

or paper clips are satisfactory. Next pour about 175 cc. of 80% alcohol into a 250-cc. beaker and add 10 cc. of the 2,6-D reagent solution. Stir well. Then add 10 cc. of the borax solution and mix well. Allow the solution to become quiet and stand the cylinder upright in the center of the beaker. Note that the cylinder should be placed in the solution within one minute or less after the borax has been added. Let the paper stand without mixing and examine it by viewing both vertically and horizontally against white backgrounds. The presence of a blue ring near the paper shows the BHA is present. A more positive test is generally obtained if the treated side is inside the cylinder, hence the preparation of two cylinders. It is again most desirable to run untreated paper along with the paper in question for a comparison of the results. The cylinder should be viewed at least at 5-, 10-, and 15-minute intervals and more often if desired. Once the blue color has formed, it diffuses slowly throughout the solution. It is necessary therefore to follow the formation of the blue rings. If

both sides of the paper are treated, the test will indicate by the rates of formation and intensities which side contains the higher concentration of BHA. Again if the paper does not contain BHA, a light pink color will form within the 15-minute test period. This test has been tried on vegetable parchment, sulfite, sulfate, and glassine with excellent results. It will not work however on waxed paper or paperboard. Simple as this solution is, it solves a problem that is frequently very baffling.

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Molar Refraction, Molar Volume, and Refractive Index of Fatty Acid Esters and Related Compounds in the Liquid State¹

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THE use of refractive index and molar refraction as aids in establishing the identity, purity, or structure of organic compounds is well known. Measured values of these constants do not appear to have been fully exploited for such purposes however in the chemistry of pure fatty acids and derivatives even though refractive index has been widely used in the characterization of natural fats and oils.

Recently in this laboratory a supposedly pure methyl docosahexaenoate, prepared from hog brain (1), was found to have a molar refraction higher than the value calculated from atomic constants. The refractive index and molar refraction were also higher than empirical values reported by Farmer and Vandenheuvel for a methyl docosahexaenoate prepared from cod liver oil (2). Moreover these authors also described a simple straight line relationship between refractive index and unsaturation for methyl esters of long chain fatty acids (3) that was consistent with, and in part dependent on their observed refractive index for the docosahexaenoate.

A study was therefore undertaken of molar refraction, molar volume, and refractive index of methyl esters of fatty acids as functions of two parameters, carbon chain length and unsaturation, with a view to developing equations that would be useful in determining identity, purity, and structure. The development of such equations for the methyl esters of common types of fatty acids (excluding those with conjugated double bond systems and those with double bonds in the *trans* configuration) is reported. Similar principles and relationships appear to hold for the fatty acids themselves and other derivatives.

General Relationships

Molar refraction, R_m , refractive index, n , and molar volume, V_m , are related by the well known equation of Lorenz (4) and Lorentz (5):

$$R_m = \frac{n^2 - 1}{n^2 + 2} V_m \quad I$$

It is also well known that R_m may be calculated to a first approximation for any compound by the summation of certain atomic "constants." The "constant" for any particular atomic component is subject to second order variations, depending on the nature of other components and molecular structural relationships. It is reasonable to expect however that for a family of compounds which differ only in carbon chain length and unsaturation, for example, methyl esters of normal fatty acids, a single set of constants could be found which would yield calculated molar refractions in very close agreement with observed values for all members of the family of compounds. The equation could take the form:

$$R_m = k_1 C + k_2 D + k_3 \quad II$$

where C is the number of carbon atoms in the fatty acid chain (not including the carbon of the alcoholic methyl group), D is the number of double bonds, and k_1 , k_2 , and k_3 are constants. The relationship to which II reduces in the absence of double bonds has been found to hold for saturated fatty acids at 80°C. by Dorinson *et al.* (6).

It has also been found previously that V_m is a linear function of the carbon chain length for saturated fatty acids and esters; further, the introduction of an isolated double bond in members of such a series of compounds has a uniform effect on molar volume (6, 7). It is to be anticipated therefore that a general equation for molar volume and density such as follows might be applicable:

$$V_m = \frac{M}{d} = k_4 C + k_5 D + k_6 \quad III$$

where M is the molecular weight, d is the density, and k_4 , k_5 , and k_6 are constants.

R_m , which is considered to be a function of the volume actually occupied by the molecules of a mol of substance, is nearly independent of temperature. This is not true of V_m , and the constants k_4 , k_5 , and k_6 will

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hold for only one temperature. Moreover the molar volume and density of fatty acids are affected by molecular association and equation III will not be accurate for the lower homologues of a series (6).

If equations II and III are substituted in equation I, solving for n gives the relation:

$$n = \frac{\sqrt{(2k_1 + k_4)C + (2k_2 + k_5)D + 2k_3 + k_6}}{\sqrt{(k_4 - k_1)C + (k_5 - k_2)D + k_6 - k_3}} \quad \text{IV}$$

Evaluation of Constants

The evaluation of constants k_1 to k_6 is necessarily dependent on empirical data. Because data from different sources for a single compound may differ quite markedly, it is necessary to be somewhat arbitrary in selecting values on which to base the constants. Although, in all that follows, efforts have been made to select data as carefully as possible, it is recognized that some errors of judgment may have been made. This will not appreciably impair the usefulness of equations based on II, III, and IV, but slight revisions of the values for the constants may become desirable as data of greater accuracy become known.

Values for constants k_1 , k_3 , k_4 , and k_6 were based on data for saturated methyl esters at 20°C. Data for saturated methyl esters are more extensive and accurate than for unsaturated esters. The temperature 20°C. was preferred because of its widespread use as a reference temperature, particularly for unsaturated fatty acid compounds. One drawback in using data for saturated methyl esters at this temperature is that those of chain length greater than myristate are normally solid at 20°C., and hence data for the liquid state at 20°C. are not available. Nevertheless values for the constants based on data for the saturated methyl esters up to myristate are judged to be more accurate than any obtainable from available data for other methyl esters.

Data pertaining to the calculation of values for constants k_4 and k_6 are given in Table I. The values for V_m^{20} in column 2 were obtained from the data of Bonhorst *et al.* (8). The increment in V_m^{20} per added $-\text{CH}_2-$ group in going from one ester to the next higher ester of column 1 is given in column 3. The value for k_4 is then taken to be the mean of the $\Delta V_m^{20}/\Delta C$ values, 16.54.

Again using the V_m^{20} values of column 2, the values of $V_m^{20} - 16.54C$ in column 4 were calculated. The mean of the latter values, 47.99, was taken as the value of k_6 .

TABLE I
Evaluation of k_4 and k_6 for Methyl Esters

Ester	V_m^{20} (observed)	$\frac{\Delta V_m^{20}}{\Delta C}$	$V_m^{20} - 16.54C$
	cc.	cc.	cc.
Caproate.....	147.20	47.96
Caprylate.....	180.32	16.56	48.00
Caprate.....	213.39	16.54	47.99
Laurate.....	246.51	16.56	48.03
Myristate.....	279.54	16.52	47.98
Mean.....		16.54	47.99

Thus for the saturated methyl esters at 20°C., III becomes

$$V_m^{20} = 16.54C + 47.99 \quad \text{V}$$

where C is the number of carbon atoms in the fatty acid portion of the molecule.

Equation V represents the observed values for V_m^{20} from which it was derived with a mean deviation ± 0.02 cc. Greater variations than this are found in the most concordant empirical values for the V_m^{20} of saturated esters given by different investigators. A comparison of columns 5 and 6, Table IV, reveals that the differences between calculated and observed values are appreciable in lower members of the saturated series up to valerate, but for higher esters the agreement, in general, falls within the limits of accuracy of the experimental data.

A somewhat similar procedure, also involving the use of data for saturated methyl esters, was followed in arriving at values for k_1 and k_3 . The values of n_D^{20} in column 2, Table II, are selected data (in some cases averaged values) from Mattil and Longenecker (9), Althouse *et al.* (10), Craig (11), and Ralston (12). Observed values for V_m^{20} are given in column 3 except for those esters that are normally solid at 20°C.; in the latter cases, values calculated from V are given. The R_m values in column 4 have been calculated from the n_D^{20} and V_m^{20} values using I.

TABLE II
Evaluation of k_1 and k_3 for Methyl Esters

Ester	n_D^{20}	V_m^{20}	R_m	$\frac{\Delta R_m}{\Delta C}$	$R_m - 4.641C$
Caproate.....	1.4052	147.20	36.094	8.248
Caprylate.....	1.4170	180.32	45.346	4.626	8.218
Caprate.....	1.4256	213.39	54.631	4.642	8.221
Laurate.....	1.4318	246.51	63.912	4.640	8.220
Myristate.....	1.4370	279.54	73.236	4.662	8.262
Palmitate.....	1.4408	312.63 ^a	82.540	4.644	8.268
Stearate.....	1.4440	345.71 ^a	91.852	4.654	8.293
Arachidate.....	1.4463	378.79 ^a	101.095	4.620	8.250
Behenate.....	1.4484	411.87 ^a	110.376	4.638	8.243
Mean.....				4.641	8.247
Standard deviation.....				± 0.005	± 0.008

^a Values calculated from V.

Column 5 gives the increments in R_m per added $-\text{CH}_2-$ group in going from one ester to the next higher ester listed in column 1; the mean of these values, 4.641 is taken as k_1 . In column 6 are given the $R_m - 4.641C$ values, the mean of which, 8.247 is taken as k_3 .

For saturated methyl esters, II then becomes

$$R_m = 4.641C + 8.247 \quad \text{VI}$$

and IV becomes

$$n_D^{20} = \sqrt{\frac{25.82C + 64.48}{11.90C + 39.74}} \quad \text{VII}$$

The mean deviation of values for n_D^{20} calculated by means of VII from the observed values used in establishing k_1 and k_3 (column 2, Table II) is ± 0.0002 ; this agreement is as good as the agreement among the more nearly concordant values from different laboratories for the same esters.

The evaluation of constants k_2 and k_5 was a more difficult problem, primarily because the sources of data for unsaturated methyl esters are more scattered and because it was more difficult to ascertain the accuracy of the data. Selected values for n_D^{20} and V_m^{20} are given in columns 3 and 4, Table III. These values, inserted in I, gave the R_m values shown in column 5. Values of R_m for the corresponding saturated methyl esters, calculated from VI, are given in column 6. The

TABLE III
 Evaluation of k_2 and k_5 for Methyl Esters

C	D	n_D^{20}	V_m^{20}	R_m^a	$R_m \text{ sat.}^b$	$\frac{\Delta R_m}{\Delta D}$	" k_5 " ^c
18	1	1.4521 (9, 13)	339.30 (14)	91.55	91.785	-0.23	-6.67
18	2	1.4614 (3, 9, 15, 16)					-6.95
18	3	1.4710 (3, 9)					-6.88
20	1	1.4539 (11)	350.67 (18)	99.60	101.067	-0.37	-6.58
20	4	1.4799 (17)					-6.81
20	5	1.4896 (19)					-6.82
22	1	1.4556 (11, 20)	405.17 (20)	110.06	110.349	-0.29	-6.70
22	5	1.4883 (19)					-6.87
22	6	1.4974 (1)	371.96 (1) (at 25°C.)	108.50 ^d	110.349	-0.31	-6.86
Mean.....						-0.30	
Mean for monoenes.....							-6.65
Mean for polyenes.....							-6.87

^a Calculated from I using observed values for V_m^{20} and n_D^{20} .

^b Calculated from VI.

^c Calculated from IV using previously obtained values for k_1 , k_2 , k_3 , k_4 , and k_6 .

^d Calculated using a value for n_D^{20} calculated from n_D^{20} employing temperature coefficient for n_D of -0.00038 per degree.

mean of the $\Delta R_m/\Delta D$ values of column 7, -0.30, was taken as k_2 .

This value is based on relatively few empirical data. However the few values of $\Delta R_m/\Delta D$ are reasonably consistent even though they were based on esters representing quite widely different degrees of unsaturation. More important, the value for k_2 is quite small, and hence is proportionately less important than some of the larger constants in relation to the accuracy of values for R_m , V_m^{20} , and n_D^{20} based on II, III, and IV. The value for k_2 is also consistent with Eisenlohr's refraction constants for ethylenic systems (21).

The use of a single k_2 value for both monoenes and polyenes appears to be justified in spite of the paucity of data on which the value for k_2 was based, inasmuch as the use of a single value leads to no serious discrepancies between calculated and empirical values for R_m .

Substitution of the values for k_2 in II gives the following equation for R_m for the methyl esters in general:

$$R_m = 4.641C - 0.30D + 8.247 \quad \text{VIII}$$

Substitution of the observed n_D^{20} values of column 3 and the derived values for k_1 , k_2 , k_3 , k_4 , and k_6 in IV yields the individual k_5 values of column 8. It is evident that the values for monoenes are lower than those for polyenes. It is therefore necessary to have two k_5 values, one for monoenes, -6.65, and the other for polyenes, -6.87, in order to achieve good agreement between calculated and observed values of V_m^{20} and n_D^{20} . If differences exist in the k_5 values for polyenes of different degrees of unsaturation, such differences are not readily apparent, and the use of a single k_5 for all polyenes appears not to lead to any serious discrepancies between calculated and observed values for V_m^{20} and n_D^{20} .

Substituting the values for all of the constants in III and IV, it is found that

$$V_m^{20} = 16.54C - 6.65D + 47.99 = 16.54C + 41.34 \text{ for monoenes} \quad \text{IX}$$

$$V_m^{20} = 16.54C - 6.87D + 47.99 \text{ for polyenes} \quad \text{X}$$

$$n_D^{20} = \sqrt{\frac{25.82C - 7.25D + 64.48}{11.90C - 6.35D + 39.74}} = \sqrt{\frac{25.82C + 57.23}{11.90C + 33.39}} \text{ for monoenes} \quad \text{XI}$$

$$n_D^{20} = \sqrt{\frac{25.82C - 7.47D + 64.48}{11.90C - 6.57D + 39.74}} \text{ for polyenes} \quad \text{XII}$$

Table IV compares values for R_m , V_m^{20} , and n_D^{20} calculated from VIII-XII with empirical or observed values. It is seen that agreement in general is very good for the saturated esters and most of the monoenes. For polyenes, data obtained in preparations involving prolonged distillation (29, 30, 31, 32) do not agree well generally with predicted values, but more recent data obtained on materials obtained by milder preparative treatments show good agreement. In particular, the calculated and observed values for n_D^{20} usually differ by only a very few units in the fourth decimal place.

It was mentioned at the outset that a primary reason for investigating molar refraction, molar volume, and refractive index in relation to carbon chain length and unsaturation was to provide an additional check on the purity of a methyl docosahexaenoate prepared in this laboratory (1). This compound is the last of those listed in Table IV. It is seen that the calculated and observed values are in good agreement.

Fatty Acids and Other Derivatives

Equations similar to VIII-XII may also be established for fatty acids, fatty alcohols, esters other than the methyl esters, and other derivatives. Calculations of the values for k_1 to k_6 for several types of derivatives have been made by the same procedures described for the methyl esters. Calculations for the free fatty acids are based on data of Garner and Ryder (33), Craig (11), Dorinson *et al.* (6) and on data collected by Ralston (12) and Timmermans (22); for fatty alcohols, data collected by Ralston (12); for ethyl esters, data collected by Timmermans (22), and Ralston (12); for propyl and isopropyl esters, data of Bonhorst *et al.* (8), and Althouse *et al.* (10); and for aldehydes, data of Powell *et al.* (34).

As was anticipated, the values for k_1 and k_4 were essentially the same in all cases as those found for the methyl esters. The values previously obtained with the methyl esters were therefore used in calculating other constants for the other derivatives. The values established for k_3 and k_6 for several types of compounds are given in Table V. Insufficient data are available for a complete evaluation of k_2 and k_5 for the various derivatives, but, from such data as were available, values were obtained for these constants

TABLE IV
 Comparison of Observed Values and Values Calculated from VIII-XII for R_m , V_m^{20} , and n_D^{20} for Methyl Esters

Ester	C	R_m^a (obs.)	R_m^b (calc.)	V_m^{20} (obs.)	V_m^{20c} (calc.)	n_D^{20d} (obs.)	n_D^{20e} (calc.)
Saturates							
Formate.....	1		12.89	61.63 (22)	64.53		1.3224
Acetate.....	2		17.53	79.35 (22)	81.07		1.3519
Propionate.....	3	22.17	22.17	96.29 (22)	97.61	1.3775 (22)	1.3717
Butyrate.....	4		26.81	113.68 (22)	114.15	1.3879 (23)	1.3859
Valerate.....	5		31.45		130.69		1.3966
Caproate.....	6	36.09	36.09	147.20 (8)	147.23	1.4052 (10, 12)	1.4050
Enanthate.....	7		40.73		163.77	1.4113 (12)	1.4117
Caprylate.....	8	45.35	45.38	180.32 (8)	180.31	1.4170 (10)	1.4173
Pelargonate.....	9		50.02		196.85		1.4219
Caprate.....	10	54.63	54.66	213.39 (8)	213.39	1.4256 (9, 10)	1.4257
Laurate.....	12	63.91	63.94	246.51 (8)	246.47	1.4318 (9, 10)	1.4320
Myristate.....	14	73.24	73.22	279.54 (8)	279.55	1.4370 (9, 10)	1.4368
Pentadecanoate.....	15		77.86		296.09	1.4390 (12) ^f	1.4388
Palmitate.....	16		82.50		312.63	1.4408 (9, 10, 11) ^f	1.4406
Stearate.....	18		91.78		345.71	1.4440 (11) ^f	1.4436
Arachidate.....	20		101.07		378.79	1.4463 (1) ^f	1.4462
Behenate.....	22		110.35		411.87	1.4484 (11) ^f	1.4483
Monoenes							
Decenoate.....	10		54.36		206.74	1.4362 (24)	1.4387
Dodecenoate.....	12	63.84	63.64	241.57 (25)	239.82	1.4414 (25)	1.4434
Tetradecenoate.....	14		72.92		272.90	1.4403 (24)	1.4469
Tsuzuate.....	14		72.92		272.90	1.4469 (26)	1.4469
Hexadecenoate.....	16		82.20		305.98	1.4500 (27)	1.4497
Oleate.....	18	91.52	91.48	339.30 (14)	339.06	1.4519 (9)	1.4520
Oleate.....	18	91.61	91.48	339.46 (12)	339.06	1.4522 (13)	1.4520
Petroselenate.....	18	90.92	91.48	338.18 (28)	339.06	1.4502 (28)	1.4520
Eicosenoate.....	20		100.77		372.14	1.4539 (11)	1.4538
Erucate.....	22	110.10	110.05	405.17 (20)	405.22	1.4558 (20)	1.4554
Erucate.....	22		110.05		405.22	1.4555 (11)	1.4554
Dienes							
Hexadecadienoate.....	16	81.12	81.90	295.97 (29, 30, 31)	278.89	1.4604 (29, 30, 31)	1.4602
Linoleate.....	18		91.18		331.97	1.4612 (15)	1.4614
Linoleate.....	18		91.18		331.97	1.4613 (3)	1.4614
Linoleate.....	18		91.18		331.97	1.4613 (16)	1.4614
Linoleate.....	18		91.18		331.97	1.4616 (9)	1.4614
Trienes							
Hexadecatrienoate.....	16	81.81	81.60	289.84 (29, 30, 31)	292.02	1.4764 (29, 30, 31)	1.4708
Hexadecatrienoate.....	16	81.84	81.60	290.22 (32)	292.02	1.4759 (32)	1.4708
Linolenate.....	18		90.88		325.10	1.4726 (15)	1.4710
Linolenate.....	18		90.88		325.10	1.4709 (3)	1.4710
Linolenate.....	18		90.88		325.10	1.4711 (9)	1.4710
Tetraenes							
Octadecatetraenoate.....	18	90.83	90.58	318.04 (32)	318.23	1.4830 (32)	1.4810
Octadecatetraenoate.....	18	90.73	90.58	317.76 (29, 30, 31)	318.23	1.4829 (29, 30, 31)	1.4810
Octadecatetraenoate.....	18	91.03	90.58	317.79 (29, 30, 31)	318.23	1.4847 (29, 30, 31)	1.4810
Eicosatetraenoate.....	20	99.95	99.87	349.74 (29, 30, 31)	351.31	1.4834 (29, 30, 31)	1.4803
Eicosatetraenoate.....	20	99.81	99.87	349.67 (32)	351.31	1.4827 (32)	1.4803
Arachidonate.....	20	99.85	99.87	350.68 (18)	351.31	1.4813 (18)	1.4803
Arachidonate.....	20		99.87		351.31	1.4799 (17)	1.4803
Pentaenes							
Eicosapentaenoate.....	20	99.47	99.57	345.79 (29, 30, 31)	344.44	1.4871 (29, 30, 31)	1.4898
Eicosapentaenoate.....	20		99.57		344.44	1.4896 (19)	1.4898
Docosapentaenoate.....	22		108.85		377.52	1.4883 (19)	1.4883
Docosapentaenoate.....	22	108.85	108.85	374.27 (29, 30, 31)	377.52	1.4934 (29, 30, 31)	1.4883
Docosapentaenoate.....	22	108.33	108.85	370.97 (29, 30, 31)	377.52	1.4958 (29, 30, 31)	1.4883
Hexaenes							
Docosahexaenoate.....	22	107.25 (2)	108.55		370.65	1.4930 (2)	1.4974
Docosahexaenoate.....	22	108.50 ^g	108.55		370.65	1.4974 (1)	1.4974

^a Calculated from I, using observed refractive index (column 7) and observed density or molar volume (column 5).

^b Calculated from VIII.

^c Calculated from IX or X.

^d Averaged values used in some cases; also, in some cases, an "observed" value for n_D^{20} was calculated from n_D obtained at some other temperature, using $0.00038/^\circ\text{C}$. as the value for $\frac{dn_D}{dt}$.

^e Calculated from XI or XII.

^f Extrapolated values; esters normally solid at 20°C .

^g Calculated on the basis of an observed density of 0.9214 g./cc. at 25°C . and n_D^{20} [see footnote (d)].

which agreed well with the values established for the methyl esters. Thus equations VIII-XII will probably yield quite accurate values for all of the derivatives listed in Table V, subject to limitations due to molecular association in the smaller molecules as previously noted for the methyl esters, provided that the k_3 and k_6 values in the equations are replaced by those given in Table V.

Interesting confirmation of the validity of using the same k_4 values for different fatty acid derivatives

 TABLE V
 Values of k_3 and k_6 for Fatty Acids and Derivatives at 20°C .

Derivative	k_3	k_6
Fatty acid.....	3.62	26.09
Methyl ester.....	8.247	47.99
Ethyl ester.....	13.19	66.41
Propyl ester.....	17.58	83.24
Isopropyl ester.....	17.65	85.44
Alcohol.....	3.57	25.50
Aldehyde.....	2.30	23.80

is found in the work of Ruhoff and Reid (35). These investigators measured the densities of a series of 15 straight chain saturated esters in which the sum of the number of carbon atoms in the alcoholic and fatty acid chains was 16. From our assumptions and the relationships based on them it would be predicted that the densities or molar volumes in such a group of compounds would all be the same, except for those esters derived from either very short chain acids or short chain alcohols. Within the limits of experimental error the data of Ruhoff and Reid confirm this prediction for all of the compounds containing acid radicals with more than three carbons and alcohol radicals with more than two carbons.

It is to be anticipated that the relationships that have been described will be applicable in some cases to mixtures consisting of two or more members of a family of fatty acid derivatives. A necessary condition in such cases will be that the volume of the mixture shall equal the sum of the volumes of the

individual components in a pure state. Data representative of the average number of carbons in the chain and of the average number of double bonds in the mixture would be required for calculations of V_m and n_D . However complications would be encountered with mixtures of monoenes and polyenes because of differences in the value of k_5 for monoenes and polyenes.

It is to be anticipated also that when adequate data become available, relationships for calculating accurate values of R_m , V_m , and n_D will be developed for individual triglycerides, but again application of the relationships to mixtures of triglycerides will be complicated by the problem of differing values for k_5 for monoenes and polyenes. However, for mixtures in which the volume is equal to the sum of the volumes of the pure individual components, it is probable that k_5 may be expressed as a straight line function of the proportion of monoene present in the mixture. Substitution of such an expression for k_5 in the relationships would then permit calculation of V_m and n_D if, in addition to data representing average values of C and D, the proportion of monoene is known. Alternatively, with some types of mixtures, measured values of V_m and n_D would permit a calculation of the average chain length and unsaturation.

An empirical equation representing an approximate straight line relationship between refractive index and iodine value is sometimes used for triglyceride oils but is not very exact and further is limited to certain types of oils (36).

Variation of n_D and V_m with Temperature

It is well known that the refractive indexes of fatty acids and their derivatives in the liquid state are approximately straight line functions of temperature. Relationships for calculating the refractive index of a given compound at any temperature have been established (9). It has been found that for various homologous series of these compounds, the temperature coefficient of n_D is approximately -0.00038 per degree C., regardless of chain length.

Craig (11) has evaluated this coefficient more carefully and has found that it varies slightly with chain length and is appreciably different for free acids and methyl esters. The effect of unsaturation on the coefficient has not been thoroughly investigated, but such data as are available indicate that it is probably not large. In general, for all compounds considered in this paper, and for temperatures not far removed from 20°C ., the coefficient -0.00038 per degree may be used to calculate to a close approximation the refractive index at a given temperature from the observed or calculated value for n_D^{20} .

The general equation III may be expected to hold for the liquid state of a family of fatty acid derivatives at any temperature although of course different sets of constants will be required at different temperatures. If one may assume that the temperature coefficients of the constants k_4 , k_5 , and k_6 are constant over a certain temperature range, then

$$V_m^t = V_m^{20} + (k_4'C + k_5'D + k_6')(t - 20) \quad \text{XIII}$$

where V_m^t equals molar volume at a temperature t , and k_4' , k_5' , and k_6' represent $\frac{dk_4}{dt}$, $\frac{dk_5}{dt}$, and $\frac{dk_6}{dt}$, respectively.

From the results of Bonhorst *et al.* (8), for saturated methyl, propyl, and isopropyl esters, it was found that k_4' is essentially constant with temperature in the range covered by their data. In addition, as shown in Table VI, the values of k_4' are approximately the same for all three groups of esters. On the other hand, k_6' was found to increase slightly with temperature. However, over a limited temperature range, k_6' may be assigned the values shown in Table VI, and the effect of temperature on k_6' compensated by adding the empirical correction

$$9.01 \times 10^{-5} (t-20)^2 + 1.53 \times 10^{-6} (t-20)^3$$

to the right hand member of equation XIII. The latter correction was found to be applicable to the methyl, propyl, and isopropyl esters and gave calculated values that agreed with observed values within the limits of experimental error up to the maximum temperatures for which data were given by Bonhorst *et al.* (8), 98.9°C .

Values for k_4' and k_6' for ethyl esters and fatty acids were calculated from data of Timmermans (22) and Dorinson *et al.* (6), respectively, but these data are more limited in scope, and the values obtained are therefore less reliable than those calculated for methyl, propyl, and isopropyl esters. The value of k_4' for the free acids is apparently quite different from those for the esters (see Table VI).

TABLE VI

Constants for the Calculation of V_m^t from V_m^{20} for Saturated Fatty Acids and Derivatives

Derivative	k_4'	k_6'
Fatty acid.....	0.006	0.0085
Methyl ester.....	0.0113	0.0974
Ethyl ester.....	0.0108	0.112
Propyl ester.....	0.0108	0.1229
Isopropyl ester.....	0.0102	0.1332

An attempt was made to evaluate k_5' using the data of Keffler and McLean (14) for oleic acid derivatives. The data did not permit an accurate evaluation but did permit the conclusion that k_5' is comparatively small, if not negligible.

Discussion

Figure 1 is a plot of the calculated n_D^{20} versus D for common unconjugated straight chain methyl es-

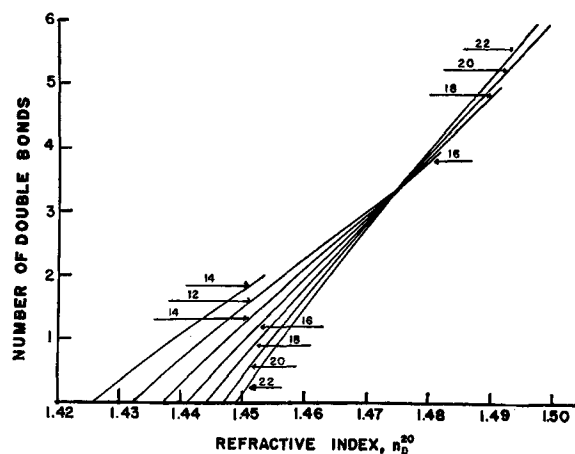


FIG. 1. Relationships between n_D^{20} and number of double bonds for methyl esters of fatty acids.

ters. Each line represents a different value of C. All of these lines cross at a single point between 3 and 4 double bonds so that with methyl esters containing three or less double bonds, the refractive index increases with chain length, and with methyl esters having four or more double bonds, the refractive index decreases with chain length.

An explanation for this may be found in XII. It will be seen that at a value of D where

$$\frac{64.48 - 7.47D}{39.74 - 6.57D} = \frac{25.82}{11.90}$$

the refractive index will be the same for all values of C.; that is, it will be $\left(\frac{25.82}{11.90}\right)^{1/2}$ or 1.4730. The value of D at which this point occurs, calculated from the foregoing relation, is 3.28. The refractive index of methyl esters with three or less double bonds will increase toward the value 1.4730 and those with four or more double bonds will decrease toward this value as C becomes larger.

Since k_1 and k_2 are the same for all compounds considered in this paper, n_D^{20} will tend to approach 1.4730 in all of the families of compounds as the chain length becomes infinitely great. However the value of D in plots such as Figure 1 at which all lines for the different chain lengths will cross will depend on the different values for k_3 and k_6 for the various families of compounds.

The ratio of D to the molecular weight M is proportional to iodine value. In Figure 2 the values of D/M versus n_D^{20} have been plotted for methyl esters of various chain lengths. It is apparent that, for a given chain length, there is an approximate straight

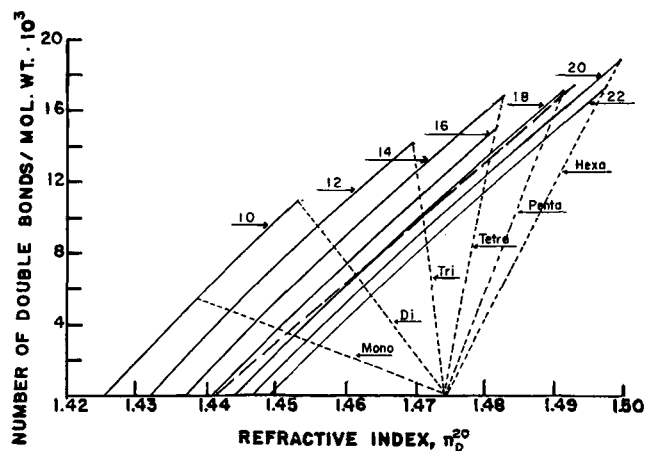


FIG. 2. Relationships between n_D^{20} and comparative unsaturation for methyl esters of fatty acids [— — — represents an empirical relationship developed by Farmer and Vandenhuevel (3)].

line relationship between iodine value and refractive index. It is also clear that a straight line relationship between refractive index and iodine value such as proposed by Farmer and Vandenhuevel (3), represented in the broken line, can have only limited

applicability; and, indeed, their relationship was developed on the basis of data for a limited group of methyl esters.

Summary

Equations have been developed for the calculation of molar refraction, molar volume or density, and refractive index for liquid fatty acids and fatty acid derivatives in relation to two parameters, carbon chain length, and unsaturation. Particular attention has been given to the applicability of the equations to families of compounds in which the unsaturated members contain only *cis* double bonds and in which the ethylene units of the polyene members are interrupted by methylene groups. The equations are sufficiently accurate to have considerable utility in establishing the identity, purity, or structure of various fatty acid derivatives.

It was indicated that, with more data, similar relationships may be developed which will be useful in characterizing some types of mixtures of fatty acid derivatives.

An approximate relationship between molar volume and temperature for fatty acids and derivatives was also devised.

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