acid, covered with a glass cover plate, and placed in a 110°C oven for 15 min. Gangliosides were quantitated by densitometry using a Kontes densitometer (Vineland, NJ, U.S.A.) with peak integration as before.

Gas chromatography of monosaccharides

Carbohydrate composition of individual *p*-nitrobenzyloxyamine-labeled gangliosides was determined by gas chromatography (GC). After drying, the labeled gangliosides were methanolyzed in 0.5 *M* methanolic hydrochloric acid (75°C, 24 h) and were transferred to microvials and dried under a stream of nitrogen. Trifluoroacetate derivatives were formed by the method of Zanetta et al. [10] in 0.2 ml of dichloromethane–trifluoroacetic anhydride (1:1). The reaction mixture was injected directly onto a 5% OV-21 6-ft. long column installed in a Perkin-Elmer (Norwalk, CT, U.S.A.) Model 3920 gas chromatograph fitted with dual flame ionization detectors. Temperature programming, initiating at 90°C and ending at 210°C, at a rate of 4°C/min achieved good resolution of the monosaccharides. Peak areas were integrated electronically.

RESULTS

Gangliosides were prepared from human brain tissue and were stored at −45°C under nitrogen until use. Our experimental design to develop a sensitive assay for individual gangliosides by HPLC involved (see Fig. 1) ozonation of the double bond in the ceramide, cleavage of the ozonide by triphenylphosphine to aldehydes, oxime bond formation to *p*-nitrobenzyloxyamine, and resolution by reversed-phase chromatography.

Derivatization

Ozonation of human brain gangliosides proceeded rapidly with the end point, as determined by the starch—iodine test, dependent upon concentration of the gangliosides and on the oxygen–ozone flow-rate. The sharp appearance of color in this test occurred at about 2 min per mg of ganglioside with an oxygen flow-rate of 10 ml/min. After cleavage of the nascent ozonide by triphenylphosphine, derivatization of the aldehyde products was also observed to progress readily. Initial experiments involving greatly excessive molar amounts of *p*-nitrobenzyloxyamine and reaction at 60°C gave two successive sets of sialic acid positive peaks (Table I). Both sets on subsequent TLC gave the complete series of ganglioside derivatives. Evidence was obtained by using extended reaction times to suggest that once formed, the derivatives can undergo some further reaction or perhaps a degradation yielding another product (Table I). During incubation of the reaction mixture at 60°C, the total sialic acid content of the more slowly eluting set of peaks was found to increase with a parallel loss of neuraminic acid in the more rapidly eluting set of peaks. As outlined in the Materials and methods section, conditions were found that allowed a single set of derivatives to be formed, i.e., reaction at 40°C for 15 min with a *p*-nitrobenzyloxyamine—aldehyde ratio of 1.3:1. See Discussion for further details on this point.

Ion-exchange chromatography

Chromatography of the reaction mixture at this stage revealed that *p*-nitrobenzyloxyamine and triphenylphosphine elute with retention times in the middle of the ganglioside peaks. To remove impurities from the derivatized gangliosides, the ion-exchange chromatographic method of Ledeen et al. [9]

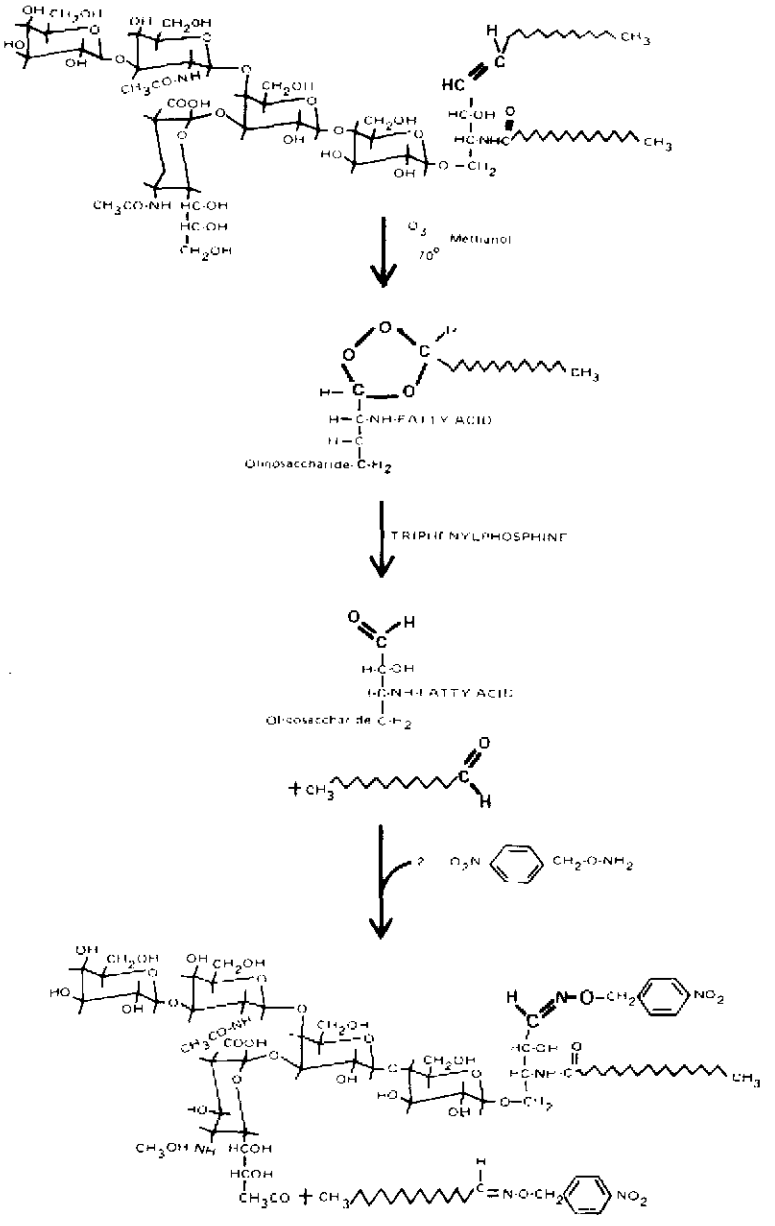

Fig. 1. Derivatization of ganglioside GM1 by *p*-nitrobenzyloxyamine. Other gangliosides are derivatized identically.

was employed. Free *p*-nitrobenzyloxyamine and *p*-nitrobenzyloxyamine-labeled long chain fatty aldehyde (primarily tetradecanal and hexadecanal) were eluted first since they are not strongly retained by the resin (Fig. 2). *p*-Nitrobenzyloxyamine-labeled gangliosides were eluted from the column following the addition of ammonium acetate to the mobile phase.

TABLE I

SECONDARY DERIVATIZATION OF HUMAN BRAIN GANGLIOSIDES IN EXCESS *p*-NITROBENZYLOXYAMINE AT 60°C

Aldehyde cleavage products of ozonolysed ganglioside (1 mg) were incubated at 60°C with a 30-fold molar excess of *p*-nitrobenzyloxyamine for the time indicated. Primary set of peaks were eluted from the HPLC system in 4—20 min. The secondary set was eluted from the column in 40—60 min. Data are presented as percent total sialic acid. Sialic acid was determined by the resorcinol–hydrochloric acid method [7].

	Time (min)			
	15	30	60	120
Primary set	83%	71%	58%	41%
Secondary set	17%	29%	42%	59%

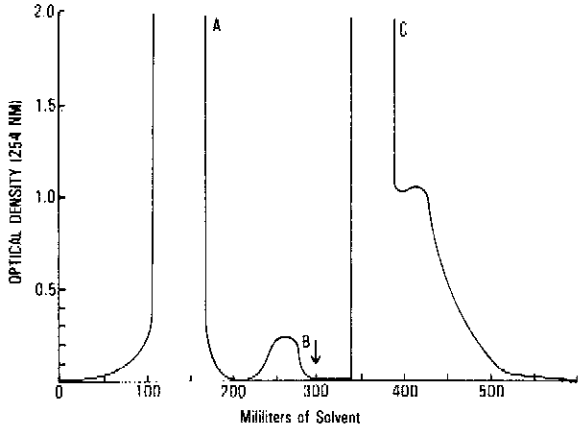

Fig. 2. DEAE-Chromatography of *p*-nitrobenzyloxyamine-labeled ganglioside, *p*-nitrobenzyloxyamine-labeled long-chain aldehyde, and other reaction components. A reaction mixture of 30 mg of ganglioside in methanol—chloroform (7:2) was placed onto a previously prepared DEAE-Sephadex column and the column was eluted with 300 ml of methanol—chloroform (7:2). *p*-Nitrobenzyloxyamine-labeled gangliosides were eluted by solvent change (at arrow) to methanol—water (7:2) that was made 0.2 *M* with ammonium acetate. Peaks: A = long-chain fatty aldehyde labeled with *p*-nitrobenzyloxyamine and unreacted *p*-nitrobenzyloxyamine; B = triphenylphosphine; C = *p*-nitrobenzyloxyamine-labeled ganglioside.

High-performance liquid chromatography

Labeled gangliosides were resolved by reversed-phase chromatography using methanol—water as mobile phase (Fig. 3). A gradient, beginning with methanol—water (50:50), held constant for 1 min, and then linearly adjusted over 15 min to methanol—water (70:30), gave good resolution. Four major peaks with retention times of A = 5.6, B = 6.6, C = 10.8, and D = 13.3 min were observed, and these were collected separately. Minor peaks C′ and D′ were also resolved but usually collected along with corresponding major peaks. Peak A consistently presented a leading shoulder.

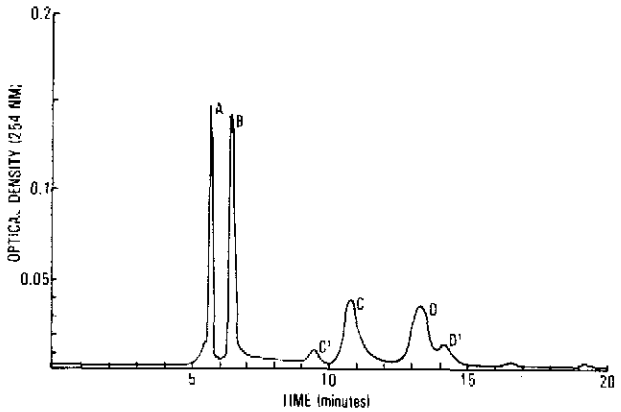

Fig. 3. Resolution of *p*-nitrobenzyloxyamine-labeled ganglioside by HPLC. Twenty-five μg NANA of *p*-nitrobenzyloxyamine-labeled ganglioside were injected onto a μBondapak C_{18} (30 cm × 2 mm) column. Initial solvent composition of methanol—water (50:50) at a flow-rate of 1 ml/min was used with linear programming over 15 min to methanol—water (70:30). For explanation of peaks see text.

Characterization of p-nitrobenzyloxyamine-labeled ganglioside

Identification of individual peaks as resolved by HPLC was undertaken by TLC and GC. *p*-Nitrobenzyloxyamine-labeled gangliosides migrated on TLC in a manner that closely resembled that of native gangliosides (Fig. 4). Indicated peaks were collected as they were eluted and were separately applied to the thin-layer plate. The sequence of elution by reversed-phase chromatography of *p*-nitrobenzyloxyamine-labeled gangliosides was the reverse of the sequence of migration on TLC. That is, on silica gel smaller monosialogangliosides migrate most rapidly, and larger more polar polysialogangliosides migrate more slowly, while on reversed phase the highly polar *p*-nitrobenzyloxyamine-labeled ganglioside GT1b is the first major ganglioside to elute from the column. GC analysis of monosaccharides [10] cleaved from labeled gangliosides after resolution by HPLC (Table II) further confirmed the ganglioside identification suggested by TLC. The monosaccharide ratios closely conform to those expected based upon the suggested identification provided by TLC.

Quantitative comparison of ganglioside analysis by HPLC with analysis by TLC

Quantitative analysis of the major *p*-nitrobenzyloxyamine-labeled gangliosides provided by UV absorption at 254 nm following their resolution by HPLC was compared (Table III) with analysis of the native ganglioside preparation as provided by TLC. Ganglioside determination after resolution by TLC involved the spraying of the plate with resorcinol—hydrochloric acid, heating, and densitometry. The two methods yielded essentially the same quantitative data.

DISCUSSION

The difficulty of consistent resolution and quantitation of the gangliosides by TLC [11–13] or by conventional column chromatography, as well as the

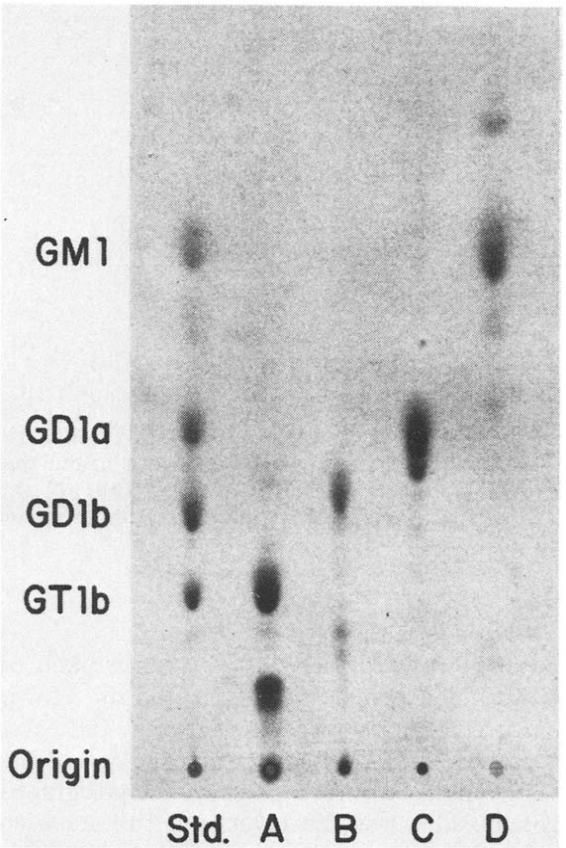

Fig. 4. Thin-layer chromatogram of *p*-nitrobenzyloxyamine-labeled gangliosides after resolution by HPLC. The standard lane was spotted with native brain gangliosides (14 μg N-acetylneuraminic acid). Lane A was spotted with the labeled ganglioside (23 μg N-acetylneuraminic acid) first to elute on HPLC (Peak A, Fig. 3). Lane B was spotted with 14 μg N-acetylneuraminic acid of *p*-nitrobenzyloxyamine-labeled ganglioside (Peak B, Fig. 3). Lane C was spotted with 21 μg N-acetylneuraminic acid of *p*-nitrobenzyloxyamine-labeled ganglioside (Peaks C and C′, Fig. 3). Lane D was spotted with 19 μg N-acetylneuraminic acid of *p*-nitrobenzyloxyamine-labeled ganglioside (Peaks D and D′, Fig. 3). The HPTLC plate was developed in chloroform—methanol—water (60:35:8) containing 20 mg $CaCl_2 \cdot 2H_2O$. The plate was sprayed with resorcinol—hydrochloric acid and placed in an 110°C oven for 15 min [6].

number of known different ganglioside species [14], make use of the superior resolving power of HPLC attractive for the resolution and quantitation of these glycolipids. Tjaden et al. [15] demonstrated the feasibility of resolving native gangliosides by HPLC expanding on methods developed for TLC, that is a silica gel column and a similar solvent system were used along with a moving wire detector. It was our goal to develop an assay system for gangliosides that would rival or exceed the sensitivity of the resorcinol—hydrochloric acid assay and so be applicable to clinical samples having low levels of gangliosides and to general experimental applications. To accomplish this goal, we chose to add a UV-absorbing compound with a high extinction coefficient to the ganglioside. We desired a method that would not alter the carbohydrate moiety in order to

TABLE II

MONOSACCHARIDE COMPOSITION OF LABELED GANGLIOSIDES AFTER RESOLUTION BY HPLC

Molar ratios of individual saccharides (relative to galactose) are given as determined by GC of trifluoracetate derivatives by the method of Zanetta et al. [10].

Peak	Retention time (min)	Major ganglioside	Monosaccharides		
			Galactose	*n*-Acetylgalactosamine	NANA
A	5–6	GT1b	2	0.91	3.20
B	6–7	GD1b	2	0.98	2.14
C, C′	9–12	GD1a	2	1.05	2.25
D, D′	12–15	GM1	2	1.12	1.26

TABLE III

COMPARISON OF HPLC QUANTITATION OF *p*-NITROBENZYLOXYAMINE-LABELED GANGLIOSIDES WITH TLC OF GANGLIOSIDES FOLLOWED BY RESORCINOL ASSAY FOR N-ACETYLNEURAMINIC ACID

Data are presented as mole percent (± S.D.) of ganglioside. After TLC, the plates were sprayed with resorcinol–hydrochloric acid [6] and quantitated by densitometry coupled with electronic integration of peak areas. For HPLC, the eluate was monitored at 254 nm using a fixed-wavelength detector, the output of which was quantitated by electronic integration of peak areas. Peaks C′ and D′ are not included. Twelve determinations were made by TLC of native human brain gangliosides. Similarly twelve determinations were made by HPLC of the same preparation of gangliosides which had been derivatized with *p*-nitrobenzyloxyamine.

Ganglioside	TLC	HPLC
GM1	30.5 ± 2.2	30.0 ± 1.6
GD1a	20.5 ± 1.5	20.4 ± 1.2
GD1b	23.7 ± 2.1	23.6 ± 1.8
GT1b + GQ	25.3 ± 3.3	25.9 ± 2.0

permit later chemical characterization of unknown or unusual peaks. Double bonds characteristically add ozone rapidly and quantitatively form ozonides (see Fig. 1). Although ozonides are unstable and give rise to multiple oxidation products when allowed to degrade randomly [16], the product can be selected by use of the appropriate reducing agent. For specific cleavage to aldehydes, we employed triphenylphosphine [17]. The nascent aldehyde group appeared to fulfill our requirements for a site for the addition of a UV label since there are no aldehyde groups on the native ganglioside molecule. The property of aldehydes to react quickly and quantitatively with hydroxylamines to form oximes is well known. A wide variety of aromatic hydroxylamines are available that absorb at 254 nm; *p*-nitrobenzyloxyamine was chosen since its oximes possess high extinction coefficients [18].

Experimental verification of the soundness of this approach was quickly attained. Ozonation in methanol proceeded quickly. Derivatization after cleavage with triphenylphosphine was carried out in the same vessel without

solvent change by simply adding an aliquot of a methanolic solution containing a carefully weighed quantity of *p*-nitrobenzyloxyamine. Derivatization conditions detailed herein should be carefully adhered to. In our initial attempts, it was found that in the presence of excessive molar amounts of *p*-nitrobenzyloxyamine (30-fold), some secondary derivatization products of the gangliosides were formed. It is not clear whether these are degradation products or follow a second addition of *p*-nitrobenzyloxyamine. In either case, it was found that the secondary derivatization proceeds slowly below 50°C. Closely controlling the molar ratio of *p*-nitrobenzyloxyamine to the aldehyde and lower temperatures eliminated detectable levels of the secondary set of peaks. The chemical nature of the secondary derivatives was not determined, but if it is assumed that a second addition is occurring, then some other carbonyl must be present since the reagent is specific for these. Perhaps an alternative canonical form of the aldose sugars exists momentarily and is able to add hydroxylamine. To remove *p*-nitrobenzyloxyamine following derivatization, the reaction mixture is immediately applied to a DEAE-Sephadex column. Triphenylphosphine, its oxidized product (both of which absorb in the ultraviolet), and *p*-nitrobenzyloxyamine-labeled long-chain fatty aldehydes are also removed in this step.

To apply this methodology to the assay of individual gangliosides, it is mandatory that their lipid structure be known. Although C_{18}-sphingosine and C_{20}-sphingosine will give rise to the same polar cleavage product, dihydrosphingosine contains no double bond and will fail to react. Fortunately, human brain gangliosides contain little reduced ganglioside sphingosine [19—21] and the amount present appears to be relatively constant. Fatty acids in human brain ganglioside are largely saturated [21—23] (stearic acid is the dominant component), but small amounts of unsaturated fatty acids have been detected. Undoubtedly, these double bonds also readily ozonate and derivatize. It is unlikely that a ganglioside derivatized once — either from an aldehyde derived from a sphingosine or from an unsaturated fatty acid — would be distinguished by the reversed-phase packing; ganglioside molecules unsaturated both in the sphingosine and the fatty acid surely add two molecules of *p*-nitrobenzyloxyamine. If such double-labeling is occurring in this procedure, then either the products do chromatograph identically with the mono-derivatized products or occur at such a low incidence they have escaped detection. Since quantitation by HPLC and by conventional TLC gave closely comparable results (Table III), it is evident that the problems just described do not hinder the application of the procedure to the assay of human brain gangliosides. The olefinic structure of the gangliosides from other sources must be known before this method can be applied with confidence for their assay. Fortunately, many studies have already been reported detailing the olefinic composition of the gangliosides isolated from various sources, e.g., the fatty acid and/or sphingosine structure of gangliosides isolated from brain of many species have been reported [23—27]; from human plasma [28]; from human adrenal [29]; from human lens [30]; from human, bovine, and rabbit retina [31]; from bovine [32] and human kidney [33]; from bovine mammary gland [34, 35]; and from rat liver [36].

Sensitivity of our assay exceeds that of TLC resolution of the gangliosides

followed by resorcinol—hydrochloric acid assay from the plate [6]. In our experience, a minimum of 10 μg of ganglioside NANA is required (15—25 μg is desirable) in order to resolve and quantitate individual gangliosides by TLC. We have resolved and quantitated less than 1 μg ganglioside NANA using this methodology; however, the ultimate sensitivity was not examined. Preliminary work (unpublished) on a derivatization method similar to that reported here but using dansyl hydrazine as a label, gave a similar resolution of gangliosides. Although we monitored UV absorption, this compound is strongly fluorescent at 525 nm, suggesting fluorescence monitoring should offer sensitivity to allow ganglioside resolution and quantitation of less than 100 ng of ganglioside NANA. Such a procedure would allow resolution and quantitation of gangliosides on 200 μg of brain tissue, which might prove useful in examining local minute areas of brain for anatomical distribution of gangliosides or following physiological or pharmacological experimentation.

Derivatization at the oxidized double bond of the ceramide by *p*-nitrobenzyloxyamine or by dansyl hydrazine can be generalized to other glycolipids. Cerebrosides, globosides, and other neutral glycolipids can be derivatized by a straightforward extension of the procedures detailed herein. It must be remembered, however, that foreknowledge of the olefinic structures of the molecules of interest must be available. Preliminary experiments suggest the retention times of *p*-nitrobenzyloxyamine-labeled neutral glycolipids are longer, and appropriate changes in the mobile phase are indicated.

HPLC of *p*-nitrobenzyloxyamine-labeled gangliosides offers a procedure that is subject to less variation than TLC, is more sensitive than the resorcinol assay (fluorescence labeling offering an even greater sensitivity), and is non-destructive of the oligosaccharide structure. The method is applicable to routine assay of ganglioside, gangliosides isolated from slow-growing cell cultures, and can also be applied to clinical analyses. The procedure is relatively rapid, the slowest steps are the ion-exchange chromatography and dialysis. Miniaturization should offer no obstacle since ozonation and derivatization can be performed in dilute solution and appropriately sized ion-exchange columns utilized. The potential of derivatization of the initial lipid extract by our procedures and then isolation by an adaption to HPLC of the ion-exchange chromatographic method of Ledeen et al. [9] has not been evaluated. Successful application, however, should provide a means to isolate and characterize gangliosides in a matter of hours rather than days or weeks as presently required.

ACKNOWLEDGEMENTS

Our studies were supported in part by the Institute Grants Program of the American Cancer Society, the Koebig Foundation and the South Carolina Appropriation for Medical Research.

REFERENCES

1 R.O. Brady and P.H. Fishman, Biochim. Biophys. Acta, 355 (1974) 122.
2 K. Sandhoff and H. Jatzkewitz, Adv. Exp. Biol. Med., 19 (1971) 305.

3 N.K. Gonatas and J. Gonatas, J. Neuropathol. Exp. Neurol., 24 (1965) 318.
4 J. Holmgren, Nature (London), 292 (1981) 413.
5 J. Folch, M. Lees and G.H. Sloane-Stanley, J. Biol. Chem., 226 (1957) 497.
6 K. Suzuki, J. Neurochem., 12 (1965) 629.
7 L. Svennerholm, Biochim. Biophys. Acta, 24 (1957) 604.
8 T. Miettinen and I. Takki-Luukkainen, Acta Chem. Scand., 13 (1959) 856.
9 R.W. Ledeen, R.K. Yu and L.F. Eng, J. Neurochem., 21 (1973) 829.
10 J.-P. Zanetta, W.C. Breckenridge and G. Vincendon, J. Chromatogr., 69 (1972) 291.
11 J.-P. Zanetta, F. Vitiello and J. Robert, J. Chromatogr., 137 (1977) 481.
12 K. Eberlein and G. Gercken, J. Chromatogr., 106 (1975) 425.
13 R.J. Penick, M.H. Meisler and R. McCluer, Biochim. Biophys. Acta, 116 (1966) 279.
14 M. Iwamori and Y. Nagai, Biochim. Biophys. Acta, 528 (1978) 257.
15 U.R. Tjaden, J.H. Krol, R.P. van Hoeven, E.P. Oomen-Meulemans and P. Emmelot, J. Chromatogr., 136 (1977) 233.
16 P.S. Bailey, Chem. Rev., 58 (1958) 925.
17 M. Beroza and B.A. Bierl, Anal. Chem., 39 (1957) 1131.
18 D.R. Knapp and S.A. Kruger, Anal. Lett., 8 (1975) 603.
19 T. Taketomi and N. Kawamura, J. Biochem. (Tokyo), 75 (1974) 1403.
20 M. Naoi and E. Klenk, Hoppe-Seyler's Z. Physiol. Chem., 353 (1972) 1677.
21 T. Abe and W.T. Norton, J. Neurochem., 23 (1974) 1025.
22 G. Rouser and A. Yamamoto, J. Neurochem., 19 (1972) 2697.
23 K. Sambasivarao and R.H. McCluer, J. Lipid Res., 5 (1964) 103.
24 E.G. Trams, L.E. Giuffrida and A. Karmen, Nature (London), 193 (1962) 680.
25 N.Z. Stanacev and E. Chargaff, Biochim. Biophys. Acta, 59 (1962) 733.
26 N.Z. Stanacev and E. Chargaff, Biochim. Biophys. Acta, 98 (1965) 168.
27 N.F. Avrova and S.A. Zabelinskii, J. Neurochem., 18 (1971) 675.
28 R.K. Yu and R.W. Ledeen, J. Lipid Res., 13 (1972) 680.
29 M. Igarashi, H.H. Schaumburg, J. Powers, Y. Kishimoto, E. Kolodny and K. Suzuki, J. Neurochem., 26 (1976) 851.
30 G.L. Feldman and L.S. Feldman, Lipids, 1 (1966) 21.
31 M. Holm, J.E. Mansson, M.T. Vanier and L. Svennerholm, Biochim. Biophys. Acta, 280 (1972) 356.
32 K. Puro and A. Keranen, Biochim. Biophys. Acta, 187 (1969) 393.
33 H. Rauvala, J. Biol. Chem., 251 (1976) 7517.
34 T.W. Keenan, Biochim. Biophys. Acta, 337 (1974) 255.
35 A.A. Bushway and T.W. Keenan, Lipids, 13 (1978) 59.
36 E.V. Dyatlovitskaya, A.M. Novikov and L.D. Bergel'son, Biokhimiya, 39 (1974) 552.