Volatile Components of Bartlett Pear

Higher Boiling Esters

R. K. Creveling and W. G. Jennings

Bartlett pear volatiles, obtained by isopentane extraction of an aqueous essence, were separated by high-resolution gas chromatography. The components were characterized by infrared, mass, and where applicable, UV and NMR spectrometry. Micro-ozonolysis was used to establish the position of double bonds, where necessary, and when sufficient material was available. The volatiles included a wide variety of esters of methyl, ethyl, propyl, butyl, and hexyl alcohols, and C-10 to C-18 fatty acids with varying degrees of unsaturation. The interrelated structures support the concept that these pear volatiles share a common biogenesis.

Earlier studies on the volatile composition of Bartlett pear (Heinz and Jennings, 1966; Jennings, 1967; Jennings *et al.*, 1964; Jennings and Sevenants, 1964) were primarily concerned with the identification of the more abundant volatiles. There were indications that these pear essences contained many other compounds, especially higher boiling volatiles, many of which were present in such low concentrations that their occurrence was easily overlooked. This paper reports the results of these studies on the higher boiling compounds from the volatile essence of Bartlett pear.

MATERIALS AND PROCEDURE

An aqueous essence of Bartlett pear was obtained from a commercial processing company producing pear puree from ripe, unblemished fruits. This material was extracted with isopentane in a continuous counter-current extractor (Heinz *et al.*, 1966). After the major portion of the solvent was removed, the concentrated extract was subjected to preparative gas-liquid chromatography (GLC). The volatiles studied were those that emerged after ethyl *trans-2, trans-4*-decadienoate on the preparative Triton X-305 column (Heinz and Jennings, 1966). Since most of these compounds were present in very limited amounts, many repetitive collections were necessary to obtain sufficient sample for spectral characterization. In several instances, the composited samples were still too small for complete characterization.

EXPERIMENTAL

Apparatus. The GLC apparatus and techniques were essentially those described by Heinz and Jennings (1966), except for the final isolation and purification steps. These utilized a dual-column Aerograph 1520 gas chromatograph with a Carle high-temperature thermistor detector. Two 500-foot × 0.03-inch (I.D.) stainless steel, open-tubular, capillary columns were used (Teranishi and Mon, 1964), one coated with OV-17, and the other with SF 96 (50) admixed with 5% (w./w.) Igepal CO 880. In most instances, fractions obtained from the initial separation were rechromatographed directly on the capillary columns under isothermal conditions most suitable for the compound(s) in question. Helium flow rates through the capillary columns were adjusted to 5 ml. per minute.

The separated components were trapped in thin-walled glass capillaries as described previously (Jennings *et al.*, 1964). Infrared spectra were taken of thin films contained between sodium chloride plates on a Beckman IR-8 infrared spectrophotometer equipped with a 5× beam condenser. Calibration marks from a polystyrene film at 3026.27, 1601.0, 1027.7, and 906.5 cm.$^{-1}$ were routinely recorded on the infrared spectra. A dual-focusing Varian M66 mass spectrometer was used to obtain the mass spectral data, using the solid-sample probe to introduce these higher boiling compounds. The NMR spectrum was obtained on a Varian HA-100 spectrometer. The apparatus described by Beroza and Bierl (1967), with minor modifications, was used for micro-ozonolysis.

RESULTS AND DISCUSSION

Chromatograms typical of the initial separations have been shown in an earlier publication (Heinz *et al.*, 1966). Except as noted, compounds will be discussed in the order of their elution (as crude fractions) from the Triton X-305 column. Methyl and ethyl esters of the same acid moiety usually will be discussed together, even though the ethyl esters emerge later on GLC. Where the spectral data are not of the same quality for the two esters, the more definitive data will be used.

Ethyl *trans*-2, *trans*-4, cis-7?-Decatrienoate. (Figure 1). The mass spectrum has a molecular ion peak at *m/e* 194, and a peak at *m/e* 149 attributable to M − OCH_2CH_3. The infrared spectrum bears marked similarity to that of ethyl *trans*-2, *trans*-4-decadienoate (Heinz and Jennings, 1966). The appearance of *cis* out-of-plane deformation between 750 and 670 cm.$^{-1}$, and the lack of absorption at 965 cm.$^{-1}$, indicate that the additional isolated double bond is *cis*. There is no evidence for a terminal vinyl group; if three double bonds were in conjugation, the fingerprint regions of this compound and ethyl *trans-2, trans*-4-decadienoate would probably exhibit greater variation than they do. Ozonolysis yielded no aldehydes greater than C-3. These data indicate the double bonds are *trans*-2, *trans*-4- and *cis*-7 or *cis*-8. The assignment of the Δ^7 position is favored because of biochemical considerations to be discussed later.

Methyl and Ethyl *trans*-2, *cis*-6-Dodecadienoate. Figure 2

Department of Food Science and Technology, University of California, Davis, Calif. 95616

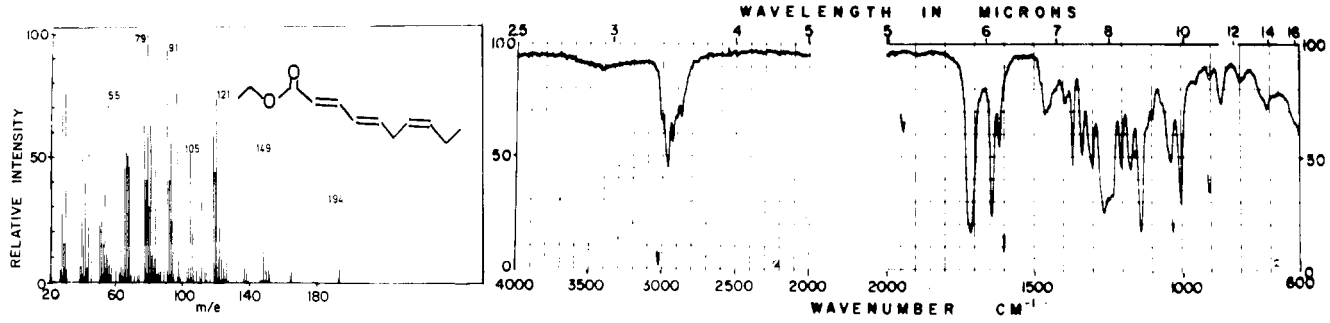

Figure 1. Mass and infrared spectra of ethyl *trans*-2, *trans*-4, *cis*-7?-decatrienoate

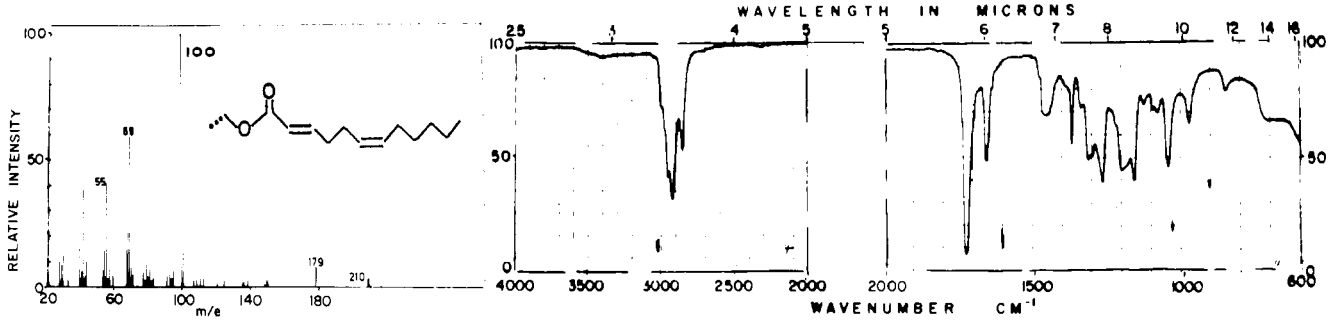

Figure 2. Mass spectrum of methyl *trans*-2, *cis*-6, dodecadienoate. The infrared spectrum (right) is of the corresponding ethyl ester

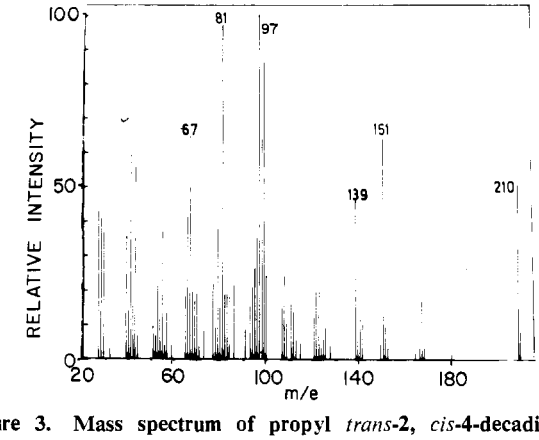

Figure 3. Mass spectrum of propyl *trans*-2, *cis*-4-decadienoate

(left) shows the mass spectrum of the methyl ester, with a molecular ion peak at *m/e* 210 and a peak at *m/e* 179 equivalent to M − OCH_3. The base peak at *m/e* 100 (*m/e* 114 in the ethyl ester) consisted of $C_5H_8O_2$, indicating 4,5 cleavage plus a hydrogen transfer to the carbonyl oxygen. The mass spectrum of the ethyl ester appears to be entirely analogous, with a molecular ion peak at *m/e* 224, and an M − OCH_2CH_3 peak at *m/e* 179. The infrared spectrum of the ethyl ester (Figure 2, right) is predictably similar to the spectrum of the methyl ester. The shift in C=O absorption to 1724 cm.$^{-1}$ is indicative of conjugation with the double bond signaled by the absorption at 1655 cm.$^{-1}$. The *trans* out-of-plane deformation band is displaced slightly to a higher frequency 972 cm.$^{-1}$, which indicates that the conjugated double bond is probably *trans*. Crombie (1952) observed that this shift

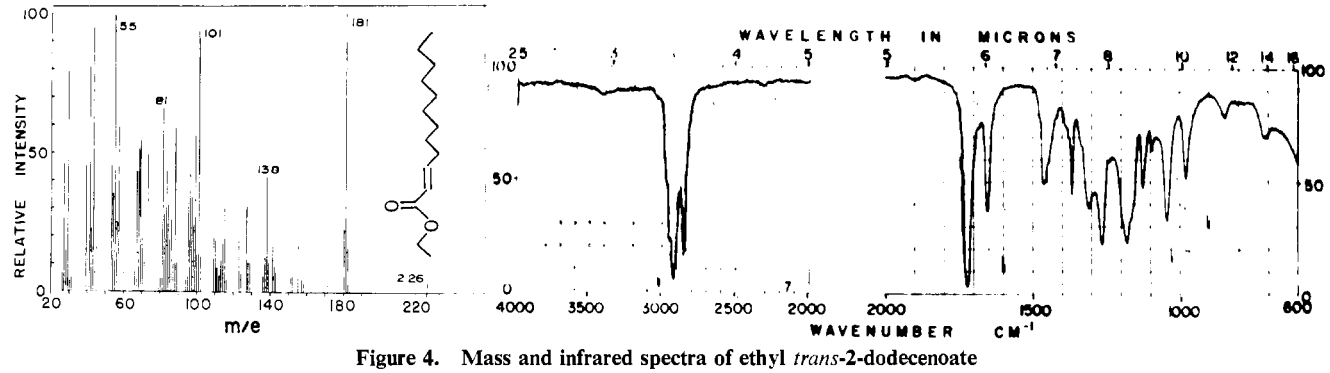

Figure 4. Mass and infrared spectra of ethyl *trans*-2-dodecenoate

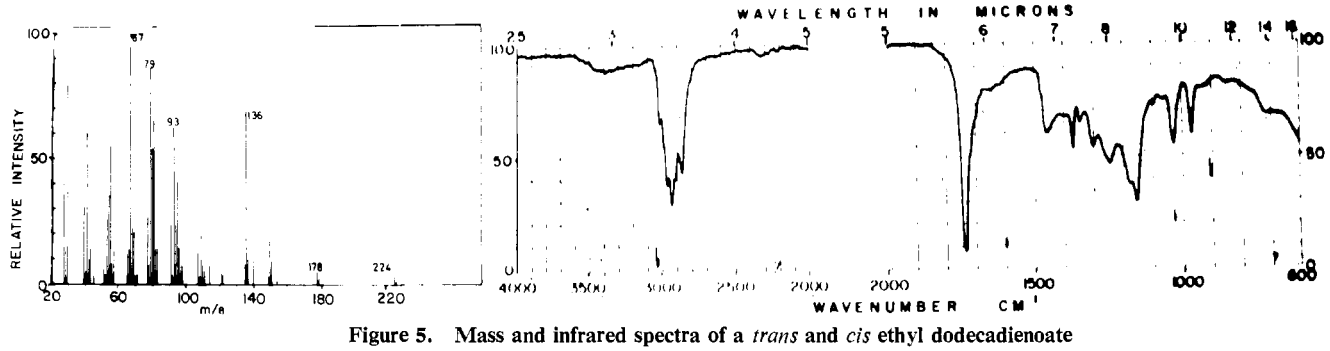

Figure 5. Mass and infrared spectra of a *trans* and *cis* ethyl dodecadienoate

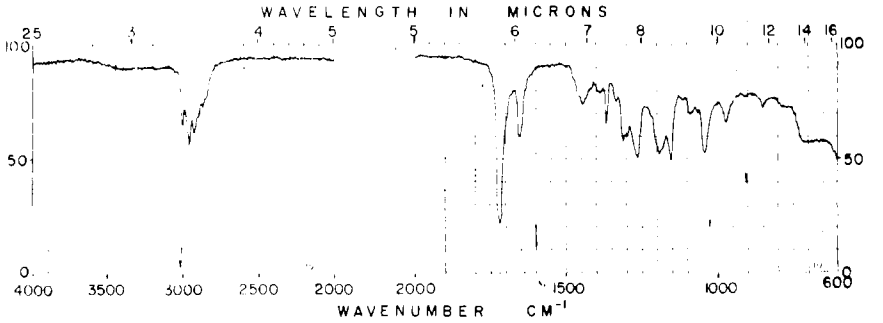

Figure 6. Infrared spectrum of an ethyl dodecatrienoate (?)

was less than anticipated in the case of the *N*-isobutylamide of *trans*-2, *cis*-8-dodecadienoic acid, in which the absorption occurred at 974 cm.$^{-1}$. According to Bellamy (1958) and Wheeler (1954), a *cis* double bond conjugated with a carbonyl group would have a characteristic absorption near 820 cm.$^{-1}$, which is not present in this spectrum. The broad band between 750 and 650 cm.$^{-1}$ indicates the presence of a *cis* double bond which, from the above considerations, is probably isolated. These same assignments also hold for the methyl ester. Ozonolysis yielded normal 6-carbon aldehydes from both esters, fixing the isolated *cis* double bond in the Δ^6 position.

Propyl *trans*-2, *cis*-4-Decadienoate. The mass spectrum (Figure 3) indicates a molecular ion peak at *m/e* 210 and an M − $O(CH_2)_2CH_3$ peak at *m/e* 151. The infrared spectrum is in agreement with that published by Jennings *et al.* (1964). On the basis of compounds isolated from pear essence hydrolysates, and on the odor of chemosynthesized products, they postulated that propyl, butyl, and hexyl esters of *trans*-2, *cis*-4-decadienoic acid were critically important to the aroma of Bartlett pear.

Methyl and Ethyl *trans*-2-Dodecenoate. The mass spectrum (Figure 4, left) indicates a molecular weight of 226 for the ethyl ester. The *m/e* 181 peak agrees with M − OCH_2CH_3. The mass spectrum of the methyl ester is in good agreement, indicating a molecular ion peak at *m/e* 212 and a M − OCH_3 peak at *m/e* 181. The infrared spectrum of the ethyl ester has a C=O absorption band at 1725 cm.$^{-1}$ indicating conjugation with the double bond absorbing at 1655 cm.$^{-1}$. The absorption at 981 cm.$^{-1}$ confirms the *trans* configuration and that the conjugation has shifted it to a higher frequency.

Ethyl Dodecadienoate. This compound, which was incompletely characterized, has a mass spectrum (Figure 5) indicating a molecular ion peak at *m/e* 224, and *m/e* 178 and 179 peaks consistent with M − $HOCH_2CH_3$ and M − OCH_2CH_3, respectively, although somewhat low in intensity. This lower intensity, which occurred at the end of the scanning period, was probably due to exhaustion of the sample. The infrared spectrum is indicative of an unconjugated ester (C=O at 1738 cm.$^{-1}$) with both *trans* (966 cm.$^{-1}$) and *cis* (752–670 cm.$^{-1}$) unsaturation. Several double bond positions could satisfy these data. Unfortunately, the fraction was small; and attempts at ozonolysis were unsuccessful.

Ethyl *trans*-2, *cis*-6, *cis*-9?-Dodecatrienoate. The strength of the 3010 cm.$^{-1}$ band in the infrared spectrum (Figure 6) indicates that this ester is more highly unsaturated than the ethyl *trans*-2, *cis*-6-dodecadienoate (Figure 4). The C=O absorption at 1722 cm.$^{-1}$ indicates conjugation with the double bond signaled at 1654 cm.$^{-1}$. The absorption band at 972 cm.$^{-1}$ is probably due to a conjugated *trans* double bond, as discussed above. The 740–680 cm.$^{-1}$ absorption indicates

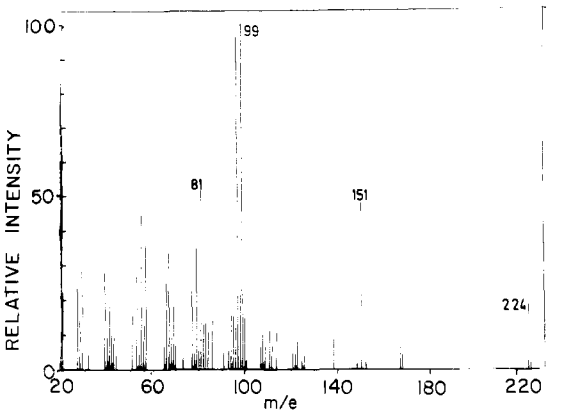

Figure 7. Mass spectrum of butyl *trans*-2, *cis*-4-decadienoate

cis double bonding. A mass spectrum was not obtained for this compound, nor was there sufficient material for ozonolysis. Ethyl *trans*-2, *cis*-6-dodecadienoate and this compound exhibited the same retention time on the SF 96(50) column, but the postulated trienoate emerged later on the more polar OV-17 column. These same retention characteristics occurred when ethyl *trans*-2, *trans*-4-decadienoate and ethyl *trans*-2, *trans*-4, *cis*-7?-decatrienoate were chromatographed.

Butyl *trans*-2, *cis*-4-Decadienoate. The infrared spectrum matches that published for the synthetic compound (Jennings *et al.*, 1964). Mass spectral data (Figure 7) indicate a molecular ion peak at *m/e* 224 and an M − $O(CH_2)_3CH_3$ peak at *m/e* 151, values consistent with this structure.

Methyl and Ethyl *cis*-5, *cis*-8-Tetradecadienoate. The mass spectral data indicate molecular ion peaks at *m/e* 238 and 252 for the methyl (Figure 8, left) and ethyl esters, respectively. Peaks at *m/e* 206 and 207 represent losses of OCH_3 and $HOCH_3$, respectively, in the methyl ester or losses of OCH_2CH_3 and $HOCH_2CH_3$ in the ethyl homolog. Rather surprising are the peaks at *m/e* 189, which were found to represent $C_{14}H_{21}$ ions, and which indicate loss of H_2O from the acylium ion as postulated by McLafferty (1967). Such a fragment is observed in the spectrum of methyl *cis*-5, *cis*-8-octadecadienoate at *m/e* 245 (Christie and Holman, 1967). The infrared spectrum of the methyl ester (Figure 8, right) reveals C=O absorption at 1740 cm.$^{-1}$, indicating nonconjugation, a band at 1435 cm.$^{-1}$ indicative of methyl ester, and broad *cis* absorption between 750 and 650 cm.$^{-1}$. Ozonolysis yielded a normal 6-carbon aldehyde, fixing the position of the second double bond at the Δ^8 position. The NMR spectrum of the ethyl ester reveals an isolated, two-proton triplet centered at 2.73 p.p.m., which can be assigned to the protons of the methylene group between the two double bonds. The triplet (actually, two overlapping doublets) compares favorably in position and shape to this particular assignment

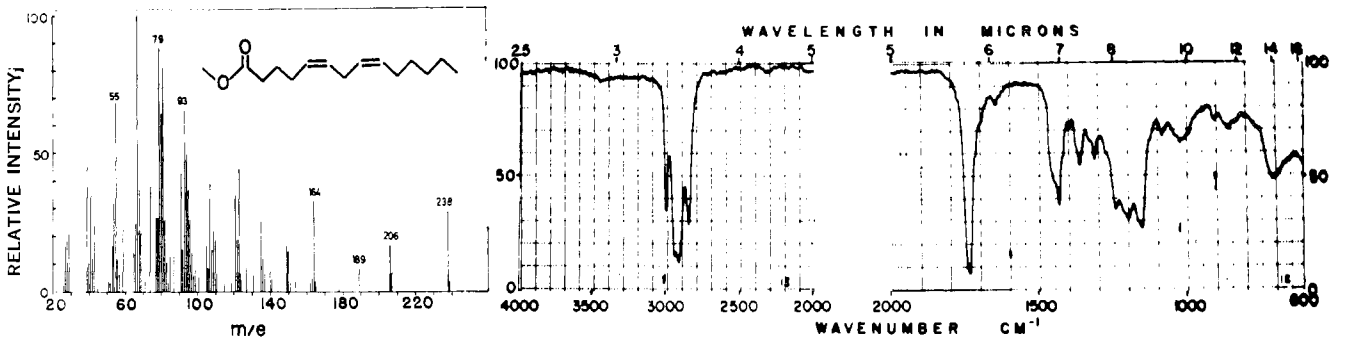

Figure 8. Mass and infrared spectra of methyl *cis*-5, *cis*-8-tetradecadienoate

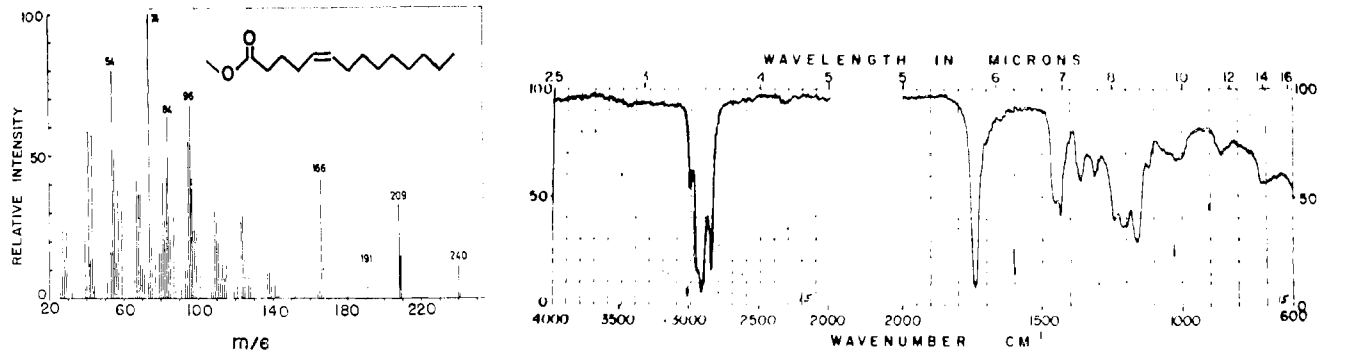

Figure 9. Mass and infrared spectra of methyl *cis*-5-tetradecenoate

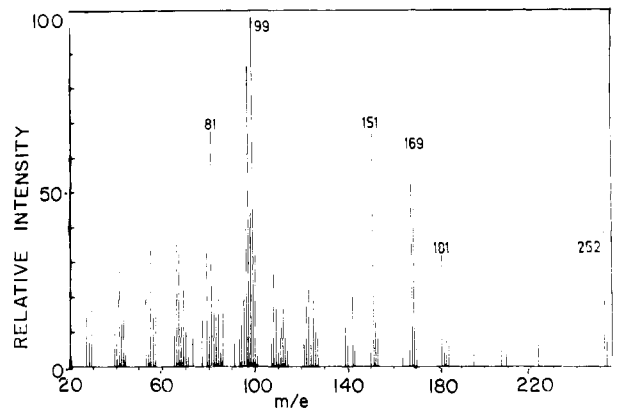

Figure 10. Mass spectrum of hexyl *trans*-2, *cis*-4-decadienoate

in the NMR spectrum of methyl linoleate (Christie and Holman, 1967), and fixes the first double bond at the Δ^5 position.

Methyl and Ethyl *cis*-5-Tetradecenoate. Figure 9 represents mass and infrared spectra of the methyl ester. The mass spectrum has a molecular ion peak at *m/e* 240 and an M − OCH_3 peak at *m/e* 209. The mass spectrum of the ethyl ester has a molecular ion peak at *m/e* 254 and an M − OCH_2CH_3 peak at *m/e* 209. Again, as in the *cis*-5, *cis*-8-tetradecadienoate esters, there are M − 49 and M − 63 fragments for the methyl and ethyl esters, respectively. The base peak in each case arises from the classical McLafferty rearrangement at *m/e* 74 and *m/e* 88 for the methyl and ethyl esters, respectively. Both compounds have *cis* absorption in the infrared between 750 and 650 $cm.^{-1}$, with no evidence of *trans* double bonding. Ozonolysis of the ethyl ester yielded a normal nine-carbon aldehyde, fixing the double bond position at Δ^5.

Ethyl Tetradecanoate. The infrared and mass spectra of this compound agree with those obtained from the authentic compound.

Hexyl *trans*-2, *cis*-4-decadienoate. The structural assignment postulated for this compound is based upon the similarities of its mass spectrum (Figure 10) with the mass spectra of the propyl and butyl esters of *trans*-2, *cis*-4-decadienoic acid (Figures 3 and 7). Diagnostic peaks are at *m/e* 252 (M); *m/e* 151 [M − $O(CH_2)_5CH_3$]; *m/e* 181, probably due to 5,6 cleavage and comparable to the *m/e* 139 and 153 peaks found in the propyl and butyl esters, respectively; and *m/e* 169 and 168, which could arise from loss of the alkyl portion of the alcohol moiety, with accompanying transfer of one and two hydrogen atoms to form the carboxylic acid ion and

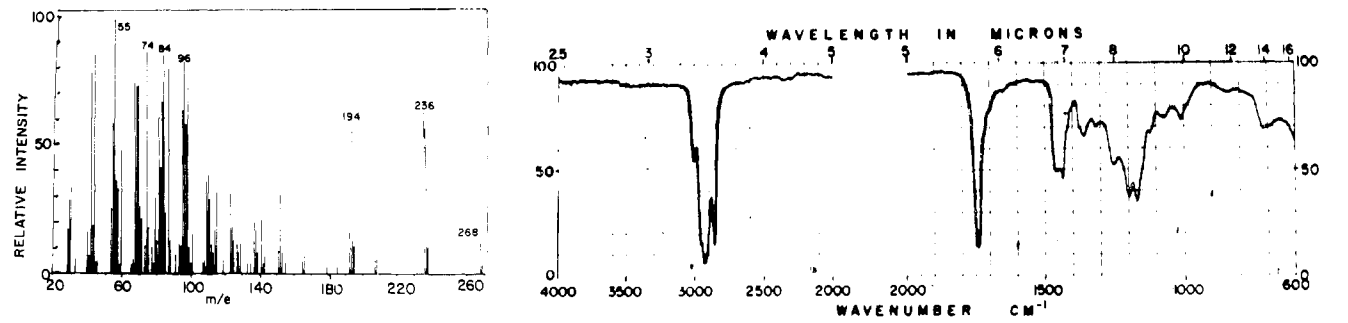

Figure 11. Mass and infrared spectra of a *cis* methyl hexadecenoate

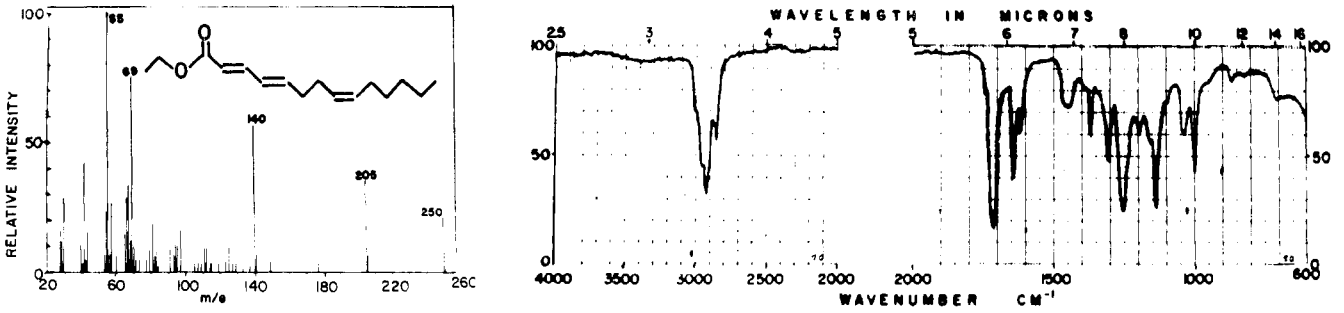

Figure 12. Mass and infrared spectra of ethyl *trans*-2, *trans*-4, *cis*-8?-tetradecatrienoate

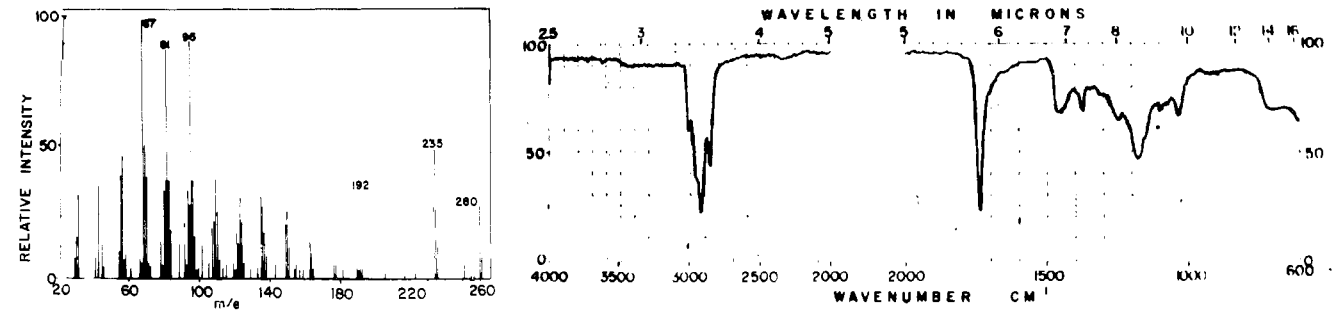

Figure 13. Mass and infrared spectra of *cis-cis* ethyl hexadecadienoate

the protonated carboxylic ion, respectively (Budzikiewicz *et al.*, 1967). Amounts isolated were not sufficient for infrared analysis.

Methyl Hexadecenoate. Figure 11 represents the mass and infrared spectra of an incompletely characterized ester. The molecular ion peak at *m/e* 268, and the peaks at *m/e* 236 and 237, are consistent with a methyl ester. The infrared spectrum reveals C=O absorption at 1742 cm.$^{-1}$; unsaturation is signaled at 3005 cm.$^{-1}$ and again weakly at 1650 cm.$^{-1}$. Bands at 1460 and 1435 cm.$^{-1}$ are consistent with a methyl ester, and the broad band between 650 and 760 cm.$^{-1}$ indicates *cis* unsaturation. Attempts at ozonolysis were unsuccessful.

Methyl Hexadecanoate. This material, a white solid at room temperature, has a molecular ion peak in its mass spectrum at *m/e* 270, and a peak at *m/e* 239 corresponding to M − OCH_3. Both the mass and infrared spectra agree with those of the authentic compound.

Methyl and Ethyl *trans*-2, *trans*-4, *cis*-8?-Tetradecatrienoate. The mass spectrum of the ethyl ester (Figure 12, left) indicates a molecular ion peak at *m/e* 250 and an M − OCH_2CH_3 peak at *m/e* 205; the methyl ester has a molecular ion peak at *m/e* 236 and an M − OCH_3 peak at *m/e* 205. The infrared spectrum of the ethyl ester reveals a C=O shift to 1715 cm.$^{-1}$ and absorptions at 1642 and 1618 cm.$^{-1}$. Such a spectral configuration could be accounted for by two conjugated double bonds in conjugation with the carbonyl. The band at 1000 cm.$^{-1}$ indicates a highly conjugated *trans* system comparable to the *trans*-2, *trans*-4-decadienoates reported by Heinz and Jennings (1966). There is also evidence of *cis* absorption between 760 and 660 cm.$^{-1}$, and an absence of isolated, *trans* double-bond absorption at 966 cm.$^{-1}$. The ultraviolet spectrum has a maximum at 257 nm., indicating that only two of the double bonds are in conjugation with the carbonyl. The position of the isolated *cis* double bond could have been firmly established by ozonolysis if sufficient material had been available. Both mass spectra had strong M − 110 peaks which could be the result of 6,7 cleavage coupled with transfer of a hydrogen atom from the acid tail. If the *cis* double bond were located in the Δ^8 position, allylic cleavage between carbon atoms 6 and 7 would be favored. Such cleavage would be analogous to the fragmentation observed in the *trans*-2, *cis*-6-dodecadienoates, where the cleavage was apparently accompanied by a hydrogen transfer from the acid tail.

Hexyl *trans*-2, *trans*-4-Decadienoate? Only traces of the compound of this presumed structure were isolated. The mass spectrum was of low intensity, but similar to that of the *trans*-2, *cis*-4-isomer previously discussed. Earlier work with the methyl esters of various isomers of this acid moiety (Heinz and Jennings, 1966) revealed the *trans*-2, *trans*-4-isomer to have a longer GLC retention than the *trans*-2, *cis*-4-isomer.

Ethyl Hexadecadienoate. The mass spectrum (Figure 13, left) indicates a molecular ion peak at *m/e* 280 and an M − OCH_2CH_3 peak at *m/e* 235. The infrared spectrum (Figure 13, right) is typical of a nonconjugated, *cis* unsaturated, straight chain, fatty acid ester.

Ethyl Hexadecenoate. This compound is probably the ethyl ester of the methyl *cis*-hexadecenoate described previously. Sample size limited analysis to mass spectroscopy. The data revealed a molecular ion peak at *m/e* 282, with major fragments at *m/e* 237 (M − OCH_2CH_3), 236 (M − $HOCH_2CH_3$), 194 (M − 88), and 88 (McLafferty rearrangement ion). The lack of a prominent *m/e* 127 peak indicates that the double bond is not conjugated with the carbonyl group (Budzikiewicz *et al.*, 1967).

Methyl *cis*-9-Octadecenoate. The mass spectrum of this compound indicates a molecular ion peak at *m/e* 296, and corresponds well with the mass spectrum of an authentic sample. The infrared spectra of the known and unknown matched precisely.

Methyl Octadecanoate. Both GLC retention characteristics and mass spectral data for this fraction, a white solid at room temperature, are in agreement with those of the authentic compound.

Heinz and Jennings (1966) postulated that several biochemical pathways might contribute to the 8- and 10-carbon

Table I. Fatty Acid Moieties Isolated from Ripe Bartlett Pear Volatiles

Length, Carbon Atoms	Degree and Type of Unsaturation[a]			
	...	Mono	Di	Tri
2	S	...	...	...
8	S[b]	t:2	...	...
10	S	t:2 & C:4	t:2–C:4 & t:2–t:4	t:2–t:4–C:(7?)
12	S	t:2	t:2–C:6 & t:(?)–C:(?)	t:2–C:6–C:(9?)
14	S	C:5	C:5–C:8	t:2–t:4–C:(8?)
16	S	C:(7?)	C:(?)–C:(?)	
18	S	C:9		

[a] S, saturated; t, *trans;* C, *cis;* ?, assignment tentative, uncertainty limited to parenthetical portion. [b] The β-hydroxy octanoate has also been isolated.

fatty acid moieties found in their investigations of Bartlett pear essence. Bartlett pears are normally harvested green, stored near 0° C. for a period of 6 to 45 days, then ripened at 20° C. because of the superior flavored fruits produced by this treatment (Claypool *et al.*, 1958). Lower environmental temperatures are reported to cause plant tissues to produce more of the unsaturated fatty acids and less of their saturated counterparts (Zill and Cheniae, 1962; Mudd, 1967). Romani *et al.* (1965) observed that C-18:2 and C-18:3 fatty acids in pear mitochondrial particles decreased in relation to the C_{16} and C_{18} saturated fatty acids during the 20° C. ripening period. Heinz *et al.* (1965) showed that the Δ^2–Δ^4 acid moieties were produced almost entirely subsequent to the preclimateric minimum, which signals the onset of ripening after a fruit is removed from cold storage.

Table I shows the fatty acid moieties of volatile esters isolated from ripe Bartlett pears in this and earlier studies (Jennings *et al.*, 1964; Jennings, 1967; Heinz and Jennings, 1966). Only straight-chain, even-numbered-carbon-atom acid moieties have been observed. Most of the double bond positions have been established, and are consistent with those that would be derived from β-oxidation (Mudd, 1967) of the unsaturated fatty acids found in pear mitochondrial particles (Romani *et al.*, 1965). The differences between the mitochondrial fatty acids and those of the volatile esters occur in the length of the aliphatic chains, and in the double bond positions, relative to the carboxylic group. The authors have used this evidence to make tentative assignments (indicated by "?") of double bond positions which were not established by other means.

These findings would be consistent with the following sequence: During cold storage there may be an accumulation of the unsaturated 18-carbon fatty acids. As a fruit ripens at 20° C., the fatty acids are volatilized by esterification instead of continuing in the "normal" metabolic route(s). The fatty acid moieties found in this work could well be β-oxidation intermediates which have been reesterified. This laboratory is currently involved in attempts to determine the pathways involved in the biogenesis of pear volatiles.

ACKNOWLEDGMENT

The authors are grateful to R. M. Silverstein, Stanford Research Institute, for his assistance in obtaining an NMR spectrum, and for valuable advice on micro-ozonolysis.

LITERATURE CITED

Bellamy, L. J., "The Infrared Spectra of Complex Molecules," p. 48, John Wiley & Sons, Inc., New York, 1958.
Beroza, M., Bierl, B. A., *Anal. Chem.* **39,** 1131 (1967).
Budzikiewicz, H., Djerassi, C., Williams, O. H., "Mass Spectrometry of Organic Compounds," pp. 184, 194, Holden-Day, Inc., San Francisco, 1967.
Christie, W. W., Holman, R. T., *Chem. Phys. Lipids* **1,** 407 (1967).
Claypool, L. L., Leonard, S., Luh, B. S., Simone, M., *Food Technol.* **12,** 375 (1958).
Crombie, L., *J. Chem. Soc.* (London), 2997 (1952).
Heinz, D. E., Creveling, R. K., Jennings, W. G., *J. Food Sci.* **30,** 641 (1965).
Heinz, D. E., Jennings, W. G., *J. Food Sci.* **31,** 69 (1966).
Heinz, D. E., Sevenants, M. R., Jennings, W. G., *J. Food Sci.* **31,** 63 (1966).
Jennings, W. G., Chem. Peaches and Pears, in "Chemistry and Physiology of Flavors," pp. 419–430, Avi Publishing Co., Westport, Conn., 1967.
Jennings, W. G., Creveling, R. K., Heinz, D. E., *J. Food Sci.* **29,** 730 (1964).
Jennings, W. G., Sevenants, M. R., *J. Food Sci.* **29,** 158 (1964).
McLafferty, F. W., "Interpretation of Mass Spectra," p. 146, W. A. Benjamin, Inc., New York, 1947.
Mudd, J. B., *Ann. Rev. Plant Physiol.* **18,** 229 (1967).
Romani, R. J., Breidenbach, R. W., van Kooy, J. G., *Plant Physiol.* **40,** 561 (1965).
Teranishi, R., Mon, T. R., *Anal. Chem.* **36,** 1490 (1964).
Wheeler, D. H., Infrared absorption spectroscopy in fats and oils, in "Progress in the Chemistry of Fats and Other Lipids," Vol. 2, pp. 268–291, Academic Press, New York, 1954.
Zill, L. P., Cheniae, G., *Ann. Rev. Plant Physiol.* **13,** 255 (1962).

Received for review May 12, 1969. Accepted October 8, 1969. This project was supported in part by Research Grant UI 00276 from the National Center for Urban and Industrial Health.