Positional distribution of isomers of monoenoic fatty acids in animal glycerolipids

H. BROCKERHOFF and R. G. ACKMAN

Fisheries Research Board of Canada, Halifax Laboratory, Halifax, Nova Scotia, Canada

ABSTRACT The distributions of the following monoenoic acids were determined [notation: (*position of double bond*)-(chain length): (no. of double bonds)]: *7*-, *9*-, and *11*-16:1; *7*-, *9*-, *11*-, and *13*-18:1; *9*-, *11*-, and *13*-20:1; *9* + *11*-22:1 and *13*-22:1. As a rule, all isomers of a group show different distribution patterns. In the phospholipids of fish and mammals, the *7*- and *13*-isomers of 18:1 accumulate in position 1. In triglycerides of mammals fed on fish they accumulate in positions 1 plus 3, and this distribution is shared by *7*-16:1 and *11*-16:1 and by the groups 20:1 and 22:1. The positional distribution of the acids seems to depend on their structure, the *9*-isomers in general accumulating in position 2; but in triglycerides, at least, the origin of the acid also seems to play a directing role, the exogenous acids being incorporated into positions 1 and 3. The variability of the distribution patterns of *9*-16:1, *9*-18:1, and *11*-18:1, which contrasts with the regularity of the patterns for saturated and polyenoic acids, may be connected with the ability of the endogenous monoenoic acids to balance fluctuations in the supply of the exogenous polyenoic acids, and with the role of the fatty acid 9,10-dehydrogenation mechanism in the maintenance of structural and physical properties of phospholipids and triglycerides.

KEY WORDS monoenoic fatty acids · isomers · positional distribution · phospholipids · triglycerides · depot fat · whale milk fat · egg lecithin · fish · mammals · 9,10-dehydrogenation system

SATURATED AND POLYUNSATURATED fatty acids occupy definitive structural positions in the phospholipids and triglycerides of animals. Saturated acids always predominate in position 1 of phospholipids, polyunsaturated acids in position 2. A similar, but less precise division is made in the triglycerides of depot fats, with exceptions confined to certain related groups of animals (1, 2). Such a general pattern is not found for the monoenoic acids. The only detectable regularity is a preference of the shorter acids, 16:1 > 18:1 > 20:1 > 22:1, for position 2 in triglycerides (1); therefore 20:1, and especially 22:1, will usually be found in positions 1 and 3. The distribution patterns of monoenes as a group, however, and especially the distribution patterns of the most common of all fatty acids, 18:1, are unpredictable. No pattern is discernible that would recur in the triglyceride or phospholipids of all animals, or of groups of related animals.

The monoenoic acids of animal lipids are mixtures of isomers. Since a rapid analysis of such mixtures can now be performed on open-tubular gas–liquid chromatographic columns, we have investigated the positional distribution of fatty acids with special attention to the monoenes. We hoped to find some regularity in the patterns for at least some of the isomers, and a confirmation of our suggestion (2) that the irregular distribution will be confined mainly to the *9*-isomers.[1]

MATERIALS AND METHODS

Isolation of Lipids

The following species and organs were analysed: cod, *Gadus morrhua*, liver; mackerel, *Scomber scombrus*, total animal; lobster, *Homarus americanus*, hepatopancreas of an animal that had been kept on a diet of herring; harp seal, *Pagophilus groenlandicus*, blubber of a 4 yr old lactating female; mink, *Mustela vison*, and white rat, *Rattus norvegicus*, the collected adipose tissues or the livers of animals that had been kept on a diet of mackerel (4); rat, kept on laboratory chow; dog, *Canis familiaris*, mesenteric fat (5); pig, *Sus scrofa*, commercial bacon; pig,

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography.

[1] The shorthand notation proposed by Holman (3) is used, in which, e.g., *cis*-vaccenic acid≡*cis*-Δ^{11}-octadecenoic acid, is written *11*-18:1.

mesenteric fat from an animal raised to a weight of 90 kg on a fat-free diet (by courtesy of R.E. Anderson, Texas A and M University); fin whale, *Balaenoptera physalus*, milk of an animal caught in Nova Scotian waters (by courtesy of M. Cawthorne, Fisheries Research Board, Montreal, Quebec); duck, *Anas*, fat from the region of the pygostyle (5).

Depot fat triglycerides were extracted with hexane in the presence of sodium sulfate and purified by chromatography on silicic acid (6) and aluminum oxide (7). Their purity was checked by TLC.

The whale milk was extracted with chloroform–methanol (8), and the triglyceride was purified by gel filtration (9).

Liver lipids were extracted with chloroform–methanol. The triglycerides were isolated by column chromatography (6), and their purity was ascertained by TLC. The phospholipids were isolated by TLC (10) with 0.03% α-tocopherol added to the solvent to suppress oxidation.

Analysis of Fatty Acid Distribution

The lipids of the mink and of the rat on the fish diet had been analyzed in connection with a different project, as reported elsewhere (4). For the purpose of the present investigation these analyses were extended to the isomeric monoenes.

Triglycerides were analyzed with pancreatic lipase (EC 3.1.1.1) (11). The phospholipids were degraded with phospholipase A (EC 3.1.1.4) from snake venom (*Ophiophagus hannah*) (12). The products, lysophosphatides (residual fatty acid in position 1) and free fatty acids (from position 2) were separated by TLC (10), with 0.03% tocopherol in the solvents. When the analytical data of the products were added for comparison with the original lipid, the agreement was always within 5%, relative, for any major component.

Gas–Liquid Chromatography

The methyl esters, prepared with BF_3–methanol (13) or by transesterification with KOH–methanol (14), were analyzed by GLC on ethylene glycol succinate–silicone copolymer columns (15, 16). Overlapping peaks were resolved, and identifications established, by GLC with liquid phases of different polarity. A hydrogen flame ionization detector was used, and percentages of components were calculated by the peak height–retention time method (17). Analyses of NIH standard mixtures were correct within ± 3% (relative) for major compounds (>10%) and ±7% (relative) for minor compounds.

Isomers of monoethylenic fatty acids were determined by analysis of total methyl esters on an open-tubular gas–liquid chromatographic column (18–21). The apparatus was a Perkin-Elmer model 226 with a hydrogen flame ionization detector. The column was of stainless steel, 0.01 inch I.D. × 150 ft long, coated with butanediol succinate polyester. Operating conditions were: column 170° C and 50 psi helium, injection port 250° C. Under these conditions the number of theoretical plates calculated for methyl palmitate (adjusted retention time, 7 min) was 10–12,000 per column. Samples were injected in carbon disulphide solution and were kept sufficiently small that ratios of peak heights could be used to determine isomer ratios (attenuation of 10× or less). In these analyses the probable error for each component is inversely proportional to its magnitude relative to other components. In most instances these errors would not exceed ±10% (relative) for small components but the poorly indicated *11*-18:1 shoulder could be subject to errors as large as ±20% in all analyses.

RESULTS

As a rule all isomers of monoenoic acids show different distribution patterns, i.e., the relative proportions of the isomers within a group differ for each position (Tables 1 and 2). Identical proportions of isomers are less common.

Phospholipids

Among the isomeric monoenoic acids (Table 1) those with the double bond nearest to the methyl end of the chain (*11*-16:1, *13*-18:1, and *13*-20:1) tend to accumulate in position 1, as does the 18:1 acid with the double bond nearest to the carboxyl group, *7*-18:1. Concomitantly, there is a higher proportion of the more "symmetrical" acids, *7*-16:1 and *9*-16:1, *9*-18:1 and *11*-18:1, and *9*-20:1 and *11*-20:1, in position 2. Most often the proportion of the *9*-isomers is found to increase at the expense of the *7*-, *11*-, and *13*-isomers (exceptions are found in the mink and in the 16:1 group). This is also true for the egg lecithin.

Triglycerides of Fish, Lobster, Duck, and Whale Milk

Compared with the phospholipids or with the mammalian triglycerides, the triglycerides of fish show little positional differentiation of isomers (Table 2, Nos. 1, 2).

The depot fat of lobster shows a decidely nonrandom pattern (Table 2, No. 3), but there seems to be no system in it. This brings to mind the failure of fats of other invertebrates (the few that have been analyzed) (2) to obey a recognizable pattern.

The duck (Table 2, No. 4) shows a random distribution in agreement with the finding that the fats of birds, as observed so far (2), are patterned more randomly than fats of other animals.

In the whale milk fat (Table 2, No. 5), the major isomeric monoenoic acids are not differentiated.

TABLE 1 COMPOSITION OF THE MIXTURES OF ISOMERIC MONOENOIC ACIDS IN THE DIFFERENT POSITIONS OF PHOSPHOLIPIDS, GIVEN AS PERCENTAGE OF EACH MEMBER IN THE GROUP OF ISOMERS

Source of Phospholipid	Phospho-lipid	Posi-tion	16:1 Total	16:1 Proportions 7	9	11	18:1 Total	18:1 Proportions 7	9	11	13	20:1 Total	20:1 Proportions 9	11	13
			% of fatty acids												
1. Mackerel, total	PC*	1	*2*	36	64		*11*	8	61	26	5	*4*	8	82	10
		2	*<1*	37	63		*4*	2	80	17	1	*<1*	21	76	3
	PE*	1	*1*	43	57		*9*	12	46	38	5	*4*	5	83	12
		2	*<1*	54	46		*3*	3	65	31	1	*<1*	12	86	2
2. Cod, flesh	PC	1	*3*	24	56	22	*11*	13	44	39	4	*2*	13	80	7
		2	*<1*	18	78	4	*6*		84	16		*<1*	42	58	
	PE	1	*1*	33	56	11	*23*	11	41	45	3	*8*	12	82	6
		2	*<1*	40	48	12	*5*	10	45	43	2	*1*	16	84	
3. Mink, liver (mackerel-fed)	PC	1	*2*	28	62	10	*12*	12	58	27	4	*2*	19	69	12
		2	*1*	12	82	6	*9*		61	38	1	*2*	20	77	3
	PE	1	*1*	40	60		*8*	9	58	28	5	*2*	32	63	5
		2	*1*	40	60		*8*	2	62	35	1	*2*	24	72	4
4. Mink, liver (chicken-fed)	PC	1	*4*	36	64		*13*		89	11		*1*			
		2	*2*	24	76		*18*		80	20		*1*			
	PE	1	*2*	68	32		*14*		90	10		*1*			
		2	*2*	66	34		*2*		84	16		*1*			
5. Rat, liver (mackerel-fed)	PC	1	*2*	47	53		*7*	7	39	45	8	*1*			
		2	*3*	19	81		*10*	2	81	16	1	*1*			
	PE	1	*3*	60	30		*9*	4	48	39	9	*1*			
		2	*1*	29	71		*3*	1	72	25	1	*1*			
6. Rat, adipose tissue (mackerel-fed)	PC	1	*1*	20	80		*11*	8	62	25	5	*3*	13	70	17
		2	*3*	31	69		*18*	2	78	18	2	*2*	32	55	13
	PE	1	*2*	11	85	4	*22*	5	75	18	2	*3*	15	74	11
		2	*2*	20	76	4	*8*	1	85	13	1	*2*	34	58	7
7. Hen's egg	PC	1	*1*	31	69		*4*		83	17					
		2	*1*	9	91		*61*		97	3					

The numbers in italics in the body of the table give the concentration (percentage of total fatty acids) of the respective group of isomers in this position.

* PC, phosphatidyl choline; PE, phosphatidyl ethanolamine.

Triglycerides of Mammals

In nearly all cases (Table 2, Nos. 6–13), the extreme members of the 16:1 and 18:1 series are preferentially located in positions 1 or 3; these are the acids 7-16:1, 11-16:1, 7-18:1, and 13-18:1. Among the remaining acids, 9-16:1, 9-18:1, and 11-18:1 (palmitoleic, oleic, and *cis*-vaccenic acid) the 9-isomers in particular predominate in position 2. There is a relative accumulation of the 9-isomer of 20:1 in position 2 in the fats of seal and rat, though not in mink; however, the groups 20:1 and 22:1, as such, tend to occupy the positions 1 and 3. In the one triglyceride subjected to a stereospecific analysis (Table 2, No. 10) the pattern is nonrandom and asymmetrical.

DISCUSSION

Distribution and Structure of Fatty Acids

At some stage in the biosynthesis of phospholipids and triglycerides the different isomers of the monoenoic acids are sifted and channeled into different positions. Such discrimination could be a result of the different chemical or physical properties of the isomers, or the result of different pools of acids being used for different reactions. As to the first possibility, we might postulate, for example, that the enzymes that acylate the 2-position select fatty acids of similar molecular shapes with the double bond at a certain preferred distance from the carboxyl group or from the methyl end. We find in the tables that in the phospholipids of fish and mammals and in the triglycerides of mammals the "extreme" isomers (11-16:1, 7-18:1, 13-18:1, 13-20:1) usually accumulate in the positions 1 or 3. The enzymes acylating position 2 could therefore be said to have a higher affinity for the more "symmetrical" isomers; however, 11-18:1 has the same degree of "asymmetry" (distance of the double bond from the middle of the chain) as 7-18:1, but it is not excluded to the same extent from position 2. The distance of the double bond from the end methyl group does not seem to be correlated with the distribution patterns. The distance from the carboxyl group, however, appears to be important: in

TABLE 2 COMPOSITION OF THE MIXTURES OF ISOMERIC MONOENOIC ACIDS IN THE DIFFERENT POSITIONS OF TRIGLYCERIDES, GIVEN AS PERCENTAGE OF EACH MEMBER IN THE GROUP OF ISOMERS

Source of Fat	Position	16:1 Total	16:1 Proportions 7	9	11	18:1 Total	18:1 Proportions 7	9	11	13	20:1 Total	20:1 Proportions 9	11	13	22:1 Total	22:1 Proportions 9 + 11	13
											% of fatty acids						
1. Mackerel, depot	1, 3	*5*	10	80	10	*15*	3	74	19	3	*12*	9	83	7	*15*	87	13
	2	*4*	19	74	7	*6*	2	65	29	3	*4*	9	84	7	*2*	79	21
2. Cod, depot	1, 3	*9*	6	90	4	*23*	9	62	28	1	*15*	14	77	9	*10*	80	20
	2	*4*	7	90	2	*9*	7	72	21	1	*8*	14	79	6	*7*	87	13
3. Lobster, depot	1, 3	*7*	7	89	4	*20*		74	24	2	*12*	20	51	29	*7*	78	22
	2	*8*	14	79	8	*21*		41	58	2	*7*	21	71	7	*1*	80	20
4. Duck, depot	1, 3	*7*	8	92		*44*		91	9								
	2	*4*	8	92		*59*		94	6								
5. Whale, milk	1, 3	*5*	2	93	5	*31*		69	30	*1*	*4*	16	94	10	*2*	83	17
	2	*11*	6	91	3	*8*		70	28	2	*<1*				tr.		
6. Seal, depot	1, 3	*10*	2	96	2	*20*	26	44	28	2	*17*	16	76	8	*3*	n.d.	n.d.
	2	*34*	1	98	1	*23*	4	80	14	2	*3*	22	70	8	*<1*		
7. Mink, depot (mackerel-fed)	1, 3	*4*	18	76	6	*28*	17	61	21	2	*17*	28	68	4	*8*	84	16
	2	*8*	2	91	6	*36*		85	15	1	*3*	28	64	7	*1*		
8. Mink, liver (mackerel-fed)	1, 3	*4*	15	78	7	*20*	12	65	20	3	*9*	31	62	7	*1*	60	40
	2	*6*	5	93	2	*39*		75	24	1	*3*	23	71	6	*1*	60	40
9. Rat, liver (mackerel-fed)	1, 3	*3*	28	63	9	*18*	23	59	17	1	*8*	22	70	8	*4*	83	17
	2	*6*	7	92	1	*50*		87	12	1	*5*	50	44	6	*1*	80	20
10. Rat, depot (mackerel-fed)	1	*6*	18	72	10	*30*	17	60	21	2	*9*	22	67	11	*4*	83	17
	2	*7*	4	94	2	*49*	1	96	2	1	*5*	54	44	2	*1*	90	10
"	3	*7*	22	74	4	*27*	20	44	34	2	*12*	23	72	5	*8*	81	19
11. Rat, depot	1, 3	*4*	16	84		*34*		88	12								
	2	*4*	5	95		*38*		94	6								
12. Dog, depot	1, 3	*4*	18	82		*46*		91	9								
	2	*7*	4	96		*35*		93	7								
13. Pig, depot	1, 3	*2*	35	65		*64*		88	12								
	2	*5*	15	85		*24*		88	12								
14. Pig, depot (fat-free diet)	1, 3	*3*	8	92		*61*		90	10								
	2	*6*	11	89		*13*		91	9								

The numbers in italics in the body of the table give the concentration (percentage of total fatty acids) of the respective group of isomers in this position. n.d., not determined.

most lipids the *9*-isomers of 16:1, 18:1, and 20:1 tend toward position 2, although several partial exceptions can be found, as in the phospholipids of mink, in addition to the major exceptions of the triglycerides of fish and lobster.

The accumulation of the *9*-isomers in position 2 may be a result of their structure, but it can also be interpreted in terms of their potentially endogenous origin, as we shall see in the following paragraph. However, a persuasive example of the dominance of structure over origin is furnished by the 20:1 group in mammalian triglycerides. In the rat, for instance, all three 20:1 acids are derived from the fish diet, but they are nevertheless differentiated in their distribution (Table 2, No. 10).

Distribution and Origin of Fatty Acids

A good case can also be made for a hypothesis in terms of different pools or different origins of fatty acids. Mammals can dehydrogenate saturated fatty acids in the 9,10-position. The preponderance of the *9*-isomers of 16:1 and 18:1 in position 2 of the glycerolipids may therefore indicate that this position receives endogenous (or partly endogenous) fatty acids from the 9,10-dehydrogenation system. Conversely, those acids which are very probably exogenous, *7*-16:1, *7*-18:1, and *13*-18:1, accumulate in the 1- or 3-positions and the acid *11*-18:1, which may be endogenous [synthesized by lengthening of *9*-16:1 (22)], shows intermediate behavior. The case of exogenous vs. endogenous acids can be demonstrated even more clearly from the data on animals of the same species but on different diets (Table 2). The fat of the pig raised on the fat-free diet (No. 14) shows random distribution. In the other pig (No. 13), there is an increase in the proportion of *7*-16:1; this increase is probably due to the diet, and it occurs mostly in the 1- or 3-position. The same acid is higher in

the rat fed on mackerel (Table 2, Nos. 9, 10) than in the other rat (No. 11), and again it accumulates in positions 1 and 3. The acids *11*-16:1, *7*-18:1, and *13*-18:1 which occupy the positions 1 or 3 not only in rat but also in mink (Table 2, No. 7, Table 1, No. 5) and seal (Table 2, No. 6) are obviously of dietary origin. The distribution patterns of the exogenous *7*-18:1 and the potentially endogenous *9*-18:1 are so dramatically different that it is difficult to believe that the slight difference in structure could be wholly responsible, especially since the *7*-isomers are not excluded from position 2 in triglycerides where they must be (Table 2, No. 14) or may be (Nos. 1–3) endogenous.

In triglycerides, then, the distribution patterns seem to be related to the endogenous or exogenous origin of the monoenoic acids, though the influence of the structure of the fatty acid cannot be discounted. In the phospholipids of the fish (Table 1, Nos. 1, 2) we find a degree of discrimination among acids and a similarity of patterns between species that are missing in the triglycerides of the same fish (Table 2, Nos. 1, 2). It seems, therefore, that the patterns of these phospholipids, and perhaps of phospholipids in general, may depend more on the structure of fatty acids and less on their origin than the corresponding patterns of the triglycerides.

Function of Monoenoic Acids

Compared with the distribution patterns of saturated and polyunsaturated acids, those of the acids 16:1 and 18:1 appear indistinct and accidental. The patterns not only differ with different species, but they also vary with the diet (Table 1, Nos. 3, 4; Table 2, Nos. 10, 11). However, further analysis of these acids, and of 20:1 and 22:1, shows that this is not true for all isomers. Some accumulate regularly in the 1- or 3-position of phospholipids and of mammalian triglycerides: *7*-16:1, *11*-16:1, *7*-18:1, *13*-18:1, *13*-20:1, and 22:1, in the few exceptions, the acids are present in traces only.

This leaves us these monoenoic acids with indeterminate or variable distribution: *9*-16:1, *9*-18:1, *11*-18:1, *9*-20:1, and *11*-20:1. All these acids are potentially endogenous, available to animals by 9,10-dehydrogenation of saturated fatty acids, followed by chain-lengthening for the *11*-isomers (22). This suggests that differences in the monoene distribution patterns are tied to differences in the activity of the desaturation system. The flexibility of this system is well known; many experiments (23–28) have shown that a deficiency in polyenoic acids is compensated for by dehydrogenation of saturated acids, and the suggestion has been made repeatedly that this mechanism serves to balance the composition and properties of lipids (25, 27, 29). The existing data, combined with our present results, lend themselves to the following interpretation.

In the biosynthesis of phospholipids and of triglycerides, fatty acids go to predetermined positions. If we may simplify the picture: saturated acids are marshalled to position 1 and polyunsaturated acids to position 2. Whereas the saturated acids are always and sufficiently available by biosynthesis, the polyenoic acids are not: they have to be acquired through the diet. Fluctuations in the supply are buffered by 9,10-dehydrogenation which supplies more monoenoic acid when it becomes necessary to make up for a deficit of polyenoic acids in the β-position [the formation of *5,8,11*-20:3 from *9*-18:1 (30) is a further extension of this buffering mechanism]. The 1-position can also be supplied with monoene, if this becomes necessary to maintain the general physical properties of the lipids (26).

In the major phospholipids of mammals and fish the acids 16:1, 20:1, and 22:1 are of minor importance; *9*-18:1 is the principal acid used to balance the structure in cases of emergency, followed by *5,8,11*-20:3. Under normal conditions, the monoenes do not play a very important role. The triglycerides of depot fat, on the other hand, always contain large amounts of monoenoic acids. These are used to fill the structural frame set by saturated and polyunsaturated acids. The balancing mechanism, nevertheless, is active also; e.g., monoenoic acids will compensate for a lack of polyenoic acids in the depot fat of rat (23) or herring (31). Perhaps the necessity here is not so much to maintain a certain molecular structure as to keep the fat liquid.

To summarize this interpretation: the different distribution patterns of endogenous monoenoic acids reflect differences in the supply or utilization of polyenoic acids, and in the equilibria of the 9,10-dehydrogenation systems in different animals or tissues. The biochemistry of the system may involve competitive inhibition of the dehydrogenase by polyenoic acids (27, 32).

Manuscript received 12 June 1967; accepted 9 August 1967.

References

1. Brockerhoff, H. 1966. *Comp. Biochem. Physiol.* **19:** 1.
2. Brockerhoff, H., R. J. Hoyle, P. C. Hwang, and C. Litchfield. 1967. *Lipids*. In press.
3. Holman, R. T. 1966. Progress in the Chemistry of Fats and Other Lipids. Pergamon Press Ltd., Oxford. **9:** 1.
4. Brockerhoff, H., R. J. Hoyle, and P. C. Hwang. 1967. *Biochim. Biophys. Acta*. In press.
5. Brockerhoff, H., R. J. Hoyle, and N. Wolmark. 1966. *Biochim. Biophys. Acta*. **116:** 67.
6. Barron, E. J., and D. J. Hanahan. 1958. *J. Biol. Chem.* **231:** 493.
7. Brockerhoff, H., R. G. Ackman, and R. J. Hoyle. 1963. *Arch. Biochem. Biophys*. **100:** 9.
8. Bligh, E. G., and W. J. Dyer. 1959. *Can. J. Biochem. Physiol*. **37:** 911.
9. Tipton, C. L., J. W. Paulis, and M. D. Pierson. 1964. *J. Chromatog*. **14:** 486.

10. Brenner, R. R., and A. M. Nervi. 1965. *J. Lipid Res.* **6:** 363.
11. Mattson, F. H., and R. A. Volpenhein. 1961. *J. Lipid Res.* **2:** 58.
12. Nutter, L. S., and O. S. Privett. 1966. *Lipids.* **1:** 258.
13. Metcalfe, L. D., and A. A. Schmitz. 1961. *Anal. Chem.* **33:** 363.
14. Brockerhoff, H. 1965. *Arch. Biochem. Biophys.* **110:** 586.
15. Ackman, R. G., and R. D. Burgher. 1965. *J. Am. Oil Chemists' Soc.* **42:** 38.
16. Ackman, R. G. 1966. *J. Gas Chromatog.* **4:** 256.
17. Carroll, K. K. 1961. *Nature.* **191:** 377.
18. Ackman, R. G. 1966. *J. Am. Oil Chemists' Soc.* **43:** 483.
19. Ackman, R. G., and J. D. Castell. 1966. *Lipids.* **1:** 341.
20. Ackman, R. G., and J. D. Castell. 1967. *J. Gas Chromatog.* In press.
21. Ackman, R. G., J. Sipos, and P. M. Jangaard. 1967. *Lipids.* In press.
22. Holloway, P. W., and S. J. Wakil. 1964. *J. Biol. Chem.* **239:** 2489.
23. Hilditch, T. P., and P. V. Williams. 1964. The Chemical Constitution of Natural Fats. Chapman and Hall Ltd., London. 93–97.
24. Longenecker, H. E. 1939. *J. Biol. Chem.* **128:** 645.
25. Mead, J. F. 1957. *J. Biol. Chem.* **227:** 1025.
26. Johnson, R. M., and T. Ito. 1965. *J. Lipid Res.* **6:** 75.
27. Mohrhauer, H., and R. T. Holman. 1963. *J. Lipid Res.* **4:** 151.
28. Catalá, A., and R. R. Brenner. 1967. *Lipids.* **2:** 114.
29. Lovern, J. A. 1942. *Gt. Brit. Dept. Sci. Ind. Res. Food Invest. Spec. Rep.* **51.**
30. Fulco, A. J., and J. F. Mead. 1959. *J. Biol. Chem.* **234:** 1411.
31. Ackman, R. G., and C. A. Eaton. 1966. *J. Fisheries Res. Board Can.* **23:** 991.
32. Brenner, R. R., and R. O. Peluffo. 1966. *J. Biol. Chem.* **241:** 5213.