gion will undoubtedly find usefulness in applications to derivatives containing these groups.

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Recent Progress in the Applications of Infrared Absorption Spectroscopy to Lipid Chemistry

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INFRARED ABSORPTION SPECTRA were introduced to the lipid chemist as an analytical tool during the decade 1940–1950. The first half of the following decade, the years 1950–1955, witnessed widespread growth with a continually increasing number of research papers in technical journals describing infrared spectral procedures for qualitative identifications, quantitative determinations, and for elucidation of structures of lipid materials (91).

Infrared absorption spectroscopy cannot therefore be introduced at this Short Course as a new tool of lipid chemistry. However there have been many advances in applications and many new techniques introduced to the fatty acid chemist during the past few years. This paper will describe some of these. Except for earlier references, where necessary to establish essential background, this review will be limited to work reported during the past five years or since the American Oil Chemists' Society's Short Course on "Analytical Techniques" held at the University of Illinois in 1955.

Cis and Trans Unsaturation

One of the most popular applications of infrared absorption spectroscopy to lipid chemistry has been the identification and measurement of *cis-trans* isomerization. It should be recalled that in 1947 Rasmussen, Brattain, and Zucco (104) showed that a strong band at 10.3 μ appears in the infrared spectra of all unsaturated compounds which contain a *trans* C=C group and furthermore this band does not appear unless this group is a *trans* isomer. In 1949 Sheppard and Sutherland showed that the band arises from a C-H deformation about a *trans* C=C in the internally unsaturated group RHC=CHR (112). From 1950 to 1953 four papers described techniques that used this band as a means for the quantitative determination of internal, isolated (that is, nonconjugated) *trans* isomers (1, 53, 113, 122).

The published methods for the determination of isolated *trans* content have been the subject of extensive study by the Spectroscopy Committee of the American Oil Chemists' Society. As a result of long collaborative testing the committee has recommended a procedure which has been adopted as a tentative official method of the Society (93). The recommended procedure provides for the determination of isolated *trans* content of long-chain fatty acid esters and of their triglycerides. Long-chain fatty acids can be analyzed directly if the *trans* content is 15% or greater. If below 15%, the recommended procedure requires conversion of the fatty acid to its methyl ester for, at these low concentrations, correction for an absorption arising from the carboxyl band becomes too great for precise measurement. The method proposes standardization of infrared instruments with secondary standards to avoid the difficulty of preparing the highly purified primary standards otherwise required. Secondary standards for the methyl esters and triglycerides are available, and similar standards for the long-chain fatty acids, with *trans* content greater than 15%, are in the process of collaborative examination.

Back in 1952 Jackson *et al.* (55) investigated the spectra of pure *trans*-10, *trans*-12, and *trans*-10, *cis*-12 conjugated linoleates. From these spectra they concluded that *trans-trans* conjugated linoleate is characterized by a band at 10.11 μ and *cis-trans* conjugated linoleate by a doublet at 10.18 and 10.55 μ. Ahlers *et al.* (1) investigated several pure compounds, many containing conjugated unsaturation. They confirmed the assignments of Jackson *et al.* and accounted for the positions of maxima in the spectra of conjugated acids containing *trans* bonds by a hypsochromic shift from the position of the isolated *trans* band, small but significant and consistent with the effect of conjugation on the C-H deformation frequency of the system. O'Conner in 1958 (92) extended this postulation to the isolated *cis*-system (Table I). However the C-H

TABLE I

1. Determination of conjugated *cis-trans* octadecadienoate
   $X = A_{10.55}/0.288b$
   X = Content in grams per liter of *cis-trans* octadecadienoate in solution examined
   A = Observed absorbance (peak absorbance—base line absorbance)
   b = Thickness of the sample cell in cm.
2. Determination of conjugated *trans-trans* octadecadienoate in absence of *cis-trans* isomer (no peak at 10.55 μ)
   $X = A_{10.11}/1.330b$
3. Determination of conjugated *trans-trans* content in presence of *cis-trans isomer*
   $$Y = \frac{(A_{peak}/b - a_x X)}{1.330}$$
   A_{peak} = Absorbance of the combination peak (peak absorbance—base line absorbance)
   NOTES Measurement is made at combination peak to avoid measurement on the steep side of the combined bands.
   a_x = The absorptivity of the *cis-trans* material at the exact wavelength of the combination peak.

deformations about the *cis* C=C groups are quite variable in position with changes in molecular environment and are in all cases very weak. They are not satisfactory for diagnostic purposes. Determination of *cis*-unsaturation by means of infrared spectra appears to be limited to measurements in the near-infrared region, as outlined in the preceding paper.

The bands representing *cis-trans* and *trans-trans* conjugated unsaturation have been the subject of several research papers during the past five years. Chipault and Hawkins (20) described in detail a quantitative procedure for the determination of conjugated *cis-trans* and *trans-trans* methyl octadecadienoates by infrared spectrophotometry. *Cis-trans* compounds alone or in the presence of *trans-trans* conjugation may be determined directly by absorption at 10.55 μ. In the absence of *cis-trans* isomers, conjugated *trans-trans* octadecadienoates may be determined directly from the absorption at 10.11 μ. However, in the presence of *cis-trans* isomers, *trans-trans* content can be obtained only after applying a correction for the contribution of the *cis-trans* isomers to the 10.11 μ absorption of the *trans-trans* material. Using absorptivity values obtained by Chipault and Hawkins from measurements with a Perkin-Elmer Model 21 infrared spectrophotometer, these three determinations can be made from the equations, Table I.

Von Mikusch (86) has shown that maleic anhydride reacts with conjugated *trans-trans* unsaturated dienes very readily but that reaction is slow and very incomplete with *cis-trans* conjugated dienes. Bickford and coworkers took advantage of this difference in reaction in establishing the structure of *alpha-* and *beta*-eleostearic acids as the *cis, trans, trans-* and *trans, trans, trans*-isomers, respectively (7). Obviously, in the preparation of adducts by the Diels-Alder type of reactions, the higher yields will be obtained more readily if the diene has the *trans-trans* configuration or if there is an equilibrium mixture between *cis-trans* and *trans-trans*-isomers. (In equilibrium the removal of the active *trans-trans* isomer by reaction with the dienophile would favor complete conversion of the *cis-trans* isomers to the *trans-trans*-configuration.)

Tolberg and Wheeler (125) described in detail a procedure for the conversion of *cis-trans* to *trans-trans* structures by the action of iodine with either heat or light. Their work showed that the three geometrically isomeric types of conjugated linoleates *(cis, cis; cis, trans;* and *trans, trans)* are readily equilibrated by dilute iodine and light to an equilibrium mixture containing 32% *cis-trans* and 64% *trans-trans*-isomer. *Cis-cis* isomer was considered to be present, but in very small amounts, probably not more than 5 to 10%, very possibly less. This reaction, to convert *cis-trans* octadecadienoates to *trans-trans* isomers, was studied in detail by Chipault and Hawkins (21). They confirmed the conclusions of Tolberg and Wheeler, that the conversion takes place easily in the presence of iodine and light, and agreed reasonably well with the composition of the equilibrium mixture, reporting 71% of the conjugation to be in the *trans-trans* form and very little, if any, *cis-cis* isomer present. These two authors studied, and reported in detail, the conditions that affect the rate and extent of the reaction with various catalysts. They concluded that the reaction is apparently pseudo-first order with respect to the substrate but complicated by side reactions which inactivate the catalyst and destroy conjugation. The reaction was found to proceed at a measurable rate with iodine in the dark or in light without iodine, but no conversion took place without iodine in darkness. Temperature affects the reaction but to a lesser degree than either the catalyst or light. The equilibrium can be attained by starting with either *cis-trans* or *trans-trans* materials.

Tolberg and Wheeler (125) showed that, besides its application to the preparation of equilibrium mixtures for reaction with dienophiles in a Diels-Alder reaction, the equilibrated mixture can be used to determine total conjugation in mixtures of conjugated geometric isomers by either infrared or ultraviolet absorption. In both of these reports infrared absorption was used to follow the reactions and to determine the *cis-trans* and *trans-trans* content of reaction mixtures.

Tolberg, Paschke, and Wheeler (124) extended the isomerization to an equilibrium to the trienoic system. Theoretically there are eight isomeric 9, 11, 13 trienoic C_{18} acids:

1. ttt	3. ttc	5. cct	7. ctc
2. ctt	4. tct	6. tcc	8. ccc

of which 2 and 3, 5 and 6 would be expected to have identical infrared and ultraviolet absorption. Tolberg and coworkers investigated the isomerization with iodine and light of a mixture of: a) *beta*-eleostearic acid, which has been assigned structure 1, ttt, from investigations of its infrared spectra (7, 96); b) *alpha*-eleostearic acid, for which structure 2, ctt, has been proposed also from the infrared spectral investigations (7, 96); and c) punicic acid, which had been assigned structure 6, tcc, by Ahlers *et al.* (2), again mainly from interpretations of its infrared absorption spectra. By means of known infrared bands, from examination of the spectra of the pure compounds, the concentrations of the three components could be determined in the equilibrium mixture and during the reaction. These measurements showed a) that as punicate isomerized, a definite maximum for *alpha*-eleostearate is observed, the concentration of the *alpha*-isomer then decreased to the equilibrium value while that of the *beta*-isomer rose steadily to the equilibrium value; b) as *alpha*-eleostearate isomerized, the *beta*-isomer increased regularly to its equilibrium value, punicate concentration apparently went through a slight, but definite, maximum greater than the final low equilibrium value; and c) as *beta*-eleostearate isomerized, the content of the *alpha* and punicate isomers increased regularly to equilibrium values. Regardless of the starting ma-

aterial, the final equilibrium mixture contains 2.6% punicate, 33% *alpha*-eleostearate, and 64% *beta*-eleostearate. No bands other than those which could be attributed to one of these three isomers appeared during the isomerization. This led Tolberg and his coworkers to postulate the ctc, structure 7, to punicate rather than the tcc, structure 6, assigned by Ahlers *et al.* for the ctc structure would be expected to give the observed maximum of the *alpha*-isomer ctt during its isomerization. The tcc structure would be expected to give a tct structure as one of the primary products of isomerization which, in turn, might be expected to have other secondary bands in the 10 to 11 μ region. This discrepancy has been resolved by the very elegant syntheses of Crombie and Jacklin (27) conclusively confirming the ttt structure for *beta*-eleostearate, ctt structure for *alpha*-eleostearate, and the ctc structure for punicate.

Applications of infrared absorption spectroscopy to identify the presence of, or quantitatively to measure the concentration of isolated *trans*-isomers or conjugated *cis*- and *trans*-configurations have been described in many research reports during the past five years.

For example, investigations of elaidinization or *trans*-isomerizations, infrared spectroscopy has become an accepted tool. Khan (61) used infrared absorption spectra to follow the *cis-trans* interconversions of methyl oleate, linoleate, and linolenate when treated with oxides of nitrogen. Huo-Ping-Pan (52) followed the *trans*-isomerization of oleic acid and potassium oleate during irradiation with cathode rays by means of infrared absorption spectra. The cyclic material obtained by prolonged heating of methyl linolenate in alkaline ethylene glycol solution was shown by Scholfield and Cowan to be free of either *trans-trans* or *cis-trans (trans-cis)* conjugation by means of measurements of infrared spectra in the 10 to 11 μ region (108). Kuhn and Luck (67) showed that the hypothesis that only ruminant anibal depot fat contains *trans*-fatty acids (47) is questionable by demonstrating, by means of infrared absorption spectra, that human fat contains 3 to 5% and the fat of the domestic cat 4.0 to 6.3% *trans*-isomers. Infrared absorption spectra showed that irradiation of lard, olive oil, and beef fat with ultraviolet radiation increased the *trans*-isomer content from between 5–8%. Radiation of hydrogenated peanut oil, containing about 30% *trans*-acids, however resulted in a slow decrease in *trans*-acid content.

Infrared absorption spectra has proven useful in investigations of natural products and their composition. Cleverly (23) has described a procedure for the examination of C_{18} fatty acid or ester mixtures prepared from natural lipids for their nonconjugated (isolated) *trans*-content by infrared absorption spectra. As part of their investigations of unsaturated hydroxy acids which interfere with epoxide determinations by the Durbetaki procedure, Morris, Holman, and Fontell (88) showed, from infrared absorption spectra of fractions separated by gas phase chromatography, that the acid from *Dimorphotheca aurantiaca* oil has a *trans-trans* diene structure while oils of *Artemisia absinthium, Calliandra eriophylla, Balanites aegyptica, Cosmos bipinnatus,* and *Helianthus annuus* contain 9-hydroxy- *trans*-10-*cis*-12- and 13-hydroxy-*cis*-9-*trans*-11-octa-decadienoic acids. Butterfat contains C_{10} to C_{18} monoethenoic acid of which the C_{12} and C_{14} are predominately *cis* and the C_{16} and C_{18} both *cis* and *trans,* according to investigations reported by Scott and his coworkers (111). Infrared absorption measurements also revealed that the nonconjugated dienoic acids were either mixtures of *cis-cis* and *cis-trans* or of *cis-cis* and *trans-trans* isomers. Conjugated dienes were either *cis-trans* or *trans-trans.* Unsaturation in the more unsaturated acids, trienoic, tetraenoic, and pentaenoic, was all-*cis.*

The Structure of Sterculic Acid

Infrared spectra have been used in many investigations of the structure of lipid materials (95, p. 453). The elucidation of the structure of sterculic acid has been selected to illustrate this application of infrared absorption spectra because it has occupied attention for several years, the configuration of this molecule has only recently been conclusively established, and interest in this compound has been augmented because its structure has shown that it is closely related to the malvalic acid responsible for the Halphen reaction of cottonseed oil (79, p. 220).

Sterculic acid comprises more than 70% of the total fatty acids of the seed fat of the Java olive, *Sterculia foetida,* family Sterculiaceae. It was isolated in 1941 by Hilditch *et al.* (49), who established its molecular formula as $C_{19}H_{34}O_2$ and proposed for it the diene conjugated structure, 12-methyl-9,11-octadecadienoic acid. Later, in 1952, Nunn (90) proposed a structure with a cyclopropene ring ω-(2-n-octylcycloprop-1-enyl octanoic acid), based mainly on inference from infrared absorption spectra of the hydrogenation product, dihydrosterculic acid, which is identical to that of lactobacillic acid, which has been assigned the cyclopropane ring.

Varma *et al.* (128) reported a detailed study of sterculic acid and proposed the structure: ω-(2-n-hexylcyclopropyl)-9-10-decenoic acid, again based on infrared absorption spectra, particularly the appearance of bands with maxima at 6.09 μ, assigned to the C=C group in the long chain and at 9.9 μ, which they assigned to a CH_2 group conjugated to a cyclopropane ring.

Dijkstra and Duin (29) supported the structure proposed by Nunn mainly on three premises, two of which are based on interpretations of infrared absorption spectra. a) Sterculic acid exhibits a band at 9.91 μ which shifts to 9.79 μ on hydrogenation. The spectrum of 2-cyclopropylpropene exhibits a band at 9.77 μ and 2-cyclopropylpropane a maximum at 9.82 μ. The shift in the spectra of the latter two compounds is explained by the assignment of the band to a $-CH_2$ in-plane-wagging vibration which on conjugation is displaced slightly to higher frequency (lower wavelength). However the shift from dihydrosterculic to sterculic acid is in the opposite direction and of greater magnitude. This shift is attributed to the increased strain in the cyclopropene ring analgous to that shown in the spectra of other compounds with strained rings and is therefore evidence for the cyclopropene grouping in the sterculic acid molecule. b) The Halphen reaction, a red color developed in amyl alcohol solution of sulfur in carbon disulfide, is positive with sterculic acid. This test appears to be attended by the appearance of a band at 9.91 μ. When oils which react positively to the Halphen test are treated to cause disappearance of the 9.91 μ band, no Halphen reaction is obtained. Neither cyclopropane derivatives nor conjugated systems give a positive

Halphen test. This constitutes further evidence of the cyclopropene structure of sterculic acid. c) Sterculic acid polymerizes very rapidly whereas cyclopropane and 9,11-octadecadienoic acid are fairly stable at room temperature. Furthermore conjugation of these two groups would be expected to increase this stability. The extreme reactivity of sterculic acid indicates again the presence of a cyclopropene ring.

Faure (34) and Faure and Smith (35) also supported the structure proposed by Nunn. They reported that they could find no evidence for a band with maximum at 6.09 μ, upon which Varma and his coworkers based their interpretation for a C=C group in the long chain, in the infrared spectra of fresh sterculic acid. As sterculic acid aged, this band appeared, and the spectra resembled that reported by Varma *et al.* Faure and Smith believed that Varma *et al.* had measured the spectrum of, and based their postulated structure on a spontaneously polymerized sterculic acid. The strong band observed in the spectra of fresh sterculic acid at 9.91 μ was assigned to a CH_2 in-plane wagging vibration of the ring CH_2 group, and the weak band at 5.35 μ to a C=C stretching in the cyclopropene ring, thus confirming the structure of Nunn.

Faure and Smith contended that the key compound in Nunn's chemical evidence for the cyclopropene structure was the dioxo acid obtained by hydrogenation of the ozonide formed from sterculic acid and deduced to be 9,11-dioxononadecanoic acid, $CH_3(CH_2)_7COCH_2CO(CH_2)_7COOH$. They prepared this compound from sterculic acid and were able to isolate and identify three of the four compounds which would be expected upon alkaline hydroylsis of a dioxo acid of this structure. They concluded that "All the spectral and chemical evidence which exists with regard to sterculic acid supports the originally proposed structure of Nunn."

However Varma and coworkers (126) continued to maintain the correctness of their originally proposed structure for sterculic acid as the only configuration which would account for the oxidation products obtained by them and the behavior of the acid toward heat (gelation). In 1957 they proposed (127) to estimate the content of sterculic acid in the seed of *Sterculia foetida Linn.* by measurement of the cyclopropane ring by means of the 9.92 μ characteristic of this group in the infrared absorption spectrum.

The controversy appears to have been resolved by two different research approaches. Brooke and Smith (8) investigated the problem of the structure of sterculic acid. They confirmed the assignments made by Faure and Smith for the band at 9.91 μ and the absence of a 6.09 μ band in the infrared absorption spectrum of fresh sterculic acid. The workers obtained an authentic sample of 9,11-dioxononadecanoic acid from Hofman, Orochena, and Yoho, who had achieved the total synthesis of this compound (51), and showed that it was identical with the product obtained by ozonolysis and hydrogenation of the ozonide of sterculic acid by the procedure of Faure and Smith. This product could be expected from the structure proposed by Nunn but is not possible from that advocated by Varma. Finally Rinehart *et al.* (105) have shown that the nuclear magnetic resonance spectra of sterculic acid exhibits no peak near $\delta = 0.0$ (H_2O Ref.) characteristic of the olefinic, =C-H, group. This group therefore cannot be present in the structure of sterculic acid, and the structure proposed by Nunn is conclusively confirmed.

The reported work on the elucidation of the structure of sterculic acid illustrates several factors involved in the proof of structure of lipid (or any other) materials by means of infrared absorption spectroscopy. a) Infrared spectra, if obtained from authentic compounds and correctly interpreted, can yield valuable information regarding configuration. The structure of sterculic acid has been proved to be that first suggested by Nunn, from infrared absorption spectra. b) It is essential that the infrared spectra represent authentic samples of the compound under investigation. If, as claimed by subsequent workers, the substance actually measured by Varma and coworkers was a spontaneously polymerized sterculic acid, it can be readily appreciated that interpretations would lead to an erroneous conclusion. c) Considerable care must be taken to verify new assignments, as illustrated by what have proven to be erroneous correlations of bands representing CH_2 deformation and C=C stretching vibrations. d) The elucidation of sterculic acid, as finally confirmed, represents a combined use of more than one instrumental procedure. The structure of sterculic acid, as proposed from infrared spectroscopy, which is an instrumental approach, was finally confirmed by nuclear magnetic spectra, another instrumental technique. This tandem use of techniques to solve single problems will be encountered more frequently. In the future it may become difficult to discuss applications of an individual instrumental approach to problems in lipid chemistry successfully, that is, it may be nearly impossible to discuss applications of infrared absorption spectroscopy in a morning lecture and, say, nuclear magnetic spectroscopy in an afternoon lecture, so interwoven will these techniques become in combined efforts to solve specific problems.

Solid State Spectra

A development in infrared absorption spectroscopy which is apparently destined to play an important role in its applications to lipid chemistry is the finding, reported by several infrared spectroscopists, that the spectra of materials when measured in the solid or crystalline state, such as crystalline films, Nujol mulls, potassium bromide disks, etc., exhibit several bands not observed in liquid or solution spectra.

Sinclair *et al.* (114) showed that the most remarkable feature in the spectra of crystalline acids and esters is a progression of bands between about 7.4 and 8.5 μ spaced at approximately equal intervals. The bands were considered to arise from wagging and/or twisting vibrations of the methylene groups. Jones *et al.* (57) investigated these band progressions and pointed out that the number of bands in the progression is proportional to the chain length of the fatty acid or ester. For every two methylene groups one progression band results, and chain length of the compound can be calculated from the formula:

$$\text{No. of carbon atoms in chain} = 2 \times \text{No. observed absorption bands in progression} + 2$$

Meyer and Schuette (85) investigated the crystalline spectra of more than 300 long-chain aliphatic compounds and concluded that the progression bands arise from inductive effects of the terminal polar groups. The bands are not observed in hydrocarbons

or other compounds with nonpolar terminal groups. In all cases where observed, the number of bands increases regularly with the number of carbon atoms in the chain, but the intensities of the bands decrease with chain length.

More recent work of Susi (117) has shown that, in the crystalline spectra of *trans*-6 through *trans*-11-octadecenoic acids, the equally spaced progression of absorption bands in the 7.4 to 8.5 μ region follow the rule of two proposed by Meiklejohn *et al.* who reported on the infrared spectra of several crystalline lipid materials (84):

Even No. carbon atoms in straight-chain fatty acid:
No. of bands in progression = n/2
Odd No. carbon atoms in straight-chain fatty acid:
No. of bands in progression = n + 1/2

where n is now the number of carbon atoms in the chain segment between the carboxyl group and the double bond, including the end atoms of the segment. In other words, a count of the progression bands can be used to determine the position of the double bond in the chain.

Susi found that hydroxylation resulted in a kind of modification of the band structure different from unsaturation. No regularities in the crystalline spectra, comparable with the band progression of unsaturated acids, could be detected, but in the region 8.7 to 10μ and about 10.8 μ there are differences between the individual members within one series which are small but sufficient to allow identification and differentiation of each individual isomer by direct comparisons with spectra of known isomers, *i.e.*, by means of the "fingerprint" technique.

Erik von Sydow (123) appears to be the first to suggest the use of crystalline spectra to differentiate and identify different crystal forms (polymorphs) of a number of normal fatty acids. He found that there are easily recognizable differences in the spectra of the different crystalline modifications of the same acid at 5.9 μ, the C=O stretching vibrations, and near 7.0 μ, CH_2 deformation. From 7.4 to 8.5 μ, the region of the progression bands, the exact positions of the absorption peaks depend upon the crystal form. Also at about 11.0 μ, the O-H out-of-plane deformation, no two crystal forms have the same absorption, and this region is therefore very suitable for identification of a specific polymorph.

To evaluate differences in infrared spectra of different polymorphic forms of the long-chain fatty acids, Susi measured the crystalline state spectra of the "C" Form of stearic acid and of vaccenic acid (*trans*-11-octadecenoic acid) [which had been shown by Lutton and Kolp from x-ray diffraction data not to exhibit polymorphic modification, (72)] and of the "C'" and "B'" Forms of heptadecanoic and the "A'" Form of tridecanoic acid (118, 121). Measurements were made with polarized infrared radiation with the electric vector along the major crystallographic axes. Susi published tables listing for each of these five acids the band positions, their polarizations, and their assignments. Vaccenic acid was shown to crystallize with orthorhombic hydrocarbon-chain packing. Infrared spectra of the "C'" Form of the fatty acids resembled that of the "C" Form except that the band associated with the CH_3 group at about 7.27 μ polarized differently for the two crystalline modifications and affords a differentiation between acids of odd and even carbon chain length. The "B'" Form of heptadecanoic acid crystallizes with orthorhombic packing, the "A'" Form of tridecanoic acid with triclinic packing.

Chapman published two papers dealing with the infrared spectra of crystalline acids, one on short-chain acids (16, 24), the second on long-chain compounds. These two papers present data on the infrared spectra of crystalline modifications of the monocarboxylic acid from C_2 to C_{20}. Chapman investigated, particularly, bands appearing in the region about 13.9 to 14.1 μ and demonstrated that compounds, the infrared absorption spectra of which exhibited two bands at 13.8 and 14.0 μ, very probably crystallized with orthorhombic packing while those which revealed only a single strong band at 13.9 μ crystallized with either hexagonal or triclinic packed hydrocarbon chains.

In 1958 Chapman reported results of an investigation of the infrared spectra of anhydrous sodium soaps (17). All of the bands could be accounted for as arising from vibrations assigned to the carboxylate group or to the paraffin chain. In soaps the usual C=O stretching (of the acid or ester) is replaced by two bands arising from symmetrical and antisymmetrical stretching vibrations of the COO^- ion. A list of the major bands and their assignments as reported by Chapman is given in Table II. In the 11 to 14 μ region these compounds exhibit a single band, indicating, by analogy with interpretations made from infrared absorption spectra of long-chain fatty acids, that they all crystallize with triclinic packed chains. Chapman discussed the infrared absorption spectra of polymorphic forms of long-n-chain alcohols, ethyl esters, and mono- and triglycerides, again demonstrating that bands in the 13 μ region correlated with the type of hydrocarbon packing in the crystal (18).

TABLE II

Infrared Absorption Bands in Spectra of Anhydrous Sodium Soaps

Wavelength maximum μ	Vibration and correlated functional group
6.37	COO^- Stretching (antisym.)
6.85	CH_2 Sym. bending
6.95	CH_2 Sym. bending (adj. carboxylate group)
7.07	COO^- Stretching (sym.)
7.25	CH_3 Sym. bending
7.41– 8.33	$=CH_2$ Wagging and/or twisting
9.05– 9.60	CH_3 Rocking
9.26–10.64	C–C Skeletal
9.91–10.87	CH_3 Rocking
11.11–13.90	CH_2 Rocking
14.4	COO^- Deformation

Susi (119) investigated the spectra of crystalline adipic acid and deuterated analogs. He made 19 band assignments to active fundamental modes of vibration and showed that the bands between 7.4 and 8.5 μ arise "from mixed modes involving carboxyl deformation, CH_2 wagging, and C-C stretching."

Between 1956 and 1958 Chapman published a series of five papers under the title "Infrared Spectra and the Polymorphism of Glycerides" (14). The first of these investigated infrared absorption spectra of the crystalline phases of 1- and 2-monoglycerides. From the infrared curves the α-, sub-α-, β-, and β'-forms of 1 monostearin could be readily distinguished from one another; this differentiation is usually accomplished by x-ray diffraction techniques. In particular, the sub-α-form could be easily differentiated from the β'-form; this distinction is difficult to achieve with x-ray powder photographs. Chapman's infrared curves

showed that the α-form is reasonably stable from its melting point down to the melting point of the sub-α-form and that the sub-α-form has a definite crystalline lattice, is quite stable at room temperature, and that the transition of the sub-α- to the α-form is reversible. All of these observations agree with conclusions from x-ray diffraction measurements by Lutton and Jackson (70) but are contrary to the observations reported by Malkin (76).

Chapman found only one polymorphic form of the 2-monoglycerides in agreement with the x-ray data of Daubert and Clarke (28) and of Filer *et al.* (38), which indicated that 2-monoglycerides do not exhibit polymorphism but, again in conflict with the postulations of Malkin (76), that two modifications, the α- and the β-forms, exist.

Chapman's second paper investigated the crystalline infrared spectra of 1,3-diglycerides and saturated triglycerides. Both β- and β'-forms were observed, in agreement with x-ray data of Bauer *et al.* (6), but no α-form, reported by Malkin *et al.* (78), on the basis of x-ray spectra, was observed. The two polymorphic forms of the 1,3-diglycerides could readily be differentiated by means of infrared absorption spectra.

Infrared absorption curves could also readily identify three polymorphic forms of the saturated triglycerides, trilaurin, tripalmitin, and tristearin. Infrared data again indicated the existence of the α-, β'-, and β-forms of Lutton (69) rather than the vitreous-, α-, β', and β-forms reported by Clarkson and Malkin (22).

Chapman's third and fourth papers described investigations of the solid state spectra of palmitodistearins and dipalmitostearins and of myristopalmitins and myristostearins. Again examination of the infrared spectra of 1-palmitodistearin and 1-stearodipalmitin revealed that α-, β'-, and β-forms could readily be differentiated by means of characteristic bands, in agreement with results from x-ray and thermal data of Lutton *et al.* (71). Infrared spectra revealed only two polymorphic modifications of 2-palmitodistearin and 2-stearodipalmitin, the α- and β- and the α- and β'-forms, respectively. Two polymorphic forms are again in agreement with results obtained by Lutton *et al.* (71) but are contrary to the reported observations of Malkin and Meara (77) and Carter and Malkin (12) that four forms of these glycerides exist. Based on x-ray short spacings however, Lutton assigned the α- and β'-forms to both of these triglycerides.

Of the four myristopalmitins and myristostearins (2-myristodistearin, 1-stearodimyristin, 2-myristodipalmitin, and 1-palmitodimyristin) the first, 2-myristodistearin, is the most interesting as it crystallizes in four polymorphic forms while most other saturated triglycerides exhibit only three crystalline modifications. Jackson and Lutton (54) reported four forms for this triglyceride while Malkin and Meara (77), Carter and Malkin (12) found only three. The spectra of the β-form of these compounds exhibited a single band at 13.93 μ like other triglycerides, *i.e.*, the β-form of tristearin. The α- and β'-forms exhibited double bonds at 13.77 and 13.91 μ, resembling the corresponding forms of other triglycerides.

In the fifth paper of this series Chapman investigated the infrared spectra of dilaurin, dimyristin, dipalmitin, and distearin. From infrared spectral data two crystalline forms are indicated, the α- and β'-modifications, in agreement with the x-ray data of Jackson and Lutton (54). The spectra of the α-form is analogous to the α-form of hydrocarbons, alcohols, esters, and saturated triglycerides and monoglycerides. 1:2 diglycerides can be readily distinguished from 1:3 diglycerides by means of infrared absorption spectra, as both the α-form, crystallizing with hexagonal packing, and the β'-form, crystallizing with orthorhombic packing, exhibit two bands at 13.72 and 13.91 μ while the corresponding α- and β'-forms of the 1:3 diglycerides, crystallizing with triclinic packing, reveal only a single band at 13.95 μ.

Chapman reviewed the wealth of information available on the infrared spectra of glycerides in the crystalline state in various polymorphic forms and concluded that "polymorphic form, chain length, type of unsaturation, and configuration are all revealed by the spectra" (15).

Solid-state infrared spectra have added a considerable fund of valuable information to lipid chemistry beyond that contributed by solution spectra. In particular, by use of infrared spectra of crystalline lipid materials, it is possible a) to determine the chain-length of acids, esters, etc.; b) to differentiate and recognize various polymorphic forms of those lipid materials which crystallize in more than one modification; c) to determine the position of the double bond in unsaturated compounds or the position of the hydroxyl group along the carbon chain in the spectra of hydroxylated fatty acids; and d) to obtain considerable information regarding the crystalline structure of specific lipid materials, *i.e.*, the type of packing of the chains, or to differentiate or identify various classes of glycerides, *i.e.*, the 1:2 diglycerides from the 1:3 diglycerides.

The story of the development of this new tool for the lipid chemist again emphasizes the potential importance of combinations of instrumental techniques. Most of the differentiations and identifications which can be made by means of infrared spectra of lipid materials in their crystalline states is restricted to measurements of either pure compounds or mixtures of only two or three components. Otherwise interferences of the numerous crystalline bands will make any interpretations impossible. Thus it appears that separations of components from natural commodities will be a prerequisite before much advantage can be taken of the information this technique is capable of providing. With the use of gas-phase chromatography at higher temperatures it appears possible, even likely, that a combination of gas-phase separation and infrared identification of specific components will increase. The development of crystalline state infrared spectra of glycerides again illustrates the advantages of the combination of more than one type of instrumental approach to a problem in lipid chemistry. From x-ray diffraction data disagreements arose regarding the number and identification of polymorphic forms of the glycerides, and no further advances from x-ray diffraction data to resolve these differences appeared possible (94). Solid-state infrared spectra however appears to have very capably furnished the additional data from which decisions as to correct interpretation of x-ray diffraction data can be based.

Hydrogenation, Oxidation, and Autoxidation

Among the many applications of infrared spectroscopy to lipid chemistry have been its uses in investigations of hydrogenation, oxidation, and autoxidation, processes known to produce *trans* bands (95,

p. 441). Feuge and coworkers used infrared absorption spectra to follow *cis* to *trans* isomerization in their investigations of the positional isomers formed during the hydrogenation of cottonseed oil (13), methyl linoleate (26), and methyl oleate (36). They reported that the selectivity of the hydrogenation reaction had more effect on the formation of *trans* isomers than it had on the formation of positional isomers. Nonselective hydrogenation of cottonseed oil (iodine value *ca.* 75) produced 9.4% *trans* isomer calculated as trielaidin. Selective conditions of hydrogenation of the same sample produced 27.3% *trans* isomer. With oils of iodine value about 48, nonselective hydrogenation resulted in 21.6% *trans* isomer while selective conditions yielded 37.7% *trans* content.

Studying the factors which effected the hydrogenation of pure methyl linoleate, Cousins, Guice, and Feuge (26) found that increasing the temperature increased the rate of formation of *trans* isomers but that increase in *trans* isomers with decrease in rate of hydrogen dispersion was very small. Palladium catalyst produced far greater proportions of *trans* isomers than did a nickel catalyst. From studies on the hydrogenation of methyl oleate Feuge and Cousins (36) concluded that hydrogenation conditions which cause an increase in the formation of positional isomers also cause an increase in the formation of geometrical isomers. The amount of *trans* isomers formed was not proportional to either the degree of hydrogenation or to the amount of migration of the double bonds. When methyl oleate was hydrogenated in solvents, the *trans* bonds varied from 20.7% (Ni catalyst, hexane solvent, and hydrogenation to an iodine value of 50.9) to 79.1% (Pd catalyst, ethanol solvent, and hydrogenation to an iodine value of 45.0). These values differ considerably from the equilibrium value of 67% *trans* which had been reported in earlier studies (37). High percentages of *trans* isomer could be accounted for by the population, for which there is some support, that the *cis* bonds are hydrogenated (destroyed). However this explanation would not account for the low percentages of *trans* found in other runs. Heretofore it had been concluded that whenever new positional isomers were formed, the *cis* to *trans* ratio of the double bonds in the new positions was 1:2. Present evidence indicates that, under certain conditions of hydrogenation, the ratio of *cis* to *trans* bonds in new positional isomers is greater than 1:2. Under these conditions either the *trans* bonds are hydrogenated preferentially or the *cis* bonds are formed preferentially.

In his investigations of the hydrogenation of fatty oils with palladium catalyst Zajcew (129) has made considerable use of infrared absorption spectra to follow the formation of *trans* isomers and the factors which influence it. Hydrogenation with palladium was found to form more *trans* isomers than nickel unless an effort was made to adjust processing conditions to control it. *Trans* formation was shown to decrease as agitation was increased, a quantitative relation was reported between speed of stirring and *trans* formation. *Trans* formation decreased as pressure increased while increasing temperature gave a higher *trans* content. Deactivation of the catalyst resulted in a drastic reduction of *trans* isomer formation. In a typical experiment an oil hydrogenated to a specific iodine value with active palladium catalyst gave 52.9% *trans* isomers. The same oil hydrogenated to essentially the same iodine value with palladium catalyst that had been deactivated by the addition of silver and bismuth gave only 35.9% *trans* isomers.

In the hydrogenation of shortening stocks the type of feed is also a factor in determining the *trans* isomer content of the product. Soybean oil forms more *trans isomers* than cottonseed oil. The maximum *trans* content occurs at about an iodine value of 65 to 70 for soybean oil, at 50 to 55 for cottonseed oil. The more unsaturated oil presumably will always give more *trans* isomers if the olefins are of similar type. Pilot-plant hydrogenated samples were found to have a somewhat higher *trans* content than obtained in the laboratory, chiefly because different kind and degree of agitation has been shown to effect *trans* formation.

During the hydrogenation of tall oil fatty acids the tendency to form *trans* isomers with platinum metals was found to increase in the order: platinum, iridium, ruthenium, rhodium, and palladium. Despite palladium's tendency to *trans* formation, its high activity and selectivity and its relatively low cost make it the most attractive of the platinum metals for fatty acid hydrogenation. Use of a partially deactivated palladium catalyst might be a practical method to obtain a higher percentage of *cis*-oleic acid and lower linoleic acid and *trans* isomer content.

The relatively high percentages of *trans* isomers produced by hydrogenation with palladium on carbon catalyst have been demonstrated by Cousins and Feuge (25). Hydrogenation of methyl oleate with palladium, with or without solvent, produced 76.6 to 79.1% *trans* bonds, significantly higher than the 67% formerly considered the equilibrium mixture (37). Hydrogenation products obtained with Raney nickel and solvents contained as little as 20.7% *trans* bonds (iodine value of about 50). Positional isomers form extensively when Raney nickel is used in the absence of solvents and when palladium catalyst is employed. However, with Raney nickel and solvents, large proportions of the double bonds were found in the original 9-position. It would appear from these data that where there is little or no migration, there is little or no *trans* formation.

In their report of the investigation of the hydrogenation of linolenate Scholfield and coworkers (109) briefly review earlier work on the hydrogenation of vegetable oils and, in particular, the formation of *trans* acids as measured by infrared absorption spectra. The hydrogenated products from methyl linoleate were separated, by countercurrent distribution, into three fractions, monoene, diene, and triene. The monoene and diene fractions were found to contain almost identical amounts of *trans* esters, 46.1% for the monoene and 44.7% for the diene. The triene fraction appears to undergo little change during hydrogenation.

Trans bonds have been shown to appear during autoxidation of fats and oils (95, p. 444). Helme and Moline (48) found that the 10.35 μ band in the infrared spectra of autoxidized raw and treated linseed oil corresponded to the formation of a highly active *trans* form in the treated oil. The *trans* form was shown to autoxidize more slowly than the *cis* form.

Khan has made several investigations of the autoxidation of pure fatty acid materials. The hydroperoxides formed by autoxidation of methyl oleate and methyl linoleate were decomposed under vacuum at 150°, and the volatile product was collected. Infrared spectra showed the disappearance of *trans* absorption in the residue of methyl oleate and the appear-

ance of *trans* absorption in the products from methyl linoleate (62). Khan (63) made infrared studies of the behavior of methylene interrupted double bonds during autoxidation at three temperatures with or without stirring. Methyl linoleate autoxidized at 0°, without stirring, and methyl linolenate autoxidized at −10° gave *cis, trans*-conjugated monohydroperoxides. *Cis-* to *trans*-interconversions of methyl oleate, and linolenate, when treated with oxides of nitrogen, were also investigated by Khan (64) with infrared absorption spectra. *Trans*-isomers from methyl linoleate and linolenate were less than 5%, but from methyl oleate they were 35%.

Ramanathan, Sakuragi, and Kummerow (103) obtained infrared absorption curves of methyl esters of fatty acids on all original and thermally oxidized samples in their investigations of thermal oxidation, and Perkins and Kummerow (97) showed that the polymers formed during thermal oxidation of corn oil were identical with methyl linoleate with the exception of hydroxyl groups, revealed by bands in the infrared spectra at 2.7 to 2.8, 7.5 to 7.9, and 9.0 μ. Maier and Tappel (75) made considerable use of infrared spectra in their investigations of the products formed from unsaturated fatty acids upon oxidation catalyzed by hermatin compounds. Bands at 2.8 to 3.0 μ indicated the presence of hydroperoxyl and hydroxyl groups and at 5.85 μ the presence of considerable amounts of carbonyl compounds. The band at 6.1 μ was interpreted as characteristic of double bonds in general, those at 10.34 of isolated *trans* double bonds, and those at 10.18 and 10.53 and 10.12 of conjugated *cis, trans,* and conjugated *trans trans* double bonds, respectively.

Frankel, Evans, and Cowan (41) used infrared absorption spectra in their investigations of the products from thermal dimerization of fatty ester hydroperoxides. An infrared procedure was used for hydroxyl determination with methyl ricinoleate as a standard. Infrared spectra were reported for various fractions from thermal decomposition of autoxidized soybean methyl esters, of the polymeric fraction in thermally decomposed autoxidized safflower methyl esters, and, for comparison, of methyl oleate and linoleate and their corresponding hydroperoxide dimers. Changes were followed by observations of the infrared bands at 2.95 μ, hydroxyl; 5.8 to 5.9 μ, carbonyl; and 10 to 11 μ, isolated *trans,* conjugated *cis-trans* and conjugated *trans-trans* groups. Dimers isolated by molecular distillation have approximately one mole of hydroxyl, 0.5 moles of carbonyl, and two double bonds per mole of dimer. The infrared spectra of the dimers were similar to those of the original fatty esters except for the band at 2.9 μ, assigned to a secondary hydroxyl group. The *cis-trans* diene in the polyunsaturated hydroperoxides was isomerized to *trans-trans* configuration on dimerization, but methyl oleate hydroperoxide showed only absorption for isolated *trans* double bond.

Infrared absorption was used in a similar fashion by Knapp and Tappel (65) to investigate products formed by *gamma* radiation and linoleate peroxidation of *alpha*-tocopherol, the major lipid antioxidant in nature.

Banks and coworkers (4) used infrared absorption spectroscopy to characterize products obtained from a continuous separation of the hydroperoxide of oxidizing methyl linoleate, and to establish the purity of the final crystallized material. The hydroperoxide exhibited bands with maxima at 2.9 μ, the hydroperoxide group, at 10.10 and 10.52 μ, *cis, trans* and *trans, trans* conjugation, and evidence for a carbonyl group in addition to the ester. After recrystallizations from petroleum ether and from ethanol, infrared spectra showed that the carbonyl decomposition product had been removed. The 10.11 μ band was strong, but the 10.52 μ band had disappeared, indicating that the product was pure *trans, trans* conjugated linoleate hydroperoxide.

A particularly interesting use of infrared absorption spectra is demonstrated by the work of Privett and Nickell on the structure of the hydroperoxides of autoxidized methy oleate (101). According to the hydroperoxide theory of Farmer *et al.* (33), four monomeric monoethenoid hydroperoxide isomers should be formed in equal proportions in the autoxidation of methyl oleate. However Knight, Eddy, and Swern (66) have suggested that the 9- and 10-hydroperoxy isomers were formed preferentially on the basis of the finding of predominately the high-melting 9,10-dihydrostearic acid and the low-melting 9,10-epoxystearic acid in autoxidized oleic acid. Privett and Nickell made a detailed investigation of this autoxidation and were able to demonstrate that all four isomers are formed in approximately equal amounts. By means of infrared spectra they were also able to prove that the hydroperoxide groups reside on the carbon atoms adjacent to the double bond, also in accordance with the hydroperoxide theory of autoxidation. The infrared absorption evidence for this hypothesis consists of the following steps.

a) Infrared spectra of O-acetyl derivatives of the hydroperoxides of methyl octadecenoate and methyl ricinoleic acid before and after fission of the double bonds exhibit maxima at 8.07 and 9.82 μ as well as the carbonyl band at 5.75 μ.

b) The band at 9.82 μ disappears after oxidative fission of the acetylated hydroperoxides but not after oxidative fission of acetylated ricinoleic acid.

c) Therefore the band at 9.82 μ must be specific for secondary acetyl compounds. The acetyl derivatives of the hydroperoxides and ricinoleic acid are secondary acetyl compounds, and the acetyl fission products of the hydroperoxides should be primary acetyl derivatives. The band at 9.82 μ is consequently specific for secondary acetyl attachment.

d) The bands at 8.07 and 5.75 μ prove the presence of acetyl derivatives among the fission products of the acetylated hydroperoxide derivatives.

e) Consequently it may be concluded that the absence of the band at 9.82 μ in the fission products of the acetylated hydroperoxide derivatives is because the acetyl groups are attached to the carbon atoms *alpha* to the carboxyl groups in these compounds. Therefore the hydroperoxy groups in the original mixture of methyl octadecenoate hydroperoxide isomers are located in the position *alpha* to the double bonds in accordance with the hydroperoxide theory of autoxidation.

Kaufmann and Thomas (59) measured the infrared spectra of tri α-eleostearin and α-eleostearodilinolein on polyethylene films during the oxidative processes that accompany drying. Conjugated *cis, trans,* measured by the infrared band at 10.37 μ, weakens in 5 hrs and disappears after 9 hrs. *Trans,*

trans conjugation, 10.09 μ, decreases while hydroxyl and hydroperoxide increase. A band at 10.2 μ, attributed to isolated *trans,* appears as the conjugated groups disappear.

Applications of infrared absorption spectra to hydrogenation studies have been limited to measurements of *trans* bonds. The considerable importance attached to the formation of *trans* bonds during hydrogenation has however prompted numerous investigations. Infrared spectra have been used as a guide in the selection of hydrogenation conditions, the degree of selectivity or nonselectivity, the choice of solvents or of catalysts and of several other factors which influence the formation of *trans* isomers.

Trans bond formation has also been demonstrated to accompany oxidation and autoxidation processes, and several studies have been concerned with its measurement, by means of infrared spectra, during various stages and conditions of oxidation. Applications of infrared spectra to investigations of oxidation or autoxidation have not been entirely limited to measurements of *trans* isomers. Measurements of the disappearance of hydroxyl groups or of the appearance of hydroperoxide, epoxy, and changes in various carbonyl bands have been used with considerable success to follow the course of such reaction or to characterize the products formed.

Applications to Investigations of Composition or to Modifications of Natural Products

Infrared absorption spectroscopy has been applied to a considerable number of investigations of either the composition of lipid-containing natural products or to the examination of products formed by chemical modifications of lipid materials to explore potential new uses.

Earle and his coworkers (32) have used infrared spectra as a tool in their screening studies as part of an extensive search for new industrial raw materials among the many plants that have had little or no study of their chemical composition. In preliminary screening of such plants for possible new oil-containing materials, infrared spectra have been obtained and checked for unusual band maxima which may indicate hydroxyl, epoxy, carbonyl, etc. Such infrared spectra have revealed considerable diversity in oils obtained from different plants. Earle *et al.* have published tables listing (with considerable other data) the positions of maxima of infrared bands which are somewhat different from those found in usual vegetable or drying oils, as obtained in their survey on 158 species representing 52 plant families in 23 orders, 138 of which have heretofore not been included in any compilations of composition.

Glass and Melvin used infrared absorption spectra to determine the composition of copolymers of soybean vinyl ethers by means of the ether band at 9.1 μ (46). Jojoba oil is composed of about 93% long-chain monoethylenoic compounds present as esters, of which 45% are C_{20} acids and alcohols (eicosenoic and eicosenol) and 48% C_{22} acids and alcohols (docosenoic and docosenol). Molaison, O'Connor, and Spadaro (87) showed that this oil can, by sodium reduction, become an excellent source of long-chain alcohols. The course of the proposed reduction and the properties of the final product alcohols were followed by means of infrared absorption spectra.

Ricinelaidic acid, methyl ricinelaidate, and several derivatives were investigated by McCutchon and coworkers (74), and the infrared procedure for the determination of isolated *trans* bonds was applied to the system ricinoleic-ricinelaidic. The equilibrium mixture indicated that the *cis* to *trans* conversion of the ethylenic bond is approximately 76% in comparison with the well-established equilibrium point of 67% for the oleic-elaidic transformation. The difference in the two systems was attributed to the influence of the hydroxyl group on the ethylenic bond of the ricinoleate either through electronic or steric effects, or possibly both. Absorptivity values of the maximum at 10.23 μ, in chloroform and at 10.27 μ in carbon disulfide, were obtained, both to establish purity criteria for both ricinelaidic acid and for its methyl ester and to provide data for the determination of ricinelaidic acid or methyl ricinelaidate in the presence of their *cis* isomers. In additional studies these same authors used infrared spectra of several derivatives of ricinelaidic acid to confirm the structure of the ricinelaidic moiety (73).

Dupuy and coworkers (31) investigated the morpholides and the products from cyanoethylation of ricinoleic acid derivatives. The products were fully characterized by infrared absorption spectra. The infrared spectra of morpholides showed characteristic absorption bands, which were suggested for diagnostic purposes and for analysis. A strong band at 5.78 μ is characteristic of the esters. An equally strong band at about 6.10 μ, present in all the morpholides, completely separated from the 5.78 μ band and absent from the spectrum of morpholine, characterizes the morpholides. The morpholides, but not morpholine, exhibit a band of moderate intensity at about 10.3 μ. Hence morpholides represent another class of compounds in which *trans* unsaturation cannot be detected by use of infrared spectra.

Five ricinoleic acid derivatives were cyanoethylated with acrylonitrile, and among other properties their infrared absorption spectra were obtained (30). A band at 4.44 μ is characteristic of the nitrile group and was found to be about 67% more intense in chloroform solution than in carbon tetrachloride. It was found to obey Beer's law over a wide range of concentrations and permits a convenient infrared analysis of this type of compound. Oxidation of methyl ricinoleate with *tert*-butyl chromate was investigated by Maruta and Suzuki (81), who used infrared spectra to follow conversion of the *cis*-methyl ricinoleate to *trans* methyl ricinelaidate.

The *alpha-* and *beta*-eleostearic acids of tung oil have also been investigated for the possibility of producing from tung oil new and improved plasticizers for the vinyl chloride copolymers. Placek and Bickford (98) described the reaction of methyl vinyl ketone with *alpha-* and *beta*-eleostearic acid; and Placek, Magne, and Bickford (100) explored the plasticizer properties of methyl vinyl ketone—methyl *alpha*-eleostearate adducts and derivatives. Infrared spectra were used to follow any *alpha-* to *beta*-conversion of the *alpha*-eleostearic acid and to confirm the fact that hydrogenation of the adducts was accomplished without attack on the carbonyl group. Placek and Bickford (99) have also investigated the reaction of divinyl sulfone and *alpha*-eleostearic acid and used infrared absorption to partially characterize the product obtained. Infrared absorp-

tion was also used by Fore and Bickford (39) to demonstrate that diborane could be added to the ethylenic bond of methyl oleate smoothly without significant reduction of the carbomethoxy group.

Fore and colleagues (40), investigating additions of various compounds to ethylenic bonds of linoleic acid and methyl linoleate, examined the reaction between mercaptoacetic acid and both of these compounds. They made considerable use of infrared spectra to characterize their final products. Calderon *et al.* (10) made similar use of infrared spectra in their preparations of vinyl ketosetarates to provide monomers for polymerization and copolymerization investigations.

Infrared absorption spectroscopy was the main tool in determining the composition and structure of the products formed by the addition of carbon monoxide to the double bonds of long-chain unsaturated compounds in sulfuric acid solution to produce branched carboxylic acids, reported by Roe and Swern (106). 3-*Cis*-hexenal was demonstrated by Hoffman (50) to be the compound responsible for the special off-flavor (odor) of soybean oil, to which the descriptive name "green bean" has been given. Volatile components from the oxidation of soybean oil in air at high temperatures were separated by gas-phase chromatography and characterized by means of infrared spectra. Carbonyl compounds were identified by characteristic bands at 3.66 and 5.77 μ (aldehyde) and 13.87 μ, $(CH_2)_4$, as short-chain aldehydes. *Cis*-hexenal was distinguished from the *trans* isomer by the absence of the band at 10.31 μ.

Susi and coworkers (120) described the infrared spectra of some long-chain fatty acid derivatives containing sulfide, sulfoxide, and sulfone groups. They made several correlations of observed bands with functional groups and recommended, for differentiations of very closely related sulfur derivatives of fatty acids, infrared measurements in the 7.4 to 8.4 μ region in solid-state spectra from KBr pellets. For differentiations among different main classes of these compounds dilute solution spectra of the corresponding methyl esters were recommended. Sasin and coworkers made a similar survey of phosphorus derivatives of fatty acids, obtaining infrared spectra of all phosphorus derivatives prepared by addition of dialkyl phosphonates to unsaturated compounds (107).

Backderf and Brown (3), by means of infrared absorption spectra, identified *trans*-16-octadecenoic acid, not before reported, as a constituent of butterfat. *Trans*-11-octadecenoic acid (vaccenic) was also identified, but no elaidic acid was found. More than 20% of the hexadecanoic acids of butterfat were found to be *trans*, but no position isomers of the 9-hexadecenoic acids were identified. Differential infrarad spectroscopy was recommended by Bartlet and Mahon (5) to detect oil adulteration. In a typical experiment the reference sample, in a double-beam infrared spectrophotometer, was a 10% w/v solution of olive oil and CCL_4. The sample beam compartment held a similar solution of olive oil admixed with rapeseed oil, often suspected as an adulterant. The differential spectra clearly reveal the adulterant, and by means of it the rapeseed can be identified and its presence conclusively demonstrated.

Chapman and coworkers (19) demonstrated the practical application of the solid-state infrared spectra of triglycerides by showing that the major glyceride of cocoa butter is 2-oleopalmitostearin, not 2-palmito-oleostearin as reported by several workers. This latter compound is however a major component in lard, in agreement with results reported by Meara (83) and by Quimby, Wille, and Lutton (102).

In the elucidation of the structure of the newly discovered palustric acid there remained some question as to whether the two double bonds were in the 5–6 and 7–8 or at the 7-8 and 13–14 positions (9). If located between carbon atoms 5–6 and 7–8, infrared spectra would be expected to resemble those of 1-abietic, levopimaric, and neoabietic acids with vibrations arising from both di- and tri-substituted ethylenic bonds. The alternate choice, with double bonds in the 7–8 and 13–14 positions only, would be expected to give rise to infrared spectra resembling that of the trisubstituted ethylenic bond only as in dihydroabietic acid. From the following considerations from infrared spectra, the double bonds were proven to be in the 7–8 and 13–14 positions.

a) A C=O stretching is seen in the spectra of abietic, levopimaric, and neoabietic acids at 6.15 to 6.17 μ. No band is exhibited in the spectra of palustric or dihydroabietic acids.

b) The =C-H out-of-plane-deformation vibration is exhibited as a single band by abietic acid at 12.65 μ and by levopimaric at 12.62 μ. The spectra of dihydroabietic, neoabietic, and palustric acids reveal bands at 12.48, 12.50, and 12.38 μ, respectively.

c) Levopimaric and neoabietic acids reveal bands at 14.35 and 14.40 μ, respectively. No bands are found at this wavelength in the spectra of dihydroabietic or palustric acids. Thus palustric acid resembles dihydroabietic acid.

Uses of Infrared Absorption Spectroscopy Combined with Other Instrumental Methods of Analysis—Applications in Biochemistry

Among the newer applications of infrared spectroscopy to lipid chemistry are numerous examples of its tandem use with other instrumental methods of analysis. In investigations of lipids in the area of biochemistry, the use of column chromatography and infrared absorption spectra has been described frequently. Freeman and his coworkers have reported several investigations of the composition of serum lipids. Separations of the components were made on silicic acid—Celite columns and eluates from the column evaporated to dryness and redissolved in CS_2 for infrared measurements (44). A method for the quantitative analysis of serum lipids by these techniques has been described (45). Three fractions were eluted from the column in succession. Fraction I contained cholesteryl esters, identified by the infrared bands at 5.8 or 8.55 μ and quantitatively measured at either of these wavelengths. Fraction II contained glycerides, unesterified fatty acids, and unesterified chloresterol, identitfied by characteristic bands at 5.75, 5.85, and 9.5 μ and quantitatively evaluated by multicomponent analysis from simultaneous equations involving measurements at these three wavelengths. Fraction III contained phosphatides, identified and quantitatively measured by the band at 9.35 μ.

Serum phospholipids were analyzed in a similar manner (89). Five fractions were obtained from the silicic acid—Celite column and analyzed as follows:

Fraction No.	Eluent	Components	I. R. analysis
I	CH_2Cl_2	Nonphospholipids Cholesterol esters and fatty acids	Not analyzed
II	Acetone	Nonphospholipids Pigments	Not analyzed
III	35% Methanol 65% CH_2Cl_2 (10 cc.)	Phosphatitdylethanolamine Phosphatidylserine	Bands at 5.8 and/or 9.3 μ (absence of lecithin established by absence of band at 10.3 μ)
IV	35% Methanol 65% CH_2Cl_2 (20 cc.)	Lecithins	Bands at 9.2 and/or 10.3 μ
V	95% Methanol 5% Water	Lecithins Sphingomyelins	Bands at 5.8 and6.1 μ as multicomponent analysis

Milk phospholipids were similarily fractionated into major phospholipid classes by successive elution from silicic acid columns with $CHCl_3$, acetone, MeOH 20% in $CHCl_3$, MeOH 40% in $CHCl_3$, and MeOH. Each fraction was analyzed by infrared absorption spectra from KBr disks and compared with pure compounds. The phospholipids of milk were found to be (116) cerebrosides, 6%, cephalins, 35%, lecithins, 32%, sphingomyelins, 24% with minor components, carbohydrate containing phospholipids, lysolecithin, and unidentified lipids. Infrared absorption of each of these separated components were obtained, as KBr disk spectra, and compared with similar spectra of pure compounds.

Infrared spectra were used to show that the double bond of the sphingosine moiety has the *trans* configuration (appearance of the 10.3 μ band) in work reported on the structure of cerebrosides in Gaucher's disease (80). The infrared spectra of sphingine obtained from hydrolysis of the reduced cerebrosides and then converted to the diacetylsphingine were identical with the spectra of an authentic sample of diacetylsphingine. Infrared absorption spectra were also used in the identification of fractions separated by silicic acid columns as cephaline, lecithins, sphingomyelin, and lysolecithin in an investigation of the composition of lipids extracted from normal human serum (56). Lakshminarayana and coworkers (68) combined not only column chromatography with infrared, but also radioactivity assay in their studies of the composition of commercial samples of triolein-1 and oleic acid-1 and the distribution of the label in human serum lipids following oral administration.

Silica gel and Hyflo supercell columns were used by Frankle (43) to prepare methyl oleate from hazelnut oil, methyl linoleate from poppyseed oil, and methyl linolenate from linseed oil; infrared absorption was used to confirm the fact that there was no isomerization of the natural *cis* to *trans* isomers during these processes. Specific bands were used in the analysis of tissue lipids by Schwarz and coworkers (110). Sphingolipids were identified by the Amide I band at 6.04 μ, cephalins and lecithins by the C=O band at 5.72 μ, and cholesterol by the band at 9.44 μ. The fractions were separated on silicic acid columns, and all infrared measurements were made from KBr disk spectra.

Frankel and coworkers (42) used partition chromatography to separate hydroperoxides in autoxidized fatty acids or their methyl esters. The percentages of methyl oleate hydroperoxide determined chromatographically agreed well with those based on spectral measurements of isolated *trans* bands in the autoxidized methyl oleate. Methyl linoleate hydroperoxide fractions were shown, by infrared spectroscopy, to have *cis-trans* and *trans-trans* conjugated configurations; infrared measurements showed that saponification of fatty ester hydroperoxides converts them to the corresponding monomeric hydroxy acids without apparent change in their conjugated diene structure. The unsaponifiable portion of fats was separated by silicic acid absorption chromatography, and infrared absorption spectra were used to identify some of the components by Capella and his coworkers (11). Eight fractions were successively eluted from the column, and the various paraffins, olefins, waxes, sterol esters, higher aliphatic alcohols, triterpenoid alcohols, and sterols were identified by comparisons of the infrared spectra with authentic samples (fingerprint technique) or by group frequency analyses.

Techniques other than column chromatography have also been used with infrared absorption spectroscopy in investigations of lipid materials. Kauffman and Lee (58) used gas-liquid partition chromatography in a study of octadecenoic acids, checking the chromatographic determinations of the *trans* acids with the infrared methods. Smith and Coffman (115) also used gas-liquid chromatography for separations and infrared spectra for identifications in their investigation of the neutral components from bread preferment liquid, and Matthews and coworkers (82) combined gas-liquid chromatography with both infrared spectra and nuclear magnetic resonance in the separation and identification of C_8 aldehydes.

Conclusions

Scores of other applications of infrared spectra to problems of lipid chemistry could be cited. No useful purpose would be served by a somewhat repetitious listing of the many applications which have been reported. An attempt has been made to select applications that would represent the various ways in which infrared absorption spectroscopy is being used for and by the fatty acid chemist. A review of this nature a decade ago could easily have included a complete bibliography with reference to all papers which appeared within the period covered. This is no longer practical as infrared spectroscopy has become a major tool of the lipid chemist. In this review the reports by several workers of very excellent research contributions have not been included.

During the past five years several review papers have appeared. In the second edition of Markley's "Fatty Acids" the subject of infrared absorption is covered to about 1958 (95). In German an excellent review has been written by Kaufmann, Volbert, and Mankel (60), including 249 literature references and again covering the period through about 1958.

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