Analysis of the core components of Alzheimer paired helical filaments A gas chromatography/mass spectrometry characterization of fatty acids, carbohydrates and long-chain bases

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Abstract **We have carried out a fatty acid and carbohydrate compositional analysis of the protease-resistant core of paired helical filaments (prcPHF) isolated from six Alzheimer's diseased brains. Fatty acids, long-chain bases and monosaccharides were characterized by gas chromatography/mass spectrometry (GC/MS) of fatty acid methyl esters, trimethylsilylated long-chain bases, peracetylated alditol acetates and trimethylsilyl methyl glycosides. Glucose and mannose were found to be the only carbohydrate components. Four of the six prcPHF samples contained only glucose while the remaining two samples contained between 30–40% mannose in addition to glucose. None of the samples were found to contain either hydroxylated fatty acids or long-chain bases. The average fatty acid profile of prcPHF was highest in stearic (C18:0) and palmitic acids (C16:0) with less than 10% unsaturated fatty acids. By comparing the carbohydrate and lipid composition of prcPHF to similar data for other brain glycolipids, it was determined that prcPHF is a unique glycolipid, distinct from cerebrosides, gangliosides or brain phospholipids. The fatty acid and carbohydrate composition of a glycolipid isolated from a population of normal brains according to the prcPHF protocol was found be identical to that of prcPHF glycolipid. It is possible that subtle differences in structure or indigenous factors are responsible for the initiation of PHF formation in vivo.**

Key words: GLC; Mass spectrometry; GC/MS; Alzheimer's disease; AD; PHF; Glycolipid

1. Introduction

Neurofibrillary tangles (NFT) and senile plaques (SP) can be found in the hippocampus of normal aged individuals, but occur with much greater density in the hippocampus and temporal cortex of patients suffering from Alzheimer's disease (AD) and Down syndrome individuals greater than 45 years of age [1–3]. The presence of NFT and SP in histological sections serves as a basis of diagnosis for AD and the density of tangles has been correlated with the severity of dementia [4,5]. The NFT occur in the perinuclear cell cytoplasm and are composed primarily of bundles of paired helical filaments (PHF) [6–11]. Since PHF are a common element to both lesions and are diagnostic of AD, it is reasonable to assume that an understanding of their composition might lead to a better understanding of the etiology of AD.

PHF isolated from AD brain NFT remain insoluble under even the most stringent conditions used to solubilize protein [12]. For these reasons, they are difficult to characterize using standard biochemical techniques. It has been shown that the outer, protease-sensitive, fuzzy coat of PHF is composed of a hyperphosphorylated form of the microtubule-associated tau protein [13–17]. Using scanning transmission electron microscopy it has been estimated that tau outer coat comprises only 17% of the PHF structure, and the composition of the remaining 83% that represents the protease resistant core remains unknown [17].

We have shown by total amino acid analysis that the protease-resistant PHF core (prcPHF) structure contains less than 5 percent protein [18]. X-ray microprobe analysis of these intact prcPHF confirmed that they were nonproteinaceous in composition [19]. An independent laboratory has previously shown that neurofibrillary tangles could be immunolabeled in situ with a monoclonal antibody to a ganglioside [20]. They have since shown by immunoelectron microscopy that this antibody will immunolabel isolated, intact PHF [21]. The high-resolution ^{1}H NMR spectrum of prcPHF in d_6-DMSO:D_2O (9:1) contains resonances which can only be attributed to either fatty acid or sugar protons [18]. No resonances are present which can be ascribed to a sphingosine long-chain base or to a glycerol backbone, suggesting the absence of phospholipid, ganglioside or cerebroside.

The goal of the present study was to characterize the fatty acid and carbohydrate components of prcPHF isolated from a population of AD individuals. Common compositional elements of the prcPHF samples are then compared to those of similarly isolated glycolipid from normal individuals in order to deduce features which might be responsible for filament formation.

2. Materials and methods

2.1. Purification of prcPHF

Frozen, AD tissue for PHF isolations was obtained from Dr. Wallace Tourtellotte of the National Neurological Research Specimen Bank (Los Angeles, CA). Tissue from normal aged individuals used for control preparations was a gift from Dr. John Richardson, Montreal General Hospital (Montreal, Quebec, Canada). All samples were provided with a clinical history and a report of the neuropathological examination. The clinical diagnosis of AD was made based on the criteria described by Khachaturian [5]. The AD and contol brains used in this study were randomly selected. AD brains with moderate to severe numbers of senile plaques and neurofibrillary tangles were used for this study, while control brains contained no neurofibrillary pathological involvement. Reagents used in prcPHF and control preparations were obtained from Sigma Chemical Co. (St. Louis, MO).

The prcPHF were isolated from gray matter rich in NFT using a modification of SDS extraction method that included incubating the

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PHF with protease to remove protein contaminants and with DNase to remove any contaminating DNA. This procedure has previously been described in detail elsewhere [18,19,22]. A control fraction was isolated using identical brain regions from the non-AD brains by the same method used to isolate the prcPHF. The quantity of each prcPHF and control fraction was visually assessed by examining each preparation by electron microscopy [22]. Each preparation was further monitored for compositional consistency by high-resolution NMR to insure that their spectrum was identical to those previously published [18].

2.2. Preparation of trimethylsilyl ethers of methyl glycosides and long-chain bases

A general procedure for the preparation of trimethylsilyl ethers of methyl glycosides and long-chain bases has been described [23–26]. Accordingly, 500 μl of 0.6 N anhydrous methanolic HCl was added to a freeze dried PHF sample aliquot (300 μl out of 1 ml total volume) or to 2 mg sugar standard. The samples were then heated at 75°C overnight in sealed tubes. The methanolic HCl was evaporated at about 40°C under a stream of argon. An N-acetylation reaction was performed by adding 200 μl of dry methanol, 20 μl of pyridine and 20 μl of acetic anhydride. The tubes were sealed again and allowed to stand at room temperature for at least 6 h. After this period, the reaction mixture was evaporated to dryness under a stream of argon. 200 μl of Sil-A reagent (Sigma) was added and the sealed tubes heated at 80°C for 20 min. The reaction mixture was cooled, centrifuged and the supernatant transferred to a sealed clean vial. The sample, dissolved in 100 μl of dichloromethane, was then ready for GC injection.

2.3. GC/MS analysis of fatty acid methyl esters, trimethylsilyl ethers of methyl glycosides and long-chain bases

To a freeze dried PHF sample aliquot (300 μl aliquot out of 1 ml total volume) 300 μl of 10% HCl in methanol was added in a sealed tube. The mixture was heated at 80°C for 2 h. The reaction mixture was cooled to room temperature and then successively extracted with two 200 μl portions of hexane. The organic phase was transferred to a clean vial, the solvent evaporated under argon, and the sample dissolved in 100 μl dichloromethane. The sample was then ready for GC injection [24,27].

The assay for fatty acid methyl esters and trimethylsilyl ether derivatives of methyl glycosides and long-chain bases was carried out on a HP-5390 Gas Chromatograph equipped with a flame ionization detector (FID), and a Nukol (Supelco) 30 m, 0.32 mm i.d. capillary column [24,27,28]. 3 μl of each sample was injected in the splitless mode while the same volume of each standard was injected in the split mode. Nitrogen (zero grade) was used as the carrier at a flow fixed by using 15 psi of pressure on the carrier gas with a split ratio of 1:85. The oven temperature was held constant at 100°C for 3 min, then increased at 10°C per min to a final temperature of 195°C. Injector and detector temperatures were 250°C. In most cases samples could be identified by comparison of their retention times to those of known standards.

A Shimadzu QP-1100 EX instrument was also used under identical conditions as above when mass detection (MSD) was needed. Saturated fatty acid methyl esters were identified on the basis of their McLaferty rearrangement product at mass 74 [29]. The remaining fragment ions present in the spectrum were used to characterize the parent hydrocarbon. Hydroxyl groups present along the hydrocarbon chain of the fatty acid will cause a change in the fragmentation pattern when compared with the non-hydroxylated form [27]. The base peak indicates the position of the hydroxyl group, since its mass corresponds to the fragment generated by cleavage of the hydrocarbon chain on either side of the hydroxyl group. For α-hydroxylated fatty acids, the base peak is the molecular ion [28].

2.4. GC/MS analysis of alditol acetates

The monosaccharide composition of prcPHF and control glycolipid samples was determined by GC/MS of alditol acetate derivatives [25]. Accordingly, a PHF or control sample aliquot (300 μl out of 1 ml total volume) was freeze dried and then hydrolyzed by resuspending in 2 M trifluoroacetic acid in a sealed tube at 75°C for 2 h. The acid was evaporated under a flow of argon and residual acetic acid was eliminated by addition of isopropanol, followed by blowing to dryness with argon (2 × 250 μl). The hydrolyzate was dissolved in 200 μl of 1 M ammonium hydroxide containing 10 mg/ml of sodium borodeuteride and kept at room temperature for at least 1 h. The reduction was stopped by the dropwise addition of glacial acetic acid. When bubbling ceased, 0.5 ml of methanol was added and the resulting mixture evaporated under a stream of argon. The addition of methanol was repeated three times and the sample evaporated to complete dryness each time. The dry residue was redissolved in 0.1 ml of acetic anhydride and 0.1 ml of dry pyridine. The tube was sealed, vortexed and heated to 120°C for 20 min. The acetylation was stopped by drying under a stream of argon and the residue dissolved in 0.5 ml of dichloromethane. 0.5 ml of water was added and the organic phase transferred to a clean tube containing a small amount of anhydrous sodium sulfate. The dry or-

Table 1
Fractions of long-chain fatty acids in prcPHF and control glycolipid[a]

		Saturated fats								
		Unsaturated fats								
	Age/sex/severity	C14	C16	C18	C20	C22	C24	C16:1	C18:1	
	C20:1	C22:1	C24:1	Sat						
		prcPHF samples								
AD1590	83/F/S	3.4	18.3	31.0	11.2	2.4	0.8	4.2	17.5	19.
AD1528	86/M/M	0.0	20.7	57.4	2.6	0.0	0.0	0.0	11.4	4
AD1519	89/F/M	7.6	39.6	17.6	22.2	0.0	0.0	0.0	3.6	9.
AD1499	70/F/S	2.9	45.6	29.1	12.2	0.0	0.0	0.0	2.9	7.
AD1937	84/M/M	12.0	16.1	67.1	6.0	1.2	0.4	0.0	4.0	4.
AD1678	87/F/S	11.6	41.1	10.1	9.9	3.2	0.6	0.0	5.4	12.
Average ± S.D.		12.0 ± 14.7	27.0 ± 13.7	32.1 ± 20.5	10.7 ± 5.6	1.0 ± 1.2	0.3 ± 0.3	3.8 ± 7.7	6.4 ± 5.6	6. 3.
		Control samples								
CT714	44/M	8.1	21.7	32.3	10.6	1.1	0.0	1.5	14.3	10.
CT92119	70/M	21.3	18.6	52.7	7.4	0.0	0.0	0.0	0.0	0.
CT9205	75/F	5.7	34.1	18.7	0.3	0.0	0.0	13.3	27.4	0.
CT661	26/F	9.2	25.3	39.6	11.5	0.0	0.0	0.0	9.9	4.
CT1226	57/M	3.6	15.0	44.0	17.0	0.0	0.0	0.7	10.0	9.
CT738	25/M	5.9	23.2	32.5	7.7	0.0	0.0	1.5	17.7	11.
CT1374		15.8	45.6	11.9	8.3	0.3	0.0	6.4	4.9	6.
CT1269		4.2	34.9	44.9	7.1	0.0	0.0	2.0	4.1	2.
Average ± S.D.		9.2 ± 5.8	27.3 ± 9.5	34.6 ± 12.9	8.7 ± 4.4	0.2 ± 0.4	0.0 ± 0.0	3.2 ± 4.3	11.0 ± 8.2	5. 5.

[a]As determined by Gc/MS of fatty acid methyl esters. Values are expressed as percent of total fatty acid.

ganic solution was transferred to a vial, evaporated and reconstituted in 100 μl of dichloromethane.

GC analysis of alditol acetates was carried out in a fashion similar to the GC analysis of fatty acid methyl esters except that the oven temperature was held constant at 170°C for 5 min, then increased at a rate of 10°C per min to a final temperature of 195°C. Alditol acetates were identified by the presence of mass fragments at 43 (base peak), 115, 103, 128, 145 and 187. GC/MS identification of trimethylsilyl derivatives of long-chain base standards was carried out as described [23].

3. Results

3.1. GC/MS characterization of fatty acid methyl esters

Fatty acids were characterized as their methyl ester derivatives by GC/MS analysis. Table 1 summarizes the results for prcPHF isolated from six AD brains and for control samples isolated from six normal brains according to the prcPHF isolation protocol. Hydroxylated and branched-chain fats were not detected. There was no recognizable correlation between the age/sex of the patient or severity of AD and the fatty acid composition of the isolated prcPHF. The fatty acids characteristic of prcPHF do not differ significantly from those of the controls.

The average fatty acid profile for the prcPHF samples is shown graphically in Fig. 1, where it is compared to the profile for fatty acids in the phospholipid components of whole brain grey and white matter [29]. Phospholipids make up approximately 60% of the fatty acids in brain tissue and like the prcPHF, they contain very little hydroxylated fatty acids. Gangliosides and cerebrosides, which contain most of the remaining fat, have between 30% and 50% of their fatty acid α-hydroxylated [31–35]. Of the non-hydroxylated fats, gangliosides contain approximately 90% stearic acid (C18:0) while cerebrosides contain about 40% C24:0 and C24:1 fatty acids. In contrast, the prcPHF and control glycolipids contain only about 30% stearic acid and only trace amounts of C24:0 or C24:1. As shown in Fig. 1, the quantities of fatty acid components also differ from fats characteristic of phospholipids. While phospholipids contain similar amounts of C16:0 and C18:0 as prcPHF, they contain substantially more C18:1 and substantially less C14:0 and C20:0 fats.

3.2. Carbohydrate and long chain base composition

Carbohydrate composition was determined by GC/MS analysis of alditol acetate derivatives formed from monosaccharides released from the acid hydrolysis of prcPHF or control glycolipid. Since sialic acid is degraded using this procedure [25], the carbohydrate composition was verified on our samples by GC/MS analysis of the trimethylsilyl derivatives of methyl glycosides. A similar derivatization procedure may also be used for the determination of long-chain bases, such as sphingosine, dihydrosphingosine and phytosphingosine [23]. Glucose and mannose were the only sugars detected in either the prcPHF or the control glycolipid samples (Table 2). Glucose occurred as the major component in all of the samples (an average of 85% of the total for the prcPHF and 96% of the total for the control) and in the majority of samples, it was the only monosaccharide detected. Three of the six control glycolipid samples contained between 5–12% mannose. The mannose fraction was significantly higher in two of the six prcPHF samples, comprising 30–40% of the total carbohydrate. No long-chain bases or sialic acid were detected in any of our samples. This finding is in agreement with the absence of proton resonances assignable to these species in the NMR of prcPHF [18].

4. Discussion

The GC/MS analysis of the lipid components of prcPHF shows the absence of long-chain base or glycerol trimethylsilyl derivatives which would be excepted had a cerebroside, ganglioside or phospholipid component been present. Our preliminary studies indicate that this result is corroborated by the absence of signals arising from these components in the ^{1}H NMR of intact or acid hydrolyzed prcPHF [18]. We have been unable to find any solvent conditions, including organic solvents, which are able to selectively extract the fatty acid component of prcPHF from the carbohydrate component. This, along with the absence of long-chain base or glycerol moieties, suggests that the fatty acids are either covalently linked to the carbohydrate or form a strongly associated complex with it.

Heterogeneity in the carbohydrate composition between prcPHF isolated from different AD brains is evident from GC/MS analysis of alditol acetates (Table 2). Four of the six prcPHF samples were found to contain glucose as their sole monosaccharide component. In two of the samples a significant fraction of mannose (up to 40%) was present in addition to glucose. As many as three independent preparations were done from many of these brains and in each case, a repeatable and consistent ratio of glucose and mannose was found. Thus, the amount of glucose and mannose in each brain fraction appear to be characteristic of that particular brain and not the result of an experimental variable in a particular preparation.

The average fatty acid profile of prcPHF glycolipid, as determined by GC/MS analysis of fatty acid methyl esters, is unique in that it is unlike the fatty acid profile of brain ganglioside,

Table 2
Carbohydrate composition of PHF from AD brains and glycolipid isolated from control brains according to PHF isolation procedure[a]

AD/PHF				Control brain			
Case	Age/sex/severity	Glucose	Mannose	Case	Age/sex	Glucose	Mannose
AD1678	87/F/S	100.0	N.D.	CT714	44/M	95.0	5.0
AD1590	83/F/S	100.0	N.D.	CT92119	70/M	100.0	N.D.
AD1528	86/M/M	60.0	40.0	CT9205	75/F	100.0	N.D.
AD1519	89/F/M	100.0	N.D.	CT661	26/F	92.0	8.0
AD1499	70/F/S	70.0	30.0	CT1226	57/M	100.0	N.D.
AD1937	84/M/M	100.0	N.D.	CT738	25/M	88.0	12.0
Average ± S.D.	85.7 ± 16.8	14.3 ± 16.8				95.8 ± 4.6	4.2 ± 4.6

[a]As determined from GC/MS of alditol acetates. N.D. = not detectable.

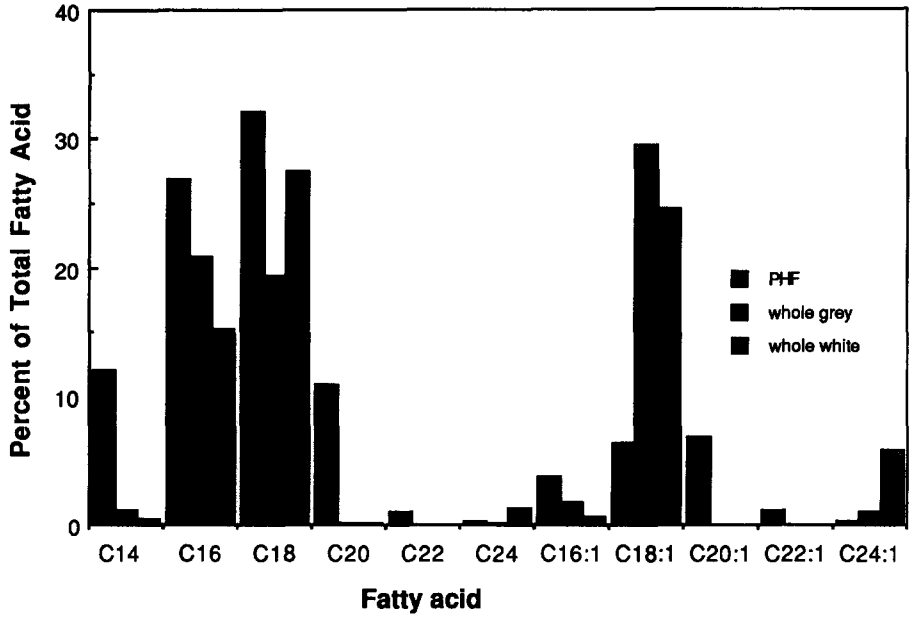

Fig. 1. Average fatty acid composition of six prcPHF samples compared to the average fatty acid compostion of phospholipids in whole brain grey and white matter [30].

cerebroside or any of the components of brain phospholipid [30–34]. It is highest in the fractions of saturated fats C18:0, C16:0 and C20:0, followed by the fractions of unsaturated fats C18:1 and C20:1 present. However, like the carbohydrate component, there appears to be considerable variation in the fraction of any specific fatty acid component between samples isolated from different individuals. Taken as a whole, our results suggests significant structural heterogeneity in both the carbohydrate and fatty acid components of prcPHF glycolipid may be tolerated, while still satisfying the criteria for filament formation.

Glycolipids similar in composition to prcPHF may be isolated from the brains of normal individuals. No significant differences were found in any of the fatty acids or monosaccharide components between prcPHF and glycolipid purified from normal brains according to the prcPHF isolation protocol. It has previously been reported that lipid from crude preparations of AD brains contains a higher degree of unsaturated fatty acid compared to lipid similarly isolated from aged-matched control brains [35]. In our purified preparations, we found that the average ratio of unsaturated to saturated fatty acid for the prcPHF did not differ significantly from that of the control lipid. Because there may be other factors which are abnormal in the neurons of AD individuals, it is difficult to solely attribute helical filament formation to the compositional characteristics of the prcPHF glycolipid. That more than a single structural factor is involved in filament formation is also suggested by the structural diversity of prcPHF glycolipid between individuals. It is likely that a combination of subtle differences in the structure and concentration of prcPHF glycolipid along with the abnormal presence of hyperphosphorylated tau or metal ion may play a role in the nucleation of filaments.

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References

[1] Alzheimer, A. (1907) Allg. Z. Psychiat. 64, 146–148.
[2] Jervis, G.A. (1948) Am. J. Psychiatry 105, 102–106.
[3] Tomlinson, B.E. and Corsellis, J.A.N. (1984) in: Greenfield's Neuropathology, 4th edn. (Adams, J.H., Corsellis, J.A.N., Duchen, L.W. Eds) pp. 951–1026, Wiley and Sons, New York.
[4] Blessed, G., Tomlinson, B.E. and Roth, M. (1968) Br. J. Psychiatry 114, 797–811.
[5] Khachaturian, Z.S. (1985) Arch. Neurol. 42, 1097–1105.
[6] Kidd, M. (1963) Nature 197, 192–193.
[7] Terry, R.D, Gonatas, N.K. and Weiss, M. (1964) Am. J. Pathol. 44, 269–297.
[8] Hirano, A., Dembitzer, H.M., Kurland, L.T. and Zimmerman, H.M. (1968) J. Neuropathol. Exp. Neurol. 27, 167–182.
[9] Metuzals, J., Montpetit, V. and Clapin, D.F. (1981) Cell Tissue Res. 214, 455–482.
[10] Yagashita, S., Itho, Y., Nan, W. and Amano, N. (1981) Acta Neuropathol. 54, 239–246.
[11] Crowther, R.A. and Wischik, C.M. (1985) EMBO J. 4, 3661–3665.
[12] Selkoe, D.J., Ihara, Y. and Salazar, F.J. (1982) Science 215, 1243–1245.
[13] Grundke-Iqbal, I., Iqbal, K., Tung, Y.-C., Quinlan, M., Wisniewski, H.M. and Binder, L.I. (1986) Proc. Natl. Acad. Sci. USA 83, 4913–4917.
[14] Kosik, K.S., Joachim, C.L. and Selkoe, D.J. (1986) Proc. Natl. Acad. Sci. USA 83, 4044–4048.
[15] Wood, J.G., Mirra, S.S., Pollock, N.J. and Binder, L.I. (1986) Proc. Natl. Acad. Sci. USA 83, 4040–4043.
[16] Wischik, C.M., Novak, M., Thogersen, H.C., Edwards, P.C., Runswick, M.J., Jakes, R., Walker, J.E., Milstein, C., Roth, M. and Klug, A. (1988) Proc. Natl. Acad. Sci. USA 85, 4506–4510.

[17] Wischik, C.M, Novak, M., Edwards, P.C., Klug, A., Tichelaar, W. and Crowther, R.A. (1988) Proc. Natl. Acad. Sci. USA 85, 4884–4888.
[18] Sparkman, D.R., Goux, W.J., Jones, C.M., White, C.L., and Hill, S.J. (1991)Biochem. Biophys. Res. Commun. 181, 771–779.
[19] Sparkman, D.R. (1993) Neurosci. Lett. 151, 153–157.
[20] Emory, C.R., Ala, T.A. and Frey II, W.H. (1987) Neurology 37, 768–772.
[21] Clements, J.R., Beitz, A.J., Emory, C.R. and Frey, W.H., II (1990) Alzheimer Dis. Assoc. Disorders 4, 35–42.
[22] Sparkman, D.R., Hammon, K.M. and White, C.L. III, (1990) J. Histochem. Cytochem. 38, 703–715.
[23] Carter, H.E. and Gaver, R.C. (1967) J. Lipid Res. 8, 391–395.
[24] Li, Y.-T., Hirabayashi, Y., DeGasperi, R., Yu, R.K., Ariga, T., Koerner, T.A.W. and Li, S.-C. (1984) J. Biol. Chem. 259, 8980–8985.
[25] York, W.S., Darvill, A.G., McNeil, M., Stevenson, T.T., and Albersheim, P. (1985) Methods Enzymol. 118, 3–40.
[26] Carpita, N.C. and Shea, E.M. (1989) in: Analysis of Carbohydrates by GLC and MS (Bierman, C.J. and McGinnis, G.D. Eds.) pp. 157–216, CRC Press, Boca Raton, FL.
[27] Ryhage, R. and Stenhagen, E. (1960) J. Lipid Res. 1, 361–390.
[28] Eglinton, G., Hunnenman, D.H. and McCormick, A. (1968) Org. Mass Spectrom. 1, 593–611.
[29] McLaferty, F.W. (1963) in: Mass Spectrometry of Organic Ions (McLafferty, F.W. Ed.) p. 309, Academic Press, New York.
[30] White, D.A. (1973) in: Form and Function of Phospholipids (Ansell, G.B., Hawthorne, J.N. and Dawson, R.M.C. Eds.) pp. 441–482, Elsevier Scientific Publishing Co., New York, NY.
[31] Radin, N.S. and Akahori, Y. (1961) J. Lipid Res. 2, 335–341.
[32] O'Brien, J.S., Fillerup, D.L. and Mead, J.F. (1964) J. Lipid Res. 5, 109–116.
[33] Sambasivarao, K. and McCluer, R.H. (1964) J. Lipid Res. 5, 103–108.
[34] Siddiqui, B. and McCluer, R.H. (1968) J. Lipid Res. 9, 366–370.
[35] Kwee, I.L., Nakada, T. and Ellis, W.G. (1991) Magn. Res. Med. 21, 49–54.