Raman Spectroscopic Analysis of the *cis/trans* Isomer Composition of Edible Vegetable Oils[1]

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ABSTRACT

A new method is presented for determining the *cis/trans* isomer content of edible vegetable oils. The intensities of Raman lines near 1656 and 1670 cm^{-1} are associated with the *cis* and *trans* configuration, respectively. A precision of ca. 1% can be obtained in the *cis/trans* isomer analysis of binary mixtures of methyl esters and triglycerides of monoenes and dienes and of hydrogenated vegetable oils. The spectroscopic data also provide the iodine value of vegetable oils or isolated fractions with precision for a single determination of ca. 1%.

INTRODUCTION

Cis/trans isomer composition of unsaturated esters in edible vegetable oils is of interest in nutrition studies (1-4) and because of altered storage, flavor and oxidative stability which accompany structural changes during hydrogenation of the oils (5-8). The IR method (9) of determining the *trans* isomer concentration, using the intensity of the 970 cm^{-1} absorption band associated with the *trans* out-of-plane ethylenic hydrogen deformation vibration (10), has been very useful, but it requires an independent determination of the *cis* isomer or total unsaturation by gas liquid chromatography (GLC) (11), UV absorption (12) or iodine absorption (13), or both. The proposed method uses the Raman scattering to directly measure the amounts of both isomers. Earlier studies (10,14) gave assignments for the vibrational spectra of simple model compounds of interest in this investigation. The integrated scattering intensity for any vibrational mode is proportional to the number of corresponding molecules in a mixture. By measuring the intensities of C=C stretching fundamentals near 1656 and 1670 cm^{-1} associated respectively with *cis* and *trans* configurations, a precision of ca. 1% can be obtained in *cis/trans* isomer analyses of pure methyl esters and triglycerides of mono-, di- and tri-unsaturates which are not conjugated in their native state.

[1]Presented at the AOCS meeting, Houston, May 1971.

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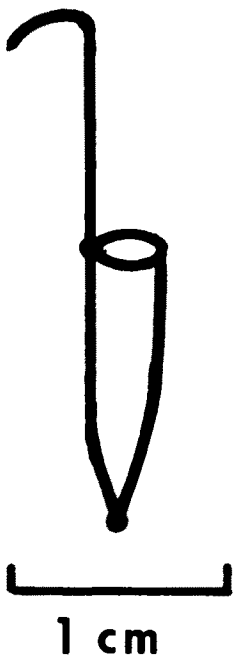

FIG. 1. Fused silica weighing and mixing vessel, 15-25 mg.

EXPERIMENTAL PROCEDURES

The Raman spectra were measured using a Spectra-physics 125 He-Ne gas laser (6328 Å) giving 50-80 mW of exciting power. The neat sample was irradiated by the laser in a 0.3 mm OD capillary cell as previously described (15). The illuminated volume on the axis of the capillary was ca. 50 μ in diameter.

A Jaegers S3205 wide-field eyepiece of 52 mm clear aperture, working at ca. *f*/0.8, imaged the illuminated sample volume on the entrance slit of the monochromator at 4.5X magnification.

A Spex 1401 double monochromator was used for all measurements.

A simple lens imaged the light from the monochromator exit slit on the cathode of an ITT FW-130 photomultiplier tube, the dark pulse rate of which was 30/sec (23 C) and <1/sec (-20 C).

The pulses from the photomultiplier were amplified to standard height and duration (16), averaged in a rate meter and recorded on a 10 mv strip chart recorder. The pulse rate and wavenumber shift were also encoded and stored in an IBM 1800 data acquisition system for convenient computation of results (17). While the results reported here were obtained and computed with a digital data acquisition system, the same results were procured from the strip chart recordings of the spectra at 20 cm^{-1}/in. using a planimeter. Where the computer results show about $\sigma = \pm 0.9\%$, with the planimeter we find $\sigma = \pm 1.3\%$. All samples were scanned at 50 cm^{-1}/min, corresponding to 6 min per sample for the analytical results.

Examples of the convenience and wide application of modern Raman instrumentation are available in many reviews and in the literature of the several Raman instrument manufacturers (18,19).

Purity of reference compounds obtained from Hormel Institute was verified by GLC (11), thin layer chromatography (TLC) and IR (9) methods. The methyl esters of lauric, oleic, elaidic, linoleic, linoelaidic and linolenic acids were analyzed on a 1.2 m x 6.3 mm column packed with 10% FFAP on Chromosorb P at 220 C and on a 24.4 m x 0.25 mm capillary column, coated with General Electric Co. SF 96-[50] Silicone oil containing 1% of General Aniline & Film Co. Igepal Co-880, which was programed from 80-175 C at 1 C per min. Chromatograms of these esters were developed on Brinkman Silica Gel G analytical plates using diethyl ether-hexane 5:95 and visualized with $K_2Cr_2O_7$ in 40% H_2SO_4 (5 gm/100 ml) to detect oxidation and polymerization products. Triglycerides of the fatty acids listed above were checked by the same TLC procedure for oxidation products except that the developing solvent was hexane-diethylether-acetic acid 89:10:1. There was no detectable impurity in any reference com-

pound except for the methyl linolenate and trilinolenin, each of which showed a very small, weak spot between the origin and the ester. IR analysis (9) of all the *cis* reference compounds showed no trace of *trans* except for the linolenates, which had a very weak band at 970 cm^{-1}. Analysis of the Raman C=C stretching lines of the *cis* reference compounds with a band resolving routine (20) showed no *trans* impurity in any but the methyl linolenate and trilinolenin, which contained 0.8 and 0.7% *trans*, respectively. Less than 10 mg quantities of pure reference compounds were weighed into counterbalanced 15-25 mg fused silica mixing vessels (Fig. 1) on a Cahn Model G Electrobalance. The samples were mixed with a 1 mm OD loop of 0.25 mm Pt wire rotated by a small stirring motor. With sample mixtures <10 mg, care was required to assure that all of each component was brought down off the vessel walls into the mixture. A heat lamp was used to keep solid samples, e.g., trielaidin, melted for weighing and mixing.

RESULTS AND DISCUSSION

Portions of the Raman spectra of some reference compounds are shown in Figure 2. As with other saturated fatty esters, methyl laurate has no Raman scattering feature between 1600 and 1710 cm^{-1}. The small feature near 1649 cm^{-1} is the Tyndall scattering by the sample of a plasma emission line, weaker than the 6328 Å laser line by a factor of ca. 10^6. This plasma line has been removed from all other spectra by insertion of a multilayer interference filter of 10 Å band width, between the laser and the sample. This reduces the intensity of the beam by about half at the sample. The *cis* monoene, diene and triene C=C stretch features are found near 1654, 1657 and 1655 cm^{-1}, respectively, compared with the *trans* monoene and diene at 1668.5 and 1670.5 cm^{-1}. These spectral features are substantially free from interference from the wings of a relatively weak carbonyl stretch band near 1745 cm^{-1} for the triglycerides or 1743 cm^{-1} for the methyl esters and of the $>CH_2$ scissor mode near 1446 cm^{-1}. The frequency shifts and band widths for the C=C and carbonyl stretch vibration of the reference compounds are given in Table I.

The Raman scattering in the C=C stretch region for triolein and trielaidin and for five different mixtures is shown in Figure 3. Since the integrated intensity of any band associated with a particular component is proportional to the concentration of that component, the fraction of the total C=C stretch scattering which is associated with the *trans* isomer is directly proportional to the *trans* isomer fraction. Referring to Figure 3, we consider the C=C stretch for these compounds falling within the limits 1626 and 1691 cm^{-1} and arbitrarily use 1664 cm^{-1} as an acceptable

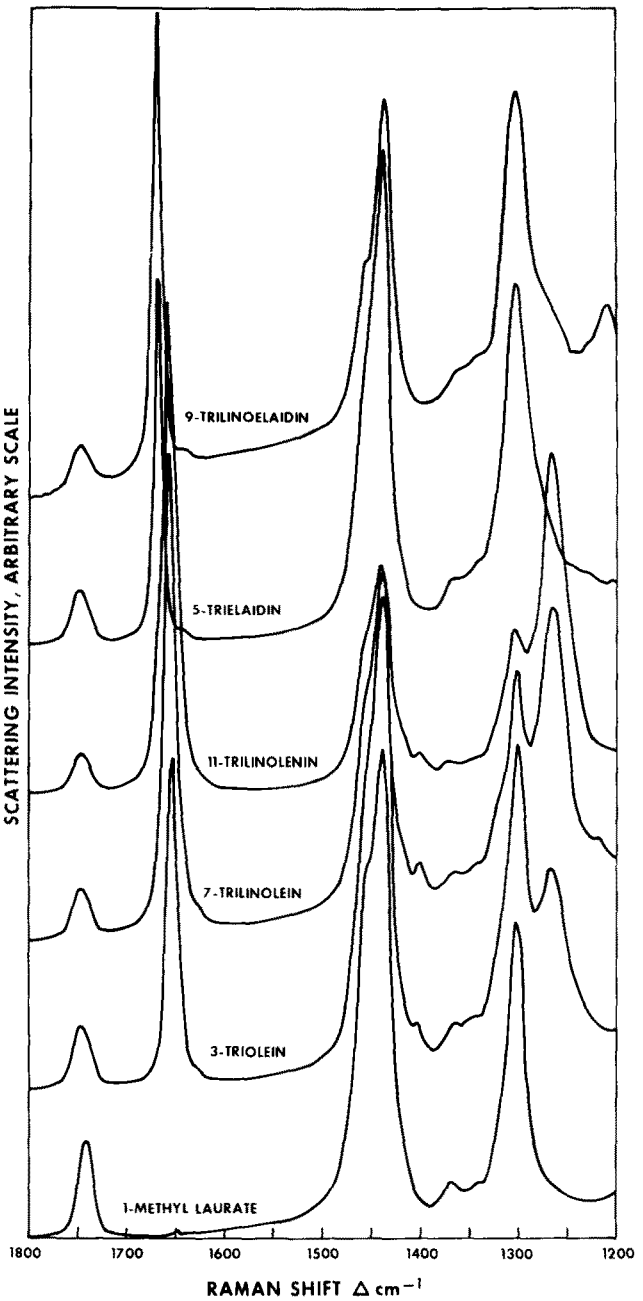

FIG. 2. Raman spectra of reference compounds, 1200-1800 cm^{-1}.

TABLE I

Raman Frequency Shifts[a] of Reference Compounds

Identifying number[b]	Compound	Class	C=C stretch cm^{-1}_{max}	C=O stretch cm^{-1}_{max}	CH_2 scissor cm^{-1}_{max}	$R_{t/U}$[c]
(1)	Methyl laurate	saturated	---	1744 (17)	1441	---
(2)	Methyl oleate	*cis*	1655.1(14.2)	1744.5(17)	1440	0.078
(3)	Triolein	*cis*	1653.8(14.5)	1747 (21)	1438	0.075
(4)	Methyl elaidate	*trans*	1669 (10)	1744 (16.5)	1439	0.768
(5)	Trielaidin	*trans*	1668.5(11)	1748 (22)	1438	0.761
(6)	Methyl linoleate	*cis,cis*	1656.1(15.7)	1741.5(17)	1439	0.149
(7)	Trilinolein	*cis,cis*	1656.4(15.4)	1747 (21)	1440	0.144
(8)	Methyl linoelaidate	*trans,trans*	1671.5(11)	1742 (17)	1437	0.826
(9)	Trilinoelaidin	*trans,trans*	1670.5(11)	1746 (22)	1437	0.821
(10)	Methyl linolenate	*cis,cis,cis*	1657 (16.2)	1741.5(16.5)	1438.5	0.210
(11)	Trilinolenin	*cis,cis,cis*	1656.4(18.6)	1745 (22)	1440	0.203

[a]The observed half-widths of the Raman lines are shown in parentheses.

[b]The numbers in column 1 are used to identify the compounds in Figures 3-6.

[c]$R_{t/U} = \int_{1626\ cm^{-1}}^{1664\ cm^{-1}} / \int_{1626\ cm^{-1}}^{1691\ cm^{-1}}$ as defined in the text.

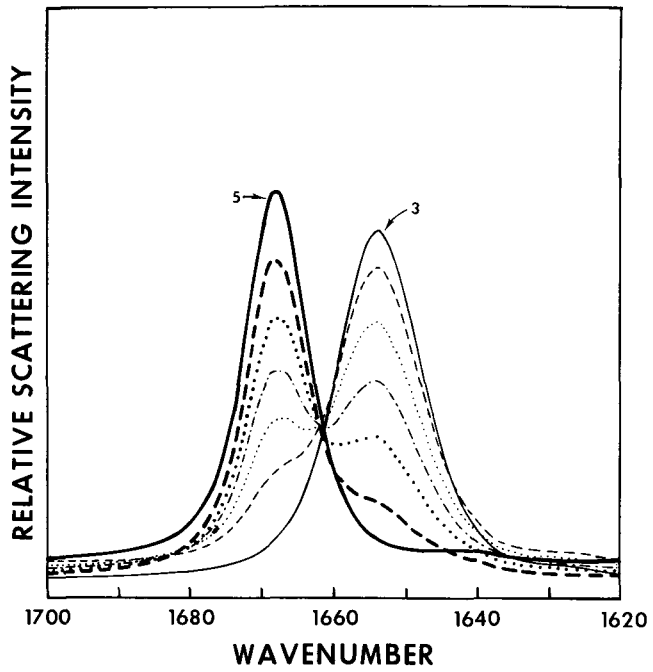

FIG. 3. Raman spectra in the C=C stretching region for Triolein (3), Trielaidin (5) and their mixtures: 15.9% Triolein, 84.1% Trielaidin, ———; 34.4% Triolein, 65.6% Trielaidin, •••; 51.3% Triolein, 48.7% Trielaidin, —•—; 67.8% Triolein, 32.2% Trielaidin, •••; and 84.0% Triolein, 16.0% Trielaidin, - - -. The isoskedastic point near 1662 cm^{-1} is somewhat obscured by the different amounts of fluorescent background, but this does not interfere with the analytical method.

compromise boundary between the *cis* and *trans* regions for the complete set of model compounds. The integrated intensities are measured with respect to a straight line background connecting the observed scattering at 1626 and 1691 cm^{-1} to remove the effects of the fluorescing impurities which may be present (Figs. 2,3). The variable fluorescence obscures the "isoskedastic" point for the binary mixtures, but does not otherwise interfere unless it is intense enough to degrade the signal-noise ratio by making the Raman scattering a small fraction of the observed spectral intensity. (Isoskedastic, from Greek *iso,* equal; *skedannumi,* to spread, disperse or scatter, as of sunlight. The usage is analogous to that for isosbestic points in absorption spectra.)

We define the intensity ratio, R_t/U, as $\int_{1664\ cm^{-1}}^{1691\ cm^{-1}} / \int_{1626\ cm^{-1}}^{1691\ cm^{-1}}$, the ratio of the *trans* intensity to the total C=C stretch scattering, measured over the straight line background specified above. For the triolein, trielaidin and mixtures shown in Figure 3, the values of R_t/U are plotted against the known composition as the lowest calibration curve in Figure 4. A measurement of the intensity ratios R_t/U for trilinolein, trilinoelaidin and their mixtures gives a similar calibration curve for the diene system in Figure 4, displaced upward as a consequence of the slightly greater frequency shift of the C=C stretching scattering for the more highly unsaturated molecules.

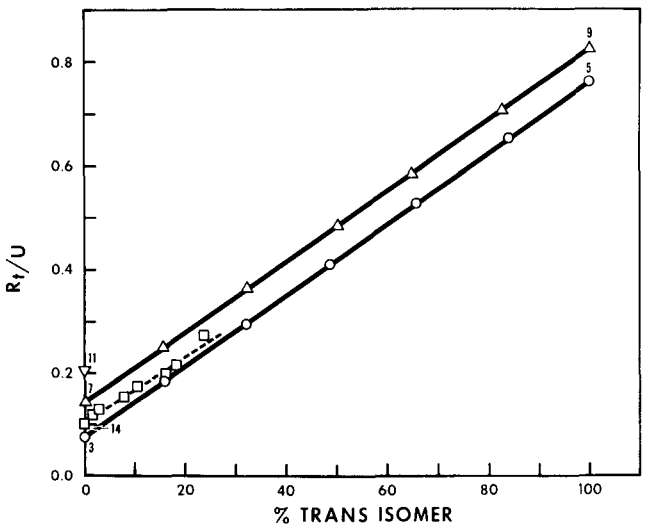

FIG. 4. Analytical calibration curves for *cis/trans* isomer analysis of edible vegetable oils. The numbers identifying the samples are in column 1 of Tables I and II.

Because of the relatively steep slopes of spectra of the *cis* and *trans* isomers near the isoskedastic points, careful control of spectrometer calibration is required. With the triolein-trielaidin mixtures we found that calibration drift introduced *cis/trans* analysis errors of ca. 5%/cm^{-1} unless they are compensated as discussed below.

A graded series of partially hydrogenated samples prepared from a refined, bleached soybean oil provided a test of the analytical procedure. The observed R_t/U values are plotted in Figure 4 against the per cent *trans* isomer as determined by the IR method (9). The scatter of the data points for the hydrogenated soybean oil samples combines the uncertainties of the IR method with those of the Raman Analysis outlined above. The trend of the data for the hydrogenated soybean oils is not parallel to the calibration curves for the monoene and diene mixtures, respectively. This may be explained by the selectivity of the

TABLE II

Comparison of Raman and IR Analyses of Per Cent *trans* Isomer

Identifying number[a]	Oil	Fatty acids 18:1	18:2	18:3	Per cent *trans* isomer Raman	IR
(12)	High oleic safflower[b]	80.7	12.2	---	0.0	0.0
(13)	Commercial safflower[b]	12.8	77.9	---	0.0	0.0
(14)	Soybean 0[c]	24.6	53.5	7.6	0.0	0.0
(15)	Soybean 1	25.9	53.2	6.0	1.5 ± .59	1.6
(16)	Soybean 2	26.9	52.8	4.3	3.3 ± .86	2.9
(17)	Soybean 4	33.6	50.0	1.2	6.4 ± .84	7.9
(18)	Soybean 5	37.2	47.1	0.6	10.3 ± .68	10.7
(19)	Soybean 7	45.0	39.8	---	14.5 ± .80	16.4
(20)	Soybean 8	48.9	36.0	---	18.2 ± 1.08	18.3
(21)	Soybean 9	58.2	26.2	---	23.0 ± .36	23.9

[a]The numbers of column 1 are used to identify the samples in Figures 4-6.

[b]Fatty acid analyses from Reference 21.

[c]Samples, fatty acid and IR analyses from Koritala (5). Precision of the IR determination of per cent *trans* isomer was estimated at ca. ± 1%.

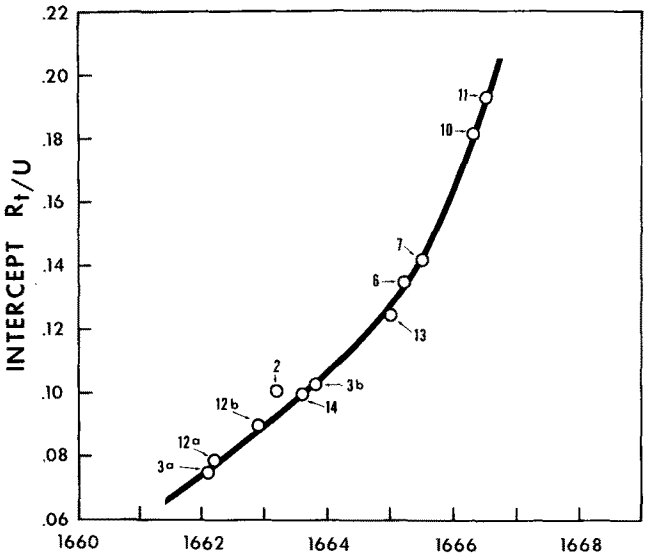

FIG. 5. Analytical Intercept R_t/U correction curve for compensation of changes of *cis* isomer spectra with hydrogenation, and for small changes in spectrometer calibration. The numbers identifying the samples are in column 1 of Tables I and II. The letter subscripts for samples 3 and 12 refer to subsets of determinations with the wave number calibration of the spectrometer shifted by thermostatting at different temperatures.

copper chromite catalysts (5-7) for hydrogenation of the more highly unsaturated components that are present in these soybean oils, as shown in Table II. We expect the composition of the product to approach the triolein-trielaidin calibration curve as a boundary value.

A correlation implied above can provide an unambiguous analysis for the general case, where the analyst has no a priori information about the fatty acid composition of the oil from which a hydrogenated analytical sample was derived. For a sample containing an arbitrary fraction of *trans* isomer, the observed R_t/U will be influenced to a degree by both the half intensity band width and frequency of the *cis* C=C stretching band, which can be determined by noting the wave number of the *cis* maximum and the half intensity point on the low frequency side of the *cis* band. Since the bands for the pure components are symmetrical (Fig. 2), the high frequency half-intensity point can be located an equal distance on the high frequency side of the *cis* maximum, even if it is obscured by the *trans* isomers in hydrogenated material. The locations of the half intensity points on the high frequency side of the C=C stretching lines, assuming a symmetrical band shape for the set of reference *cis* materials, is shown in Figure 5. Five replicate spectra each of seven hydrogenated soybean oil samples were evaluated by this procedure, and the intercept R_t/U for the *cis* isomer fraction of each, estimated from Figure 5. The per cent *trans* was then estimated from the location of the observed R_t/U on a line on Figure 4, parallel to the monoene and diene calibration lines and passing through the derived intercept R_t/U for the *cis* fraction. The results of this Raman determination of the per cent *trans* isomer are given in Table II and are comparable with the IR analysis.

While we have not yet tested hydrogenated vegetable oils with more than 25% *trans* isomer, the procedure given above gives reliable estimates of the intercept R_t/U for known mixtures containing as high as 65% of the unsaturation as the *trans* isomer. Detailed analyses of the kinetics of hydrogenation (5-7,22) show that the selectivity of copper-chromite, nickel, platinum or palladium catalysts leads to mixtures of molecules containing isolated *cis* or *trans* double bonds wherever hydrogenation has proceeded to the point where the unsaturation is more than 50% *trans* isomer. In this case the C=C stretching intensities correspond to those for mixtures of oleic, elaidic and stearic oils, and the *cis/trans* ratio can be determined from the triolein-trielaidin calibration curve.

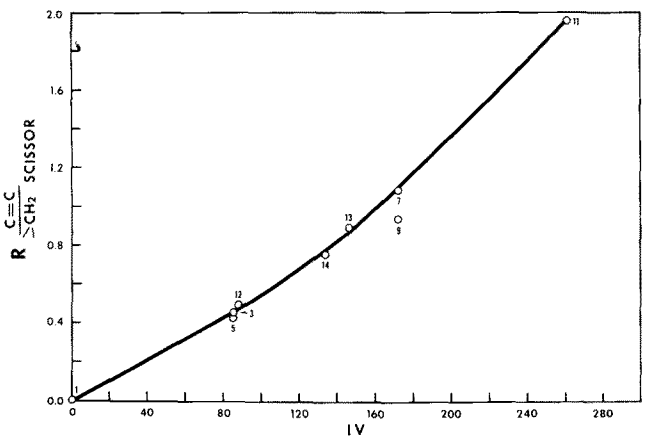

FIG. 6. Relation between $R\frac{C=C}{CH_2}$ and iodine values of reference triglycerides and unconjugated vegetable oils. The numbers identifying the samples are in column one of Tables I and II.

A useful consequence of this procedure for compensating for the changes of spectra of the *cis* isomer fraction of hydrogenated oils is that it also cancels out drifts in spectrometer calibration which are small enough to keep the analytical system in the region where the slope in Figure 5 does not greatly change. Changes during the recording of a spectrum cannot be tolerated, but 1.0 cm^{-1} shift from day to day has not interfered so long as recording time constants and scan rates are reproducible. For samples having relatively little *cis* isomer, any necessary spectrometer frequency correction can be obtained from measurements of the *trans* band location.

The direct proportionality between the Raman band intensity and the mole fraction of molecules undergoing the corresponding molecular vibration suggests a convenient analysis for the total unsaturation of these oils. We compare the scattering intensity arising from the C=C stretching vibration with that from the $>CH_2$ scissor mode,

$$R\frac{C=C}{CH_2} = \frac{\int_{1626\ cm^{-1}}^{1691\ cm^{-1}}}{\int_{1420\ cm^{-1}}^{1478\ cm^{-1}}}$$

to derive an arbitrary band intensity ratio. Part of the wings of the $>CH_2$ scissor are excluded in order to reject the weak bands near 1400 cm^{-1} in the spectra of the *cis* isomers. The $R\frac{C=C}{CH_2}$ values for reference compounds and vegetable oils are plotted in Figure 6 against their respective iodine values. The vegetable oils examined so far fall close to the curve defined by the pure triglycerides, in agreement with a recent report on unconjugated *cis* drying oils (23). For oils which contain substantial amounts of fatty acids with chain lengths other than C_{18}, correction must be made for the effect on scattering from the $>CH_2$ scissoring vibration. Drying oils which contain major amounts of conjugated *trans, trans, cis* fatty acid chains require a different calibration curve because of conjugation effects on the frequency and intensity of scattering from the C=C stretching mode (23).

ACKNOWLEDGMENT

S. Koritala, N. Market. Nutr. Res. Div., ARS, USDA, Peoria, Ill., supplied the soybean oil and the series of its hydrogenation products, as well as the fatty acid composition and IR *trans* isomer analysis. F. Stitt, J.R. Scherer and S. Kint, W. Market. Nutr. Res. Div., ARS, USDA, Berkeley, Calif., provided stimulating discussion.

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[Received April 5, 1972]