Characterization of Unsaturated Fatty Acids by Gas-Liquid Chromatography[1]

H. H. HOFSTETTER,[2] N. SEN and R. T. HOLMAN, The Hormel Institute, University of Minnesota, Austin, Minnesota

Abstract

The equivalent chain length (ECL) has been determined on 79 methyl esters of unsaturated fatty acids and on 7 ethyl esters by gas chromatography. Ethylene glycol succinate (EGS), diethylene glycol succinate, β-cyclodextrin acetate and Apiezon L were chosen as the liquid phases to be used. For methyl esters of mono- and polyenoic acids, the differences between ECL on EGS and ECL on Apiezon L approximate 0.84 per double bond. For positional isomers, the ECL on both EGS and Apiezon L are usually greater for the isomer having the longer proximal end of the molecule (smallest ω value). In these terms a triple bond is approximately equal to three double bonds. Esters of nonconjugated dienoic and trienoic acids of the same chain length are not separable on Apiezon L if their proximal structures are the same. This also applies to tetraenoic and pentaenoic acids of the same chain length and the same proximal structure. Conjugation of double bonds, either with the ester carbonyl group or with themselves, yields ECL values on Apiezon L greater than the number of carbon atoms in the acid. Monounsaturated and nonconjugated polyunsaturated esters have ECL values on Apiezon L lower than the number of carbon atoms of the acid. The ECL values of ethyl esters of 18 and 20 carbon acids are greater than the corresponding methyl esters on Apiezon L.

Introduction

IN THE COURSE of several investigations in this Institute, a number of well-characterized unsaturated fatty acids have been accumulated. These investigations have involved primarily the characterization of the lipids of beef testes (1), tetramethylene-interrupted polyunsaturated acids (2) and polyunsaturated acids of odd-chain length (3). The structures of the unsaturated acids were determined by ozonolysis-reduction (4) and hydrogenation to check the proper chain length. The temporary existence of such a collection of unsaturated acids offered the opportunity for a comparative study in one laboratory of their gas liquid chromatographic (GLC) behavior. Inasmuch as most lipid analyses are currently being performed by GLC, a table of retention time data of the esters should be helpful in the tentative identification of fatty acids in other laboratories. Ethyl esters are sometimes used in biochemical investigations and they may appear as a consequence of the use of ethanol in extraction procedures. Therefore several ethyl esters have been included in this study to illustrate where they may appear when compared with methyl ester standards. In order to include as many unsaturated acids as possible, a number of unusual unsaturated acids of proven structure have been obtained from other laboratories.

Experimental

The retention times for the individual esters were determined on four phases under conditions described in Table I. The retention times on the three polyesters are, in general, quite similar. Ethylene glycol succinate (EGS) and diethylene glycol succinate (DEGS) were both included here because they are currently popular for fatty acid analyses. β-Cyclodextrin acetate (5) was also included because its lifetime is much greater than that of EGS and DEGS, and it may be used more generally in the future. Apiezon L was chosen as the nonpolar phase because it is widely used and because, in our hands, it gave better separations of polyunsaturated esters than did Apiezon M.

The retention time data are presented as equivalent chain length (ECL) (6) which is basically similar to the carbon numbers as calculated by Woodford and van Gent (7). Such values are rather independent of operating conditions such as carrier gas flow, column dimensions and proportion of liquid phase. They are only slightly altered by temp, and in this study column age caused variations less than 0.1 unit. The reproducibility of ECL values was ±0.07. For such precision it was necessary to chromatograph the individual esters one at a time followed immediately by a proper standard or together with an internal standard. When ester mixtures were employed in which some components were overloaded, the apparent ECL values were increased slightly and gave values outside the range quoted above.

Results and Discussion

The ECL values on the four phases are presented in Table II. The shorthand notations used in this report indicate the chain length and the number and position of the double bonds in unsaturated fatty acids and esters. Both systems use as root the current shorthand which gives chain length and number of double bonds. Used alone, the root notation does not designate double bond position. In the notation directly derived from the Geneva system, double bond positions counting from the carboxyl group are used as prefixes. Thus 5,8,11,14-eicosatetraenoic acid becomes 5,8,11,14–20:4. In the ω-notation which has primary use in showing metabolic relationships, the double bond positions, counting from the terminal methyl group are given as suffixes. Thus the above example becomes 20:4ω6,9,12,15. Because in polyunsaturated fatty acid (PUFA) metabolism double bonds are methylene-interrupted and the tail structure is a metabolic unit, the formula may be more simply written as 20:4ω6. In both systems double bonds are assumed to be *cis* unless otherwise noted. If one double bond is *trans* the geometry of all double bonds is indicated. Thus *trans-9-cis*-12-octadecadienoic acid becomes 9t,12c–18:2 or 18:2ω6c,9t.

Because EGS is the most commonly used polyester phase for GLC of polyunsaturated esters, discussion shall be limited to the data obtained with it and Apiezon L. The spread of ECL values for a substance obtained on these two phases has proven to be a useful parameter to deduce the number of double

[1] Presented at the Chicago meeting of the American Oil Chemists' Society, Oct. 13, 1964.
[2] Present address: Forschungsinstitut Hohenstein, 7121, Hohenstein, Germany.

TABLE I

Conditions of Gas-Liquid Chromatography Used in this Study[a]

	β-CDX-Ac		DEGS	EGS			Apiezon L
	A	B		A	B	C	
Instrument	GC–2A	GC–2A	GC–2A	GC–2A	RS	BC10	GC–2A
Detector	TC	TC	TC	HF	β ion	β ion	TC
Carrier gas	He	He	He	He	Ar	Ar	He
Flow rate ml/min	120–150	120–150	60–80	60–80	40–60	60–80	60–80
Column size mm x mm	3000 x 7.5	3600 x 3	2000 x 3	1200 x 3	2000 x 4	2000 x 4	2500 x 3
Solid support	GP	GP	GP	GP	GP	CW	GP
Mesh size	30–60	30–60	80–100	80–100	100–120	80–100	100–140
Liquid phase content %	20	20	20	20	15	20	20
Temp °C	234	234	226	180	180	200	240

[a] Abbreviations used in this table are: GC–2A, Beckman GC–2A; RS, Research Specialties; BC 10, Barber-Coleman Model 10; TC, thermal conductivity; HF, hydrogen flame; β ion, β ionization detector; GP, Gaschrom P; CW, Chromosorb W; β-CDX-Ac, β-cyclodextrin acetate; DEGC, dicthylene glycol succinate; and EGS, ethylene glycol succinate.

bonds per molecule, and to indicate something of the positions of the double bonds. The data herein presented are in fair agreement with these reported by Farquhar et al. (8), Miwa et al. (6), Woodford and van Gent (7) and Ackman (9–11). The latter related retention time data to double bond structure in unsaturated esters with considerable success. Using data from the literature as well as form his laboratory, he showed that the logarithms of retention times of a series of related fatty acid esters plotted against the number of carbon atoms of the acids yielded a straight line for each family of acids having the same terminal structure. He postulated that from accurately determined retention time data, one can distinguish isomeric structures. In our study we have screened a larger series of unsaturated esters than was available to Ackman, and some discrepancies have been noted.

When ECL values of esters (EGS) were plotted against carbon atoms of the acids, parallel straight lines were obtained for the families n:1ω7, n:1ω8, n:1ω9, n:2ω5, n:2ω6, n:3ω5, n:3ω6 and n:5ω3. The lines for n:3ω9, n:4ω6 and n:4ω5 were not parallel to the others nor to themselves, but they were straight lines. However, to distinguish between isomers using retention time data on one phase may require higher precision than is afforded by common GLC practice.

The identification of unsaturated fatty acids from retention time data is more certain when done on two phases, one polar and one nonpolar. From our results we conclude that determination of ECL values on two unlike phases is preferable to the use of separation factors (9). Even this type of identification is not certain, and structure determination should be performed whenever possible. For example, isomers of methyl oleate are not sufficiently separable to permit identification, and 6,9,12–18:3 and 7,10,13–18:3 could not be expected to be distinguishable with certainty. The choice whether structural analysis should be performed is limited by the amt of sample available and is mediated by the judgement of the investigator. Since it is not practical to determine structure by ozonolysis-reduction on all samples, the ECL data compiled in this study are offered as a means of tentative identification of polyunsaturated acids.

Phosphoric acid treated EGS columns (12) also have been used for separation of unsaturated esters. This column gave better separation than the plain EGS column, but it suffers the disadvantage that it causes excessive corrosion on the filaments used in thermal conductivity detectors.

The ECL value contains two principal components: one contributed by London force due mainly to the hydrocarbon moiety, and another caused by polar attraction of double bonds and ester groups to the liquid phase. With a polar phase such as EGS, these effects are additive. The ECL measured on Apiezon L has likewise a positive component contributed by chain length, but a negative component contributed by the unsaturation. The difference between ECL_{EGS} and ECL_{AP} therefore minimizes the chain length component and maximizes the polarity component due to unsaturation. When such subtractions of ECL values were carried out, it became clear that the differences were roughly proportional to the number of double bonds present in nonconjugated unsaturated fatty acids. The increment per double bond in long chain esters was found to be *approximately* 0.84 regardless of the positions of the double bonds in the molecules. In short chain esters the increment is somewhat increased. In long chain esters, triple bonds contribute an increment roughly equal to three double bonds. This was true for monoynes, for methylene-interrupted ene-ynes and for tetramethylene-interrupted diynes, but the increment increases in the methylene-interrupted diynes and triynes. In the case of methyl n-decadiene-2,8-diyne-4,6-oate in which carbonyl, diene and diyne are all conjugated, the increment is even higher.

In groups of isomers of esters such as 15:2, 16:2, 18:3, 19:2, 20:3, 22:3 and 22:5, the isomer having the larger ω value emerges first in most instances on both polar and nonpolar columns. The exceptions to this rule are found in the 18:2 series which is the largest studied, and this casts some doubt on the identity of some of the substances studied.

By using columns with plate values (stearate) of >4000 for Apiezon L and >2200 for EGS, separation of *cis* and *trans* isomers was observed (13). On Apiezon L the *cis* isomer emerges first, and on EGS the *trans* isomer emerges first. In instances of conjugation with ester carbonyl, the behavior on Apiezon L is atypical. The ECL is greater than the number of carbon atoms in the acid. This was also observed in the case of diene conjugation (9,11–18:2).

Because ethyl esters can be inadvertently encountered in biological studies, it is well to know where they occur in gas chromatograms relative to the methyl esters usually used as standards. Ethyl esters of seven widely different fatty acids were investigated. In general, they mimic the behavior of methyl esters, but their ECL values are increased on both phases. The increment is not regular, but is influenced by the degree of unsaturation. Caution must be exercised in the tentative identification of methyl esters, for unsuspected ethyl esters could be wrongly identified as uncommon methyl esters. Thus, 18:3ω3 ethyl ester could easily be confused with 19:3ω5 methyl ester. This can be readily checked by hydrogenation, and if the unidentified saturated ester has n + 0.36 ECL value on EGS and n + 0.62 on Apiezon L, it

TABLE II

Equivalent Chain Lengths of Unsaturated Acids on Four Different Phases Used for Gas-Liquid Chromatography

Acid in form of methyl ester		Source	β-CDX-Ac	DEGS	EGS	Apiezon L	$ECL_{EGS}-ECL_{AP}$
2–6:1	6:1ω4	K & K Chemicals	7.52	7.43	7.60	6.50	1.10
3–6:1	6:1ω3	K & K Chemicals	6.77	7.08	7.06	6.00	1.06
2–8:1	8:1ω6	K & K Chemicals	9.57	9.62	9.60	8.55	1.05
2–9:1	9:1ω7	K & K Chemicals	10.53	10.42	10.52	9.63	0.89
2–10:1	10:1ω8	K & K Chemicals	11.53	11.43	11.55	10.63	0.92
n-Decadiene-2,8-diyne-4,6-oic		Sørensen (19)	19.35	19.15	19.10	9.93	9.17
9–14:1	14:1ω5	Schlenk (21)	14.71	14.80	14.75	13.83	0.92
7–14:1	14:1ω7	Schlenk (21)					
6,9–15:2	15:2ω6	Schlenk (3)	16.30	16.43	16.42	14.60	1.82
9,12-15:2	15:2ω3	Schlenk (3)	16.50	16.77	16.70	14.77	1.93
9–16:1	16:1ω7	(1)	16.55	16.55	16.56	15.70	0.86
6,9–16:2	16:2ω7	Schlenk (3)	17.25	17.50	17.32	15.47	1.85
7,10–16:2	16:2ω6	Schlenk (3)					
6,9,12–16:3	16:3ω4	Schlenk (3)	18.10	18.52	18.33	15.47	2.86
7,10,13–16:3	16:3ω3	Schlenk (3)					
9–17:1	17:1ω8	Schlenk (3)	17.56	17.60	17.55	16.73	0.82
9,12–17:2	17:2ω5	Schlenk (3)	18.32	18.50	18.40	16.62	1.78
6,9,12–17:3	17:3ω5	Schlenk (3)	18.93	19.23	19.00	16.40	2.60
6c–18:1	18:1ω12	Hormel Institute	18.52	18.57	18.54	17.71	0.83
6t–18:1	18:1ω12	——	18.45	18.47	18.47	17.75	0.72
8t–18:1	18:1ω10	——	18.49	18.50	18.52	17.73	0.84
9c–18:1	18:1ω9	Hormel Institute	18.55	18.51	18.50	17.71	0.79
9t–18:1	18:1ω9	Hormel Institute	18.47	18.47	18.43	17.76	0.67
10t–18:1	18:1ω8	——	18.50	18.51	18.50	17.78	0.72
11t–18:1	18:1ω7	Hormel Institute	18.60	18.58	18.53	17.80	0.73
12c–18:1	18:1ω6	Hormel Institute	18.60	18.75	18.64	17.75	0.82
12t–18:1	18:1ω6	——	18.54	18.65	18.58	17.80	0.78
17–18:1	18:1ω1	——	18.75	19.00	18.82	17.90	0.92
Octadeca–6–ynoic		Khan (20)	20.03	20.33	20.40	17.84	2.56
Octadeca–7–ynoic		Khan (20)	20.04	20.35	20.45	17.82	2.63
Octadeca–8–ynoic		Khan (20)	20.04	20.40	20.45	17.80	2.65
Octadeca–9–ynoic		Khan (20)	20.12	20.44	20.48	17.70	2.78
Octadeca–11–ynoic		Khan (20)	20.17	20.53	20.60	17.87	2.73
5,11–18:2	——	Schlenk (2)	18.93	19.03	18.90	17.40	1.50
8,11–18:2	18:2ω7	Gensler (18)	19.40	19.46	19.43	17.48	1.95
9,12–18:2	18:2ω6	(1)	19.23	19.30	19.22	17.53	1.69
9,15–18:2	——	Dutton (14)	19.33	19.46	19.43	17.60	1.83
10,13–18:2	18:2ω5	Gensler (18)	19.37	19.55	19.55	17.57	1.98
11,14–18:2	18:2ω4	Gensler (18)	19.47	19.60	19.73	17.62	2.11
12,15–18:2	18:2ω3	Dutton (14)	19.50	19.63	19.63	17.75	1.88
9t,11t–18:2	——	——	20.60	20.70	21.00	18.68	2.32
Octadeca-9-ene-12-ynoic		Wolff (15)	20.97	21.48	21.23	17.90	3.33
Octadeca-9,12-diynoic		Hoffmann La Roche	23.75	24.10	23.74	18.30	5.44
3,9,12–18:3	——	Wolff (23)	20.00	20.33	20.32	17.42	2.90
3,11,14–18:3	——	Schlenk (2)	19.60	19.86	19.63	17.24	2.39
6,9,12–18:3	18:3ω6	——	19.70	20.00	19.78	17.30	2.48
9,12,15–18:3	18:3ω3	Hormel Institute	20.10	20.40	20.13	17.51	2.62
Octadeca-6,9,12-triynoic		Hoffmann La Roche	27.33	27.80	27.90	18.70	9.20
6,9,12,15–18:4	18:4ω3	Wolff (22)	20.73	21.00	21.15	17.30	3.85
11–19:1	19:1ω8	Schlenk (3)	19.53	19.60	19.50	18.60	0.90
8,11–19:2	19:2ω8	Schlenk (3)	20.10	20.28	20.10	18.25	1.85
9,12–19:2	19:2ω7	Schlenk (3)					
11,14–19:2	19:2ω5	Schlenk (3)		20.43	20.27	18.32	1.85
8,11,14–19:3	19:3ω5	Schlenk (16)	20.80	21.05	20.85	18.25	2.60
5,8,11,14–19:4	19:4ω5	Schlenk (16)	21.27	21.54	21.30	18.02	3.28
11–20:1	20:1ω9	(1)	20.32	20.44	20.38	19.78	0.60
5,11–20:2	——	Schlenk (2)	20.77	21.00	20.80	19.32	1.48
11,14–20:2	20:2ω6	(1)	21.13	21.36	21.13	19.48	1.65
7,13–20:2	——	Gunstone (17)	21.00	21.17	20.93	19.30	1.63
Eicosa-7-ene-13-ynoic		Gunstone (17)	22.63	23.10	22.75	19.50	3.25
Eicosa-13-ene-7-ynoic		Gunstone (17)					
Eicosa-7,13-diynoic		Gunstone (17)	24.20	24.92	24.47	19.77	4.70
5,8,11–20:3	20:3ω9	(1)	21.53	21.65	21.57	19.15	2.42
8,11,14–20:3	20:3ω6	(1)	21.72	22.13	21.65	19.23	2.42
5,11,14–20:3	——	Schlenk (2)	21.50	21.75	21.60	19.15	2.45
7,10,13–20:3	20:3ω7	——	—	—	21.63	19.23	2.40
5,8,11,14–20:4	20:4ω6	(1)	22.15	22.43	22.25	19.00	3.25
5,8,11,14,17–20:5	20:5ω3	(1)	22.88	23.45	22.92	19.00	3.92
7,10,13,16–21:4	21:4ω5	Schlenk (16)	23.27	23.50	23.45	20.00	3.45
4,7,10,13,16–21:5	21:5ω5	Schlenk (16)	23.70	24.05	23.97	19.78	4.19
13–22:1	22:1ω9	Hormel Institute	22.27	22.28	22.30	21.57	0.73
7,10,13–22:3	22:3ω9	(1)	23.30	23.73	23.17	21.15	2.02
10,13,16–22:3	22:3ω6	(1)	23.36	23.94	23.38	21.23	2.15
7,10,13,16–22:4	22:4ω6	(1)	24.03	24.58	23.85	20.93	2.92
4,7,10,13,16–22:5	22:5ω6	(1)	24.43	24.97	24.37	20.87	3.50
7,10,13,16,19–22:5	22:5ω3	(1)	24.87	25.38	24.93	21.00	3.93
4,7,10,13,16,19–22:6	22:6ω3	(1)	25.35	26.03	25.40	20.73	4.67
15–24:1	24:1ω9	Hormel Institute	24.20	24.27	24.40	23.67	0.73
9,12,15,18–24:4	24:4ω6	(1)	25.87	26.50	25.73	22.87	2.86
Acid in Form of Ethyl Ester							
18:0	——	Hormel Institute	18.30	18.23	18.36	18.62	—
9–18:1	18:1ω9	Hormel Institute	18.75	18.62	18.75	18.30	0.45
Octadeca-9-ynoic		Khan (20)	20.53	20.57	20.60	18.33	2.27
9,12–18:2	18:2ω6	Hormel Institute	19.60	19.65	19.70	18.13	1.57
9,12,15–18:3	18:3ω3	Hormel Institute	20.46	20.63	20.75	18.17	2.58
5,8,11,14–20:4	20:4ω6	——	22.43	22.63	22.60	19.60	3.00
4,7,10,13,16–22:5	22:5ω6	Hoffman La Roche	24.97	25.04	25.18	21.24	3.94

is an ethyl ester of an acid containing n carbon atoms.

Some generalizations have been developed from the data in Table II. Presently they pertain only to esters of unsaturated straight chain acids but they may be more widely applicable. The ECL data given for a series of unsaturated esters should be used as an indication of, not a proof of, structure. We consider that although a series of isomers may reveal a consistent and proportional effect upon the ECL value, in most instances the magnitude of change between isomers is of the order of present experimental error, and that identification cannot be based upon gas chromatographic data alone. Nevertheless, measurement of ECL value on a polar and on a nonpolar phase sharply limits the range of structures which need be considered for an unknown polyunsaturated

fatty acid. With further improvements in techniques, this method of identification of structure may come to be used with even greater confidence.

The values for ECL presented here may be applied to old data and to data from other laboratories without the use of a contemporary standard, provided that the same type of column packing was used in the experimental situation as was used in deriving the ECL value of the authentic standard, that other chromatographic parameters were approximately the same and that at least two peaks on the chromatogram are identifiable. Thus, for example, ECL values on EGS presented here were used to identify a component in a chromatogram run more than two years previously. On the chromatogram an unknown substance lay between 20:4ω6 and 22:6ω3 which were identifiable through knowledge of the history of the sample and an extensive experience with GLC analysis of tissue fatty acids. The log retention time values for 20:4ω6 and 22:6ω3 were measured from the old chromatogram, these were plotted against the ECL values taken from Table II and the straight line between them was constructed. The log retention time for the unknown was measured and the ECL value found from the straight line was equal to that of 22:3ω9 found in the table. Thus the tentative identification of the substance could be made. The value of this technique lies also in the elimination of the need to run standards frequently after familiarity with the GLC system and the series of samples allows quick identification of some of the major components.

ACKNOWLEDGMENT

This investigation was supported in part by National Institutes of Health research grants No. HE 03559 and HE 05363.

The donors listed in Table II provided samples of unusual fatty acids.

REFERENCES

1. Holman, R. T., and H. H. Hofstetter, JAOCS *42*, 540–544 (1965).
2. Gellerman J. L., and H. Schlenk, Experientia *19*, 522 (1963).
3. Sen, N., and H. Schlenk, JAOCS *41*, 241 (1964).
4. Privett, O. S., and C. Nickell, JAOCS *39*, 414 (1962).
5. Schlenk, H., J. L. Gellerman and D. M. Sand, Anal. Chem. *34*, 1529 (1962).
6. Miwa, T. K., K. L. Mikolajczak, E. R. Fontaine and I. A. Wolff, Anal. Chem. *32*, 1739 (1960).
7. Woodford, F. P., and C. M. van Gent, J. Lipid Res. *1*, 188 (1960).
8. Farquhar, J. W., W. Insull, P. Rosen, W. Stoffel and E. H. Ahrens. Nutr. Rev. Suppl. *17*, 1 (1951).
9. Ackman, R. G., JAOCS *40*, 558, 564 (1963).
10. Ackman, R. G., Nature *194*, 970 (1962).
11. Ackman, R. G., and R. D. Burgher, J. Chromatog. *11*, 185 (1963).
12. Metcalfe, L. D., J. Gas Chromatog. *1*, 7 (1963).
13. Litchfield, C., R. Reiser, and A. F. Isbell, JAOCS *40*, 302 (1963).
14. Scholfield, C. R., E. P. Jones, Janina Nowakowska, E. Selke and H. J. Dutton, JAOCS *38*, 208 (1961).
15. Mikolajczak, K. L., C. R. Smith, Jr., M. O. Bagby and I. A. Wolff, J. Org. Chem. *29*, 318 (1964).
16. Schlenk, H., D. M. Sand and N. Sen, Biochem, Biophys. Acta *84*, 361 (1964).
17. Gunstone, F. D., and P. J. Sykes, J. Chem. Soc. 3055 (1962).
18. Gensler, W. J., and J. J. Bruno, J. Org. Chem. *28*, 1254 (1963).
19. Holman, R. T., and N. A. Sørensen, Acta Chem. Scand. *4*, 416 (1950).
20. Khan, N. A., JAOCS *30*, 355 (1953).
21. Schlenk, H., et al., JAOCS, in preparation.
22. Smith, C. R., Jr., J. W. Hagemann and I. A. Wolff, JAOCS *41*, 290 (1964).
23. Bagby, M. O., W. O. Siegl and I. A. Wolff, JAOCS *42*, 50, (1965).

[Received September 8, 1964—Accepted January 29, 1964]

The Fatty Acid Composition of the Lipids from Bovine and Porcine Reproductive Tissues[1]

RALPH T. HOLMAN and H. H. HOFSTETTER,[2] The Hormel Institute, University of Minnesota, Austin, Minnesota

Abstract

Beef and pork testes, graafian follicles and the residual ovaries were extracted and the lipids from each were separated into lipid classes by thin-layer chromatography. The fatty acids from each class were analyzed as their methyl esters by gas-liquid chromatography. The lipids from the reproductive tissues were found to be relatively rich in polyunsaturated acids, many of which did not correspond to the more commonly encountered unsaturated acids. These less familiar acids were identified by comparing their chromatographic characteristics with standards of established composition.

The polyunsaturated acids of lipids of the reproductive tissues examined are predominantly of the linoleate family. Only in the phospholipids of ovarian tissues did the linolenate family of acids reach high proportions of the total polyunsaturates. Nine members of the linoleate family were identified in the lipids of reproductive tissues. Five higher metabolites of oleate were identified as normal components of these tissues. Diglycerides were found as a significant lipid class only in testis tissue. The diglycerides and cholesteryl esters of beef testis contain tetracosatetraenoic acid as major fatty acid. The triglycerides of reproducitve tissues are notably rich in polyunsaturated acids. In the study, 16 polyunsaturated acids were identified by ozonolysis-reduction and several others were tentatively identified by retention time data. Two acids, previously unreported, are 10,13,16-docosatrienoic acid and 9,12,15,18-tetracosatetraenoic acid.

Introduction

THE DEFICIENCY of essential fatty acids (EFA) is known to result in sterility in both the male and the female rat (1,2). The ovary and the testis which produce reproductive cells contain lipids which are especially rich in polyunsaturated acids (3). The content of polyunsaturated fatty acids (PUFA) in the the rat testis decreases as essential fatty acid deficiency sets in (4). Semen (5) and eggs (6) contain lipids rich in polyunsaturates, and the cause of the sterility of EFA-deficient animals may be the insufficiency of the polyunsaturates for the synthesis of the fertile reproductive cells.

The lipids of the testes, ovaries, ovarian follicles and corpora lutea have been studied by many investigators using classical methods of lipid biochemistry (7–13). These investigations produced information concerning the content of phospholipid, cholesterol, neutral fat and unsaponifiable matter in these tissues. In some the approximate fatty acid composition was given for the lipids, and evidence exists for the presence of highly unsaturated acids in lipids. However, precise information concerning the fatty acid composition of the several lipids present in these tissues gathered using the newer, more discriminating, techniques of

[1] Presented in part at the First World Fat Congress, Hamburg, October, 1964, and at the meeting of the American Oil Chemists' Society, Chicago, October, 1964.

[2] Present address: Forschungsinstitut Hohenstein, 7121 Hohenstein, Germany.