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DETERMINATION OF THE DISTRIBUTION OF THE ALIPHATIC GROUPS OF GLYCERYL ETHERS BY GAS–LIQUID CHROMATOGRAPHY OF THE DIACETYL DERIVATIVES

P. W. ALBRO AND J. C. DITTMER
Department of Biochemistry, St. Louis University Medical School, St. Louis, Mo. 63104 (U.S.A.)
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SUMMARY

The alk-1-enyl and alkyl glyceryl ethers produced by hydrogenolysis of the parent lipids were separated from each other and from other hydrogenolysis products by silicic acid chromatography. The isolated ethers were acetylated and the diacetates fractionated by gas–liquid chromatography on the basis of the different aliphatic groups. The free and acetylated alk-1-enyl glyceryl ethers were characterized by thin-layer chromatography, chemical analysis and infrared spectroscopy in addition to gas–liquid chromatography. The feasibility of the method for the determination of the distribution of the aliphatic groups in the alk-1-enyl glyceryl ethers of rat brain and of the alk-1-enyl and alkyl glyceryl ethers in ox brain ethanolamine phosphoglycerides was demonstrated.

INTRODUCTION

The distribution of the types of aliphatic groups found in the aldehydogenic moiety of plasmalogens has been determined in the past by gas–liquid chromatography of the dimethyl acetals[1–6], alcohols[2], acetylated alcohols[2,7], cyclic acetals of diols[8], and fatty acid methyl esters[2,9] derived from this portion of the plasmalogens. The most frequently used derivative, the dimethyl acetal, has the advantage of being simply prepared but there has been a recurring question in regard to the occurrence of artefacts[2,5,6,10]. They also have the disadvantage that fatty acids are methylated under the same conditions used in their preparation, and the fatty acid methyl esters are poorly resolved in most chromatographic systems. The preparation of the other derivatives cited employs more complicated preparative procedures but otherwise apparently gives satisfactory data except that the oxidation of double bonds in the preparation of fatty acid derivatives prevents the analysis of unsaturated aliphatic groups[2]. We present here another method of analysis in which the vinyl or alk-1-enyl glyceryl ethers derived from plasmalogens by hydrogenolysis are separated as the diacetates. The gas–liquid chromatographic separation of the diacetates of synthetic *cis* and *trans* hexadec-1-enyl glyceryl ethers has been described[11].

METHODS

Hydrogenolysis of lipids

Hydrogenolysis was carried out by a modification of the method described by THOMPSON[12]. A sample with the equivalent of up to 40 μmoles of lipid phosphorus (approximately 50 mg of lipid) was dissolved in 3 ml of dry diethyl ether and cooled in an ice bath. Twenty milligrams of $LiAlH_4$ was added, the reaction mixture was brought up to room temperature and then refluxed for 30 min. The mixture was returned to the ice bath and excess $LiAlH_4$ decomposed by the addition of 0.5 ml of ice cold water. The salts formed were centrifuged down and the ether supernatant solution decanted. The precipitate was washed in succession once with 2 ml of acetone and twice with 2 ml of diethyl ether. The hydrogenolysis products have been reported to be difficult to recover from the precipitate[12] and we are indebted to Dr. M. A. WELLS, University of Arizona, for the details of this quantitative extraction procedure. The washes were combined with the original ether solution and the mixture dried under reduced pressure. This solution contained sterols, long-chain alcohols derived from the fatty acids of the original lipids, alk-1-enyl ethers derived from the plasmalogens and the alkyl glyceryl ethers derived from both simple and complex parent lipids.

Column chromatographic fractionation of hydrogenolysis products

The hydrogenolysis products were separated into three major fractions by chromatography on silicic acid. The dried products were dissolved in hexane and chromatographed on a silicic acid column (Mallinkrodt, 100 mesh) made up in hexane. A loading of up to 20 mg of lipid per g of silicic acid was used. Sterols and fatty alcohols were eluted with 40 ml of 20 % ether in hexane. Alk-1-enyl glyceryl ethers were eluted with 15 ml of 45 % ether in hexane and a final fraction which was primarily alkyl glyceryl ethers was eluted with 20 ml of ether saturated with water. The volumes of solvents given are for a one-gram column. The "activity" of the silicic acid is important and for reproducible results it was heated at 100° overnight before use. We found it advisable to routinely monitor the fractionation by chromatographing samples of each fraction on silica gel thin-layer plates with solvent 3 (Table III).

While the alkenyl ethers obtained by silicic acid fractionation were satisfactory for gas–liquid chromatographic analysis of the diacetate derivatives, for purposes of characterization some preparations were subjected to further purification after conversion to the diacetates. The diacetate derivatives prepared as described below were taken to dryness under reduced pressure, dissolved in hexane and chromatographed on Florisil (60/100 mesh from Varian Aerograph, Walnut Creek, Calif.) columns prepared in hexane and eluted in succession with 15 ml of 5 % ether in hexane and 15 ml of 20 % ether in hexane. The Florisil was deactivated before use by mixing with 7 % its weight of water and allowing it to stand for several days in a closed container. The volumes given were for a one-gram column, and a loading of 10 to 20 mg of lipid per gram of adsorbent was used. The diacetyl alk-1-enyl glyceryl ethers were recovered with the second solvent.

Acetylation of hydrogenolysis products

Up to 100 mg of the lipid to be acetylated was taken to dryness under reduced

pressure, 1 ml of pyridine and 0.4 ml of acetic anhydride were added and the mixture left to stand overnight at room temperature in the dark. Two milliliters of water were added and the lipid extracted with two 3 ml aliquots of petroleum ether. The petroleum ether phase was washed once with 0.1 M Na_2CO_3, once with water and taken to dryness under reduced pressure. A similar procedure was used for the preparation of the dipalmityl alk-1-enyl ethers except the reaction was run in CCl_4–pyridine with palmitoyl chloride. We are indebted to Dr. M. A. WELLS for details of the latter preparation not yet published.

Conversion of the vinyl ethers to long-chain alcohols

An aliquot of approximately 2 μmoles of the alk-1-enyl glyceryl ethers was treated by a modification of the procedure described by FARQUHAR[2] for conversion of dimethyl acetals. The lipid was dried under reduced pressure in a screw-capped tube and hydrolized for 3 h at 50° in 5 ml of 90 % acetic acid to which 0.1 ml of saturated mercuric chloride solution was added. The aldehydes were isolated by pouring the reaction mixture into 25 ml of hexane, adding 25 ml of water and removing the upper hexane phase. This phase was washed twice with 25 ml of water and taken to dryness under reduced pressure. Alkaline conditions were avoided in order to guard against polymerization of the aldehydes. The isolated aldehydes were reduced by dissolving in 10 ml of dry diethyl ether and then adding dropwise to 15 ml of a 7 mg/ml suspension of $LiAlH_4$ in ether. From this point the mixture was handled as described in the hydrogenolysis procedure given above.

Gas–liquid chromatography

The acetyl derivatives were chromatographed in a Barber-Colman series 5000 apparatus with an argon ionization detector on a 122 × 0.5 cm glass column packed with 3 % SE-30 on 80–100 mesh acid-washed Chromosorb W (Applied Sciences Laboratory, College Station, Pa.) treated with hexamethyldisilazane. Runs were made with the column at 230°, the injection port at 260° and the detector at 275° with a flow rate of 60 ml of argon/min. Samples were injected dissolved in hexane. Acetates of fatty alcohols were chromatographed on a 0.5 × 180 cm column of 10 % ethylene glycol adipate on 80–100 mesh acid-washed Chromosorb W with a column temperature of 185°. The sample injection port and detector were maintained at 260 and 270°, respectively. A flow rate of 60 ml/min was used.

Materials

An ether-soluble fraction was prepared from a chloroform–methanol extract of beef brain and an ethanolamine glycerophosphatide fraction was prepared from it by chromatography on Florisil followed by further purification on alumina[13]. The ethanolamine phosphoglycerides were eluted from Florisil with chloroform–methanol–water (10:5:1.2) and from alumina with chloroform–methanol–water (30:20:2.5). The product appeared homogeneous on silica gel thin-layer plates[14], and analysis by differential hydrolysis[15] showed it to consist of 48 % plasmalogen and 6 % alkyl glyceryl ether. Rat brain lipid extracts were obtained as described elsewhere[15].

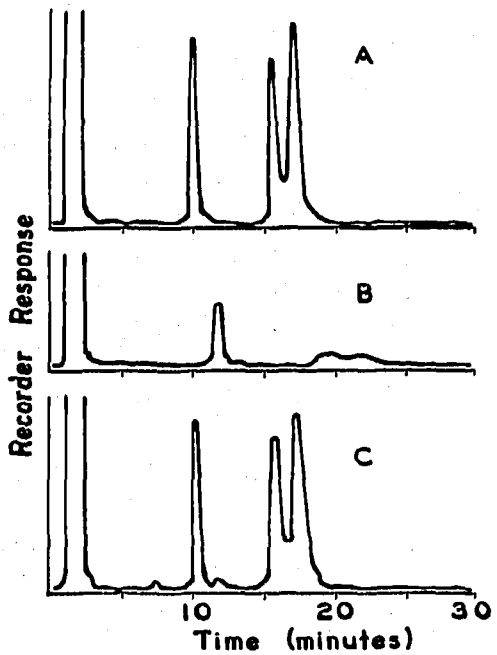
All solvents and other chemicals were analytical grade and were used without further purification except the hexane was redistilled from $KMnO_4$ and the fraction boiling between 67.5 and 68.5 was used. Diethyl ether was dried over Al_2O_3 before

use. Most standards for gas–liquid and thin-layer chromatography were either commercially available products or, as in the case of acetate derivatives and alcohols, were prepared from commercially available material by the methods indicated above. The exceptions are the palmital cyclic acetal of glycerol, which was a gift from Dr. D. N. RHODES, and the dimethyl acetal of palmitaldehyde, which was prepared from commercially available palmitaldehyde bisulfite[16].

RESULTS

Gas–liquid chromatography of the diacetyl derivatives of alkyl and alk-1-enyl glyceryl ethers

The glyceryl ethers prepared by hydrogenolysis of 180 mg samples of rat brain lipids were separated by silicic acid fractionation, acetylated and used to establish the conditions for gas–liquid chromatography described above (Fig. 1 and Table I). Diacetyl alkyl glyceryl ethers prepared from commercially available ethers were used



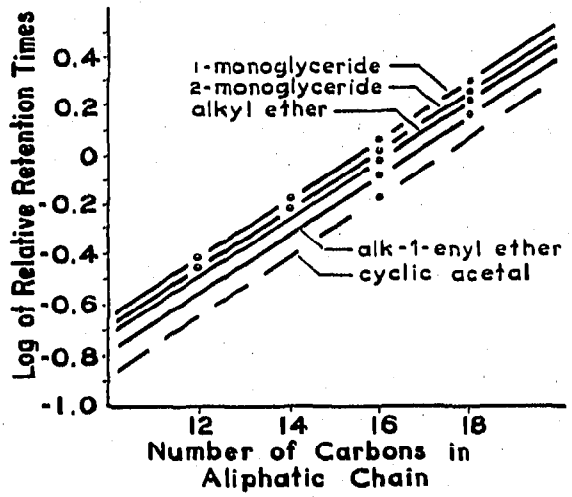


Fig. 1. Gas–liquid chromatograms on 3% SE-30 columns of the diacetate derivatives of (A) the alk-1-enyl, (B) the alkyl, and (C) the alk-1-enyl and alkyl glyceryl ethers prepared from rat brain lipids.

Fig. 2. Resolution on SE-30 columns of various acetylated lipid derivatives. The relative retention times were calculated on the basis of diacetyl chimyl alcohol equal to one.

to establish the identity of these compounds derived from brain lipids, and the identities of the diacetyl derivatives of the alk-1-enyl ethers were established by independent means described below. The acetate derivatives of α- and β-monoglycerides and the acetyl derivative of palmital cyclic acetal of glycerol and fatty alcohol acetates were also chromatographed. All of these compounds were potential contaminants of the fractions chromatographed. Fig. 2 shows the resolution obtained with the various compounds, except the alcohol acetates, which were eluted with the solvent front when they contained up to 18 carbon atoms.

TABLE I

RELATIVE RETENTION TIMES AND DISTRIBUTION OF THE DIACETATES OF ALK-1-ENYL AND ALKYL GLYCERYL ETHERS DERIVED FROM RAT BRAIN LIPIDS

Structure of aliphatic moiety[a]	*Relative retention times*[b]	*% distribution*	
		Sample 1	*Sample 2*
Alk-1-enyl ether			
16:0	0.87	24.2	24.3
18:0	1.56	39.7	41.1
18:1	1.45	36.0	34.4
Alkyl ethers			
16:0	1.00	39.2	37.5
18:0	1.80	26.4	27.0
18:1	1.64	34.4	35.3

[a] The first number indicates the number of carbons in the aliphatic chain and the second the number of double bonds exclusive of the vinyl ether.
[b] Calculated relative to the retention time of chimyl diacetate.

Identification of the diacetyl alk-1-enyl glyceryl ethers

Since standards of individual alk-1-enyl glyceryl ethers were not available to us, it was necessary to establish the identity of the peaks obtained by gas–liquid chromatography by converting to a derivative for which standards were available. Fatty alcohols corresponding to the aliphatic chain of the ethers were prepared by hydrolyzing the vinyl ethers to the aldehyde and reducing the aldehyde. For this purpose the alk-1-enyl glyceryl ether fraction from ox brain ethanolamine glycerophosphatides was used. Prior to reduction, the fraction was shown to be free of alcohols by thin-layer chromatography as described below. The alcohols were acetylated by the method described for glyceryl ethers and the retention times of the acetates were compared with standard fatty alcohol acetates prepared by reducing the corresponding fatty acid methyl esters with $LiAlH_4$ and acetylating. Table II gives the retention times of the alcohol acetates derived from ox brain ethanolamine phosphoglycerides and the relative distribution of the alcohol acetates and the alk-1-enyl glyceryl ether diacetates from the same source. On the basis of these data an

TABLE II

CHROMATOGRAPHIC CHARACTERIZATION OF THE ALCOHOL ACETATES PREPARED FROM THE ALK-1-ENYL GLYCERYL ETHERS DERIVED FROM OX BRAIN ETHANOLAMINE PLASMALOGENS

Structure of aliphatic chain	*Relative retention times of alcohol acetates*	*% distribution*	
		Alcohols derived from ethers[a]	*Alk-1-enyl ethers as diacetates*
16:0	1.0	34.0	33.0
18:0	1.89	26.8	28.2
18:1	2.12	38.0	38.6

[a] Traces of several components were observed and identified on the basis of retention times as most probably 12:0, 14:0, 16:1, 17:0 and 18:2.

TABLE III

THIN-LAYER CHROMATOGRAPHY OF GLYCERYL ETHERS, ACYL DERIVATIVES AND RELATED STANDARDS

Chromatograms were run on 0.5 mm Silica Gel G plates with the following solvent systems: (1) *n*-heptane–ethyl acetate (20:3); (2) *n*-hexane–diethyl ether–methanol (90:20:4); (3) chloroform–methanol (95:5); (4) benzene–chloroform (1:3).

Compound	R_F *value*			
	Solvent 1	*Solvent 2*	*Solvent 3*	*Solvent 4*
Heptacos-13-ene	0.97	0.98	0.98	0.90
Cholesterol			0.81	0.13
Cholesterol stearate	0.91	0.96	0.98	0.97
Methyl palmitate	0.85	0.96	0.98	0.77
12-Tricosanone	0.81	0.95	0.98	0.89
Chimyl dipalmitate	0.61	0.94	0.96	0.57
Alk-1-enyl glyceryl ether dipalmitate (brain)	0.61	0.93	0.95	0.33
Tripalmitin	0.52	0.89	0.97	0.46
Tricosan-12-ol	0.41	0.58	0.91	0.57
Behenyl alcohol	0.21	0.33	0.69	0.23
Behenyl acetate			0.70	0.79
Oleyl alcohol	0.18	0.31	0.67	0.25
Oleyl acetate			0.70	0.78
1,3-Dipalmitin	0.13	0.31	0.87	0.08
1,2-Dipalmitin	0.07	0.24	0.87	0.08
Palmital cyclic glyceryl acetal	0.06	0.20	0.63	0.73
Palmital cyclic glyceryl acetal acetate				0.24
Monopalmitin	0.00	0.05	0.30	0.00
Stearic acid	0.00	0.00	0.15	0.06
Alk-1-enyl glyceryl ether	0.00	0.12	0.37	0.32
Alk-1-enyl glyceryl ether diacetate			0.32	0.25
Batyl alcohol	0.00	0.07	0.27	0.02
Batyl diacetate			0.92	0.23
Chimyl diacetate			0.92	0.23

assignment of the structures indicated in Tables I and II was made for the peaks observed in the chromatography of the diacetate derivatives of the alk-1-enyl ethers.

Thin-layer chromatography of glyceryl ethers, their acyl derivatives and associated compounds

The mobilities of a number of compounds on Silica Gel G (Merck) thin-layer plates developed in four different solvent systems are given in Table III. The standards were selected primarily because they could appear as lipid-soluble products after hydrogenolysis of lipids. The compounds were detected by spraying in succession with acidic dinitrophenylhydrazine (saturated 2 *N* HCl solution) and Rhodamine 6G (0.005 % aqueous solution) as carbonyl specific and general reagents, respectively. The freshly prepared free alk-1-enyl ethers and their diacetates showed only one spot which reacted strongly with dinitrophenylhydrazine. Both the free ether and diacetates showed additional spots after storage for a few days. The alkyl ethers from brain lipids generally appeared homogeneous and chromatographed similarly to standards. In some preparations of alkyl ethers from total rat brain, a second minor component was detected with solvent 3 (Table III), which had a slower mobility than the main component. This compound was not identified.

Vinyl ether and aldehyde content of the alk-1-enyl glyceryl ethers and derivatives

The alk-1-enyl ethers isolated from ox brain ethanolamine phosphoglycerides and their acyl derivatives were used for these analyses. The vinyl ether content of the free ethers, the diacetyl ethers and dipalmitoyl ethers was analyzed by the specific iodination assay of GOTTFRIED AND RAPPORT[17] and the values obtained were compared with the total aldehyde content as determined in the free ethers by the dinitrophenylhydrazine method of SCHWARTZ *et al.*[18] and in the diacetate by the *p*-nitrophenylhydrazine method of PRIES AND BÖTTCHER[19]. With the latter assays, the ratio of moles of aldehyde determined/moles of aldehyde calculated from mass was found to be 0.98 for both the free ether and dipalmitoyl derivatives and 1.0 for the diacetate derivative. The iodination assay gave somewhat equivocal data. The free ethers reproducibly gave values of only 17 % that expected from the aldehyde content, and the dipalmitin derivatives would not take up any iodine under the usual assay conditions. When the reaction with the dipalmitin derivatives was run by adding 1 ml of 6×10^{-4} *N* methanolic I_2 to 1 ml of a chloroform–methanol (2:1) solution of the compound followed by the addition of 0.5 ml of 3 % aqueous KI, an iodine uptake equivalent to 80 % of the aldehyde content was found. Unlike the free ethers and dipalmityl derivatives, the diacetates reacted to give an iodine uptake with the standard assay procedure which was 92 % of the theoretical uptake based on aldehyde content. This is in good agreement with the uptake found with intact phospholipids with this method.

While these experiments suggest that the isolated free ethers and dipalmitate derivatives may not be entirely in the vinyl ether form, we believe that the discrepancies are due to limitations of the iodination assay system. The differences observed in reactivity of plasmalogen preparations from different sources[20] support this. The possibility that the free ether preparation is in fact the cyclic acetal was ruled out on the basis of gas–liquid and thin-layer chromatographic analyses and, of course, this structure is impossible with the dipalmitate derivative. The possibility that the vinyl ether may be in equilibrium with the hemiacetal and that this structure is favored in the free ether and dipalmitate cannot be entirely ruled out.

Infrared analyses

Spectra of thin films dried on NaCl plates were obtained with a Perkin Elmer Model 21 Spectrometer. Reference spectra were run on chimyl alcohol, chimyl diacetate, palmital cyclic glyceryl acetal and its acetate, palmitaldehyde, tripalmitin and oleyl acetate. The spectra of the free alk-1-enyl ethers, the diacetates and dipalmitates were very similar to the spectra of chimyl alcohol and its diacetates except the vinyl ethers showed a strong peak at 6.0 μ and a peak at 13.55 μ which are probably due to the *cis* double bond and =C–H, respectively[21]. The free ethers showed a stronger band at 8.95 μ which is presumably evidence for a predominance of the ether linkage on the primary hydroxyl group[22].

Hydrogenation of the alk-1-enyl ethers

Reduction of the alk-1-enyl ethers to the alkyl analogues would provide a convenient route to the identification of the original compounds. Numerous reports of the hydrogenation of vinyl ethers have appeared. The conditions used for reduction have varied from hydrogen at atmospheric pressure with platinum oxide as

the catalyst, under which conditions 96.6 % conversion in 20 min was reported[23], to the use of 4.0 atm of pressure for 3 h with the same catalyst[24]. We were limited to working at or near atmospheric pressure and under such conditions attempted to reduce ox brain ethanolamine phosphatides, the free alk-1-enyl glyceryl ethers and their diacetates with platinum oxide, palladium on charcoal or palladium on $BaSO_4$ as catalyst. Gas–liquid chromatography of the acetylated products failed to show any saturated ethers except, as in the case of the ethanolamine phosphatides, those present in the unreduced lipids. Free aldehydes and fatty alcohols were observed by infrared spectroscopy and gas–liquid chromatography, respectively, and the former predominated with short reaction times. We are unable to account for our failure to obtain conversion under essentially the same conditions as used by SCHOGT *et al.*[23] but point out that because of reduction to the alcohol, the disappearance of dinitrophenylhydrazine-reactive material as used by these workers as a criterion for conversion to the saturated ether is not reliable.

Periodate oxidation of the alk-1-enyl glyceryl ethers

One goal of preparing the saturated analogues of the vinyl ethers was so that periodate oxidation, selective for vicinal hydroxy groups, could be used to determine the proportion of 1- (3-) and 2- ethers. Having failed in the preparation of the alkyl ethers, periodate oxidation was carried out on the vinyl ethers directly at pH 7 (ref. 25). An aliquot of the alk-1-enyl ether fraction from ox brain ethanolamine phosphoglycerides which contained the equivalent of 1.16 μmoles of aldehyde was taken to dryness and dissolved in 1 ml of 95 % ethanol. One ml of 0.1 *M*, pH 7.0 acetate buffer and 0.5 ml of 0.1 *M* $NaIO_4$ were added and the mixture was incubated in the dark at 25° until no further decrease in absorption at 300 mμ could be detected over a 15 min interval. The total reaction time was 3 h. One half milliliter of 10 % aqueous $NaHSO_3$ was added and 1 ml aliquots were taken for the analysis of formaldehyde[26]. The equivalent of 1.19 μmoles of formaldehyde was formed or 103 % of the yield expected if all of the alk-1-enyl ethers were linked with the primary hydroxyl group. A sample of chimyl alcohol, 0.235 μmoles, yielded exactly 0.235 μmoles of formaldehyde under the same conditions of oxidation.

Quantitative recovery and precision of the method

Samples of ox brain ethanolamine phosphoglyceride and rat brain total lipid were assayed for total aldehyde content by Schiff's assay[16], and it was shown by the same assay that from 94 to 96 % of the total aldehyde was recovered after hydrogenolysis and of that recovered from 90 to 95 % was recovered in the alk-1-enyl glyceryl ether fraction isolated from silicic acid. When lipid fractions which had been stored for several weeks were assayed, the aldehyde content as determined by Schiff's assay was as much as two times the values consistent with the plasmalogen content determined by selective hydrolysis[15]. The aldehyde values were restored to a consistent value when the lipid was chromatographed on a silicic acid column eluted in succession with 20 ml/g of chloroform and 10 ml/g of methanol. The phospholipids were eluted with the methanol. Aldehyde assays on aged samples after hydrogenolysis also were consistent with the plasmalogen values of the original extract which indicates that whatever gives the high aldehyde values with Schiff's reagent is lost during the hydrogenolysis or subsequent isolation of the products.

TABLE IV

REPRODUCIBILITY OF THE ASSAY FOR DISTRIBUTION OF BRAIN GLYCERYL ETHERS

Sample	*% distribution ± S. D.*[a]		
	16:0	*18:0*	*18:1*
Rat brain lipid alk-1-enyl ether	24.1 ± 0.6(6)	39.2 ± 1.4(6)	36.2 ± 1.3(6)
Ox brain ethanolamine phosphoglycerides			
alk-1-enyl ether	30.8 ± 1.9(4)	31.7 ± 1.8 (4)	36.7 ± 1.4(4)
alkyl ether	41.3 ± 1.7(3)	19.7 ± 0.5(3)	37.7 ± 1.2(3)

[a] The designation of the aliphatic group is the same as used in Table I. The number in parentheses after the standard deviation indicates the number of samples assayed.

The precision of the method was tested by assaying the distribution of aliphatic groups in the alk-1-enyl glyceryl ether fraction from 25 mg samples of rat brain lipids and from samples of ox brain ethanolamine phosphoglycerides that contained 40 μmoles of phosphorus. The alkyl ethers from the latter samples were also assayed. At least five times the quantity of rat brain lipid would have been needed to obtain satisfactory data on the alkyl ethers. As can be seen from the data in Table IV, the method is generally reproducible within approximately 5 %.

DISCUSSION

The conversion of plasmalogens and glyceryl ether derivatives to the alk-1-enyl and alkyl glyceryl ethers by hydrogenolysis was used by THOMPSON[12,27] to measure tissue content and the extent of incorporation of various labeled precursors into the parent lipids. More recently, WOOD AND SNYDER[22] have used densitometry of thin-layer chromatograms of the alkyl and alk-1-enyl glyceryl ethers derived from lipids by hydrogenolysis as a measure of the tissue content of the parent lipids. We have extended these applications by use of a simple silicic acid fractionation and acetylation of the ethers to permit the gas–liquid chromatographic analysis of the distribution of the various types of aliphatic groups. We have affirmed the identification and extended the characterization of the hydrogenolysis products.

The feasibility of using the diacetates to characterize the ether fractions by gas–liquid chromatography has been demonstrated by a check on the recovery of the total aldehydogenic material in lipid extracts in the appropriate fractions before and after acetylation and by a combination of chromatographic, chemical and infrared spectroscopic characterization of the isolated diacetate derivatives. As applied to rat brain lipids and ox brain ethanolamine phosphoglycerides, the method gives reproducible results and the identification of the major aliphatic groups in the alkyl ethers of rat brain lipids is in agreement with the identification made on the basis of the isopropylidine derivatives[15]. No previous information is available on the distribution of aliphatic groups in the alk-1-enyl ethers of rat brain, but the similarity to the aliphatic groups of the alkyl ethers is consistent with the metabolic interrelationship of alkyl and alk-1-enyl ethers in rat brain proposed by HORROCK AND ANSELL[28] on the basis of ethanolamine incorporation into the parent compounds.

Studies on the interrelationship of these two classes of lipids and those studies concerned with the origin of the aliphatic groups[12] can be made more definitive by the isolation and examination of the individual ethers by the method described here.

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