Determination of *trans*-Octadecenoic Acids, Esters, and Alcohols in Mixtures

Infrared Spectrophotometric Method

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An infrared spectrophotometric method, based on differences in absorption at 10.36 microns, is described for determination of *trans*-octadecenoic acids, esters (including glycerides), and alcohols in the presence of the corresponding cis and saturated compounds. Extinction coefficients at 10.36 microns are reported for seventeen pure cis and trans monounsaturated and saturated acids, esters, and alcohols. The method is rapid, accurate, and directly applicable to determination of trans isomers in acid, ester, or alcohol mixtures. Only small samples are required, and they can be recovered. The authors know of no other method for determination of trans alcohols or for direct determination of trans esters in mixtures, although approximate and time-consuming chemical methods are available for the acids. The accuracy and precision of the method are indicated from analyses of nineteen synthetic mixtures of known composition. The infrared method is suggested as a valuable tool to investigators conducting research on oxidation, isomerization, polymerization, composition, and hydrogenation of fats and their components and derivatives, and on the preparation of pure unsaturated acids and esters.

THE two methods now employed for determining trans components in acid or ester mixtures are not based on any unique characteristic of trans compounds, but either on the preferential insolubility of lead (*1, 22, 30*) or other salts (*5, 8, 9, 16, 27, 29*) of trans acids in organic solvents, or on the difference between the equilibrium constants of cis and trans acids in their reaction with iodine in carbon tetrachloride solution (*4, 21*). These chemical methods are generally laborious and time-consuming, they require large quantities of sample, and they are of doubtful reliability in many applications (*25*). The authors know of no previously reported method for determination of trans alcohols in mixtures.

Published infrared absorption spectra (*17, 19*) for pure elaidic (*trans*-9-octadecenoic), petroselaidic (*trans*-6-octadecenoic), and vaccenic (*trans*-11-octadecenoic) acid, methyl elaidate and petroselaidate, elaidyl alcohol (*trans*-9-octadecenol), and trielaidin show a strong absorption maximum at 10.36 microns, which is absent in the corresponding cis and saturated compounds. No marked differences exist, however, between corresponding trans, cis, and saturated compounds in other regions of the infrared spectrum.

Although several groups of workers have reported that olefins with an internal unsubstituted double bond (R_1—CH=CH—R_2) have an absorption band at about 10.36 microns (*7, 11, 13, 14, 28*), Rasmussen, Brattain, and Zucco (*18*) were apparently the first to point out that this absorption is due to trans configuration of this group. Absorption at this wave length has since been employed for both the qualitative and quantitative analyses of a variety of materials (*2, 3, 10, 11, 14, 15*), but not for the quantitative determination of *trans*-octadecenoic acids, esters, and alcohols in mixtures. An infrared spectrophotometric method based on the difference at 10.36 microns has been developed by the authors for

this type of determination. In developing the method, extinction coefficients were determined on the compounds mentioned above as well as on pure oleic (*cis*-9-octadecenoic), petroselinic (*cis*-6-octadecenoic), palmitic (hexadecanoic), and stearic (octadecanoic) acids, methyl oleate and stearate, triolein, trimyristin (glyceryl tritetradecanoate), and oleyl (*cis*-9-octadecenol) and stearyl (octadecanol) alcohols. In addition, mixed palmitostearins were obtained by crystallization of completely hydrogenated vegetable oil triglycerides. The infrared method is rapid, specific, and accurate, it is directly applicable to determination of trans components in acid, ester, or alcohol mixtures, only small samples are required, and the sample can be recovered.

APPARATUS, MATERIALS USED, AND PROCEDURE

Apparatus. This has been reported (*25*).

Materials Used. The methods of preparation of most of the reference compounds have been described (*25*). Oleyl alcohol, boiling point 180.1–180.4° C. at 3.6 mm., n_D^{30} 1.4562, and iodine number 93.7 (calculated 94.5), was prepared by repeated fractional distillation and low temperature crystallization of a good commercial grade (*24*). Elaidyl alcohol, melting point 35.7–35.9° and iodine number 93.7, was prepared by isomerization of oleyl alcohol with powdered selenium at 220–225° (*23*). Stearyl alcohol, melting point 58°, was obtained by recrystallization of the purest commercial grade three times from 95% ethyl alcohol and once from methanol at 0° (7 to 10 ml. of solvent per gram of solute).

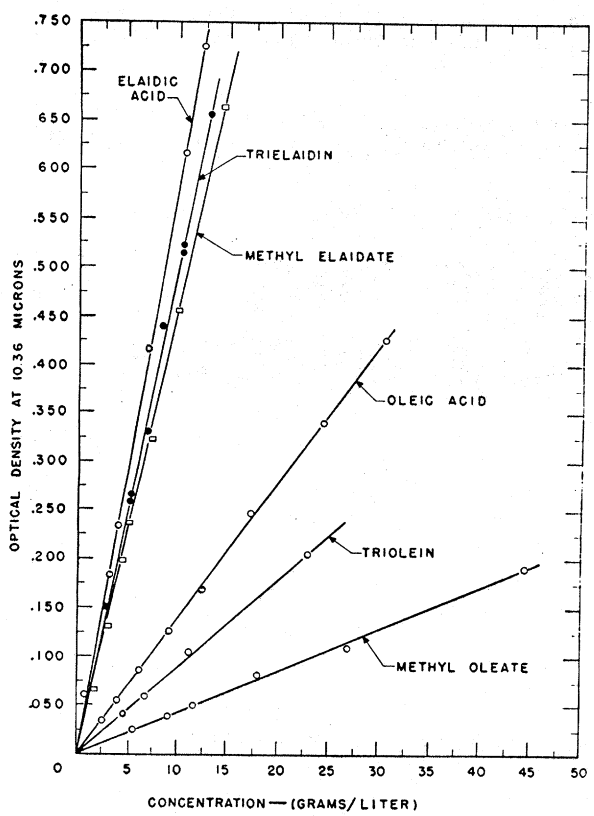

Figure 1. Optical Density at 10.36 Microns as Function of Concentration in Carbon Disulfide

Determination of Extinction Coefficients of Pure Compounds. The procedure employed has been described (*25*). Concentration ranges covered were 0 to approximately 15 grams per liter for all trans compounds (strong absorbers); 0 to approximately 30 grams per liter for the cis monounsaturated and saturated acids, glycerides, and alcohols (moderate to weak absorbers); and 0 to approximately 45 grams per liter for the cis monounsaturated and saturated methyl esters (extremely weak absorbers). Plots of optical density as a function of concentration in grams per liter showed excellent adherence to Beer's law over the concentration range studied. Figure 1 shows some representative plots. The extinction coefficients, k, to be used in analysis were obtained by dividing the slopes of the straight-line Beer's law plots (on an expanded scale) by the cell thickness (*20*) in centimeters. Thus $k = \frac{\text{optical density at } 10.36\mu}{(\text{concn., grams/liter})(0.1054 \text{ cm.})}$. Table I lists the k values and values for the molar extinction coefficient, ϵ ($k \times$ molecular weight).

Table I. Extinction Coefficients at 10.36 Microns for Pure cis and trans Monounsaturated and Saturated Acids, Methyl Esters, Triglycerides, and Alcohols

Compound	Extinction Coefficients k^a	ϵ^b	No. of Determinations
Oleic acid	0.133	37.6	8
Petroselinic acid	0.129	36.4	7
Palmitic acid	0.129	33.1	7
Stearic acid	0.123	35.0	7
Elaidic acid	0.552	155.9	7
Petroselaidic acid	0.560	158.2	5
Methyl oleate	0.041	12.2	7
Methyl stearate	0.028	8.4	7
Methyl elaidate	0.442	131.1	7
Methyl petroselaidate	0.454	134.6	6
Triolein	0.084	74.4	4
Trimyristin	0.087	62.9	5
Palmitostearins	0.078	...	7
Trielaidin	0.475	420.6	8
Oleyl alcohol	0.069	18.5	6
Stearyl alcohol	0.058	15.7	6
Elaidyl alcohol	0.523	140.4	6

[a] $k_{10.36} = \frac{\text{optical density at } 10.36\mu}{(\text{concn., grams/liter})\ (0.1054 \text{ cm.})}$

[b] $\epsilon_{10.36} = \frac{\text{optical density at } 10.36\mu}{(\text{concn., moles/liter})\ (0.1054 \text{ cm.})}$

Analysis of Known and Unknown Mixtures. A weighed sample of the mixture is diluted to 10 ml. with carbon bisulfide in a volumetric flask, and the optical density of the solute at 10.36 microns is determined under conditions identical with those described above for the pure compounds. In general, the total concentration of the mixture should be adjusted to give a solute optical density between 0.2 and 0.6. The concentration selected will depend on the approximate content of trans component expected and whether an acid, methyl ester, glyceride, or alcohol mixture is being analyzed. For mixtures containing substantial percentages of trans component, 0.1 to 0.2 gram diluted to 10 ml. will usually give satisfactory results. Higher total concentrations are employed when the trans content of the mixture is low. In any case the sample can be greatly reduced by semimicro weighing and dilution techniques.

Calculation of Results. The following formulas are used in calculating the weight per cent of trans component in mixtures:

MIXTURES CONTAINING *cis*- AND *trans*-OCTADECENOIC COMPONENTS ONLY.

$$\text{trans component, weight } \% = \frac{100\ (k_{ob} - k_c)}{k_T - k_c} \qquad (1)$$

where k_{ob} = "observed extinction coefficient" for the mixture = $\frac{\text{optical density at } 10.36\mu}{(\text{total concn., grams/liter})(\text{cell thickness, cm.})}$

k_c = extinction coefficient of pure cis compound (Table I)
k_T = extinction coefficient of pure trans compound (Table I)

MIXTURES CONTAINING *trans*-OCTADECENOIC AND SATURATED COMPONENTS ONLY.

$$\text{trans component, weight } \% = \frac{100\ (k_{ob} - k_s)}{k_T - k_s} \qquad (2)$$

where k_s is the extinction coefficient of the pure saturated compound (Table I).

MIXTURES CONTAINING *cis*- AND *trans*-OCTADECENOIC AND SATURATED COMPONENTS.

$$\text{trans component, weight } \% = \frac{100\,(k_{ob} - k_cY - k_sZ)}{k_T - k_c} \quad (3)$$

where the k values are as defined above; Y is the total weight fraction of octadecenoic components (cis plus trans), and Z is the weight fraction of saturated components. Y is calculated from the iodine value of the mixture and Z is obtained by difference.

In practical applications to unknown mixtures, the *cis*- and *trans*-octadecenoic fractions may each consist of one or more compounds in which the exact position of the double bond is unknown. Also the relative proportions of long-chain saturated compounds in the saturated fraction are often unknown. Therefore, the following facts, evident from the data of Table I, are fortunate.

Shifting the double bond from the Δ^9 to the Δ^6 position in either the *cis*- or *trans*-octadecenoic acids produces only a small change in extinction coefficient. The same holds for the *trans*-methyl esters, and therefore might be expected to hold for the *cis*-methyl esters, and the *cis*- and *trans*-glycerides and alcohols. Furthermore, it seems safe to assume that an equivalent shift in the opposite direction to Δ^{12} or to any intermediate position would likewise produce little change. (The effect of more drastic shifts remains to be investigated.)

The extinction coefficients for the various *cis*-octadecenoic and saturated acids studied are all approximately the same. This relation also holds for the cis and saturated glycerides and alcohols and to a somewhat lesser degree for the cis and saturated methyl esters.

Table II. Analyses of Synthetic Mixtures for trans Component

Composition of Mixture, %[a]			trans Component Found, %			
ME	MO	MS	1	2	Av.	Difference
66.91	33.09	0	66.52	66.81	66.66	−0.25
49.03	50.97	0	49.52	49.10	49.31	0.28
3.12	96.63	0	3.20	3.38	3.29	0.17
26.12	23.87	50.01	25.47	25.68	25.58	−0.54
9.96	16.16	73.88	9.67	9.15	9.41	−0.55
3.17	15.00	81.83	3.42	3.28	3.35	0.18
EA	OA	SA				
67.48	32.52	0	67.11	67.52	67.32	−0.16
18.10	81.90	0	17.59	18.40	18.00	−0.10
10.41	89.59	0	10.52	11.11	10.82	0.41
3.31	96.69	0	3.50	3.80	3.65	0.34
65.17	20.11	14.72	66.21	65.77	65.99	0.82
39.44	32.28	28.28	38.33	38.61	38.47	−0.97
10.32	7.07	82.61	10.51	10.73	10.63	0.31
4.44	20.30	75.30	3.73	3.92	3.83	−0.61
EAL	OAL	SAL				
65.87	34.13	0	65.72	65.84	65.78	−0.09
50.13	49.87	0	50.22	49.98	50.10	−0.03
19.95	80.05	0	20.00	19.85	19.92	−0.03
19.48	39.22	41.30	19.07	19.41	19.24	−0.24
2.99	51.14	45.87	3.00	3.11	3.05	0.06

[a] ME = methyl elaidate; MO = methyl oleate; MS = methyl stearate; EA = elaidic acid; OA = oleic acid; SA = saturated acids; EAL = elaidyl alcohol; OAL = oleyl alcohol; SAL = stearyl alcohol.

In unknown acid mixtures that contain no octadecenoic acids outside the Δ^6 to Δ^{12} range, the total percentage of trans acids present can be calculated with acceptable accuracy by using the average k value of the two trans acids from Table I for k_T, that of the two cis acids for k_c, and that of the two saturated acids for k_s in the appropriate formula above. Results on unknown methyl ester, glyceride, or alcohol mixtures may be similarly calculated by using appropriate average or single values in Table I. In view of the above statement, determination of iodine number in a trans-cis-saturated mixture (of either of the four types) can be eliminated if desired, and results calculated from the following simplified formula:

$$\text{trans component, weight } \% = \frac{100\,(k_{ob} - k_{\text{av.}})}{k_T - k_{\text{av.}}}$$

where k_T is the appropriate average or single k value for trans compounds and $k_{\text{av.}}$ is the average k value for both cis and saturated compounds.

For given selected values of k_c and k_s, Formulas 3 and 4 will yield identical results (expressed as per cent trans, to the nearest 0.01%) when $Y = Z = 0.5$—that is, when total per cent octadecenoic components equal total per cent saturated components. As Y departs from 0.5 in either direction, the absolute error entailed by use of Formula 4 increases. In the most unfavorable cases likely to be encountered in analyzing acid mixtures (Y = 0.03 or Y = 0.97), this error is about 0.5% when the average value for the two trans acids is used for k_T, that of the two cis acids for k_c, that of the two saturated acids for k_s, and that of the latter for $k_{\text{av.}}$ In most cases, the error will be much less.

RESULTS

Table II gives the percentage of trans component obtained on nineteen known synthetic mixtures of pure compounds. The results show that in general the infrared method has a satisfactory degree of precision and accuracy. The best oleic acid from the chemical identity standpoint is that obtained from olive oil (*6*). Oleic acid obtained from animal fat sources (*12, 24, 26*) contains varying amounts of trans isomers (4 to 45%), depending on the starting material and the preparative process employed (*25*). This is probably not disadvantageous for many technical applications, but it suggests that these oleic acids should not be used when chemical homogeneity is important.

GENERAL COMMENTS

Because of the present state of infrared spectrophotometry, extinction coefficients reported in this paper cannot be employed directly by other workers. Variations in such factors as scattered radiation, wave-length calibration, slit-width settings, and accuracy of cell-thickness measurements make it necessary to determine the required extinction coefficients on the instrument being used under the exact conditions to be employed in the analysis. It may be unnecessary, however, to determine the extinction coefficients of all the compounds reported in Table I—for example, from redeterminations of one trans and one cis or saturated compound, in conjunction with relative values calculated from those in Table I, it should be possible to calculate sufficiently accurate values for the remaining compounds.

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