J. Lipid Research, October 1963
Volume 4, Number 4

Structures of the normal unsaturated fatty acids of brain sphingolipids*

YASUO KISHIMOTO and NORMAN S. RADIN

Mental Health Research Institute,
University of Michigan,
Ann Arbor, Michigan

[Manuscript received March 29, 1963; accepted June 11, 1963.]

SUMMARY

A description is presented of the structural characterization of the normal unsaturated fatty acids of pig brain sphingolipids. The acids were isolated with the use of silver nitrate-impregnated silica gel and gas-liquid chromatography, and the positions of the double bonds were determined by a simplified ozonolysis procedure. In this method, the methyl esters were treated consecutively with ozone, performic acid, and diazomethane. Gas-liquid chromatography with a temperature program permitted analysis of the degradation products without preliminary separation of the mono- and dicarboxylic acids. Twenty-seven chemically-defined unsaturated acids were found, 25 of which have not been previously reported. These included the odd- and even-numbered monoenoic acids 22–26 carbon atoms long, as well as C_{24}, C_{25}, and C_{26} dienes. Each acid consisted of 2–4 positional isomers, which appear to have been derived from oleic, palmitoleic, linoleic, or an odd-numbered acid by chain elongation. Some of the acids appear to have been derived also by a 1-carbon degradation reaction from the longer homologue. Small amounts of trienoic acids were also found.

Until recently, nervonic acid ($24:1^{15}$)[1] was the only unsaturated normal acid of sphingolipids that had been characterized structurally (1). Carroll has recently identified $20:1^{11}$ and $22:1^{13}$ as occurring in beef spinal cord sphingolipids (2). These three acids have the double bond in the 9-10 position, counting from the methyl end, and thus resemble oleic acid ($18:1^{9}$). As the result of a study with isotopic acetate in rats, Fulco and Mead (3) suggested that nervonate is synthesized from oleate by chain elongation. Gas-liquid chromatographic (GLC) studies in this (4, 5) and other (2, 6–8) laboratories have demonstrated the existence of other unsaturated acids in the brain sphingolipids. Since recent work (9–12) has shown that the *saturated* cerebroside acids are made enzymatically by chain elongation and by a 1-carbon degradation system, we thought that we might obtain evidence for a similar set of enzymes in the case of the unsaturated acids by determining the position of their double bonds. This paper describes the isolation and characterization of pig brain sphingolipid acids, 22:1 to 26:1, and 24:2 to 26:2.

* Supported in part by PHS Research Grant NB-03192 from the National Institute of Neurological Diseases and Blindness, U.S. Public Health Service.

EXPERIMENTAL METHODS

All solvents were distilled except U.S.P. ether. Spectrograde MeOH[1] was used for the ozonolysis. Unsaturated fatty ester standards were obtained from the Hormel Foundation, Austin, Minn., and from the National Heart Institute, U.S. Public Health Service.

Preparation of Sphingolipid Fatty Acids. The sphingolipids were isolated from two pig brains,[2] total weight 204 g, by a method (12) involving exposure to mild alkali to cleave the esters, exposure to mild acid to cleave the alkenyl ether linkages, and silica gel chromatography.[3] The yield was 7.51 g, or 3.7% of the wet weight.

Thin-layer chromatography of this material with chloroform–methanol–water 24:7:1 (v/v) (13) showed

[1] Abbreviations used: MeOH, methanol; DEGS, a polymeric form of diethyleneglycol succinate; SE-30, a General Electric silicone gum; SK, Skellysolve B, a commercial hexane. In fatty acid abbreviations, the first number indicates the chain length, the second number indicates the number of double bonds, the superscripts indicate the positions of the double bonds, d indicates the acid is an α,ω-dicarboxylic acid, and h a hydroxy acid.

[2] Gift of Peters Sausage Company, Ann Arbor, Mich.

[3] Unisil silicic acid, Clarkson Chemical Company, Inc., Williamsport, Pa.

spots corresponding to cerebrosides, cerebroside sulfate, sphingomyelin, and a small amount of acidic material at the origin.

The methyl esters were obtained from the sphingolipids as previously described (14). The normal esters weighed 1.50 g and the hydroxy esters 1.13 g (total weight is 35% of the sphingolipids). All the hydrolysis and esterification reactions were carried out under nitrogen. Thin-layer chromatography of the normal esters, using SK–absolute ether 85:15 (15),[1] showed the presence of a small amount of slower moving material, possibly dimethyl acetals derived from the plasmalogens.

Separation of the Esters by the Degree of Unsaturation. The method of de Vries (16) was adapted.[4] Silica gel (100 g) was mixed with a silver nitrate solution (25 g in 190 ml water) and dried in a tray at 120° for 4.5 hr. The powder was passed through a 140-mesh sieve and stored in the dark.

Fractionation of the normal esters was accomplished with columns packed in Skellysolve and eluted with various mixtures of Skellysolve and benzene. Each fraction was analyzed by GLC, using an instrument[5] equipped with a flame ionization detector and DEGS[1] as stationary phase. The peaks were identified by comparison with standards.

Skellysolve alone eluted the saturated esters, SK–benzene 9:1 and 8:2 the monounsaturated esters, and SK–benzene 6:4 the diunsaturated and triunsaturated esters. The sharpness of separation was reduced by the fact, previously unreported, that the longer chain esters within each class came out in part before the shorter esters. However, by rechromatographing the various fractions, we obtained rather sharply separated groups. The yields of the saturated, monoenoic, and dienoic esters were 56, 45, and 2.7%, respectively.

Isolation of the Individual Esters. The GLC methods have been described before (17). Here we used a steel column, 80 in. long and 1/4 in. o.d., containing 9% of the polar silicone SE-30[1] on Chromosorb W (60–80 mesh). Each run was made with a sample of 5–7 μl of the appropriate silica gel–silver nitrate fraction, without solvent, and took 20 min. The column temperature was about 260°.

The condensed esters were washed out of the collection tubes with Skellysolve and pooled. Although the chromatograph readily separated esters differing by one carbon atom, 25:2 and 26:2 were collected together because so little was present. The dienes proved to be contaminated with about 10% of monoenes so they were rechromatographed on small silica gel–silver nitrate columns. Each ester was stored in Skellysolve at −20° in screw-cap test tubes after flushing with nitrogen.

The individual esters thus isolated were examined in four ways: by GLC on DEGS, by thin-layer chromatography, by infrared analysis, and by ozonolysis. The samples were prepared for spectral analysis by grinding 40 mg KBr with 20 μl of ester solution (10 mg/ml in Skellysolve) in a Mullite mortar. Discs of 5-mm diam were pressed in an evacuated die and examined with a Perkin-Elmer Model 237 spectrophotometer.

Ozonolysis of the Individual Esters. A simple ozone generator was prepared[6] by sealing off the bottom end of a Pyrex Liebig condenser (jacket 30-cm long). A length of stainless steel wire (1-mm diam), hung by a cork stopper from the top of the inner tube, served as one electrode. The wire was centered at the bottom by a lump of wax. Oxygen was passed through the outer jacket by means of Tygon tubing at 30 ml/min. The whole condenser was immersed in a polyethylene cylinder containing ice and salt water and the other electrode, a plain copper wire, was inserted in the salt water close to the generator. A 10,000-volt neon lamp transformer was connected to the two electrodes. While this generator is relatively inefficient (ozone concentration in oxygen = 1.5%), it gives ample amounts of ozone for micro work.

The ozonolysis procedure was a micro modification of the method of Ackman et al. (14, 18). We used 1.5–3 mg of each ester in 0.6 ml of chloroform–methanol 1:2 and decomposed the ozonide with 100 μl of 98% formic acid and 30 μl of 30% hydrogen peroxide. The resultant mixture of mono- and dicarboxylic acids was mixed with 0.5 ml of absolute ether–MeOH 9:1 (19) and anhydrous sodium sulfate. After 1 hr, the dried solution was transferred to a 15-ml conical centrifuge tube fitted with a 2-hole rubber stopper. One hole contained a short L-tube for carrying in gaseous diazomethane; the other hole contained a long Vigreux tube, reaching down to the bottom of the liquid (20). Diazomethane was generated from N-methyl-N-nitroso-*p*-toluenesulfonamide[7] (21) and carried to the reaction mixture with ether-saturated nitrogen. By this arrangement, methylation took place inside the Vigreux tube in efficient contact with the gas.

Some evaporation of the reaction mixture took place

[4] We are indebted to Dr. L. J. Morris, Brunel College of Technology, and Dr. B. de Vries, Unilever Research Laboratory, Vlaardingen, Holland, for their assistance in the use of the silver nitrate–silica gel.

[5] Model 609, F & M Scientific Corporation, Avondale, Pa.

[6] We are grateful to Dr. A. L. Henne, Ohio State University, for furnishing the design for a simple ozone generator.

[7] "Diazald", Aldrich Chemical Company, Inc., Milwaukee, Wis.

during the 30 min required for completion of the reaction, so small portions of ether were added periodically to the top of the reaction tube. When the solution turned yellow, the diazomethane generator was disconnected and nitrogen was passed through to remove the excess reagent. The solution thus obtained was used directly for GLC with the flame ionization detector (22). Each sample was examined with a DEGS column and an SE-30 column, using 5 μl of solution. The column was started at room temperature, with the oven cover off, and the temperature raised to 210° at 4.6°/min. The main identification standard mixture was made from commercially available acids, namely 6:0, 7:0, 9:0, 12:0, 3d:0, 8d:0, 12d:0, and 14d:0. Areas were determined by triangulation.

Results

Validation of the Isolation Methodology. The silver nitrate column was first tested with a standard mixture of 18:0, 18:1, 18:2, and 18:3 methyl esters and was found to give nearly 100% recovery and excellent separations. Our finding that chain length affects the elution rate has subsequently been extended to glycerides in thin-layer chromatography (23).

The GLC procedure was checked by comparing the weights of recovered esters and the peak areas. Although the peak shapes for the major esters indicated heavy loading of the column, the weights agreed very well with the observed areas. Analysis of the final products with DEGS showed the presence of 2.2% or less of any homologous ester. It could be readily calculated that such contamination was too small to confuse the ozonolysis results.

Thin-layer chromatography of the isolated esters showed the presence in most samples of very faint spots at the solvent front, possibly derived from products "bleeding" from the silicone column. Only 22:1 and 26:1 revealed a few extra faint spots with lower mobilities; the amounts seemed so small that further purification was not performed.

The infrared spectra of the monoenes and 24:2 were examined and compared with those of known esters. The spectra were very similar, showing no *trans* double bond but a peak of 3.35 μ. The intensity of this peak, which corresponds to CH stretching of double bonds, was similar to that of standard 24:1, except in the case of 24:2, which showed much stronger absorption. (The standard 24:1 did show a small peak for *trans* double bonds at 10.3 μ.) Peaks for silicone "bleeding" products, which might have been expected, could not be seen.

Validation of the Ozonolysis Procedure. Using our ozonolytic analysis method with highly purified nervonate and linoleate, we found small amounts of overoxidation and side products, as reported by Ackman et al. (18). Thin-layer chromatography of the methyl esters thus produced disclosed numbers of faint spots more polar than the dicarboxylic esters. Gas chromatography showed that nearly all of these by-products yield very small peaks. Where by-products were appreciable, comparison on DEGS and SE-30 columns served to eliminate them from consideration as the abnormal products did not appear in the same relative position in both recordings. In this way, we were able to show that overoxidation amounted to no more than 1.7%.

It was observed that malonic acid was largely decomposed in the procedure, and only a tiny malonate peak appeared on the gas chromatograms of the dienoic acids. An attempt to reduce the destruction by heating with performic acid for only 15 min did not help. Klenk and Bongard (24) have shown that the malonic acid can be somewhat preserved by decomposing the ozonide with peracetic acid at 38° for 36 hr. However, since the double bonds of most naturally occurring nonconjugated dienoic acids are arranged in the divinylmethane rhythm, we made no further attempts to improve the procedure.

Another difficulty in the method was the finding that the peak areas of the mono- and dicarboxylic esters differed from the expected values, and the ratio of the areas depended on which column was used. Presumably this is the result of using a flame detector. Thus, the ratio of 9:0 to 15d:0[1] from nervonate is theroetically 10/17 = 0.59 on the basis of the number of carbon atoms in the two esters. The observed ratios were 0.82 with the silicone column and 0.72 with the DEGS. The ratio of 6:0 to 9d:0 from linoleate (theoretically 0.64) was 0.67 with silicone and 0.61 with DEGS. The lower ratio in the latter example is probably due to a slight loss of the 6:0 ester by evaporation during the methylation step.

Each isolated ester proved to be composed of several positional isomers. The GLC data were treated in the following way to calculate the amount of each isomer: (1) each peak area was corrected for overoxidation; (2) any peak whose retention time did not fall on the standard time curve with both columns was disregarded; (3) each isomer was required to yield a pair of peaks, corresponding to a mono- and a dicarboxylic acid, with carbon numbers totaling that of the parent acid (or 3 less in the case of dienoic acids); (4) the ratio of areas of the pair of peaks had to be close to that observed for the standards, as described above.

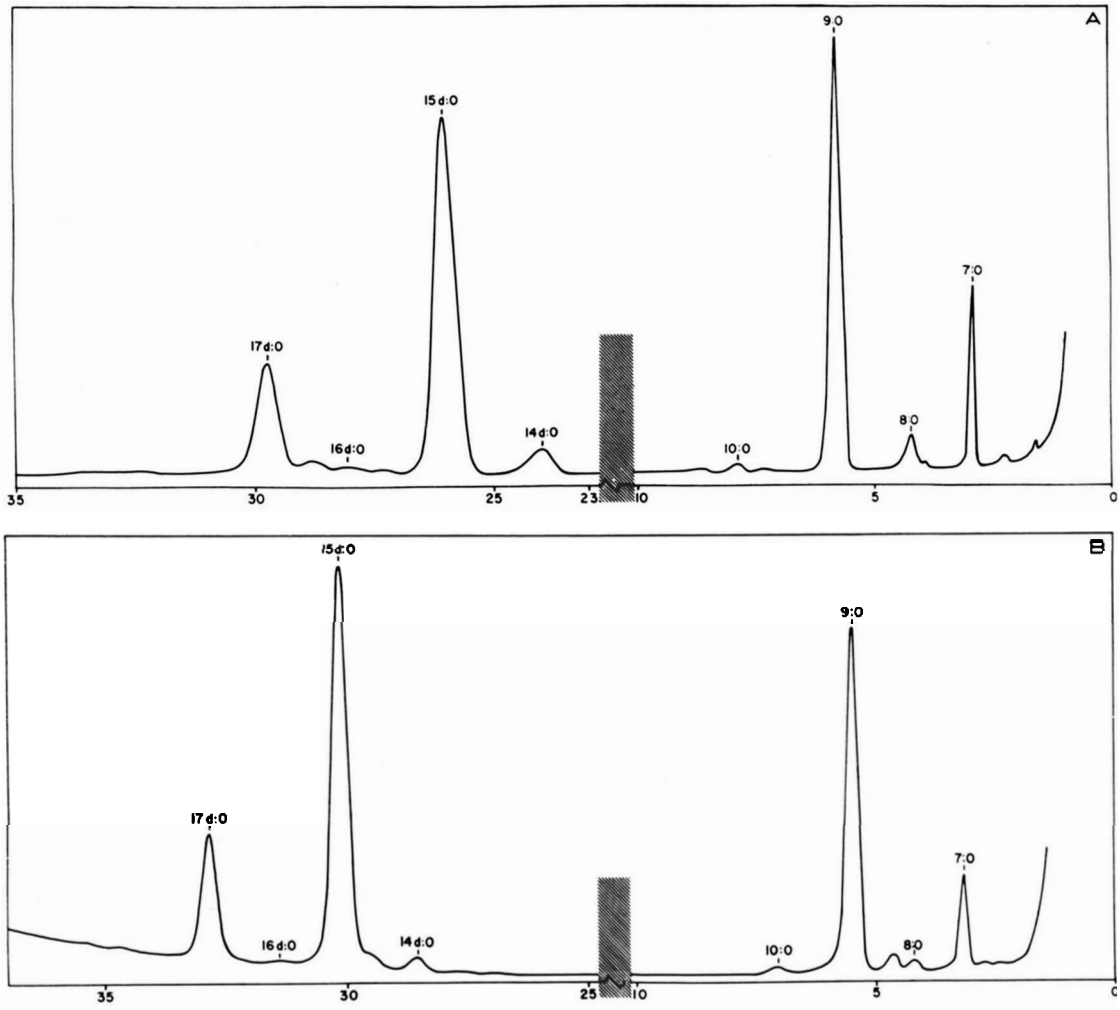

FIG. 1. Gas-liquid chromatograms obtained with degradation products from pig brain 24:1 methyl ester. The abscissa indicates elution time in minutes. **Chromatogram A was obtained with the SE-30 column;** B, with the DEGS column. Note the unidentified trace peaks just following the 8:0 and 14d:0 peaks in B; these are apparently eluted earlier in A, overlapping the 8:0 and 14d:0 peaks.

Figure 1 shows the two chromatograms obtained with pig brain 24:1. The major isomer was previously found and characterized by Klenk, but the 24:1[14] and 24:1[17] are new. While the peaks for 8:0 and 16d:0 suggest the existence of a trace amount of 24:1[16], these are primarily due to overoxidation.

In the case of 25:2 and 26:2, which were ozonized together, the calculations were more involved. Table 1 shows the observed areas from the DEGS column for the various peaks, corrected for overoxidation. The problem is simplified by the reasoning, assuming the divinylmethane rhythm, that certain dicarboxylic esters can be assigned exclusively to 25:2 (15d:0 and 12d:0) and to 26:2 (17d:0 and 14d:0). (The appearance of 15d:0 from a hypothetical 15,18-dienoic C_{26} would require the simultaneous appearance of 8:0 among the monocarboxylic acids, and this is absent. Similar reasoning applies to the other three dicarboxylic acids mentioned.) Knowing these relative areas, we could calculate the areas in the corresponding monocarboxylic esters assignable to these isomers.

The remaining areas in those monocarboxylic areas were then used to calculate the composition of the remaining isomers. For example, the observed area of 2.16 for 15d:0 meant that $2.16 \times 0.57 = 1.24$ in the 7.0 peak was derived from 25:2[15, 18]. (The ratio, 0.57, was calculated from the areas obtained by ozonolysis of 22:1[15].) Since the observed area for 7:0 was 3.85, part of this $(3.85 - 1.24 = 2.61)$ must have come from 26:2[16, 19]. The area of 16d:0 thus assignable to this isomer must be $2.61/0.49 = 5.30$. (The ratio, 0.49, comes from the results with 23:1[16].) As a check on the validity of these calculations, we added up the amounts of the various isomers (as shown in Table

2) and found excellent agreement with the relative amounts of 25:2 and 26:2 as shown by the silicone gas chromatogram of the original isolated mixture.

Occurrence of Positional Isomers. The results of application of the ozonolysis method to the isolated pig brain esters are shown in Table 2. The ratios of isomers were calculated from the molar ratios of the corresponding dicarboxylic esters, rather than the monocarboxylic esters, since errors due to partial volatilization during methylation are thereby eliminated. The molar ratios agreed well between the SE-30 and DEGS chromatograms.

The column in Table 2 showing the amount of each isomer per 100 g brain reveals that nervonic acid is distinctly the most common acid, with its isomer, $24{:}1^{17}$, being the next most common. Third most common is $22{:}1^{15}$, and the next most common are $25{:}1^{16}$, $26{:}1^{19}$, and $26{:}1^{17}$. From our previous work, one would expect the actual and relative amounts per brain to change with age. The two major 26:1 acids were probably included in the 26:1 acid isolated by Klenk and Leupold (25).

Analysis by GLC of the fractions coming off late from the silver nitrate column revealed the presence of 22:2, 23:2, and trienes, but there was too little of the material to permit characterization.

DISCUSSION

The Ozonolysis Method. The merits of this method of ozonolytic analysis are: (1) it is simple to perform and requires only simple apparatus; (2) it is not necessary to separate the degradation products into two classes, low- and high-molecular weight, before chromatographic analysis; (3) there is no potential danger from organic phosphines or their by-products (26); (4) there is no need to run the ozonolysis twice, in two different solvents, except when very short-chain esters are expected; (5) chromatographic standards for identification are readily available (compare the reductive ozonolysis methods); (6) unlike the aldehydic degradation products, the esters can be stored a long time before GLC analysis is carried out.

The Even-Numbered Monounsaturated Acids. Column 3 in Table 2 lists the positions of the double bonds, counting from the methyl end. On this basis, palmitoleic acid ($16{:}1^{9}$) would yield a 7 and oleic acid would yield a 9. It can be seen that each even-numbered monounsaturated sphingolipid fatty acid occurs in both isomeric forms, in the 7 and in the 9 groups. Apparently these isomers are derived from palmitoleate and oleate by a chain-elongating enzyme system, possibly the same one that forms the saturated sphingolipid acids. Such elongating enzymes do not seem to be restricted to the very long acids, for $18{:}1^{11}$ exists in brain (27), presumably made from palmitole-

TABLE 1. GLC PEAK AREAS OF OZONOLYSIS PRODUCTS OF MIXTURE OF 25:2 AND 26:2*

Fragment Ester	Chromatogram Peak Area
	sq cm
6:0	3.44
7:0	3.85
9:0	3.63
10:0	0.51
12d:0	0.18
13d:0	2.00
14d:0	3.01
15d:0	2.16
16d:0	8.74
17d:0	3.49

* Data from GLC analysis on a DEGS column. 12d:0 = saturated C_{12} dicarboxylic diester.

TABLE 2. ISOMERIC COMPOSITION OF EACH UNSATURATED SPHINGOLIPID ESTER

Ester	Position of Double Bond: From COOH	Position of Double Bond: From CH_3	Proportion of Each Isomer	Concentration in Fresh Brain	Precursor*
			%	*mg/100 g*	
22:1	15	7	85	21	P
	13	9	15	3.7	O
23:1	16	7	51	4.5	P
	15	8	31	2.7	H
	14	9	17	1.5	O
	13	10	1	0.1	N
24:1	17	7	20	44	P
	15	9	78	170	O
	14	10	2	4.4	N
25:1	18	7	22	3.7	P
	17	8	8	1.3	H
	16	9	62	10	O
	15	10	8	1.3	N
26:1	19	7	49	13	P
	17	9	40	10	O
	16	10	11	2.9	N
24:2	15, 18	6, 9	72	1.9	L
	14, 17	7, 10	13	0.3	P
	12, 15	9, 12	15	0.4	O
25:2	16, 19	6, 9	50	0.14	L
	15, 18	7, 10	25	0.07	P
	13, 16	9, 12	22	0.06	O
	12, 15	10, 13	3	0.01	N
26:2	17, 20	6, 9	29	0.14	L
	16, 19	7, 10	43	0.22	P
	14, 17	9, 12	25	0.13	O
	13, 16	10, 13	3	0.02	N

* Indicates possible precursor of the isomer. P = palmitoleic, O = oleic, L = linoleic, H = heptadecenoic ($17{:}1^{9}$), N = nonadecenoic ($19{:}1^{9}$).

TABLE 3. CHAIN-LENGTH DISTRIBUTION OF ESTERS HAVING THE SAME DEGREE OF UNSATURATION*

Group	Carbon Number 22	23	24	25	26
	%	%	%	%	%
Saturated	30.3	12.0	51.9	3.4	2.4
Monounsaturated	7.2	2.9	76.0	5.2	8.7
Diunsaturated	5.3	1.0	70.7	8.5	14.5

* Data from gas chromatographic analysis of the three groups isolated by silver nitrate–silica gel chromatography.

ate; $20:1^{11}$ in spinal cord (2), from oleate; and certain bacteria can lengthen 8:1, 10:1, and 12:1 to 18:1 (28). Brain plasmalogen also contains the $18:1^{11}$ and $18:1^{9}$ alkenyl ethers, evidently derived from palmitoleate and oleate (29).

It is interesting to note the presence of a 10 isomer in 24:1 and in 26:1. If these were derived by chain lengthening, the precursor might be $18:1^{8}$, which has been found in hair fat (30) and olive oil (31). A more likely explanation is that this isomer is derived by α-oxidation of an odd-numbered acid, possibly by the same enzyme system that appears to form 23h:0 from 24h:0.[1] An isotopic study from this laboratory (12) offered tentative evidence that the 1-carbon degradation system also acts on odd-numbered acids. Odd-numbered 10 isomers are indeed found in our unsaturated acids. The $27:1^{17}$ acid that might be the precusor of $26:1^{16}$ has not yet been found, although traces of a 27:1 acid have been reported to occur (6).

The Odd-Numbered Monounsaturated Acids. Column 3 shows that these acids consist of 4 isomers, 2 of which are related structurally to palmitoleate and oleate. This is evidence that the α-oxidation system acts on unsaturated acids as well as saturated acids, for the $23:1^{16}$ could be made from $24:1^{17}$, $23:1^{14}$ from $24:1^{15}$, and so forth.

The odd-numbered acids of the 10 group are presumably made by chain elongation of $17:1^{7}$ or $19:1^{9}$, neither yet found in nature. A 17:1 does exist in brain (7) and skin fat (32). Our gas chromatograms generally show the presence of traces of a 19:1.

The odd-numbered acids of the 8 group also exist, in analogy with the derivatives of palmitoleic acid. The most likely precursor is $17:1^{9}$, which has been isolated from lamb caul fat (33) and musk ox fat (34). All three of the potential precursor odd-numbered acids are presumably made from propionate by elongation with acetate. The existence of the 8 and 10 series in the sphingolipid odd-numbered acids supports our earlier conclusion that the *saturated* odd-numbered cerebroside acids are made by two routes, elongation and 1-carbon degradation.

The Diunsaturated Acids. If the monoenoic acids are made by chain lengthening, the dienoic acids should be made in a similar fashion from linoleic acid. This dienoic acid is a member of the 6 series, counting from the methyl end, and we do indeed find an isomer in each sphingolipid dienoic acid assignable to this series. In 24:2 and 25:2, this is the major acid; in 26:2, it is of secondary importance. In the case of 25:2, we may assume that this isomer is derived from $26:2^{17,\ 20}$ by a 1-carbon degradation. Arachidonic acid is a tetraenoic acid in the 6 series and has been shown to be formed from linoleate by desaturation and chain elongation (35, 36).

Considerable proportions of other isomers are found in the 7 and 9 series, presumably synthesized from palmitoleate and oleate by elongation as well as desaturation. This series of reactions is similar to that found in hens (37), where $8:1^{2}$ can be lengthened and desaturated to form linoleate; and to the reactions found in fat-deficient rats (38), where oleate is lengthened and desaturated to form $20:3^{5,\ 8,\ 11}$. In the latter study, evidence was also found for a similar reaction with palmitoleate. Klenk and co-workers have found polyenoic acids in brain phosphatides that appear to be derived from oleate (39). The in vitro work of Harlan and Wakil (40) has shown that oleate can be desaturated and lengthened to form polyenoic acids. The sequence of steps in the biosynthesis of the sphingolipid dienes may involve, first, a desaturation, followed by elongation, for shorter-chain dienes of the 7 and 9 series have been found in various natural lipids (menhaden fish oil, rat tissues, and hen egg yolk). In the 7 series are $16:2^{6,\ 9}$ (41) and $18:2^{8,\ 11}$ (37, 42); in the oleate series is $18:2^{6,\ 9}$ (41).

An additional isomeric group, the 10 series, was found in the 25:2 and 26:2. In analogy with the conclusions drawn from the 10 series in the monoenes, we may conclude that these acids are made from an odd-numbered acid by lengthening, desaturation and, in the case of 26:2, 1-carbon degradation from the C_{27} acid.

Our earlier experiments on the fatty acid patterns of brain cerebrosides (4) had shown the distribution of chain lengths to be somewhat different for saturated and unsaturated acids. Moreover the changes with age in these two groups were somewhat different (43). These findings support the suggestion in this paper that the unsaturated acids are made from shorter *unsaturated* acids rather than from the analogous *saturated* acids.

The Abundance of the Various Fatty Acid Series. Examination of column 5 in Table 2 allows comparison of the abundance of the various families of acids. The 6th column is added as an aid in identifying families.

The palmitoleate-derived monoenoic acids are somewhat more evenly distributed between C_{22}, C_{24}, and C_{26} than are those derived from oleate. The ratio in the 22:1, 24:1, 26:1 isomers is 21:44:13, while the corresponding ratio for the oleate series is 4:170:10. In the diene acids, however, the ratios are rather similar for both series.

There may be a clue to the specificity of the α-degradation enzyme system in the comparison of the relative abundances of the isomers (column 4) presumed to come from 1-carbon degradation. The odd-numbered isomers having the double bond in the 16 position (counting from the COOH end) are more common than the other isomers (14 and 18 carbons from the COOH) derived in this way. Thus $23:1^{16}$ is the major isomer in the C_{23} group, derived from $24:1^{17}$; $25:1^{16}$ is the major C_{25} acid, derived from $26:1^{17}$; and $26:1^{16}$, derived from a hypothetical $27:1^{17}$, occurs in unexpectedly high relative amount. There is no $22:1^{17}$, which may explain why we could not find appreciable amounts of C_{21} acid. The same tendency of the degrading system to attack an acid with a double bond in the 17 position is seen in the dienes: the major C_{25} acid is apparently derived from $26:2^{14,\ 17}$.

Another type of comparison of abundances is shown in Table 3, which gives the relative amounts of each ester within a given saturation group. It can be seen that the average molecular weight of each group goes up with increasing number of double bonds. This seems to be a general rule in the *ester* lipids, and apparently it is followed also in the brain sphingolipids.

Additional studies of this type with the hydroxy acids of sphingolipids are under way. We also plan a search for the $17:1^{7}$, $17:1^{9}$, and $19:1^{9}$ acids postulated to exist in brain.

REFERENCES

1. Klenk, E. *Z. Physiol. Chem.* **166**: 287, 1927.
2. Carroll, K. K. *J. Lipid Res.* **3**: 263, 1962.
3. Fulco, A. J., and J. F. Mead. *J. Biol. Chem.* **236**: 2416, 1961.
4. Kishimoto, Y., and N. S. Radin. *J. Lipid Res.* **1**: 72, 1959.
5. Radin, N. S., and Y. Akahori. *J. Lipid Res.* **2**: 335, 1961.
6. O'Brien, J., and G. Rouser. *Federation Proc.* **21**: 284, 1962.
7. Johnston, P. V., and F. A. Kummerow. *Proc. Soc. Exptl. Biol. Med.* **104**: 201, 1960.
8. Bernhard, K., A. Hany, L. Hausheer, and W. Pedersen. *Helv. Chim. Acta* **45**: 1786, 1962.
9. Hajra, A. K., and N. S. Radin. *Federation Proc.* **22**: 300, 1963.
10. Mead, J. F., and G. M. Levis. *Biochem. Biophys. Res. Commun.* **9**: 231, 1962.
11. Hajra, A. K., and N. S. Radin. *Biochem. Biophys. Acta* **70**: 97, 1963.
12. Hajra, A. K., and N. S. Radin. *J. Lipid Res.* **4**: 270, 1963.
13. Honegger, C. G. *Helv. Chim. Acta* **45**: 281, 1962.
14. Hajra, A. K., and N. S. Radin. *J. Lipid Res.* **3**: 327, 1962.
15. Vioque, E., and R. T. Holman. *J. Am. Oil Chemists' Soc.* **39**: 63, 1962.
16. de Vries, B. *Chem. & Ind. (London)* no vol: 1049, 1962.
17. Hajra, A. K., and N. S. Radin. *J. Lipid Res.* **3**: 131, 1962.
18. Ackman, R. G., M. E. Retson, L. R. Gallay, and F. A. VandenHeuvel. *Can. J. Chem.* **39**: 1956, 1961.
19. Schlenk, H., and J. L. Gellerman. *Anal. Chem.* **32**: 1412, 1960.
20. Ames, D. P., and J. E. Willard. *J. Am. Chem. Soc.* **73**: 164, 1951.
21. deBoer, Th. J., and H. J. Backer. *Rec. Trav. Chim.* **73**: 229, 1954.
22. Kishimoto, Y., and N. S. Radin. *J. Lipid Res.* **4**: 130, 1963.
23. *Gas-Chrom Newsletter*, Applied Science Laboratories, State College, Pa., 1962, **3**: no. 4, p. 1.
24. Klenk, E., and W. Bongard. *Z. Physiol. Chem.* **290**: 181, 1952.
25. Klenk, E., and F. Leupold. *Z. Physiol. Chem.* **281**: 208, 1944.
26. Stein, R. A., and N. Nicolaides. *J. Lipid Res.* **3**: 476, 1962.
27. Morton, I. D., and A. R. Todd. *Biochem. J.* **47**: 327, 1950.
28. Scheuerbrandt, G., and K. Bloch. *J. Biol. Chem.* **237**: 2064, 1962.
29. Leupold, F. *Z. Physiol. Chem.* **285**: 182, 1950.
30. Weitkamp, A. W., A. M. Smiljanic, and S. Rothman. *J. Am. Chem. Soc.* **69**: 1936, 1947.
31. Allen, R. R., and A. A. Kiess. *J. Am. Oil Chemists' Soc.* **32**: 400, 1955.
32. Wheatley, V. R. *J. Invest. Dermatol.* **29**: 445, 1957.
33. Shorland, F. B., and A. S. Jessop. *Nature* **176**: 737, 1955.
34. Chisholm, M. J., and C. Y. Hopkins. *Can. J. Chem.* **35**: 1434, 1957.
35. Steinberg, G., W. H. Slaton, Jr., D. R. Howton, and J. F. Mead. *J. Biol. Chem.* **220**: 257, 1956.
36. Howton, D. R., and J. F. Mead. *J. Biol. Chem.* **235**: 3385, 1960.
37. Reiser, R., N. L. Murty, and H. Rakoff. *J. Lipid Res.* **3**: 56, 1962.
38. Fulco, A. J., and J. F. Mead. *J. Biol. Chem.* **234**: 1411, 1959.
39. Klenk, E. In *Drugs Affecting Lipid Metabolism*, edited by S. Garattini and R. Paoletti, Amsterdam, Elsevier Publishing Company, 1960, p. 21.
40. Harlan, W. R., Jr., and S. J. Wakil. *Biochem. Biophys. Res. Commun.* **8**: 121, 1962.
41. Stoffel, W., and E. H. Ahrens, Jr. *J. Lipid Res.* **1**: 139, 1960.
42. Fulco, A. J., and J. F. Mead. *J. Biol. Chem.* **235**: 3379, 1960.
43. Kishimoto, Y., and N. S. Radin. *J. Lipid Res.* **1**: 79, 1959.