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Application of Infrared Spectrophotometry to Fatty Acid Derivatives[1]

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DURING the last four years the technical literature of fats and oils has included a number of papers dealing with the use of infrared spectroscopy as a tool for qualitative identification, quantitative determinations, and the elucidation of chemical structures of fatty acid materials. A chronological listing of the most important dates in the history of the development of the applications of infrared absorption spectroscopy to fatty acid derivatives has been prepared to illustrate the phenomenal growth in this development, particularly since 1950 (Table I).

TABLE I

Historical Developments in the Application of Infrared Spectrophotometry to Fatty Acid Derivatives

1800	Herschel discovers infrared radiation.
1892	Julius illustrates application of infrared spectra to analytical chemistry.
1905	Coblentz publishes first library of infrared spectra.
1920	Gibson publishes first technical article devoted exclusively to applications of infrared spectroscopy to fatty acid materials.
1940–1950	23 publications dealing with applications of infrared spectroscopy to fatty acids appear.
1950–1954	104 articles devoted to applications of infrared spectroscopy in the field of fats and oils are published—49 or 47% since January 1953.

The earliest date in such a table, the beginning of time in the science of infrared spectrophotometry, is, of course, the discovery by Sir William Herschel, a little more than 150 years ago, that there were radiations beyond the red limit of visible light. The discovery occurred in 1800 but did not immediately provide a new tool for analytical chemistry. Just as the science of emission analysis did not start with the discovery of the spectrum by Sir Isaac Newton in 1666 but had to await the work of Kirchoff and Bunsen nearly 200 years later, so the use of infrared radiation had to await the work of Julius in 1892 (60). Julius was probably the first to show that the infrared spectra of all compounds containing a specific group exhibited that same absorption maxima, *i.e.*, the infrared spectra of compounds containing the methyl group always exhibit a band with maximum at 3.45 microns. Such empirical correlations of vibrating groups with specifically observed absorption maxima suggested to Julius the possibility of chemical identification and even of quantitative determinations.

The fact that infrared spectra were capable of identifying specific vibrating groups was confirmed and reconfirmed, but little progress was made in forging a tool for analytical chemistry of fats and oils. Only two publications appeared during the almost half century between 1892 and 1940. In 1905 Coblentz published his famous collection of the infrared spectra of 131 substances, which included the spectra of several fatty acids and vegetable oils (29). In 1920 K. S. Gibson published a paper entitled "The Infrared Spectra of Vegetable Oils," which appears to be the first publication devoted exclusively to the infrared spectra of fatty acid materials (46).

The World War II decade, 1940–1950, witnessed the first evidence of actual applications of infrared absorption spectroscopy of fat and oil chemistry. Since 1950 these applications have been widespread.

It is not intended to include in this review the definition or principles of infrared spectroscopy or to describe the instrumentation, or to provide details of infrared procedures and techniques. These subjects have been adequately covered in standard texts and in general reviews on the subject of infrared spectroscopy which are readily available. The theme of this paper is to answer the question, what can infrared spectroscopy do for the fatty acid chemist? by showing what infrared spectroscopy is doing in fatty acid chemistry, with, perhaps, some postulation as to what might reasonably be expected in the immediate future.

During even the short time that fatty acid chemists have been using infrared spectra, several reviews have appeared dealing with the applications and potentialities in fatty acid chemistry. These include reviews in standard texts or monographs on fatty acids (8, 81, 87, 96, 124), a review in this Journal (21), and reviews covering specific applications as to paint research (3, 48), coating industry (23, 74), the graphic arts and waxes (82), cosmetics (125), surface-active compounds (35), and waxes and polishes (101, 102).

Reviews have also appeared in foreign languages, including Hashimoto's in Japanese (53), Volbert's in German (121), a contribution from the U.S.S.R. (17), and reviews by Lecomte in German (70) and in French (71).

Survey of the Infrared Spectra of Fatty Acid Materials

We can, for convenience, divide the efforts being made to apply infrared absorption to the analysis of fatty acid materials, as represented by published technical articles, into various, more or less arbitrar-

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ily defined, classifications according to their major emphasis. Such a classification is attempted in Table II. Obviously, several papers cross such arbitrary classification lines, dealing with two or more of these main subjects.

The first group includes the review type of articles already discussed. A second classification contains

TABLE II
Applications of Infrared Spectrophotometry to Fatty Acid Chemistry

Subject	No. of technical papers
General review articles	26
General survey of spectra	11
Cis-trans configuration	33
Autoxidation	14
Drying oils	6
Glycerides	3
Polystyrene copolymers	3
Lipides and phospholipides	5
Elucidation of structure	10
Miscellaneous applications to qualitative or quantitative analyses	5
Total	116

general surveys of the infrared spectra of fatty acids and related materials with discussions of prominent absorption bands and correlations with the vibrating groups which give rise to them. These papers, while somewhat preliminary in character, are very important as they furnish the data needed for any extensive use of infrared spectra as a tool for the analytical chemistry of fats and oils.

The very early collection of Coblentz (29) and the first paper devoted entirely to infrared spectra of fatty acid materials by K. S. Gibson (46), already referred to, were contributions of this nature. Barnes, Gore, Liddel, and Williams include spectra of several fatty acid materials in their well-known library of infrared curves (13). Another early contribution is that of Gamble and Barnett (45), who reported the spectra of drying oils and their glyceryl esters. These authors reported some band correlations including the interesting observation that a band with maximum at 10.0 microns arises from C=C—C=C conjugation. Barceló and Bellanato have published one of the most recent surveys (12). They report the infrared spectra of several vegetable oils and, for comparison, the spectra of long chain fatty acids and glycerides over the entire range of both the NaCl and the KBr regions, 2.5 to 28.0 microns. Absorption maxima exhibited by the oils, but not found in the spectra of the fatty acids, were observed at about 9.13, 8.97, 8.77, and 7.87 microns.

During the short period from late 1950 into early 1952 several independent studies were made of the spectra of long chain fatty acids and esters. Shreve, Heether, Knight, and Swern (105) were the first to publish spectra of several long chain fatty acids, esters, and alcohols in CS_2 solution. They made several band correlations including an assignment of the 3.7 micron band as a bonded O—H . . . . O fused with the 3.4 micron band arising from C—H stretching. O'Connor, Field, and Singleton (89) published quantitative spectra of the fatty acids and their methyl and ethyl esters in $CHCl_3$ solution, reporting absorptivities at significant wavelengths, which permitted relative quantitative comparisons. They, too, made several correlations between observed maxima and vibrating groups giving rise to them, and observed that a band at 9.0 microns may be used to distinguish ethyl esters from methyl esters.

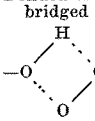
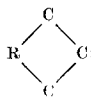
Sinclair, McKay, and Jones (110) reported on the spectra of saturated fatty acids and esters, and Sinclair, McKay, Myers, and Jones (111) described the spectra of unsaturated fatty acids and esters. These authors made several correlations between observed bands and vibrating groups and compared the spectra of solutions with those of solid films or mulls. They found solution spectra very similar for the different chain length acids and esters. The intensity of a band at 13.0 microns in the spectra of saturated fatty acids and esters, attributed to a CH_2 rocking vibration, was shown to increase progressively with chain length. This may be significant in estimations of chain lengths of unknown saturated fatty acids. In the spectra of the solutions of unsaturated fatty acids the intensity of the band at 3.3 microns, attributed to a C—H stretching vibration of the C=C—H group, was shown to increase with increasing degree of unsaturation while the intensity of the bands at 3.43 and 3.51 microns, arising from $-CH_2$ stretching vibrations, decreased in the same series. These intensity measurements require an instrument capable of high resolution at these wavelengths.

The spectra of crystalline acids (films or mulls) showed much more structure. The C=O stretching vibration occurs at 5.88 to 5.89 microns in the spectra of the even carbon acids but at 5.87 microns in the spectra of the odd carbon acids, providing a method for distinguishing between carbon chains of odd and even numbers of carbon atoms. A progression of bands, spaced at fairly even intervals of 0.05 micron, appear in the solid spectra between 7.4 and 8.5 microns, where solution spectra exhibit only broad diffuse bands. These bands, which apparently arise from CH_2 wagging and/or twisting were further studied by Jones, McKay, and Sinclair (59). In the series C_{12} to C_{21} there is a regular increase in the number of bands in the progressions with no alteration between acids with odd and even number of carbon atoms. The relative number of bands may be useful to estimate chain length of unknown fatty acids or esters.

Branched long chain fatty acids have been studied by Freeman (40), who reported on 27 of these compounds in an attempt to correlate features of their infrared spectra with types of chain branching. Freeman emphasizes the provisional nature of many of his assignments. However several characteristics of the spectra, which he points out, can be of considerable usefulness to research on the structure of branched chain acids and related compounds if judiciously used and correlated with other means of structural identification. Freeman considered position of branching, length of branches, and number of branches. Branching on the *alpha* carbon or near the carboxyl group can be detected by observation of the 7.78 and 8.00 micron bands. In straight chain compounds the 7.78 micron band is the more intense of this pair. Substitution on the *alpha* carbon reverses this relation. Substitution within five carbons of the carboxyl group can be recognized by shifts in wavelength positions of the 8.00 micron band. Lengths of branches are indicated for the ethyl group by a band at 12.95 microns, for the *n*-propyl group by a band at 13.5 microns, and for isopropyl and *t*-butyl by splitting of the band at about 7.25 microns into two components. The num-

TABLE III

One Hundred Absorption Bands Employed in the Applications of Infrared Spectroscopy to Fatty Acid Chemistry [a]

No.	Wavelength position of observed absorption band	Vibrating group giving rise to observed absorption band
	microns	
		I. O—H, C—H, N—H, C—D, P—OH, and P—H stretching vibrations. Regions 2 to 5 microns
		A. O—H stretching
1	2.75–2.80	Free —O—H
2	2.82–2.90	Bonded —O—H . . . O of single-bridged dimer
3	2.95–3.25	Bonded H . . . O—H . . . O— of double-bridged polymer or cyclic  dimer
		B. C—H stretching
4	3.00–3.05	R≡C—H
5	3.22–3.25	R_2=C—H_2
6	3.28–3.32	R_2=C—HR
7	3.40–3.45	R—C—H_3
8	3.42–3.50	R_2—C—H_2
9	3.45–3.48	R_3—CH
10	3.50–3.70	R—C(=O)—H
11	3.70	C—H and bonded O—H . . . O combination band
		C. N—H stretching
12	2.85 and 2.95	Free N—H primary amide
13	3.00 and 3.15	Bonded N—H . . . primary amide
14	2.90–2.95	Free N—H secondary amide
15	3.00–3.05	Bonded N—H . . . secondary amide, single bridge *(trans)*
16	3.15–3.18	Bonded N—H . . . secondary amide, single bridge *(cis)*
17	3.22–3.25	Bonded N—H . . . secondary amide cyclic dimer
18	2.85 and 3.02	N—H primary amine
19	2.85–3.02	N—H secondary amine
20	2.95–3.12	N—H imines
21	3.20–3.30	N—H_3^+ amino acids
		D. C—D stretching
22	4.64	C—D
23	4.84	C—D
		E. P—H, P—OH stretchings
24	4.05–4.25	P—H
25	3.70–3.90	P—OH
		II. C=O and C=C, C≡C stretching vibrations. Region 3.0 to 6.0 microns
		A. Aldehydes
26	5.75–5.80	RC(H)=O, saturated
27	5.83–5.90	PhC(H)=O, aryl
28	5.85–5.95	R—CH=CH—C(H)=O, α,β unsaturated
		B. Ketones
29	5.80–5.85	RCH_2—C(=O)—CH_2R, saturated
30	5.90–5.95	Ph—C(=O)—CH_3, aryl-alkyl
31	6.00–6.02	Ph—C(=O)—Ph, diaryl
32	6.00–6.05	R—CH=CH—C(=O)—R, α,β unsaturated
33	5.63	 C=O, 4-membered, saturated ring

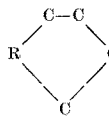
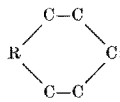
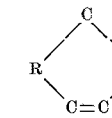
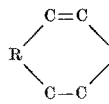
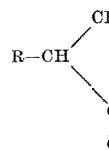
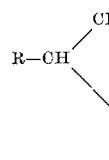
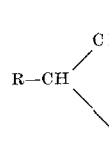
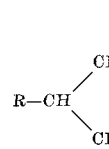
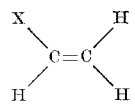
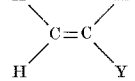
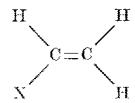
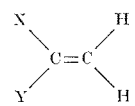
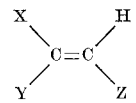
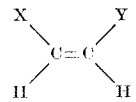
No.	Wavelength position of observed absorption band	Vibrating group giving rise to observed absorption band
	microns	
34	5.73	 C=O, 5-membered, saturated ring
35	5.81	 C=O, 6-membered, saturated ring, or  C=O, 5-membered, α,β unsaturated ring
36	5.95	 C=O, 6- (or 7-) membered, α,β unsaturated ring
37	5.90–6.00	O=Ph=O, quinone, 2C=O's on 1 ring
38	6.05–6.10	O=Ph—Ph=O, quinone, 2 C=O's on 2 rings
		C. Acids
39	5.68	R—C(=O)—OH, saturated monomer
40	5.80–5.88	R—C(=O . . . HO)—OH . . . O=C—R, saturated dimer
41	5.90–5.92	R—C=C—C(OH)=O, α,β unsaturated
42	5.90–5.95	Ph—C(OH)=O, aryl
43	6.00–6.05	Chelated hydroxy-acids, some dicarboxylic acids
		D. Esters
44	5.65	H_2—C=C—C(OCH_3)=O, vinyl ester
45	5.75	R—C(OCH_3)=O, saturated
46	5.80–5.82	R—C=C—C(OCH_3)=O, α,β unsaturated, or R—COOPh, aryl
		E. Lactones
47	5.50	 O, β, or 4-membered saturated ring
48	5.65	 O, γ, or 5-membered saturated ring
49	5.72	 O, γ, or 5 membered α,β unsaturated ring
50	5.75	 O, ϵ, or 6-membered saturated ring

TABLE III (Continued)

No.	Wavelength position of observed absorption band	Vibrating group giving rise to observed absorption band
	microns	
		F. C=C, C≡C stretching
51	6.0–6.1	C=C *cis* only (weak when internal in symmetrical molecules)
52	3.03	HC≡CH
53	4.67–4.76	RC≡CH
54	4.44–4.58	RC≡CR
55	5.14 and 9.45	C=C=C
		III. C—H deformations, saturated groups. Region 6 to 7 microns
56	6.7–6.9	—C—H_2— group
57	6.8–7.0	—C—C—H_3 group, asymmetrical deformation
58	7.15–7.20	—C—$(C—H_3)_3$ group
59	7.20–7.25	—C—$(C—H_3)_2$ group
60	7.25–7.30	C—C—H_3 group symmetrical deformation
61	7.30–7.35	—C—$(C—H_3)_2$ group
62	7.45–7.50	—C—H— group
		IV. C—O stretching and C—OH bending. Region 7.7 to 10.0 microns
		A. Alcohols
63	7.2–8.6	Phenols
64	8.3–8.9	Tertiary open-chain saturated
65	8.9–9.2	Secondary open-chain saturated
66	9.2–9.5	Primary open-chain saturated
67	8.3–8.9	Highly symmetrically-branched secondary
68	8.9–9.2	α-Unsaturated or cyclic tertiary
69	9.1–9.2	Secondary with branching on one α-carbon
70	9.2–9.5	Secondary, α-unsaturated or alicyclic 5- or 6-membered ring
71	9.5 –10.0	Secondary: di-unsaturated, α-branched and unsaturated, or 7- or 8-membered ring Primary: α-branched and/or unsaturated Tertiary: highly unsaturated
		B. Acids
72	7.75–7.80	C—O
73	8.40–8.45	C—O
		C. Esters
74	7.90–8.00	C—O
75	8.40–8.50	C—O
		D. Ethers
76	8.7–9.4	CH_3—O—CH_2, alkyl
77	7.8–8.1	Ph—O—Ph, aryl or =C—O, unsaturated
		E. Anhydrides
78	7.7–8.3	Cyclic
79	8.5–9.5	Open chain
		F. Phosphorus
80	6.90 and 10.0	P—O—Ph, aromatic
81	9.52	P—O—CH_3 aliphatic
		V. C—H deformation about a C=C and skeletal and "breathing" vibrations. Region 10.0 to 15.0 microns.
		A. C—H bending
82	10.05–10.15	
83	10.20–10.36	 (*trans* only*)
84	10.90–11.05	
85	11.17–11.30	
86	11.90–12.50	
87	13.0 > 15.0	 (*cis* only*)

* See text for table of wavelength positions of various combinations of conjugations involving these two internal groups.

No.	Wavelength position of observed absorption band	Vibrating group giving rise to observed absorption band
	microns	
		B. Skeletal and "breathing"
88	9.75 and 11.55	Cyclopropane
89	10.9 and 11.3	Cyclobutane
90	10.31 and 11.16	Cyclopentane
91	9.63, 9.86, 11.05 and 11.60	Cyclohexane
92	11.2	Epoxy-oxirane ring derived from internal R—C=C—R (*trans* only)
93	12.0	Epoxy-oxirane ring derived from internal R—C=C—R (*cis* only)
94	11.8, 12.9, and 14.7	Benzene ring
95	12.0	Hydroperoxide
96	12.95	Ethyl
97	13.0	CH_2 rocking on long carbon chain
98	13.5	n-Propyl
99	13.8	Hydroperoxide
100	7.5–8.5	Progression of bands in solid state spectra, probably due to wagging and/or bending mode of vibration of the C—H bonds of methylene groups. The number of bands in the progression is indicative of chain length.

[a] The exact position of maximum absorption depends upon whether the measurements were made on the pure liquid, solid, mull, or solvent and on the nature of the particular solvent. Several band positions are also critically dependent upon neighboring groups.

The value and range given in this table are from the author's collection of these bands in fatty acid materials mostly from original reports in technical journals. They represent average values of ranges of the various data that have been reported for the specific absorption.

ber of branches can be detected by determination of terminal methyl groups.

Sobotka and Stynler (113) reported on the infrared spectra of *iso*-(—CH_3 group on the next-to-the-end carbon), *anteiso*-(—CH_3 group on the second-from-the-end carbon), and *neo*-(two —CH_3 groups on the next-to-the-end carbon). *Iso* acids can be distinguished by a splitting of the 7.25 micron band into two components of about equal intensity, "*iso*-propyl splitting." *Neo* acids exhibit the same splitting of the 7.25 micron band, but the longer wavelength component is the more intense, characteristic of *t*-butyl splitting. *Anteiso* acids showed no splitting, but the 7.25 micron band is much stronger than in the spectra of normal acids.

The spectra of aluminum soaps of fatty acids from C_6 to C_{18} have been investigated by Harple, Wiberly, and Bauer (49) and have been compared with the spectra of fatty acids. The spectra of mono- and disoaps differ from trisoaps in that they exhibit no "fatty acid" band at 5.82 microns (C=O stretching of COOH group). These results were interpreted to indicate that trisoaps do not exist as chemical compounds but as mixtures of loose compounds of a disoap and fatty acid. This interpretation is consistent with the fact that trisoaps contain fatty acids extractable with cold isooctane in contrast with mono- and disoaps which contain almost no extractable acid. Monosoaps can be distinguished from disoaps by their

infrared spectra as they exhibit a broad band at 3.0 microns, indicating bonded O—H . . . O groups while the spectra of disoaps show a band at 2.7 microns indicating unbonded O—H groups. Spectra of soaps of a composition between mono- and disoaps exhibit both 2.7 and 3.0 micron bands, indicating that the monosoap in a disoap exists as a discrete compound. McGehee and Barr (77) have reported on the infrared spectra of tung oil, Barr on the spectra of peanut oil and its component acids (14), Barr and Hungerford on citrus oils (16), and Barr and Harp (15) on vegetable oils. In none of these last four papers were any attempts made to correlate observed bands with vibrating groups giving rise to them. Fowler and Smith (39) have studied the infrared spectra of 11 saturated monocarboxylic acids and their methyl esters in the regions of C=O stretching, 5.7 to 5.8 microns, and C—O—C stretching, 7 to 9 microns.

These papers, surveying the spectral properties of long chain fatty acids and related compounds, have resulted in the accumulation of several correlations between observed absorption bands and vibrating groups which give rise to them. The more important are shown in Table III. These data are the "working tools" for all applications of infrared spectra to the chemistry of fatty acid compounds. It is interesting to observe that practically all of these correlations are to be found in eight of the papers reviewed which appeared between November 1950 and May 1952, a period of only 18 months.

Applications to Cis-, Trans-Isomerization Studies

The most useful application of infrared spectra to fatty acid materials thus far developed has been a method to distinguish between *cis* and *trans* unsaturation and to determine *trans* bonds in the presence of *cis* bonds. Rasmussen, Brattain, and Zucco (98), in a paper published in the Journal of Chemical Physics, made the incidental observation (actually in a footnote) that a strong band at 10.3 microns in the spectra of unsaturated compounds appears to arise from a *trans* C=C group. The band has since been shown to be due to a C—H deformation about a *trans* C=C bond (103). In the hands of Shreve and coworkers this band was destined to make an important contribution to the application of infrared spectra to fatty acid chemistry. Prior to their work however McCutcheon, Crawford, and Welsh (76) had used infrared spectra to distinguish between *cis* and *trans* bonds. They measured the spectra from 5 to 6.5 microns and used the very weak band at 6.0 microns, assigned to a C=C stretching. From theoretical considerations it was shown that the *cis* double bond should absorb at 6.0 microns while the *trans* bond should not. Using ethyl stearate as a blank to cancel the very strong C=O absorption in this region, it could be shown that oleic acid has a *cis*-configuration, linoleate a *cis, cis,* and linolenate a *cis, cis, cis* structure. Elaidic acid similarly was shown to be a *trans*-isomer and linolelaidate to have a *trans, trans*-configuration. Raman spectra confirmed these results. However, due to the fact that the C=C stretching vibration band at 6.0 microns is very weak and that there is interfering absorption from the strong C=O stretching vibration, this method has not proven very satisfactory.

Shreve, Heether, Knight, and Swern (104) developed a method for the determination of isolated *trans* bond concentration in saturated and monounsaturated octadecenoic acids and related compounds based on quantitative measurements of the 10.3 micron band. Absorptivities were reported for saturated and for *cis* and *trans* monounsaturated free acids, esters, triglycerides, and derived alcohols. The method was illustrated by the analysis of mixtures containing oleic, elaidic, and saturated acids and mixtures of the corresponding methyl esters and derived alcohols.

Paschke, Jackson, and Wheeler (91), studying the thermal polymerization of isomers of methyl linoleate by means of infrared spectra, reported that the absorption band at 10.3 microns in the spectra of methyl *cis*-9, *trans*-12-linoleate was very nearly the same intensity as in the spectra of methyl elaidate and that absorptivity of the band in the spectra of methyl linolelaidate (*trans*-9, *trans*-12) was very nearly twice this value. These data strongly indicate that the absorptivities of the isolated *trans* band at 10.3 microns are additive in nonconjugated compounds and that the method of Shreve *et al.* can be extended to the analysis of polyunsaturated, nonconjugated olefinic acids and related compounds. Jackson, Paschke, Tolberg, Boyd, and Wheeler (58) reported infrared spectral studies on conjugated and nonconjugated isomers of methyl linoleate in this region of olefinic C—H bending. A weak band with maximum at 10.95 microns was found only in the spectra of nonconjugated *cis, cis*-linoleate. A single, very strong band at 10.12 microns was shown to be characteristic of conjugated *trans, trans*-linoleate, confirming earlier assignment of Gamble and Barnett (45). Conjugated *cis, trans*-linoleate exhibited two maxima, a strong band at 10.18 microns, and a somewhat weaker band at 10.52 microns.

In their studies using infrared spectra to confirm the *cis, trans*-configuration of *alpha*- and *beta*-eleostearic acids, Bickford, DuPré, Mack, and O'Connor (20) found that under their methods of measurement in chloroform solutions the absorptivity of the 10.1 micron band in *alpha*-eleostetaric acid (*cis*-9, *trans*-11, *trans*-13-octadecatrienoic) was 0.8, very nearly twice that of elaidic acid, and that of beta-eleostearic (*trans*-9, *trans*-11, *trans*-13-octadecatrienoic) acid was 1.2, almost three times that of elaidic acid. These data strongly indicate that the intensities of the C—H deformation about the C=C at about 10.0 to 10.3 microns are additive in conjugated as well as nonconjugated compounds.

Ahlers, Brett, and McTaggart (4) published results of a detailed quantitative study of some 19 fatty acids including monounsaturated and conjugated and nonconjugated polyunsaturated compounds. They confirmed the earlier work regarding the positions of band maxima for polyunsaturated acids, both conjugated and nonconjugated, and reported absorptivities for the 19 acids which were in reasonably good agreement with previously reported values for the few cases which had been reported earlier.

Ahlers, Brett, and McTaggart (4) account for the position of maxima in the spectra of conjugated acids containing *trans* bonds as 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. If we assume that a *cis* bond causes a somewhat smaller hypsochromic effect, the experimentally observed positions of maxima can be accounted for rather nicely:

Unsaturated System	Experimentally Determined Wavelength Position of Maxima
Isolated trans	10.34 microns
Cis, trans-conjugated	10.17 microns
Trans, trans-conjugated	10.12 microns
Cis, cis, trans-conjugated	10.11 microns
Cis, trans, trans-conjugated	10.09 microns
Trans, trans, trans-conjugated	10.06 microns

The shift from the position of an isolated *trans* band is least for conjugation with a single *cis* bond, somewhat greater for conjugation with a single *trans* bond, still greater for conjugation with both a *cis* and a *trans* bond, and greatest when conjugated with two *trans* bonds. This "explanation" does not account for the appearance of two bands in the spectra of *cis, trans*-conjugated acids. A comparable explanation might be found by postulating a hypsochromic effect of conjugation on the isolated *cis* band:

Unsaturated System	Experimentally Determined Wavelength Position of Maxima
Isolated *cis*	10.95 microns
Cis, cis-conjugated	?
Cis, trans-conjugated	10.53 microns

Cis, cis-conjugation, as yet unreported as far as we have been able to ascertain, would be expected at about 10.7 microns. The hypsochromic effect in the *cis* system is somewhat greater than in the *trans* system, but this is consistent with the greater observed variation in the position of isolated *cis* bands, indicating greater sensitivity to "molecular environment." It should be observed that, in such measurements as these, high resolution is required to determine accurately the exact positions of maxima. We shall see from time to time that as more precise data are made available the need for higher resolution becomes more urgent.

As an appendix to their paper Ahlers *et al.* give a detailed mathematical treatment for the estimation of isolated *trans*-unsaturated acids in the presence of *cis*-unsaturated and saturated acids, illustrated by the analysis of a mixture resulting from the elaidinization of oleic acid. This is the method of Shreve *et al.* (104). The analysis of mixtures containing conjugated *cis*- and *trans*-unsaturated acids, as well as *cis*- and *trans*-monounsaturated and saturated components for isolated *trans*, is also considered and illustrated by the analysis of a mixture which might arise during the course of dehydration of castor oil. From the data presented by Ahlers and his coworkers it would seem possible to extend the method to the quantitative determination of other components such as conjugated *cis, trans* or conjugated *trans, trans* content.

Detailed studies of the C—H bending about the C=C group have resulted in the compilation of a variety of correlations between observed bands and the exact groupings whose vibrations give rise to them. These correlations are included in Table III. The various types include bendings about terminal double bonds, only occasionally encountered in fatty acid chemistry (see, however, reference 112) and the variety of deformations of internal unsaturations discussed. These data offer the fatty acid chemist a special tool for the study of molecular structure or for following the course of chemical processes. They have, as we shall see, already been put to considerable use.

The infrared spectrophotometric method for the determination of isolated *trans* bonds was compared with the lead salt-alcohol method of determining *trans*-octadecenoic acids by Swern, Knight, Shreve, and Heether (118). The infrared method was more rapid, more specific, and more accurate. The same comparison was made by Jackson and Callen (57), who confirmed the greater accuracy and convenience of the infrared method. They reported that hydrogenated oils contained *trans* or "iso" oleic acid. Swern, Knight, and Eddy (117) found that a sample of freshly rendered edible beef fat, and of edible oleo oil and oleo stearin obtained from it, contained substantial quantities (5 to 10%) of *trans* material believed to be mainly, if not exclusively, monounsaturated. *Trans*-9-octadecenoic (elaidic) and *trans*-11-octadecenoic (vaccenic) acids were isolated from oleo oil, the former, apparently, for the first time.

Vaccenic acid was also prepared from beef tallow by Rao and Daubert (97). By comparing its infrared spectra with that of oleic and elaidic acids they showed that vaccenic acid is the *trans*-11-octadecenoic acid. Ahmed, Bumpas, and Strong (9) synthesized *cis*-11- and *trans*-11-octadecenioc acids and showed by infrared curves that the *trans* acids were *trans* and identical with Rao and Daubert's vaccenic acid from beef tallow.

The method of Shreve *et al.* was used by Fusari, Greenlee, and Brown (44) to check on the *cis-trans* configuration of *cis*- and *trans*-6, 7-, 8-, 9-, and 11-octadecenoic acids. They reported absorptivities of these 10 compounds at 10.36 microns in CS_2 solution. Ahlers and McTaggart (7) describe an interesting application of infrared spectra. Using the rules developed for the position of the *trans* bond when conjugated with either *cis* or *trans* bonds, they showed that punicic acid in pomegranate seed oil is *cis- cis, trans* without even isolating the acid from the oil. Snake gourd oil *(Trichosanthes cucumeroides)* and Indian gourd oil *(Trichosanthes anguina)*, which have been reported to contain trichosanic acid, were similarly studied by Ahlers and Dennison (5), who showed that these oils resemble pomegranate seed oil and contain punicic acid. The absence of the 10.3 micron band in the infrared spectra of C_{16} and C_{18} tetraene fatty acids isolated from fresh water alga *Chlorella pyrenoidosa* was used by Paschke and Wheeler (93) to prove that they did not contain *trans* double bonds.

Khan, Deatherage, and Brown (63) and Max and Deatherage (83) demonstrated, by means of infrared spectra, the complete absence of *trans* bonds in synthetic methyl 9,10-dideuterooleate and 8,8,11,11-tetradeutero-*cis*-9-octadecene, respectively. Infrared spectra were also used to confirm the presence of the deuterium by the appearance of the doublet at 4.64 and 4.84 microns arising from C—D stretching vibrations.

Paschke, Tolberg, and Wheeler (92) used infrared spectra in a study of the *cis, trans*-configuration of *alpha*- and *beta*-eleostearic acids. Both isomers reveal a strong band at about 10.1 microns, indicating a *trans, trans*-conjugated configuration, in agreement with the fact that they both form maleic anhydride adducts readily below 100°C. The spectra of the

alpha-eleostearic acid adduct exhibited no band at 10.3 microns, indicating that the third unsaturated C=C bond, not affected by the maleic anhydride addition, is a *cis* bond. The spectra of the maleic anhydride adduct of *beta*-eleostearic acid however did exhibit a band at 10.36 microns, indicating that the third unsaturated bond in this molecule is *trans*. Thus *alpha*-eleostearic acid has two *trans* and one *cis* bond, and *beta*-eleostearic acid is the *trans, trans, trans*-isomer. *Pseudo*-eleostearic acid was shown to have the same structure as *beta*-eleostearic acid, and *alpha*- and *beta*-licanic acids (4-keto-9,11,13-octadecatrienoic acids) the same *cis, trans*-configurations as *alpha*- and *beta*-eleostearic acids. Punicic acid is a *cis, trans*-isomer of *alpha*- and *beta*-eleostearic acids, not identical with either. We have already seen that Ahlers *et al.* (7) later showed it to have a *cis, cis, trans*-configuration. The *cis, trans*-configurations of *alpha*- and *beta*-eleostearic acids were investigated independently by Bickford, DuPré, Mack, and O'Connor (20). They confirmed the results of Paschke *et al.* A band in the spectra of the maleic anhydride addition product with butadiene at 10.40 microns was assigned as one of the symmetrical "breathing" vibrations of a substituted maleic anhydride ring. As a similar band might interfere with the 10.36 micron band, to be used as evidence for the presence or absence of an isolated *trans* bond in the spectra of the *alpha*- and *beta*-eleostearic acid maleic anhydride addition products, the adducts were converted to the corresponding tri-n-butyl esters. The spectra of these esters, which do not have maleic anhydride rings, would not be expected to exhibit the interfering band at 10.40 microns. The infrared spectra of the tri-n-butyl ester of the *beta*-eleostearic acid adduct exhibited a pronounced band at 10.36 microns while no trace of a corresponding band was found in the spectra of the ester of the *alpha*-eleostearic acid adduct. Thus infrared spectra established the presence of one *cis* and two *trans* bonds in alpha-eleostearic acid and three *trans* bonds in the *beta*-eleostearic acid molecule. The intensities of the 10.1 micron band in the spectra of chloroform solutions of *alpha*- and *beta*-eleostearic acid were 0.8 and 1.2, respectively, under the conditions of the measurements. Under identical conditions the absorptivity of elaidic acid is 0.40. The exact multiples of two and three for the *alpha*- and *beta*-eleostearic acids further confirm the presence of two *trans* bonds in the former and three in the latter and, as pointed out earlier, indicate that the absorptivities of the *trans* bond are additive even when conjugated.

Shreve *et al.* (106) have shown that in the spectra of epoxy compounds a band with maximum at 11.2 microns is exhibited if the oxirane ring is derived from an internal monounsaturated compound having the *trans*-configuration at the double bond. The corresponding *cis*-configuration gives rise, in the spectra of the epoxy derivative, to a weak band at 12.0 microns only. These correlations afforded Bickford, DuPré, Mack, and O'Connor further opportunities to confirm the *cis-trans* configuration of the *alpha*- and *beta*-eleostearic acids. The maleic anhydrides were selectively epoxidized under conditions whereby the monoepoxides with the oxirane at the exocyclic double bond only were formed. It was impossible to crystallize the epoxide of the maleic anhydride adduct of *alpha*-eleostearic acid, but two crystalline monoepoxides of the *beta*-isomer were prepared. The fact that two monoepoxides of the *beta* adduct are formed is further evidence for the presence of *trans, trans, trans* conjugation for this configuration would be expected to yield two maleic anhydride adducts below 100°C., one across the 9- and 11-*trans* bonds and the other at the 11,13-*trans* pair. To permit comparisons of the epoxidized compounds, the tri-n-butyl esters of the adducts were selectively epoxidized and their spectra measured. The epoxidized ester of the *alpha*-eleostearic acid adduct exhibited a band at 12.0 microns and no bands at 10.1 or 11.2 microns, indicating an oxirane ring formed at a *cis* exocyclic bond. The two epoxides of the esters of the *beta*-eleostearic acid adducts both exhibited bands at 11.2 microns and no bands at either 10.1 or 12.0 microns, verifying that both were epoxides formed at a *trans* exocyclic bond. These results, together with those from oxidation studies, now establish firmly the structure of *alpha*-eleostearic acid as *cis, trans, trans,* and the *beta*-isomer as *trans, trans, trans.*

Use of Cis-Trans Infrared Data in Hydrogenation Studies. Correlations of infrared absorption data and deformation vibrations about C=C bonds, as listed in Table III, have been found to be very useful in studies of selective hydrogenation of fatty acids and related materials. Lemon and Cross (73) showed that hydrogenation is accompanied by a *cis* to *trans* change in some of the double bonds of the unsaturated fatty acids, by the appearance and the intensity of the maximum at 10.3 microns in the spectra of the hydrogenated products. The hydrogenation of methyl *beta*-eleostearate was followed by infrared spectra by Woltemate and Daubert (127). The principal changes were disappearance of the band at 10.0 microns, which is now known to arise from *trans, trans*-conjugation, and the appearance of the band at 10.3 microns, due to isolated *trans* bonds. *Trans*-11-octadecenoic acid was isolated as the principal monounsaturated acid formed, indicating that the hydrogenation was somewhat selective. The partial hydrogenation of methyl linolenate yields a mixture of at least three isomers, the 8,14-, 9,15-, and 10,14-isolinoleic acids, according to Rebello and Daubert (99). Infrared absorption measurements indicate a *trans*-configuration for at least one of the double bonds.

Benedict and Daubert (18) used infrared spectra to show that the 8- and 9-octadecenoic acids, produced during hydrogenation of triolein, are the *trans*-isomers. Lemon (72) showed graphically the formation and disappearance of *trans* double bonds during the hydrogenation of vegetable oils. Sims and Hilfman repeated some of this work and extended it to animal fats (109). They found that conditions which favor selective hydrogenation of glyceride oils also favor the development of *trans*-isomers and that unusually high percentages of *trans* linkages develop during hydrogenation of tallow.

Feuge, Pepper, O'Connor, and Field (38) reported a quantitative study of the hydrogenation of methyl oleate and triolein. Infrared absorption curves were obtained of samples withdrawn periodically during the hydrogenation and measurements reported for the development of the isolated *trans* bond at 10.3 microns. These infrared studies showed that, during the hydrogenation of methyl oleate, a) *trans*-isomer are formed at very rapid rates, as much as 38% of

trans-isomers being formed while the first 10% of methyl stearate is formed, b) the rate of formation of *trans*-isomers is increased by increasing the temperature, increasing the catalyst concentration, and decreasing the degree of dispersion of hydrogen during the hydrogenation, and c) the hydrogenation of methyl oleate results in establishment of an equilibrium between *cis*- and *trans*-isomers, and, irrespective of the conditions employed, the concentration of the *trans*-isomer was always 67% calculated on the basis of total unsaturated constituents. During hydrogenation of triolein, *trans*-isomers are formed at a slightly lower rate. An equilibrium is established between *cis*- and *trans*-isomers but at values lower than 67% *trans*-constituents. The equilibrium concentration was found to vary with the condition of hydrogenation. Feuge, Cousins, Fore, DuPré, and O'Connor (37) followed the hydrogenation of methyl linoleate and cottonseed oil. Again samples were withdrawn periodically during the hydrogenations and examined by infrared spectra. Isolated *trans*-isomers were calculated, assuming that the absorptivities of the isolated *trans* bands at 10.3 microns are additive. A series of infrared curves showed a) progressive decrease in the intensity of the band at 10.95 microns arising from nonconjugated *cis*, *cis* bonds, b) early appearance of bonds at 10.1 and 10.3 microns attributed to *trans, trans*-conjugation and to isolated *trans* bonds respectively, and c) very rapid disappearance of conjugation and slower decrease of isolated *trans* bonds as the hydrogenation proceeds toward formation of methyl stearate. Maximum amounts of both diene conjugation and isolated *trans* absorption are obtained with increase in temperature, increase in catalyst concentration, and decrease in the dispersion of hydrogen during the hydrogenation.

Application of Cis-Trans Infrared Data to Oxidation Studies. Eight papers have appeared presenting infrared spectral evidence that autoxidation of fatty acid materials is accompanied by isomerizations of *cis*- to *trans*-configurations.

Knight, Eddy, and Swern (65) demonstrated that *trans* double bonds are formed during the autoxidation of methyl oleate in the presence of ultraviolet light. The increase in absorption at 10.3 microns, relative to the peroxide value, suggests that most of the peroxides formed are *trans*-peroxides. Swern, Coleman, Knight, Ricciuti, Willits, and Eddy (116) showed, by use of infrared spectra, that, in the autoxidation of methyl oleate between 35 and 120°, most, if not all, of the hydroperoxide formed has the *trans*-configuration, regardless of whether the autoxidation is conducted in the dark or in the presence of ultraviolet radiation. The autoxidation of methyl linoleate at 0° leads to the formation of about 90% *cis, trans*-hydroperoxide while at room temperature the *trans, trans*-hydroperoxide predominates, according to Cannon, Zilch, Burket, and Dutton (24). Their infrared spectra also indicate that unconjugated monohydroperoxides are formed to only a minor extent.

Use of infrared absorption data, along with ultraviolet, lead Privett, Lundberg, Khan, Tolberg, and Wheeler (94) to conclude that, of the conjugated hydroperoxides formed from normal methyl linoleate at 0°C., at least 90% were *cis, trans*-conjugated. At higher temperatures a considerable amount of the hydroperoxide goes to the *trans* form exclusively. The peroxide isolated from a sample oxidized at 24° showed a considerable amount of *trans, trans*-conjugation. Harrison and Wheeler (52) studied the products formed in the reaction of methyl linoleate and methyl linolelaidate with di-t-butyl peroxide at 125°C. With the aid of infrared analyses it was found that the products were mixtures of isomers, largely dehydro-dimers of the fatty esters, differing in the number of conjugated double bonds and in the *cis, trans*-configuration of these double bonds. Selected fractions from distillation all showed about 90% of the theoretical value for two double bonds per linoleate unit, or four per dimer, but some fractions were rich in *cis, trans*-conjugated configurations, others were mostly *trans, trans*-conjugated, while still others had relatively large amounts of isolated *trans* double bonds. Privett, Nickell, Tolberg, Paschke, Wheeler, and Lundberg (95) showed that both methyl linoleate and methyl linolenate formed a *cis, trans*-conjugated monomeric monohydroperoxide as the major initial product of autoxidation at 0°C.

Khan and Privett reported studies which showed that, on autoxidation of methyl linoleate at 0°C., at least 90% of the initially formed hydroperoxide has a *cis, trans*-conjugated arrangement of double bonds (64). At room temperature less *cis, trans* and some *trans, trans*-conjugation was found, indicating to these investigators that the *cis, trans*-diene is labile and that at higher temperatures a further isomerization to *trans, trans*-form occurs. Khan (61) used infrared absorption spectra to show that the enzymatic oxidation of linoleic acid in the presence of lipoxidase follows the same reaction as ordinary autoxidation to yield 9,11- and 11,13-conjugated hydroperoxides. He found both *cis, trans*- and *trans, trans*-conjugated peroxides as the major oxidation products.

It is thus well established, from infrared absorption studies, that the initial product of the autoxidation of monounsaturated *cis*-compounds is the isomeric *trans*-hydroperoxide, and that di-unsaturated *cis*-compounds result in the formation of conjugated *cis-trans*-hydroperoxides at 0°C. and conjugated *trans, trans*-hydroperoxides above room temperature.

Studies of Autoxidation and Rancidity

Studies on the mechanism of the reactions of unsaturated compounds with oxygen and of the products formed are essential to an understanding of the important problem of rancidity of fatty acid materials. Such studies have consequently concerned many investigators. Morris (84) had reviewed the recent studies on the mechanisms of fat oxidation and its relation to rancidity and has included a discussion of the applications of infrared spectra to this problem. While studies of *cis, trans*-isomerization occurring during oxidation have, as described, found infrared absorption spectra very useful, oxidation studies using this tool have not been limited to such *cis, trans*-configuration studies. A method which can identify and quantitatively follow the fate of such groups as hydroxyl, hydroperoxido, epoxy, carboxy, carbonyl, etc., affords many possible means to increase our knowledge of these complicated mechanisms. Only scant advantage has as yet been taken of these new opportunities.

Shreve, Heether, Knight, and Swern published two papers reporting a general survey of the infrared spectra of epoxidized and peroxidized acids and derived alcohols to serve as reference data in the application

of infrared spectral methods to the analysis of autoxidation mixtures (106, 107). Epoxidation of long chain internally monounsaturated compounds having a *cis*-configuration at the double bond causes an absorption, attributed to the oxirane ring, at about 12.0 microns. The ring in the corresponding *trans*-configuration gives rise to a band near 11.2 microns. If the oxirane compound is derived from a terminally unsaturated compound, bands appear near both wavelengths. As described earlier, Bickford, DuPré, Mack, and O'Connor (20) made application of this correlation in their work on the *cis, trans*-configuration of *alpha*- and *beta*-eleostearic acids. Hydroperoxides formed during autoxidation of unsaturated materials derived from fats and other substances were shown to give rise to characteristic bands also at about 12.0 microns. The characteristic —O—H stretching vibration at 2.8 microns, arising from —O—O—H groups, is also observed in the spectra of these compounds. Shreve and his coworkers comment that the potentialities of infrared absorption spectra can be realized only when more appropriate pure compounds have been measured and evaluated.

O'Connor, Mack, DuPré, and Bickford (90) described the infrared spectra of 10- and 12-hydroxystearic acids, their methyl esters and of 9,10-epoxystearic acids derived from oleic and elaidic acids. They reported absorptivities and correlations of the most prominent bands in these spectra with molecular groups most likely responsible for them. Infrared spectra were used to support a hypothesis explaining the formation of 10-hydroxystearic acid rather than a mixture of the 9- and 10-isomers upon hydrogenation of 9,10-epoxystearic acid, and to support an explanation for the gelling tendencies of 12-hydroxystearic acid as compared to the nongelling tendencies of 10-hydroxystearic acid.

The —O—H stretching band at 2.9 microns was used by Honn, Bezman, and Daubert (56) to study the autoxidation of linseed oil. A nonuniform increase in the intensity of this band is noted, very slow during an initial induction period, then very rapid as the various oxidation reactions proceed at accelerated pace. This increase in the intensity of the 2.9 micron band during autoxidation was accounted for by formation of hydroperoxide, —O—O—H, carboxyl —COOH, and alcohol or water ROH groups. By a combination of chemical and spectrophotometric methods the increase in concentrations of each of these groups was computed. Absorptivities were reported for three hydroxy acids. The fact that the intensities of the band would be greatly influenced by degree of hydrogen bonding does not seem to have been considered in calculations involving measurements at various concentrations.

The peracetic and performic acid oxidation of linoleic acid was studied by McKay, Levitin, and Jones (78). Besides the isomeric sativic acids two new oxidation products were isolated but only partially characterized. Infrared absorption spectra established that the compound formed by oxidation with performic acid contained hydroxy groups in the chain. A weak band at about 3.27 microns indicated either an unsaturated linkage or possibly a cyclopropyl group. The latter appears more probable as the spectra fail to show any evidence of unsaturation at either the region of C=C stretching, about 6.0 to 6.2 microns, or in the region of C—H bending about the C=C group between 10 and 11 microns. Using lithium fluoride optics to obtain higher resolution, Dugan, Beadle, and Henick (36) studied the infrared spectra of autoxidized methyl linoleate in the regions of —O—H stretching and C=O stretching vibrations, 2.8 to 3.0 microns and 5.6 to 6.05 microns, respectively. In the —O—H stretching vibration region two bands were observed, both increasing with increased peroxide value, one sharp and distinct at 2.88 microns, the other broad with maximum about 2.92 microns. Reduction of the oxidized samples with KI reagent resulted in the disappearance of the band with maximum at 2.92 microns and appearance of a new band with maximum at 2.86 microns. The band with maximum at 2.92 microns was attributed to the —O—O—H group associated by hydrogen bonding. The bands at 2.86 and 2.88 microns were attributed to —O—H stretching vibrations. This conclusion is different from that of Shreve *et al.* (107), who considered the hydroperoxide and the hydroxyl stretching vibrations to be identical. However only under conditions of highest resolution would the differences reported by Dugan, Beadle, and Henick be observed. Even at their higher resolution they report that absorption arising from keto and aldehyde carbonyl appeared only as indefinite shoulders on the strong bands due to ester carbonyl.

Chang and Kummerow (28) used infrared spectra to detect the ketonic carbonyl group at 5.83 microns in the presence of the ester carbonyl group at 5.75 microns, thereby settling a controversial point by proving that oxidation polymers of ethyl linoleate are linked through carbon to oxygen bonds rather than carbon to carbon bonds. Fractionated products of methyl esters of peanut oil fatty acids autoxidized at temperatures of 22 to 100° were studied by means of infrared spectra by Lemon, Kirby, and Knapp (75). In the region of —O—H stretching vibration a band develops in the early stages of autoxidation and was believed to be associated with the —O—O—H group. Later two bands at longer wavelengths appear, believed to be due to decomposition of the hydroperoxide to other compounds containing —O—H groups. Increasing the temperature increased the rate of both hydroperoxide formation and decomposition. The presence of iron stearate catalyzed only the decomposition. In the region of C=O stretching vibration three bands appeared and in the region of C—H bending about the C=C group two bands were found and assigned to diene conjugation and to *cis, trans*-isomerization. Catravas and Krafo (26) found from examination of infrared spectra that autoxidation of methyl oleate and methyl linoleate give unstable alcohols which are subsequently further oxidized to ketones.

Methyl linoleate hydroperoxide, produced by ultraviolet light catalyzed oxidation of methyl linoleate, was thermally decomposed in the presence of the methyl linoleate by Williamson (126). The thermal decomposition products, consisting of monomers, dimers, and trimers, were studied by means of infrared absorption spectra. The monomeric material consisted of hydroxyl and keto methyl linoleate. Dimers retaining a large proportion of the original unsaturation of the linoleate monomer but with half of the double bonds conjugated, and dimers linked by carbon to carbon bonds and having a low degree of unsaturation but containing an appreciable amount

of —O—H groups were described. The trimeric substances appeared to consist of two monomers linked by carbon to carbon bonds, then linked to another monomer by carbon to oxygen bonds.

Wheeler has also reported work on the thermal polymerization of esters of isomeric linoleic and linolenic acids (123). Dimerization of the nonconjugated linoleic isomers involves first conjugation of the double bonds, which then combine with a nonconjugated sompound through a Diels-Alder addition in which one of the nonconjugated double bonds acts as the dienophile. Trimers are probably formed by the conjugated diene reacting with a double bond of the dimer. *Cis, trans*-conjugated isomers polymerize about six times as rapidly as nonconjugated compounds, and *trans, trans*-conjugated isomers five times as rapidly as the *cis, trans*-conjugated isomers. Linolenates react in a similar manner, but the rate of polymerization is much more rapid than for the corresponding dienes.

The course of autoxidation of milk fat was followed by observation of changes in the infrared spectra of the volatile components by A. S. Henick (54). An off-flavor, off-odor sample of milk was steam-distilled *in vacuo,* and the infrared spectra of the distilled fractions were examined in CS_2 solution. At about 6.0 microns, bands appeared at 5.77, 5.81, 5.91, and 6.11 microns. The last two of these bands indicate conjugated ketones or aldehydes. (Aldehydes were eliminated by negative Schiff tests.) With this reference spectrum a sample of fresh milk was similarly studied as off-flavor and off-odor developed. The spectra of fresh milk exhibited only two bands in this region at 5.80 and 5.75 microns. Upon storage the 5.80 micron band remains unchanged, but the 5.75 micron band shifts to 5.70 microns. With longer storage new peaks appear at 5.86 and 5.91 microns, and still longer storage results in a band with maximum at 6.10 microns, which increases in intensity as the storage period is increased. Infrared spectrophotometry was found to be more sensitive to changes than a qualified taste panel and considerably more sensitive than peroxide value determinations.

Smith, Freeman, and Jack (112) obtained infrared absorption curves of monoethenoid methyl ester fractions of milk fat in the range C_{10} to C_{20} and compared them to available spectra of pure saturated and unsaturated esters of long chain fatty acids. Absence of a band at 2.8 to 2.9 microns indicated no oxidation to give hydroperoxidic hydroxyl formation. A band at 10.35 microns indicated *trans* bond formation, estimated from 14 to 27% in the various monoethenoid methyl esters. Spectra of the C_{18} to C_{20} fractions indicated conjugation entirely of the *cis, trans*-configuration. The C_{10} fraction exhibited infrared evidence for terminal bonds between the 9 and 10 carbon atoms.

Infrared Spectra of Drying Oils

The work of Gamble and Barnett (45), which has already been referred to, may be considered as the first use of infrared spectra to investigate the drying mechanism of oils. Several of the papers discussed in the section dealing with the use of infrared spectra in the study of autoxidation could also be considered as applications to drying oil chemistry.

In a preliminary study Kolb and Hauser (66) explored spectrographic methods of examination to determine their applicability in providing fundamental data on resin-bodying reactions. They found that neither Raman spectra (because of strong interfering continuous fluorescence) nor ultraviolet (because of no satisfactory differentiation) offered any promise. Infrared absorption spectra, it was concluded, particularly if measured at sufficiently high resolution, was a potentially valuable tool.

Adams and Auxier (1) reported changes in the infrared spectra of synthetic oil as a function of drying time. The intensity of a band at about 2.8 microns, attributed to formation of the hydroperoxide group, and of a band at 3.27 microns, assigned to a C—H stretching on a carbon atom adjacent to a double bond, were followed as drying proceeded. The 2.8 micron band increased in intensity and the 3.27 micron band decreased until finally a reasonably stable value was reached by each at about the same time. This is the effect to be expected if one band represents increase in hydroperoxide formation and the other decrease in unsaturation during the drying process. The slope of curves of intensity *versus* drying time was of the same order as drying rates. Linolenates had steeper slopes than linoleates, which were in turn steeper than oleates, again in agreement with known drying rates. This was interpreted as support for the theory that the carbon group *alpha* to a double bond is particularly susceptible to oxidation and that a methylene carbon between two double bonds is more so. Adams and Auxier were puzzled by a constant shift in the wavelength position of the band at about 2.8 microns. We now believe that this may be interpreted, not as a shift in wavelength position but as appearance of a new band at longer wavelengths arising from O—H stretching vibrations of other compounds formed during the autoxidation and incompletely resolved in their measurements. Adams, Auxier, and Wilson (2) interpreted their studies of the infrared absorption curves of dipentaerythritol esters of oleic, linoleic, and linolenic acids in a similar manner. They also found that the band at about 2.8 microns increased and the band at 3.27 microns decreased during the drying process and interpreted this observation as support for the theory that during the initial stages of autoxidation of drying oils the first step is the formation of hydroperoxides on the carbon atom *alpha* to the double bonds.

Nicholls and Hoffman (85) also studied the infrared spectra of pentaerythritol esters of linseed oil fatty acids, blown linseed oil, heat polymerized linseed oil, and other substances. They reported position of maxima and vibration correlations for several bands which they followed during the course of autoxidation. The O—H stretching band at about 2.9 microns progressively increased, indicating formation of O—H or O—O—H groups. The ester carboxy band at 5.75 microns increased and widened, indicating formation of compounds containing other C=O groups not completely resolved in their spectra from the ester carboxy groups. Increase in the intensity of a band at about 7.9 to 8.0 microns, ascribed to C—O stretching, was attributed to either an increase in ester concentration or to epoxide formation. Between 10 and 11 microns a band appeared which was attributed to either *trans* double bonds or to epoxide groups. The changes occurring during drying at increased temperatures or with the use of driers were very similar but more rapid.

In a report which showed that dilution with mineral oil permitted, in linseed oil, the polymerization

of acyl groups, Sims (108) presented complete infrared spectra of CCl_4 solutions of the heated and unheated oils. In the 10 micron region two bands are observed in the spectra of the unheated oil, at 10.3 and 10.7 microns, while the spectra of the heated oil exhibits only the 10.7 micron band. No bands at 10.1 microns (*trans, trans*-conjugation) nor at 10.2 and 10.5 microns (*cis, trans*-conjugation) are observed. The curves are interpreted as evidence that "all samples of heated oils except the zero hour sample contain *trans* double bonds" although the 10.3 micron band, isolated *trans,* is observed only in the spectra of the unheated sample. Kronstein (67) described the application of infrared spectroscopy to the study of drying oils and certain plastics. The spectra were limited to the 9- to 11-micron region, and no correlations of observed band maxima with vibrating groups were reported.

Crecelius, Kagarise, and Alexander (31) credit infrared spectroscopy, along with ultraviolet, with considerably stepping up progress toward understanding of drying oil oxidation mechanism. In their excellent review on the "Studies of the Mechanisms of Drying Oil Oxidation" they discuss in detail the interpretations of infrared spectra and changes in infrared spectra during drying.

The significance of the contributions of infrared absorption spectroscopy to drying oil mechanisms can be briefly summarized.

a) The appearance of bands at about 2.93 microns indicates formation of hydroperoxides. Appearance of additional bands at about this wavelength probably indicates alcoholic hydroxyl groups arising from further decomposition products during the oxidation.

b) The disappearance of the band at 3.2 microns indicates the replacement of the hydrogen on a double bond with some other radical, probably indicating polymerization.

c) The appearance of additional bands at about the 5.72 micron ester C=O stretching indicates the formation of aldehydes, ketones, or acids.

d) Changes in bands in the region 10 to 11 microns indicate *cis, trans*-isomerizations and probably formation of conjugated linkages.

Applications to Glycerides

Only three papers have been found describing the infrared spectra of glycerides. Kuhrt, Welch, Blum, Perry, and Weber (68) identified the monoglycerides isolated from lard and bread by means of infrared spectra, and Kuhrt, Welch, Blum, Perry, Weber, and Nasset (69) similarly used infrared to characterize the monoglycerides from triglycerides in the intestinal tract of humans. The infrared identifications were made essentially by the direct "fingerprint" comparison of spectra, but these authors include a rather complete table correlating the observed bands with vibrational groups giving rise to them.

O'Connor, DuPré, and Feuge (88) compared the infrared spectra of mono-, di-, and triglycerides. They found three regions of the spectra in particular which might be useful in the development of analytical procedures. The O—H stretching region at about 3.0 microns can be used to confirm the absence of mono- or diglycerides in a preparation of triglycerides by the complete absence of the characteristic O—H stretching vibration band. In the region of C—O stretching, about 9 microns, monoglycerides exhibit a characteristic band at 9.5 microns, and diglycerides a band with maximum at 9.6 microns. Triglycerides exhibit no bands at these wavelengths. Both of these bands were assigned to C—O stretching of *alpha* substituted secondary alcoholic groups, the difference in wavelength position of the maxima being accounted for by the difference in the *alpha* substituent groups. O'Connor and coworkers showed that qualitative detection and probably quantitative determination of mono-, di-, and triglycerides in admixtures could be made by use of these bands. In the region of C—H bending about *trans* C=C groups, above 10 microns, triglycerides of mixed long and short chain fatty acids, *i.e.,* acetoolein, dibutyroolein, etc., exhibit a band with maximum at 10.4 microns. The intensity of this band must be considered in applying the method of Shreve *et al.* (104) to determine the concentration of *trans*-isomers in such triglycerides by including, in the "background" corrections, values obtained from the spectra of the appropriate pure triglycerides.

Polymerization of Oil-Styrene Copolymers

Infrared spectra can be used to detect and determine concentration of oil content in oil-styrene copolymers by use of the strong ester C=O band at about 5.8 microns, a region where polystyrene is relatively transparent. Styrene can be detected and probably determined quantitatively by use of the benzenoid nuclei "breathing" bands at 13.2 and 14.3 microns. Brunner and Tucker (22) made use of both of these absorptions in their studies which showed interpolymer formation of styrene in the presence of tung oil but not in the presence of dehydrated castor oil. Bezman and Browning (19) used the 5.8 micron band quantitatively to measure the oil content of copolymers made in aqueous emulsions of styrene with linseed or soybean oils. They showed that untreated, nonconjugated oil does not form polymers but that copolymerization does occur in treated, *i.e.,* blown, thermally polymerized, or oxidized, oils containing conjugated linkages. They concluded that "in systems as complex as natural drying oils this" (*i.e.,* infrared spectra) "may indeed be the only reliable method for determining reaction of oil and changes in chemical groups."

The effect of several different fatty acids on the polymerization of styrene was investigated by Harrison and Tolberg (51). They established a multicomponent system for analysis of the benzoate, fatty ester, and polystyrene. Methyl and ethyl benzoate, measured by the band at 5.70 microns, were used as the standard for the benzoate. Methyl stearate was used for the fatty esters and measured at 5.75 microns. Heat polymerized carboxyl-free polystyrene was used for the polystyrene. Measurements were made at only the two carboxy frequencies as the contribution of polystyrene was small and constant. Methyl stearate and methyl olelate acted largely as diluents, slowing the rate slightly but not reducing the molecular weight of the polymer and not combining with the polymer to any appreciable extent. Methyl linoleate and linolenate acted as chain terminators, reducing the molecular weight and the rate of formation of the polymer but entering into the polymer only to the extent of 2 to 3 moles per mole of polymer. The conjugated linoleates copolymerized with the styrene, without affecting the molecular weight or rate of formation of the polymer, but

combining to the extent of 12 to 15 moles per mole of polymer.

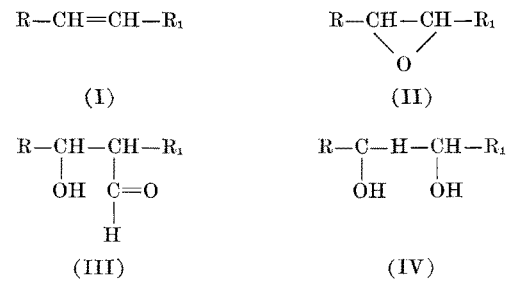
Applications to Lipides and Phospholipides

Baer (10) presented the spectra of three synthetic L-*alpha*-lecithins (dimyristoyl, dipalmitoyl, and distearoyl), which constitute a homologous series of pure phospholipides. For use in future comparisons he included wavelength positions of maxima, relative intensities and probable assignments for several of the more prominent bands. The infrared spectra of *alpha*-cephalins of the L-series were studied by Baer, Maurukas, and Russell (11). The spectra of distearoyl-, dipalmitoyl-, and dimyristoylcephalin were measured and several band correlations reported. The spectra are not identical, as in the case of the corresponding straight chain fatty acids, but several rather large and unexplained differences are found in the region from 11.5 to 14 microns.

The infrared spectra of lipoproteins isolated from human blood serum were compared with similar spectra of reference compounds including vegetable oil, ovalbumin, cholesteryl, egg lecithin, etc., by Freeman, Lindgren, Ng, and Nichols (42). Correlations of the observed bands with component molecules making up the lipoprotein were reported, and preliminary methods for quantitative analyses were suggested. Estimation of the intensity of the C=O ester carbonyl band can be used to determine lipide content; the band at 9.5 microns can be used to determine unesterified cholesterol; and the protein content can be estimated by consideration of the ratio of the intensities of the absorption bands at 5.8 and 6.1 microns. Again the need for more reference standards was emphasized. The infrared spectra of serum lipides from normal individuals and from young and old diabetics were compared by Renkonen and Koulumies (100) with the spectra of pure lipides. Considerable use of infrared spectra was made by Marinetti and Stotz (79, 80) in their studies of the structure of phospholipides. They report bands at 10.3 microns arising from the covalent phosphate P—O—C group in saturated lecithin and for the *trans* double bond and the P—O—C group in unsaturated phospholipides. A band at 9.2 microns was also believed to arise from P—O—C vibration and one at 8.2 microns from a C—O—C linkage. All phospholipides exhibited a band near 13.80 microns, due to long carbon to carbon chains, and a band at 14.4 microns, unassigned. Glycerophosphides exhibited the very strong ester C=O stretching band at 5.70 to 5.78 microns, and sphingolipides were characterized by a strong C=O stretching band at 6.1 microns. In addition, these latter compounds exhibited the N—H and O—H stretching bands in the region of 3.0–3.4 microns. The glycerophosphides and the sphingolipides can be determined quantitatively in admixture by measurements of the bands at 5.76–5.78 and 6.1 microns.

Intermediates which form during the hydroxylation of sphingomyelin were postulated by Marinetti and Stotz (79, 80), and their postulations were substantiated by means of infrared absorption spectra. These authors also studied the infrared spectra of sphingomyelin and related lipides in relation to the configuration of the double bond.

Performic acid oxidation is postulated to result first in formation of an epoxide at the double bond, then a hydroxyl and aldehyde group, and finally conversion by action of base to a dihydroxy:

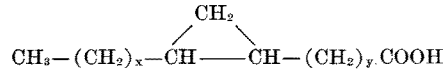


The infrared spectra of I or II exhibit no C=O at 5.81 microns, and III reveals such a band which disappears in IV. In the region of O—H stretching, 3.0 microns, no bands are observed in the spectra of I or II, but a band at 3.00–3.10 microns appears in the spectra of III and increases in intensity in IV. The spectrum of I exhibits a band at 10.3 microns, indicating that the double bond is a *trans* bond. However this band does not disappear on hydroxylation but only becomes less intense, indicating that a portion of the absorption is due to the P—O—C portion of the molecule which also gives rise to a band at 10.3 microns. Marinetti and Stotz studied the spectra of lipides which contain no interfering P—O—C linkage and found that the 10.3 micron band completely disappears on hydroxylation. They present the spectra of several lipides with a complete analysis of the bands and the groups giving rise to them.

The infrared spectra of dipalmitoleyl-L-*alpha*-glycerylphosphorglcholine from yeast was compared to that of its hydrogenation product, dipalmitoyl-L-*alpha*-lecithin, and to the spectra of palmitoleic acid by Hanahan and Jayko (47). The spectra of the two lecithins were similar, the unsaturated compound exhibiting a band at 14.7 microns not observed in the spectra of the saturated compound and attributed to a *cis* double bond. The infrared spectra of palmitoleic acid is very similar to that of oleic acid with a *cis* band at 13.17 microns, and no evidence of a *trans* bond at 10.36 microns.

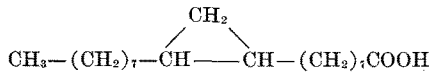
Elucidation of Molecular Structure by Means of Infrared Spectra

In the course of their search for the hypotensive principle of tung oil Davis, Conroy, and Shakespeare (34) separated a crystalline fraction by anion active ion exchange resin and low temperature crystallization. Infrared spectra were used, along with chemical data, to prove that the structure of this unsaturated hydroxylated monocarboxylic acid was 9,14-dihydroxy-10,12-octadecadienoic acid. Lactobacillic acid $C_{19}H_{36}O_2$, isolated by Hofmann, Lucas, and Sax (55), is a saturated acid yet contains 2 less hydrogens than a normal C_{19} saturated acid, suggesting a cyclic structure. Infrared absorption spectra revealed a band with maximum at 9.8 microns, characteristic of alkyl substituted cyclopropanes. Hence lactobacillic acid has the structure:



where $x + y = 14$. Similarly sterculic acid $C_{19}H_{34}O_2$, isolated by Nunn (86), was shown by appearance in

the infrared spectra of the band with maximum at 9.85 microns, to have the structure:

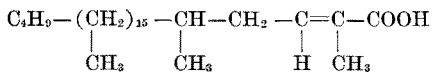


which is converted by ozonization to a 9,11-diketononadecanoic acid.

An infrared absorption curve was used by Khan (62) to observe, in his preparation of pure stearolic acid, the band due to the triple bond. A weak absorption only with a maximum at 4.42 was interpreted as indicating that the triple bond was flanked on either side by balanced groups:

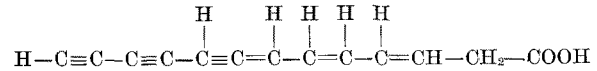
$$CH_3-(CH_2)_7-C\equiv C-(CH_2)_7COOH$$

Freeman (41) and Cason, Freeman, and Sumrell (24) resorted to infrared spectra to prove the structure of the C_{27} phthenoic acid isolated from crude methyl phthioate. Chemical and ultraviolet absorption data indicated that the acid was a 2-alkyl-2-alkenoic acid with a second substituent in the 4 or 5 position and a third substituent on a carbon more remote from the carboxyl. The infrared spectra exhibited a band at 6.08 microns, indicating a C=C group. The C=O band appeared at 5.91 microns, a position somewhat longer than the usual C=O stretching of saturated esters, indicating that the C=O was conjugated with the C=C group. Bands at 10.06, 12.50, 13.20, and 14.12 microns were attributed to C—H deformations about C=C groups, indicating that the C=C had at least one hydrogen atom attached to it. Comparisons with data reported earlier by Freeman (40) and the work of Sobotka and Stynler (113) on the spectra of isopalmitic and isostearic acid showed that there were no *iso* nor *neo* configurations. Possibility of a quaternary carbon atom more remote from the carboxyl than the *alpha* position is eliminated by absence of a band at 8.8 microns, and absence of bands at about 12.95 and 13.5 microns eliminated both ethyl and propyl groups. Consideration of bands at 7.8 and 8.1 microns and Freeman's study of the spectra of branched chain fatty acids (40) indicate a methyl group *alpha* to the carboxyl and a second methyl group not more removed than the *delta* position. Intensities of the methyl band at 7.3 microns indicate four methyl groups. From these considerations, coupled with chemical evidence, the structure of phthenoic acid was considered to be:



Walborsky, Davis, and Howton (122) used infrared spectra to confirm the absence of allene structure conjugated with an acetylenic group (5.13 microns) and the absence of *trans*-isomers (10.3 microns) in their total synthesis of linoleic acid. Friedberg, Marshall, and Newman (43) used infrared absorption spectra in an attempt to characterize an unknown acid isolated from brain extract by means of chromatography. Infrared spectra at the regions of C=O stretching (6.0 mcirons) and of C—H deformation about a *trans* C=C (10.0 microns) were studied in particular by Crombie (32) to verify the structure of *cis*- and *trans*-isomers of N-isobutylundeca-8:7 diene-1 carboxyamides.

A fascinating use of infrared spectra in the elucidation of molecular structure is the work of Celmer and Solomon (27) who, in a series of four papers, describe the proof of the structure of mycomycin and isomycomycin as:



and

$$CH_3-C\equiv C-C\equiv C-C\equiv C-CH=CH-CH=CH-CH_2-COOH,$$

respectively.

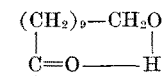
-3,5,n-tridecadiene-7,9,11-triyonic.

The essential steps in the use of infrared spectra in their proof of structure have been outlined by Wheeler (124).

Miscellaneous Applications of Infrared Spectra to Problems in Fatty Acid Chemistry

Sonntag, Trowbridge, and Krems (114) used infrared spectra to characterize fatty acid anhydrides prepared through the corresponding acid chlorides with acetic anhydride. Spectra indicated the quantitative absence of free fatty acids in each saturated anhydride. Oleic anhydride, from oleoyl chloride, prepared in turn from oxalyl chloride, exhibited a band with maximum at 5.8 microns, indicating a trace of oleic acid. Harrison and Daubert (50) prepared pure methyl isolinoleate and confirmed, by means of infrared absorption spectra, that this compound has one and probably two isolated *trans* double bonds. Autoxidation of methyl isolinoleate gave derivatives of saturated and unsaturated carbonyls similar to those obtained from soybean oil, indicating that isolinoleic acid may be one of the precursors of reversion compounds. Two of these autoxidation products, 2-hexanol and 2,6-decadienol, were identified by direct "fingerprint" comparison of the spectra of their dinitrophenylhydrozones with the dinitrophenylhydrozones of authentic samples. A sudy of iodolactonization of acids lead van Tamelen and Shamma (120) to suggest a method based on infrared spectra for the determination of the position of double bonds. A band at 5.6 microns in the infrared absorption spectra of the iodolactone indicates that the starting acid was probably *beta-gamma* or *gamma-delta* unsaturated. Absorption at 5.75 microns demonstrates the probable presence of a *delta-epsilon* double bond.

Thomas (119) showed that, even when the two groups are present in the same molecule as in *omega*-hydroxypalmitic acid, it is possible to determine the concentration of the alcoholic —OH and the carboxyl COOH groups quantitatively. In very dilute solution the O—H stretching vibration occurs at 2.74 microns, the position of free —OH. The —OH stretching of the COOH group however, even in very dilute solution, is bonded, probably as a single bridged dimer, and the band appears at 2.84 microns. *Omega*-hydroxyundecanoic acid, $CH_2OH\cdot(CH_2)_9COOH$, in chloroform solution exists as a dimer or complexes of at least three or four times the normal molecular weight. Even if sufficiently dilute, —OH bonding still exists (20%) according to Davies (33) by reason of intracyclization:



Ahlers and McTaggart (6) have suggested infrared spectroscopic methods for quantitative determinations

of hydroxyl, ketone, and ester groups and have described their use in the analysis of autoxidized materials. Hydrogen bonding effects are avoided by use of dilute solutions. Hydroxyl values, by the infrared method, agreed very well with determinations by an acetic anhydride method; ketone content agreed with a hydroxylamine technique; and ester carbonyl concentration was very close to values obtained by a chemical saponification procedure.

Conclusion

In every field of chemistry into which infrared absorption spectra have been making rapid strides in the solution of problems of identification and of quantitative analysis, advances have not been without some difficulties. The applications of infrared absorption spectra to fat and oil chemistry have been no exception, and the problems encountered have been mainly those found in its application to other fields.

One of the problems which has acted as a brake on the rapid advance of infrared spectra is the need for more and better spectral data on more highly purified compounds. As has been pointed out, many studies have indicated beyond doubt the potential value of infrared spectra in obtaining quantitative data, but actually only one or two specific quantitative methods have been described in detail. This lag has been due to lack of good quantitative data on essential compounds required to establish a precise detailed, quantitative method. Thus the infrared method for the determination of *trans* acids in the presence of monounsaturated *cis*-isomers and saturated components has already proven to be very useful in several applications. But the method is in a strict sense restricted to monounsaturated components. No quantitative data have been applied to a specific procedure for the determination of various *cis* and *trans* polyunsaturated conjugated and nonconjugated constituents although specific characteristic absorption bands to permit such determinations have been described. A similar lack of good quantitative data prohibits quantitative studies of drying oil reactions, autoxidation processes, etc.

Another problem which accompanies an increased application of infrared spectra is that of adequate resolution. As pointed out at various opportunities in this review, resolution greater than that available to many workers is required for several potentially useful applications. In the region of 3.4 microns, C—H stretching can be used to differentiate and probably quantitatively to determine such groups as methyl, methylene, and methenyl, but good resolution in this region will be required. Similarly, the differentiation and quantitative measurement of carbonyl groups such as ester, acid, aldehyde, ketone, quinone, and conjugated carbonyl by the C=O stretching band about 5.8 to 6.0 microns require very high resolution in this region. The suggested identification and measurement of various conjugated and nonconjugated *cis-trans* compounds by C—H bending vibrations at about 10.0 microns will also require good resolution out at this region of the spectra.

A third perplexing problem is the question of interchange of quantitative infrared absorption data between laboratories. Values of specific absorptivities from measurements on pure compounds in the infrared are critically dependent upon the entrance slit width and upon other physical characteristics of the particular instrument with which they were determined. Hence values obtained in one laboratory cannot be used in another laboratory for quantitative work of highest precision. Williams (126) has recently discussed this problem of interchange of infrared absorption data in considerable detail. Shreve (104) has made the practical suggestion that it may not be necessary for every laboratory to redetermine every absorptivity required for a specific quantitative determination. Redetermination of a critical coefficient and recalculation of the others by calibration should result in satisfactory agreement between laboratories.

The rapid manner in which infrared absorption techniques are finding useful applications in the field of fat and oil chemistry argues well that problems such as these will be solved and that the tool will continue to become more and more important in fatty acid research and in product control. We can conclude that the fatty acid chemist has caught up to Sir William Herschel, he has discovered infrared spectroscopy.

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Biological vs. Chemical Evaluation of Toxicity and Protein Quality of Cottonseed Meals[1]

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THE DESIRABILITY of rapid chemical tests for evaluating the toxicity and the protein quality of cottonseed meals in lieu of expensive and time-consuming biological methods can hardly be questioned. At the present time however, the chemical methods commonly used for such purposes have enjoyed questionable success.

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Toxicity. The earliest recorded statement on the harmful effect of cottonseed is attributed to Voelker in England in 1859 (1). In the intervening years many materials were blamed for the adverse findings in animals after cottonseed feeding until Withers and Carruth (2, 3, 4) and Carruth (5) published a series of papers between 1915 and 1918, attributing the toxicity of cottonseed to gossypol, a yellow polyphenolic pigment which originally had been isolated from cot-