Physical Properties of Fatty Acid Methyl Esters. I. Density and Molar Volume

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Abstract

Saturated fatty acid methyl esters from acetate to arachidate, methyl oleate, linoleate, linolenate, and erucate have been prepared in high purity. Densities, refractive indices, dispersions, ultrasonic sound velocities, and dielectric constants have been measured in the liquid state at 20 and 40C. In this first communication, the densities of the saturated compounds have been correlated with the Smittenberg relation. The following relations were derived: $d_4^{20} = 0.85407 + 0.18494/(n + 0.096)$ and $d_4^{40} = 0.84225 + 0.12904/(n - 0.408)$. Molar volumes have been computed and checked for additivity.

Introduction

TO INVESTIGATE the relationships between various physical and chemical constants of fatty oils and their derivatives (31), accurate data of pure model compounds are necessary to compute reliable group increments of additive functions. Besides finding application as basis for deriving these relationships, additive functions such as the molar volume and refraction are also used as aids in the establishment of the structure and identity of organic compounds. It is, therefore, essential that the applied increments are trustworthy.

In 1954 Hammond and Lundberg (11) published a paper on the molar refraction, molar volume, and refractive index of fatty acid esters and related compounds in the liquid state. Without detracting anything from the great value of this paper, it must be noted that the applied data have been obtained from several sources, some of doubtful reliability. And although a substantial amount of data was collected there were, for instance, no values available for the higher saturated fatty acids with an odd number of carbon atoms.

It was the purpose of these investigations to obtain some deficient data on the fatty acid methyl esters and to check the existing values on their reliability. The fatty acid methyl esters were chosen because they form the ideal model compounds for the study of triglycerides and fatty oils.

Experimental

Distillation columns. For the preparation of our model compounds we had at our disposal a spinning band column with 28 theoretical plates at an average boil-up rate of 300 ml/hr (26) and several packed columns with separating powers ranging from 20–50 theoretical plates at boil-up rates between 750–1500 ml/hr.

Esterification. Methylation of the free fatty acid was usually carried out with purified methanol in the presence of concn H_2SO_4 or, where objections may be present to this procedure, with diazomethane in ether solution.

Acetone-permanganate oxidation. This procedure, which is used to remove small amounts of unsaturates from saturated compounds, is carried out in essentially the manner described by Kartha (13) for the determination of fully saturated triglycerides.

Materials. The crude saturated fatty acid methyl esters were generally commercial samples, or have been obtained from commercial fatty acids by methylation. Purification procedures consisted of a combination of distillation from P_2O_5 (for the lower members), acetone-permanganate treatment (if the presence of unsaturated material is suspected), several distillations through the packed columns and through the spinning band column, and recrystallizations from different solvents at different temperatures (10).

Fatty acids which were not commercially available have been synthesized. Tridecanoic acid was obtained from lauryl alcohol over lauryl bromide and the corresponding Grignard compound.

The starting material for the preparation of methyl pentadecanoate was myristic acid, which had been obtained from purified methyl myristate by saponification. The acid was first refluxed with purified thionylchloride to form myristoylchloride. This product was added to diazomethane in ether and allowed to stand for 24 hr at room temp after which it was cooled to −20C to precipitate the formed 1-diazopentadecanone. This ketone was subsequently subjected to the Arndt-Eistert reaction (3, 38) by the addition of a large excess of purified methanol and small amounts of Ag_2O powder during 30 hr at 57–60C.

$$CH_3(CH_2)_{12}COCl + CH_2N_2 \longrightarrow CH_3(CH_2)_{12}COCHN_2 + HCl$$

$$CH_3(CH_2)_{12}COCHN_2 + CH_3OH \xrightarrow[Ag_2O]{} CH_3(CH_2)_{12}CH_2COOCH_3 + N_2$$

Methyl heptadecanoate was prepared from palmitic acid in an analogous reaction sequence except that the last rearrangement was carried out over 45 hr, the last 20 hr under reflux.

With the exception of the last stage, methyl nonadecanoate was prepared from stearic acid in a comparable reaction sequence. As rearrangement with methanol will give poor yields the Arndt-Eistert rearrangement was, therefore, carried out with superdry absolute ethanol to yield the corresponding ethyl ester (35). The acid was obtained by saponification and the methyl ester by treatment of the acid with diazomethane.

Methyl arachidate was obtained starting from stearoyl alcohol which was converted into the corresponding iodide by treatment with red phosphorus and iodine. This iodide was reacted with purified malonic ester, hydrolyzed with a benzenic solution of HCl and decarboxylized in vacuum at 140–150C. The crude acid was subsequently esterified with diazomethane to yield the methyl ester.

$$CH_3(CH_2)_{17}I + CH_2(COOC_2H_5)_2 \xrightarrow[NaOC_4H_9]{} CH_3(CH_2)_{17}CH(COOC_2H_5)_2 \xrightarrow[benzene + HCl]{} CH_3(CH_2)_{17}CH(COOH)_2 \xrightarrow[decarboxylation]{} CH_3(CH_2)_{17}CH_2COOH$$

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TABLE I
Density and Molar Volume of Fatty Acid Methyl Esters

Compound	d_4^{20}	d_4^{40}	Literature values			V_m^{20}	V_m^{40}
			d_4^{20}	d_4^{40}	Ref.		
Acetate	0.9338	0.9079	0.9338	0.9075	(39)	79.33	81.59
			0.9337		(18)		
			0.9330		(9)		
			0.93347		(23)		
			0.9342		(19)		
			0.93382	0.9080	(32)		
Propionate	0.9153	0.8915	0.9151	0.8912	(39)	96.26	98.83
			0.9150	0.8917	(36)		
			0.9148		(25)		
			0.91522	0.8916	(33)		
Butyrate	0.8982	0.8763	0.8984	0.8760	(39)	113.70	116.55
			0.8973		(25)		
			0.8981	0.8778	(36)		
Valerate	0.8901	0.8699	0.8899		(1)	130.50	133.53
			0.8895	0.8703	(36)		
Caproate	0.8844	0.8652[5]	0.8844	0.8652	(5)	147.20	150.46
			0.8850	0.8664	(36)		
			0.88466	0.86596	(4)		
Oenanthate	0.8801[5]	0.8618	0.8800		(1)	163.85	167.33
			0.88011	0.86220	(4)		
			0.8815	0.8636	(36)		
Caprylate	0.8769	0.8592[5]	0.8775	0.8595	(5)	180.45	184.15
			0.8770		(1)		
			0.8784	0.8613	(36)		
Pelargonate	0.8743	0.8573[5]	0.87694		(2)	197.03	200.92
			0.8751		(11)		
			0.8745		(1)		
			0.8748		(20)		
Caprate	0.8724	0.8558	0.8730	0.8563	(5)	213.53	217.67
			0.8725		(1)		
			0.8733	0.8571	(36)		
Undecanoate	0.8708[5]	0.8545	0.8712		(11)	230.02	234.42
			0.8708		(1)		
Laurate	0.8694	0.8533[5]	0.8695	0.8535[5]	(5)	246.54	251.17
			0.8695	0.8538	(21)		
			0.8702	0.8545	(36)		
Tridecanoate	0.8681	0.8524	0.8682		(11)	263.06	267.91
Myristate	0.8671[5]	0.8517	0.8671	0.8517	(5)	279.53	284.60
Pentadecanoate	0.8663[5]	0.8511[5]	0.8662		(1)	295.97	301.26
			0.8660		(11)		
			0.8657	0.8503	(24)		
Palmitate		0.8505		0.8504	(5)		317.98
Heptadecanoate		0.8499[5]					334.69
Stearate		0.8496		0.8508	(5)		351.33
Nonadecanoate		0.8493[a]					367.97
Arachidate		0.8488[a]					384.71
Oleate	0.8740[5]	0.8596[5]	0.8739		(37)	339.20	344.88
			0.8738	0.8594	(14)		
			0.8734		(22)		
			0.8728		(16)		
			0.8744		(11)		
Linoleate	0.8866	0.8720[5]	0.8870		(11)	332.12	337.67
Linolenate[b]	0.8979	0.8834	0.8995		(11)	325.70	331.05
Erucate	0.8706[5]	0.8565[5]	0.8702		(34)	404.96	411.63
			0.8701		(11)		

[a] Extrapolated values from measurements at higher temp.
[b] Contains 27.5% *trans* double bonds.

Unsaturated compounds. An additional procedure has been used for the preparation of unsaturated compounds, i.e., low temp crystallization (6).

Methyl oleate was obtained by methanolysis of olive oil in the presence of ignited K_2CO_3 (12). The esters were subjected to two urea complex precipitations (29) and subsequently distilled through a short packed column. Further purification was carried out by low temp crystallizations in purified acetone at several temp (28,29). The last traces of saturates were removed by keeping a solution of 150 g methyl oleate in 6 liters acetone at −25C for three weeks and discarding the formed crystals. The last traces of higher unsaturates were removed by a maleic anhydride treatment in decalin (15).

Linoleic acid was obtained from poppy seed oil fatty acids. After removal of the lower unsaturates by three urea crystallizations (29) the acid was isolated by low temp crystallizations from acetone and from pentane (6).

Linolenic acid was obtained from hexabromostearic acid, which was repeatedly recrystallized until a mp of 184.6–184.8C was obtained. Debromination was carried out according to the procedure described by Frankel and Brown (8).

Methyl erucate was obtained from the methyl esters of rape oil fatty acids. A crude vacuum distillation yield an erucate fraction which was further purified by low temp crystallizations according to the procedure described by Kolb (17).

Methylation of linoleic and linolenic acid was carried out with diazomethane. The final purification of all unsaturated fatty acid methyl esters consisted of a careful distillation through the spinning band column.

Purity determinations. Gas chromatography has been used to determine the purity of the prepared compounds. The utilized gas chromatograph has been developed by ourselves and was based on total combustion of the organic vapors over CuO rods and detection of CO_2 in a thermal conductivity detector. A 120 x 0.4 cm copper column with 25 w/w% polyethylene glycol adipate on 175–210 μ Chromosorb P and a 292 x 0.4 cm copper column with 25 w/w% Apiezon L on 150–250 μ Embacel kieselguhr were used in these investigations. Nitrogen was used as carrier gas. Optimum conditions were determined for each compound and test mixtures with known amounts of added impurities were first analyzed.

All compounds proved to be more than 99.7% pure, with the exception of C_{13} (99.5%), C_{15} (98.8%), C_{17} (97%), C_{19} (97%), and C_{20} (97%). Methyl linoleate was 99.0%, linolenate 98.5%, and erucate 98% pure.

Measurement of densities. Densities of the products were determined in small (5–6 ml) modified Sprengel pycnometers at 20 and 40C. These pycnometers have been carefully calibrated with distilled water at both temp. Measurements have been carried out with the normal precautions and the obtained values are estimated to possess a SD of less than 0.0001 g/ml.

TABLE II

Numerical Values of the Smittenberg Relation for Saturated Fatty Acid Methyl Esters

Terms considered	Constant	20C		40C
		a	b	
$n \geqq 2$.....	$d^t_{4,\infty}$	0.85335	0.8513	0.84171
	A	0.19839	0.23627	0.14156
	B	0.38405	1.0370	0.04323
	σ	0.00133	0.00308	0.00114
$n \geqq 5$.....	$d^t_{4,\infty}$	0.85387	0.8513	0.84212
	A	0.18921	0.24471	0.13246
	B	0.21243	1.4800	−0.25888
	σ	0.000076	0.00043	0.000082
$n \geqq 6$.....	$d^t_{4,\infty}$	0.85407	0.8513	0.84225
	A	0.18494	0.24757	0.12904
	B	0.09634	1.6374	−0.4079
	σ	0.000071	0.00031	0.000077

Results

The values obtained are presented in Table I. Large differences in the reported density values by different authors should be noted. In many cases the described preparation suggests the presence of large amounts of impurities and those values differing strongly from the accepted magnitude have, therefore, been omitted.

The data on Bonhorst et al. (5), for many years the accepted values for these compounds, are generally slightly higher. On the other hand, an excellent consistency is observed with the values of Adriaanse (1) who asserted his compounds to be better than 99.6% pure as determined from melting graphs.

Smittenberg and Mulder (27) applied the relation:

$$y = y_\infty + A/(B + n)$$

to correlate the density and the refractive index of several homologous series of hydrocarbons. In this equation y is a physical constant, y_∞ is the limiting value of the physical constant, i.e., the value of the physical constant of the term with infinite carbon atoms, A and B are constants, and n is the term number.

In the application of these equations to homologous series, two distinct cases may be distinguished:

1. A, B, and y_∞ are calculated from the obtained data.
2. y_∞ is a set value obtained from other sources, and only A and B are calculated.

For the density of the limiting hydrocarbon Smittenberg and Mulder obtained 0.8513. One may expect the same value for the limiting methyl ester of the saturated fatty acids.

From the measured data the values of A, B, and y_∞ have been computed by the method of the least squares. A and B have also been computed assuming $d^{20}_{4,\infty}$ to be 0.8513. Numerical values are presented in Table II.

σ stands for the standard deviation between predicted and observed values and has been calculated from

$$\sigma^2 = \delta^2/(m - 3)$$

where σ is the difference between predicted and measured value, and m the number of terms considered.

The major contributions to the error are furnished by the first two terms, viz, methyl acetate and methyl propionate.

In the majority of the cases in the literature this equation has only been applied to series commencing with C_5 or higher. The values of $d^t_{4,\infty}$ in the lower half of Table II have been obtained by omitting the primary homologs. Application of the Variance Ratio Test to these sets of data indicates unequivocally that the lower members of the series belong to another population.

Application of this test between the values obtained with a predetermined limiting density and those values, where A, B, and $d^{20}_{4,\infty}$ have all been computed from the observed data, indicates a probably significant difference for $n \geqq 2$ and a significant difference for $n \geqq 6$.

A significant improvement is therefore attained by using 0.85407 as limiting density instead of 0.8513. It is of interest to note that Adriaanse (1) calculated 0.8532 as his best value, while Tatevskii et al. (30) obtained 0.84957 for the *n*-alkanes and 0.85262 for the 2-methyl alkanes. These large disparities are partially explained by the fact that the limiting value is obtained by extrapolation of a limited number of terms to infinity. Small variations within the measuring error are sufficient to alter the result considerably.

Molar Volumes

The computed molar volumes have been included in Table I. For the members of a homologous series the relation between the molar volume, V_m, and the term number, n, may be represented by

$$V_m = C + n R_{CH_2}$$

where C is the basic constant of the series, and R_{CH_2} the increment of the CH_2 group.

This relation has been applied by Dorinson et al. (7) to the saturated acids. Two sets of values were applied: one for the higher, and one for the lower homologs. Hammond and Lundberg (11) have applied this expression in the derivation of their ultimate equation for the prediction of the refractive index of fatty acid esters and related compounds.

From our data one may easily observe that R_{CH_2} is not a constant, but that it decreases with increasing chain length. For the higher homologs, however, this increment is constant within the accuracy of our measurements.

The increment of the double bond in a monoethenoid system appears to be lower than the contribution of additional non-conjugated double bonds in the same chain. This phenomenon has already been noted previously (11), and may be ascribed to slight interactionary forces. The small difference between methyl linoleate and methyl linolenate is due to the influence of the *trans* double bonds in methyl linolenate. *Trans* double bonds will, in comparison to *cis* double bonds, induce a lower density and consequently a higher molar volume.

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Essential Fatty Acid Contents of Various Fats: Interpretations of Values by Physico-Chemical Tests

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Abstract

Biological assays of oil and fat products, free from isomers of the naturally-occurring *cis*-9, *cis*-12 linoleic acid, have been shown to provide estimates of essential fatty acid content which agree well with values obtained by spectrophotometric analysis. However, when partially hydrogenated fats, such as those used in margarines, are bio-assayed the estimates obtained are only about 60% of those derived by spectrophotometric tests.

In a blended corn oil margarine, good agreement was obtained for linoleic acid content by using biological assay or spectrophotometry, thiocyanometric procedure, column chromatography for saturates plus iodine value, and gas liquid partition (GLP) chromatography. This margarine fat contained about 29% of the essential form of linoleic acid, and had a ratio to saturated fatty acids of 1.6:1.

The hydrogenated corn oil margarine is unlike conventional margarines in providing high amounts of the isomeric forms of linoleic acid which lack essential fatty acid activity. For this reason, poor agreement was obtained between biological assay results and those by physicochemical measurements of linoleic acid content. Such fat contains only about 6% of the essential form of linoleic acid, with a ratio to saturated fatty acids of ca. 0.3:1.

From this study it is now possible to characterize, even without bio-assay data, the fatty acid composition of a highly isomerized fat, such as is found in hydrogenated corn oil margarine. The characterization groups the fatty acids into saturates and total linoleic acids, with the latter including estimates of the positional isomers of linoleic acid with widely spaced double bonds, *trans* forms of linoleic acid with methylene-interrupted double bonds, linoleic acids with the double bonds in conjugated position, and *cis*-9, *cis*-12 linoleic acid. The combined use of the spectrophotometric and thiocyanometric procedures makes it possible to estimate the essential fatty acid content of hydrogenated fats containing residual dienes.

Introduction

THE REQUIREMENT for essential fatty acids (EFA) by many animals is well documented (1–7). Not only are they required for proper growth, reproduction, lactation, longevity, and tissue structure, but also for regulation of plasma and liver cholesterol levels and liver lipid levels (7). However, the human requirement is less well understood. It has been shown that essential fatty acid deficiency in the human infant causes a dry and scaly skin which responds to essential fatty acid therapy (8); linoleic acid is also required for optimum utilization of the total calories ingested (8).

Values for the human requirement for essential fatty acids (viz., *cis*-9, *cis*-12-linoleic acid) have been reported to range from 1–4% of the caloric intake (8–10). Holman (9), in proposing his value of 2% of caloric intake as the minimal requirement, points out that "a judicious selection of foods should provide many times 2% linoleate calories, but diets containing high proportions of hard fats and sugars could lead to relative EFA deficiency." He also emphasizes that "for some physiological functions the requirement for EFA may be greater than 2% of calories. For example, if hypocholesterolemia is a desirable condition, the amount of highly unsaturated fatty acids required to maintain it appears to be several fold that amount." The fact that other polyunsaturated fatty acids, in addition to the essential fatty acids, exert hypocholesterolemic effects (10,11) should not lead one to regard the value of dietary linoleic acid as limited only to those functions for which it is essential. The hypocholesterolemic activity of the essential fatty acids indicates another important function, even though non-specific, of these nutrients in human nutrition.

Three essential fatty acids are now recognized: linoleic (*cis*-9, *cis*-12-octadecadienoic acid), linolenic (*cis*-9, *cis*-12, *cis*-15-octadecatrienoic acid), and arachidonic (*cis*-5, *cis*-8, *cis*-11, *cis*-14-eicosatetraenoic acid). Arachidonic acid has been reported ca. three times as effective as linoleic in promoting growth (12), and linoleic acid is more effective than linolenic in relieving EFA deficiency symptoms (13). Since linoleic acid can form arachidonic acid in the animal body (14,15), investigators have concentrated atten-