Application of High Vacuum Fractional Distillation to Complex Mixtures of Methyl Esters of Polyunsaturated Fatty Acids

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Abstract

A technique for the high vacuum fractional distillation, with a spinning band column, of methyl esters of polyunsaturated fatty acids employing a carrier of long chain acetates is described. The carrier is used to facilitate the fractionation of minor components and minimize artifact formation in mixtures of methyl esters containing up to six double bonds. The technique is demonstrated on a standard mixture of methyl esters and applied to a concentrate of unsaturated methyl esters containing a large number of minor components as well as methyl docosahexaenoate.

Introduction

APPARATUS AND TECHNIQUES have been developed for the fractional distillation of less than 1 g to over 20 kg of fatty acid methyl esters with columns that develop an efficiency of up to 100 theoretical plates (12,15,28). However, long-chain polyunsaturated methyl esters with 4, 5 or 6 double bonds cannot be distilled through high efficiency packed columns without considerable decomposition which is generally evidenced by the formation of an appreciable amount of nondistillable residue. Farmer and van den Heuvel (5) were among the first to recognize alteration of the structures of polyunsaturated methyl esters during fractional distillation. A number of investigators have made similar observations since then, and it is now generally regarded that heating methyl esters of polyunsaturated fatty acids for prolonged periods above 200 C will alter their structures (1,6,10,17). The distillation of long-chain methyl esters through packed columns generally requires still-pot temperatures well above 200 C. In order to minimize alteration of polyunsaturated fatty esters, high vacuums of the order of 10^{-4} mm have been employed (8,9). Lowering the pressure lowers the boiling point (11), and so permits the use of lower still-pot temperatures. However, the large hold-up and, as a consequence, high back-pressure at which most packed columns operate limits the application of this relationship from a practical standpoint. In contrast, columns of the spinning band type have low hold-up, and therefore, may be operated at maximum efficiency at relatively low pressures; 0.5 mm is a typical back pressure for these columns (2,15,24). In a previous communication (20) we demonstrated the value of these columns for the fractional distillation of methyl esters of polyunsaturated fatty acids, and showed that efficient fractionation could be achieved with pressures of the order of 10^{-2} mm. Nevertheless, even with these columns, appreciable decomposition of polyunsaturated esters containing 4, 5 and 6 double bonds may occur as also evidenced by the presence of a nondistillable residue in the still-pot at the end of the distillation (1,20). Described here is the use of a carrier consisting of a mixture of long chain acetates for the amplified distillation of methyl esters and application of the technique to minimize alteration of the structures of the methyl esters of polyunsaturated fatty acids.

Experimental Procedures

Materials and Methods

Long chain acetates and methyl esters were purchased from the Lipids Preparation Laboratory of The Hormel Institute, Austin, Minnesota. Gas-liquid and thin-layer chromatography showed that these compounds contained only traces of impurities, and were greater than 99% pure.

GLC was carried out with an F&M Model 1609 flame ionization instrument with a 6 ft × ¼ in. column packed with 10% EGSSX on Gas Chrom P (Applied Science Laboratory, State College, Pa.). Most analyses were carried out at 185 C using nitrogen as the carrier gas at a flow rate of 75 ml/min. A temperature of 205 C was used with some samples to speed up the analyses of fractions containing 22:6.

TLC was carried out on chromatoplates coated with 0.25 mm of Silica Gel G (Merck AG, Darmstadt, Germany) or Silica Gel G impregnated with silver nitrate (20A). Silica Gel G plates were used for the separation of the methyl esters as a fraction and were developed with petroleum ether-ethyl ether-acetic acid (90:10:1). The silver nitrate plates were used for the fractionation of methyl esters by degree of unsaturation and were developed with chloroform containing 1%, 2% or 3% methanol depending on the spectrum of the unsaturation of the sample.

Fractional distillations were carried out as previously described (20) with a spinning band column (0.8 × 60 cm) (Podbielniak, Inc., Chicago, Illinois) using GLC to monitor the composition of the distillate at approximately 20 min intervals. Fractions were collected on the basis of the GLC analyses. Generally distillations were conducted on 30–40 g samples consisting of 10 g of methyl esters and 20–30 g of acetates. Formulation of the composition of the acetates was based on the chain length composition of the methyl esters and generally was made up so as to have approximately 2 to 3 times as much acetate as corresponding methyl ester. For distillation purposes the 16-carbon acetate is considered to correspond to the 18-carbon methyl ester fraction, for example. Generally the acetates do not interfere with the GLC analysis of methyl esters of the same boiling range and are used as markers for the fractionation. However, in the analysis of fractions containing complex mixtures of methyl esters, some of the components may be masked by some acetates. In this case, one of the methyl esters that separates completely from the acetate in each fraction may be used to monitor the fractionation as well as the acetates.

Finally, the acetates are removed from the methyl esters in each fraction via saponification. Alcohols produced from the acetates by saponification are extracted from the soaps as nonsaponifiable matter;

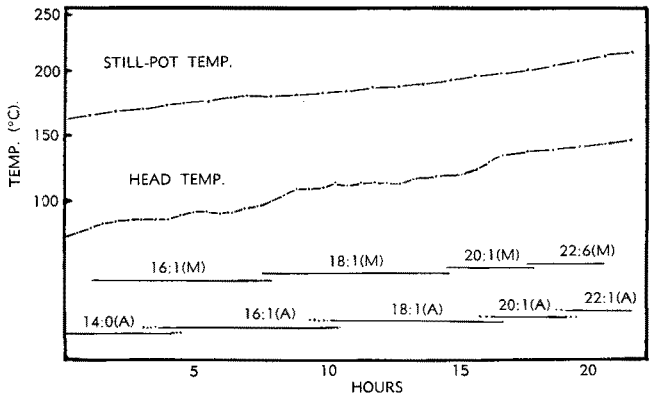

FIG. 1. General course of carrier distillation of a standard mixture of methyl esters—16:1 M, 18:1 M, 20:1 M and 22:6 M (M after short hand designation = methyl ester)—with a carrier consisting of myristyl (14:0 A), palmitoleyl (16:1 A), oleyl (18:1 A), eicosenoyl (20:1 A) and erucyl (22:1 A) acetates.

sodium acetate is converted to acetic acid by acidification and separated from the liberated fatty acids by extraction with distilled water. The fatty acids remain in the ether phase. The solution is dried with anhydrous sodium sulfate and, after filtration, is recovered by evaporation of the solvent and esterified with methanol. The solutions should be kept air-free by bubbling nitrogen through them in all operations in order to avoid autoxidation.

Results

The general course of a typical distillation of a mixture of methyl esters and long chain acetates is shown in Figure 1. The sample employed in this distillation was a standard mixture consisting of equal amounts (2.5 g) of methyl palmitoleate (16:1 M), oleate (18:1 M), 11-eicosenoate (20:1 M) and docosahexaenoate (22:6 M). Although it is generally advisable to make the carrier up so as to have a two- to three-fold excess of acetate corresponding to each ester chain length fraction, the particular mixture used in this case is one commonly used in preliminary fractionations of natural mixtures. It consisted of 2.5 g of myristyl (14:0 A), 5.0 g of palmitoleyl (16:1 A), 10.0 g of oleyl (18:1 A), 10.0 g of 11-eicosenoyl (20:1 A), and 2.5 g of erucyl (22:1 A) acetates. Acetates have boiling points between corresponding methyl ester fractions. Thus, the head temperature does not generally show sharp stepwise increases unless the acetates are in large excess. The still-pot temperature is adjusted upwards as the distillation proceeds so as to maintain a slow reflux. This adjustment is made manually based on observations of the rate of condensation of distillate from the head condenser. For large fractions, the still-pot temperature may be held constant for fairly long periods; head temperature will also remain constant under these conditions. Should the adjustment of the still-pot temperature be delayed, the head temperature may decrease as the reflux slows down. Therefore, head temperatures generally give only a rough indication of the course of fractionation in this type of distillation. GLC serves very well as a means for monitoring the fractionation and a special sample collector has been devised to permit sampling of the distillate for analysis without interrupting the distillation (20). Determination of the course of the distillation by GLC is simplified in carrier distillations because the acetates serve as markers and standardize the pattern of the fractions as illustrated in Figure 2. Under ideal conditions the acetates distill in pure form between each ester fraction. For example, in Figure 2 the first analysis in A, B and C consists of virtually pure acetates that signal the start of the distillation of each corresponding ester fraction. The last analysis in each section (A, B and C) is the end marker of one fraction as well as the beginning marker for the next or succeeding ester fraction. Between the markers the methyl esters build up to a maximum concentration. This pattern

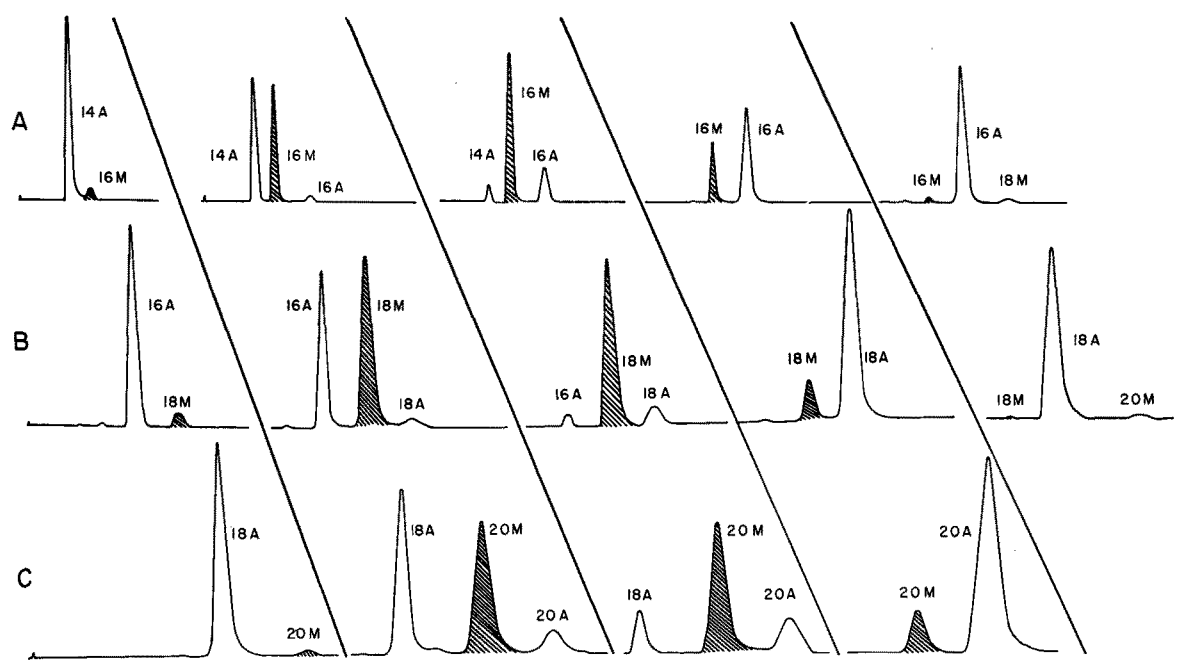

FIG. 2. Selected GLC analyses taken during the distillation of a standard mixture of methyl esters with acetates as carriers as listed in Figure 1. Section A = fraction marked off by the boiling range of 14 to 16 carbon chain acetates, B = boiling range of 16 to 18 carbon chain acetates, C = boiling range of 18 to 20 carbon chain acetates. A indicates acetate; M = methyl ester.

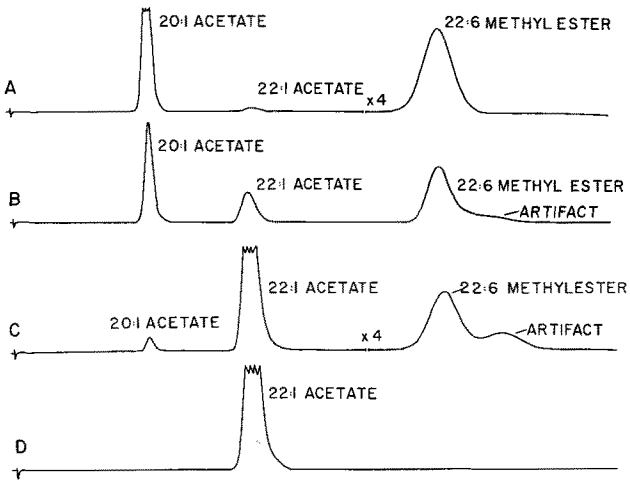

FIG. 3. Selected GLC analyses taken during the fractional distillation of the 22:6 M component of the standard mixture with corresponding boiling range acetates, A, B and C are first, main and last fraction. D = material in still-pot at end of distillation.

may be changed by changing the relative concentration of the acetates in special cases or for specific purposes. However, in general usage, the boiling ranges established by the acetates provide a convenient means for cutting the distillate into fractions.

Figure 3 shows selected analyses of the distillate containing the 22:6 component of the standard mixture. The pattern of the distillation of this fraction with respect to the 20:1 and 22:1 acetates is the same as that of the other fractions and their corresponding acetates (Fig. 2). The last analysis (Fig. 3) showed that no methyl esters were left in the still-pot at the end of the distillation. The 22:6 was included in the standard mixture because, in addition to its wide occurrence in natural fats, it is highly susceptible to structural alteration. Evidence of its alteration is indicated in Figure 3 by the small peak in the chromatogram of the GLC analysis with a slightly longer retention time than that for the parent 22:6 ester. That no polymerization of 22:6 occurred in this distillation was demonstrated by TLC analysis of the still-pot residue as well as GLC analysis which showed that it contained only erucyl acetate (Fig. 3). Thus, the carrier permitted quantitative distillation inasmuch as no sample was lost in the still-pot, and all compounds including artifact may be quantified by GLC.

In contrast, Figure 4 shows selected analyses of distillates containing the 22:6 component of the standard mixture from a normal distillation (without carrier) in the same still operated under essentially the same conditions. The build-up of the artifact in the distillate as well as in the residue in the still-pot is demonstrated by these analyses. TLC of the material in the still-pot at the end of the distillation showed that it contained nondistillable material, presumably polymers, in addition to simple methyl esters. In normal distillations so much 22:6 undergoes alteration that it is difficult and impractical to purify that which remains unaltered in distillate fractions. The amount of material remaining in the still-pot at the end of a normal distillation will depend on the size of the charge, the hold-up of the column as well as the amount of polymerization.

In order to demonstrate the utility of a carrier distillation for the fractionation of minor components

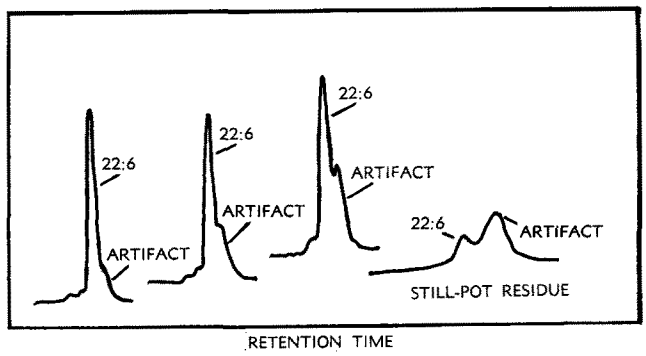

FIG. 4. Selected GLC analyses of the distillate containing the 22:6 component of a standard mixture of methyl esters (16:1, 18:1, 20:1 and 22:6) and of the material remaining in the still-pot in a normal (without carrier) distillation.

and to minimize artifact formation, the technique was applied to a concentrate of unsaturated methyl esters prepared from tuna oil by preferential removal of the saturated fraction by urea adduct formation. It consisted of approximately 68% of the original oil. This distillation was carried out on a 10 g sample of the methyl esters with a carrier consisting of 3 g of myristyl, 7 g of palmitoleyl, 7 g of oleyl, 5 g of 11-eicosenoyl and 3 g of erucyl acetates. With highly complex mixtures of methyl esters, generally more than one distillation will be required to resolve all the components. Nevertheless, a carrier with a fairly simple composition, as described above, may be employed in the initial distillation and fractions may be collected on the basis of the acetate markers as illustrated in Figure 5 for the fraction corresponding to the boiling range of the 16–18 carbon chain acetates. In addition to normal straight chain methyl esters, other fatty acids with closely similar boiling points will also separate in the same fraction with complex mixtures. Thus, in the initial distillation it may be desirable to collect fractions within those marked out by the acetates as indicated by GLC analyses on the distillate. This practice was followed in the distillation of the concentrate of unsaturated methyl esters prepared from tuna oil as illustrated in Table I. Only five acetates were used in the carrier in the fractionation of this concentrate, but eleven fractions with different fatty

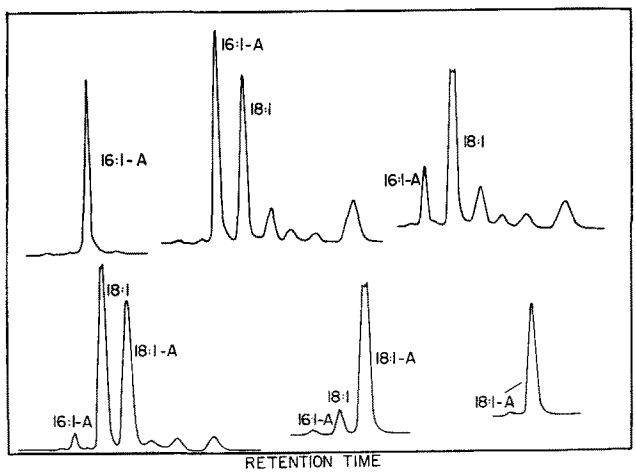

FIG. 5. Selected analyses of the distillate of the 16 to 18 acetate boiling range fraction of the distillation of a concentrate of methyl esters of unsaturated fatty acids prepared from tuna oil by urea fractionation.

TABLE I

Fatty Acid Composition of Fractions Collected in the Fractional Distillation of a Concentrate of Unsaturated Methyl Esters Prepared From Tuna Oil (10 g sample)[a,b,c]

Fractions	1	2	3	4	5[d]	6[d]	7[d]	8	9	10	11
wt (g)	0.90	0.42	0.75	0.97	1.72	0.93	0.14	1.16	0.20	2.03	2.09
Fatty acid (% wt)						Fatty acid (% wt)					
12:1	0.73					19B:1	1.30				
						19:1	1.30	1.15			
13B:1	0.48					19:2		2.75			
13:1	1.32					19:3			1.48		
14B:1	1.26					19:4			1.19		
14B:2	1.73					20B:1	53.38				
14:1	41.30	1.26				20B:2	6.35				
14:2	20.60	0.84				20B:4	2.47				
14:3	.....	1.15				20B:5		3.54			
15B:1	20.20	0.91				20:1	19.52	1.77	4.37	6.40	0.16	
15:1	4.39	1.82				20:2	1.48		1.73	2.02		
16B:1	4.28	2.61	1.76			20:3		2.72	9.50	4.95	0.55	
16B:2	1.64	3.79	2.93			20:4		12.30	4.30			
16:1	0.96	68.30	22.50	8.48		20:5	7.20	73.40	72.10	25.50	0.63	
16:2	1.45	5.05	11.75	11.78		20:5X			4.03	7 25		
16:3		6.31				21B:3			1.20			
17B:1		0.63				21B:5				0.35		
						21:1					0.22	0.98
17B:2		1.88				21:2				2.96		
17:1			6.40	4.72		21:3						1.39
17:2				10.58		21:4					0.71	
17:3		2.52				21:5				3.90	0.17	1.67
18B:1			11.01	10.12	5.9	22B:3						0.84
18B:2		2.31	39.40	46.28	4.3	22B:4						1.63
18B:3			0.92			22:1			0.50			
18:1			2.45	5.52	51.00	22:3			0.44	5.65		
18:2			0.92	2.50	10.15	22:4					2.64	
18:3				tr.	6.34	22:5				2.35	2.77	3.10
18:4				tr.	12.81	22:6			1.69	34.70	84.25	58.20
						22:6X				1.20	7.84	22.10
						23B:1					7.80	
						23:1					1.98	

[a] Total weight recovered = 10.5 g = 105% recovery.
[b] X indicates artifact.
[c] B indicates branched chain.
[d] Fraction 5 also contained 6.97% and 2.39% of 20B:1 and 20:2, respectively. Fraction 6 also contained 0.42%, 1.02%, 4.44% and 1.02% of 18B:1, 18B:2, 18:1 and 18:2, respectively. Fraction 7 also contained 1.47% and 1.41% of 18:1 and 18:2, respectively.

acid compositions were collected on the basis of GLC analyses of the distillate at approximately 20 min intervals. The final analyses of the fractions were carried out after removal of the acetates on the basis of ECL values (7) determined by GLC directly on the sample, after hydrogenation to determine chain length and on fractions separated by argentation-TLC (21A) to determine degree of unsaturation when required. From consideration of the percentage composition and amount of each fraction (Table I) a total fatty acid composition was calculated and is presented in Table II. As with the standard mixture, no esters or residue of esters (polymers) were left in the still-pot at the end of the distillation. Small amounts of artifact were formed from 22:6 and 20:5. These were detected and analyzed as separate fatty acids inasmuch as they gave separate peaks in the chromatogram of the GLC analyses. Although the method employed for the identification of the fatty acid is generally highly reliable it is evident that further analyses, possibly mass spectrometry, should be applied to those fatty acids of uncommon structure for positive identification.

TABLE II

Fatty Acid Composition of a Concentrate of Unsaturated Methyl Esters Prepared From Tuna Oil Determined Via GLC Analyses of Distillate Fractions

	%		%		%		%
12:1	tr.	16B:1	0.2	19B:1	0.1	21B:3	0.1
		16B:2	0.4	19:1	0.1	21B:5	tr.
13B:1	tr.	16:1	5.0	19:2	tr.	21:1	tr.
13:1	tr.	16:2	2.5	19:3	tr.	21:2	tr.
		16:3	0.2	19:4	tr.	21:3	0.4
14B:1	tr.					21:4	0.1
14B:2	tr.	17:1	0.9	20B:1	5.9	21:5	0.3
14:1	0.4	17:2	1.0	20B:2	0.6		
14:2	0.2	17:3	0.1	20B:4	0.2	22B:3	tr.
14:3	tr.			20B:5	tr.	22B:4	0.3
		18B:1	2.7	20:1	2.6	22:1	0.1
15B:1	0.2	18B:2	8.0	20:2	0.8	22:3	0.2
15:1	0.1	18B:3	tr.	20:3	1.3	22:4	0.5
		18:1	9.3	20:4	0.5	22:5	1.2
		18:2	2.0	20:5	10.2	22:6	28.8
		18:3	1.0	20:5X	0.6	22:6X	6.2
		18:4	2.1				
						23B:1	1.6
						23:1	0.4

B indicates branched.
X indicates artifact of distillation.

An indication of the amount of the fatty acids listed in Table II in tuna oil may be obtained from consideration that the concentrate consisted of about 68% of the original oil. However, even though the saturated fatty acids were removed preferentially by urea adduct formation some of the monoene fraction was also removed and some minor constituents probably were separated also with the urea adducts.

Discussion

The carrier technique of distillation employed here differs mainly from amplified distillation (25,26) in its mode of application and the carrier used. The classical technique of amplified distillation was developed mainly for the fractional distillation of small samples, 1 g or less, and the carrier, usually a mixture of hydrocarbons, was used in large excess—up to 30- to 40-fold the amount of sample.

According to Weitkamp (25,26), carriers for amplified distillation should have a range of boiling points covering that of the sample, be separated easily and not form azeotropes with any components of the sample. The long chain acetates satisfy all these requirements. Carriers with even closer boiling points than those used here may be obtained by employing odd as well as even numbered carbon long chain acetates. It is apparent that some overlapping will occur with a complex mixture of methyl esters consisting of odd, even and branched chains. Thus, for more precise fractionation of a particular methyl ester fraction, especially a minor component, a second distillation of specific fractions may be carried out. In this case the composition of the carrier can be selected very specifically and may be made up of both even and odd carbon chain acetates. Such a procedure could be well employed to fractionate more

precisely some of the fractions shown in Table I, for example.

If the purpose of the distillation is to separate one particular methyl ester as sharply as possible, then the composition of the carrier should be adjusted so as to have a large concentration of the appropriate acetate. In such a case the acetate to be selected should have a boiling point slightly lower than that of the methyl ester. This technique insures a slow but steady distillation of the methyl esters, conditions that are conducive to sharp fractionation. Regardless of the technique employed, there is little doubt concerning the value of the carrier technique for the fractionation of minor components of complex mixtures of polyunsaturated methyl esters in accord with elegant applications of amplified distillation (4,11,26,27).

The work reported here also shows that the use of a carrier reduces artifact formation and permits quantitative fractional distillations of mixtures containing higher polyunsaturated methyl esters. The effect of the carrier in this regard is to prevent the rapid rise in temperature that normally occurs at the end of the distillation and even at the end of each fraction. Generally the distillation of 22 carbon chain methyl esters can be carried out at still-pot temperatures below 200 C. By keeping the temperature almost constant during the distillation of an ester fraction, sharper separations are possible (by the carrier technique) because it is the rise in temperature towards the end of the fraction that results in the premature distillation of the next higher boiling fraction.

The structures of artifacts of fractional distillation have not received a great deal of attention. However, with the formation of polymers as evidenced by a nondistillable residue in the still-pot at the end of the distillation, the formation of conjugated (18,19) and cyclic compounds (13,14,16) may also be formed on the basis of the theories of polymerization (21,22). These compounds may be distilled because they are monomeric and thus distillate fractions that have elevated values for *trans* unsaturation or diene conjugation, or both, as well as spurious peaks in GLC analysis may be considered to contain artifacts.

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REFERENCES

1. Abu-Nasr, A., and R. T. Holman, JAOCS *31*, 41 (1954).
2. Birch, S. F., V. Gripp and W. S. Nathan, J. Soc. Chem. Ind. *66*, 33 (1947).
3. Chapman, C. J., J. D. Nadenicek, F. J. Pusch and O. S. Privett, manuscript in preparation.
4. Dauben, W. G., E. Hoerger and W. G. Petersen, J. Am. Chem. Soc. *75*, 2347 (1953).
5. Farmer, E. H. and F. A. van den Heuvel, J. Soc. Chem. Ind. *57*, 24 (1938).
6. Hilditch, T. P. and P. N. Williams, "The Chemical Constitute of Natural Fats," 4th ed., John Wiley & Sons, New York, 1964, p. 688.
7. Hofstetter, H. H., N. Sen and R. T. Holman, JAOCS *42*, 537 (1965).
8. Klenk, E., F. Hoppe-Seylers and H. Thierfelder, "Handbuch der Physiologisch und Pathologisch-chemischen und Analyse für Hertse und Studierende," 10th ed., Vol. 3, Berlin, 1955, p. 445.
9. Klenk, E. and W. Bingard, Z. Physiol. Chem. *291*, 104 (1952).
10. Kyte, R. M., JAOCS *33*, 146 (1956).
11. Masoro, E. J., J. L. Charhoff and W. G. Dauben, J. Biol. Chem. *179*, 1117 (1949).
12. Markley, K. S., "Fatty Acids, Their Chemistry, Properties, Production and Uses," Part 3, Interscience Publishers, New York, 1964, p. 2016.
13. Miyakawa, T., and H. Nomiza, Fette Seifen Anstrichmittel *64*, 593 (1962).
14. Michael, W. R., C. J. Alexander and N. R. Artman, Lipids *1*, 353 (1966).
15. Murry, K. E., "Progress in the Chemistry of Fats and Other Lipids," Vol. 3, Pergamon Press, New York and London, 1955, p. 243.
16. Nagano, Y., and T. Tanaka, J. Oil Chemists' Soc. Japan, *11*, 119 (1962).
17. Norris, F. A., I. I. Rusoff, E. S. Miller and G. O. Burr, J. Biol. Chem. *139*, 199 (1941); Ibid. *147*, 273 (1943).
18. Paschke, R. F., J. E. Jackson and D. H. Wheeler, Ind. Eng. Chem. *44*, 1113 (1952).
19. Paschke, R. F., and D. H. Wheeler, JAOCS *26*, 278 (1949).
20. Privett, O. S., R. P. Weber and E. C. Nickell, Ibid. *36*, 443 (1959).
20A. Privett, O. S., M. L. Blank and O. Romanus, J. Lipid Res. *4*, 260 (1963).
21. Rushman, D. G. and E. N. G. Simpson, Trans. Faraday Soc. *51*, 230 (1955).
22. Severn, D., "Bailey's Industrial Oil and Fat Products," 3rd ed., Interscience Publishers, Inc., New York, 1964, pp. 497–499.
23. Scott, T. A., Jr., D. Macmillan and E. H. Melvin, Ind. Eng. Chem. *44*, 172 (1952).
24. Syman, F. W., and C. Barkenbus, Ind. Eng. Chem., Anal. Ed., *12*, 658 (1940).
25. Weitkamp, A. W., JAOCS *24*, 236 (1947).
26. Weitkamp, A. W., J. Am. Chem. Soc. *67*, 447 (1945).
27. Weitkamp, A. W., A. M. Smiljanic and S. Rathman, Ibid. *69*, 1936 (1947).
28. Weissberger, A., "Techniques of Organic Chemistry," Vol. 2, Interscience Publishers, Inc., New York, 1951, p. 284.

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