# VOLATILES FROM STRAWBERRIES

## II. COMBINED MASS SPECTROMETRY AND GAS CHROMATOGRAPHY ON COMPLEX MIXTURES

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Extraction of hundreds of pounds, or even tons, of natural products for determination of the chemical constituents of odors and flavors generally yields only a few milligrams of complicated essence. The extracts will often contain more than 150 components having wide variations in chemical functionality. Such mixtures can usually be separated satisfactorily only by capillary gas chromatography, and the submicrogram quantities thus purified are not easily collected and identified.

The most promising method for obtaining analytical information on such mixtures appears to be the direct introduction of the chromatographic eluate into a fast-scan mass spectrometer. Recent developments in this field have demonstrated that sufficiently good mass spectra can be obtained even when scanning is limited to seconds and only submicrogram quantities enter the machine[1-3].

Research on a strawberry essence revealed three important features of this type of analysis. First, the extent of mass spectral interpretation was shown by the large number of compounds easily identified, and the limitation by the large number not identified. Second, the presence of two or more components in a single chromatographic peak was revealed by changes in the mass spectra of successive scans. Third, pump-out of the mass spectrometer is shown to be fast enough to cause no decrease in chromatographic efficiency for oxygenated materials eluted at 200°.

The identification of many of the strawberry oil components was, in itself, of considerable fundamental interest.

EXPERIMENTAL METHODS AND RESULTS

Part I of this series describes the method of concentrating to a few milliliters the ten tons of vapor condensate from strawberry jam pot stills[4]. The chromatographic equipment has been described[5], and several papers have outlined the techniques used to combine a capillary chromatograph with a fast-scan mass spectrometer[6-8]. In the present work, the simple method of direct introduction was employed, in which the capillary column operates with the exit at a pressure of about $10^{-3}$ torr and the inlet at a pressure reduced by one atmosphere from the usual inlet pressure. Comprehensive studies have shown that this method does not lead to any significant loss of chromato-

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graphic efficiency[9]. The eluate was monitored by observing the mass spectral pattern
on an oscilloscope and by recording the ionization due to mass 15 ($CH_3^+$ formed by
electron impact fragmentation) as a chromatogram.

The chromatogram of the strawberry oil (Fig. 1) was obtained with a program-
med-temperature capillary column, 200 ft. long and 0.01 in. I.D., coated with Tween 20
(Atlas Powder Co.)*. Normal exit pressure was 1 atm. A ^{90}Sr argon detector was used.

A chromatogram obtained during a mass spectral run using the ionization due
to $CH_3^+$ is not presented here because different amounts of $CH_3^+$ were formed by
different substances. Such a chromatogram is necessary, however, in order to match
the mass spectral charts with the peaks on the standard chromatogram. Several
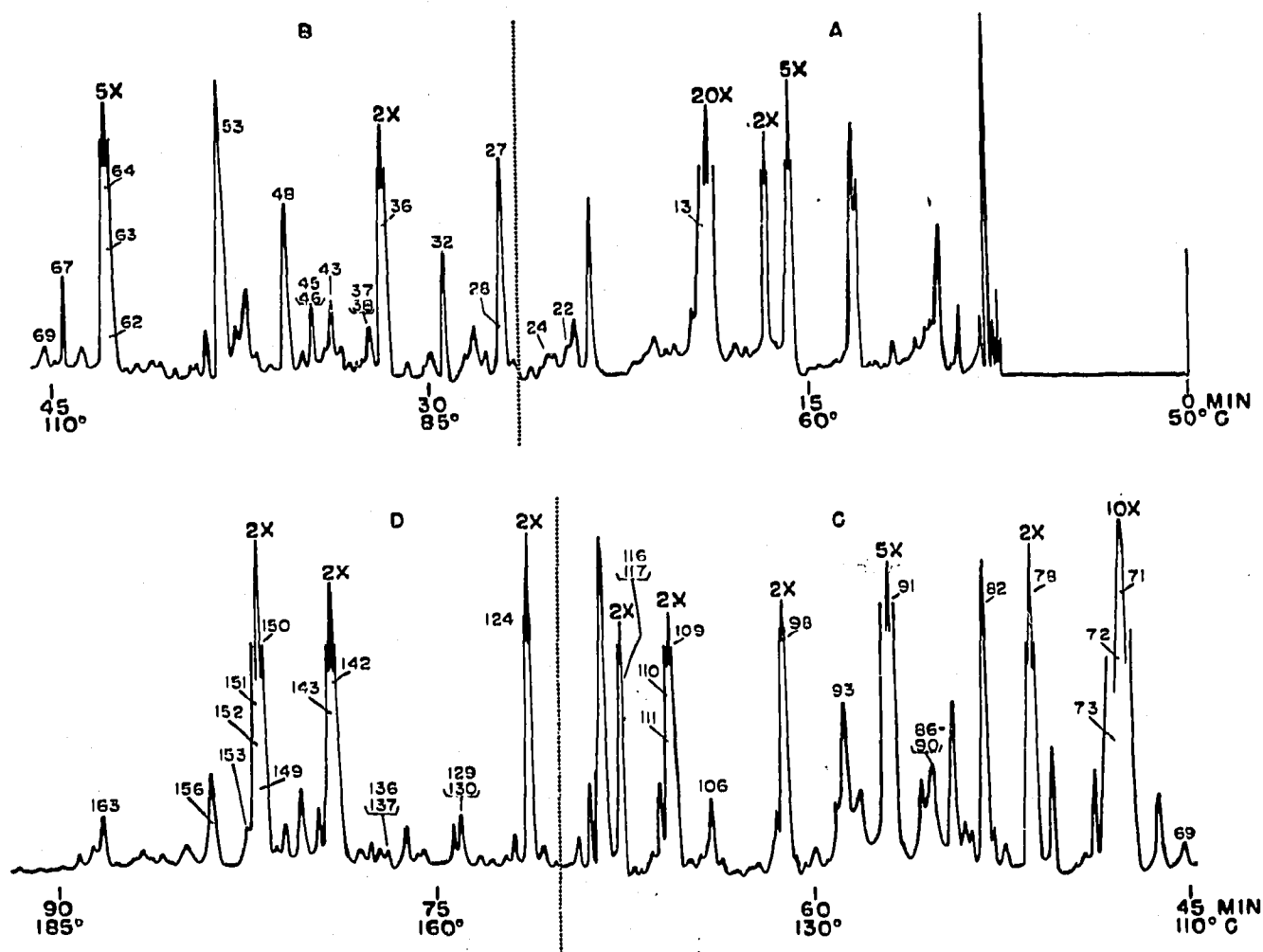such chromatograms have been shown in previous studies[4,10,11].



Fig. 1. Chromatogram of strawberry oil. Capillary column, 200 ft. long and 0.01 in. I.D., coated
with Tween 20. The numbers identify the components of Table I.

Each component observed by gas chromatography (GC) and/or mass spectro-
metry was assigned a number. If a peak contained more than one component as
revealed by mass spectrometry, each component was numbered. For a few of the

* Reference to a company or product name does not imply approval or recommendation of
the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

very dilute components, a chromatographic peak may have been observed but no mass spectral chart taken, or, alternately, a weak mass spectral chart may have been obtained but no chromatographic peak observed. These peaks also have generally been assigned numbers. (Zone A in Fig. 1 does not adhere to these rules. Because it was published earlier, the numbers used at that time are retained.)

Although all peak numbers are not printed in Fig. 1, it is easy to assign each peak its identification listed in Table I which presents the compounds not listed in the previous publication. (Peak 13 contains about 65 % diethoxyethane and 35 % ethanol. Mention of ethanol was accidentally omitted in Part I[4].) In addition to the components apparent in Fig. 1, about 15 minor components were eluted up to 230°. Those that were identified are also listed in Table I.

Three classes of identification are used to illustrate the certainty of analysis. The first is a good comparison of the unknown spectrum with the spectrum of an authentic compound, and also a match of the GC retention time of authentic compounds. Experience has shown that identification of even a simple mixture by its mass spectrum alone may be in error. A retention-time check is desirable whenever possible. In the work presented here, all retention times were obtained by enriching the strawberry oil with the authentic compound and running the sample through the entire temperature program. Thus, modest variations in experimental conditions

TABLE I

COMPOUNDS IDENTIFIED IN STRAWBERRY OIL*

| Peak No. | Mass spec. and GC retention | Mass spec. confirmation | Mass spec. indication |
|---|---|---|---|
| 13 | Ethanol <br> 1,1-Diethoxyethane | | |
| 22 | | | 1,1-Diethoxypropane <br> 1,1-Methoxybutoxyethane |
| 24 | 1,1-Ethoxypropoxyethane | | |
| 27 | 2-Methylbutane-2-ol | | |
| 28 | Methyl α-methylbutanoate | | |
| 29 | | | Isoamyl formate <br> Amyl formate |
| 31 | | 1,1-Ethoxybutoxyethane | |
| 32 | Ethyl n-butyrate | | |
| 33 | | 1,1-Methoxybutoxyethane | |
| 36 | Ethyl α-methylbutanoate | | |
| 37 | n-Butyl acetate | | |
| 38 | | | 1,1-Diethoxybutane |
| 42 | | | 2-Hydroxy-3-methylbutane |
| 43 | | | Ethyl isovalerate |
| 45 | | 1,1-Ethoxybutoxyethane | |
| 46 | | | 1,1-Methoxypentoxyethane |
| 47 | | 1,1-Methoxypentoxyethane | |
| 48 | | | 2-Hexyl acetate |
| 49 | | | 2-Pentanol |
| 50 | | 1,1-Diethoxypentane | |
| 51 | | n-Butanol | |
| 52 | Isoamyl acetate | | |
| 53 | | 1,1-Ethoxypentoxyethane | |
| 62 | | (3-Methyl-1-butanol) | |
| 63 | | (2-Methyl-1-butanol) | |

For footnote see p. 13.

TABLE I (*continued*)

| Peak No. | Mass spec. and GC retention | Mass spec. confirmation | Mass spec. indication |
|---|---|---|---|
| 64 | Methyl *n*-hexanoate | | |
| 67 | 2-Hexenal | | |
| 71 | Ethyl *n*-hexanoate | | |
| 72 | | | 1,1-Methoxyhexoxyethane |
| 73 | | | Isopropyl hexanoate |
| 75 | | | 1,1-Diethoxyhexane |
| 77 | *n*-Hexyl acetate | | |
| 78 | 1,1-Ethoxyhexoxyethane | | |
| 79 | | Ethyl heptanoate | |
| 83 | | Ethyl heptanoate | |
| 84 | | Hexenyl acetate | |
| 85 | | 1,1-Ethoxyhex-3-enoxyethane | |
| 86 | *n*-Hexanol | | |
| 88 | | Ethyl heptanoate | |
| 89 | | 3-Hexene-1-ol | |
| 91 | | 2-Hexene-1-ol | |
| 92 | | Butyl hexanoate | |
| 98 | Furfural | | |
| 99 | Methyl octanoate | | |
| 106 | *n*-Butyl *n*-hexanoate | | |
| 107 | *n*-Hexyl *n*-butyrate | | |
| 109 | | | 2-Acetylfuran |
| 112 | Ethyl octanoate | | |
| 113 | | Butyl α-methylbutyrate | |
| 115 | 1,1-Diethoxypentane | | |
| 117 | Benzaldehyde | | |
| 119 | | Pentyl hexanoate | |
| 122 | | | Methylfurfural |
| 124 | Linaloöl | | |
| 125 | Pentyl hexanoate | | |
| 132 | | | Pentenyl hexanoate |
| 134 | | | Pentenyl hexanoate |
| 137 | Acetophenone | | |
| 138 | Methyl *n*-decanoate | | |
| 142 | Ethyl benzoate | | |
| 143 | *n*-Hexyl *n*-hexanoate | | |
| 145 | 1,1-Di-*n*-hexoxyethane | | |
| 146 | *cis*-3-Hexen-1-yl caproate | | |
| 147 | Ethyl *n*-decanoate | | |
| 149 | | *trans*-3-Hexen-1-yl caproate | |
| 150 | Benzyl acetate | | |
| 151 | α-Terpineol | | |
| 152 | | 2-Hexen-1-yl caproate | |
| 153 | | Pentyl octanoate | |
| 155 | | | (Naphthalene) |
| 157 | | | 1,1-Diethoxyoctane |
| 162 | β-Phenylethyl acetate | | |
| 163 | | | 1,1-Dihexenoxyethane |

Others not on Fig. 1

| | | | |
|---|---|---|---|
| | | | 2-Methylnaphthalene |
| | | | Hexyl octanoate |
| | | | 1-Methylnaphthalene |
| | | | Ethyl dodecanoate |
| | | | Hexenyl octanoate |
| | | *cis*-Ethyl cinnamate | |
| | | *trans*-Methyl cinnamate | |
| | | *trans*-Ethyl cinnamate | |

* Duplicate listings are isomeric species not distinguished by available mass spectral data

would not lead to erroneous results. This technique is desirable whenever a sufficient quantity of the sample is available.

The second class of identification is mass spectral comparison or correlation only. When the authentic compound is not available, a fairly certain identification can frequently be made by comparison with tabulated mass spectra[12-14] or by using correlations suggested by studies on series of compounds (for a list of these, see ref. 15). Thus, the identification of most of the compounds so classified is fairly certain, although in some cases a specific isomer cannot be suggested. For example, component 119 is certainly a pentyl hexanoate. The molecular ion, mass 186, is weak, but it is observed. The ions $C_5H_{10}^+$ and $C_6H_{11}O^+$ are strong, as expected. The characteristic ester rearrangement ions, $(CH_2COOC_5H_{11})H^+$ (mass 130) and $(C_5H_{11}COO)H_2^+$ (mass 117), are both present. However, from the mass spectral data, the only isomer information that can be accepted with confidence is that the acid moiety is not $\alpha$-substituted.

The third class of identification consists of structures suggested by mass spectra, but not certain. Various reasons may exist for this uncertainty. The spectrum may be too weak for confident interpretation; the compound may have been eluted with another so that the spectrum is partially obscured; or the suggestion may be based on comparison with only one or two similar compounds.

Three components listed in Table I have been placed in parentheses. Components 62 and 63 gave a good mass spectral confirmation but the retention times of the authentic samples did not give a good check (about 45 sec late). Component 155 was indicated by the mass spectrum but the retention time of authentic naphthalene was 1 min early.

Two additional classes of identification represent the components not listed in Table I. The fourth includes cases where the mass spectra give some definitive information but not enough to assign a structure. Thus, a component may appear to be a terpene, a secondary alcohol, a possibly unsaturated ester, etc., but without structure assignment. The fifth class is that in which the mass spectrum does not yield any suggestion, even tentatively, as to the identity of the component.

INTERPRETATION OF DATA

The nature of the data obtainable and the general interpretation are illustrated by Fig. 2, which shows the mass spectral charts taken during the latter part of the chromatographic run, after 80–85 min and at about 175°.

The first chart in Fig. 2 was taken towards the top of the chromatographic peak and shows the mass spectral pattern of component 142, ethyl benzoate (the mass peaks shown at higher intensity), and a small amount of component 143. The identification of ethyl benzoate can be made quite readily by comparing the ratios of the mass peaks obtained with those of ethyl benzoate as listed in the mass spectral catalogues. Other compounds of parent mass 150 would have different ratios and, in many cases, some peaks would be absent and others present. The next chart was taken a few seconds later but shows very little ethyl benzoate. Component 143 is readily identified as hexyl caproate by means of the established mass spectral correlations already discussed[16]. Again, the only isomer information obtainable from the mass spectrum is that the acid moiety is not $\alpha$-methyl pentanoate.
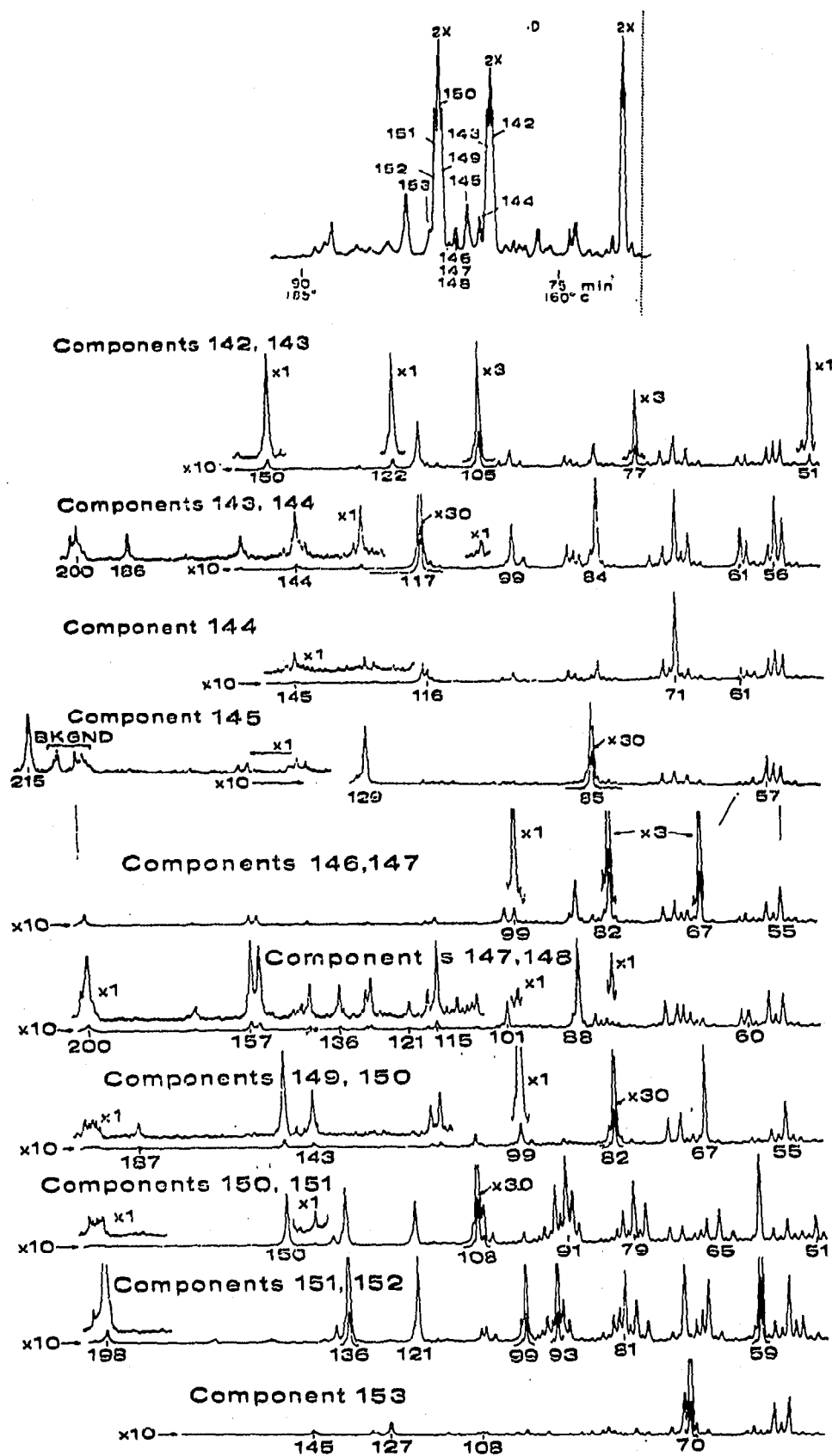
Fig. 2. Mass spectra obtained during latter part of run.

The mass spectrum obtained on the small adjacent peak is weaker and not too clear. The persistence of the mass 71 ionization is not easily explained since the ionization at masses 84, 99 and 117 has dropped as a result of effective elution of the hexyl caproate. Ionization at masses 56, 116 and 145, which might also be present in the previous mass spectral chart, may indicate a butyl octanoate. However, the spectrum does not clearly define these features so that listing this even in the third classification does not seem warranted.

The next chromatographic peak appears to be the single component dihexoxy-ethane. This compound is quite easily identified tentatively by the ion peaks at mass 215 [CH(OC$_6$H$_{13}$)$_2$$^+$] and at mass 129 [CH$_3$CHOC$_6$H$_{13}$$^+$]. Comparison with known acetal spectra[17] confirms this identification but there is not enough mass spectral data in the literature to permit any isomer assignment.

The next two mass spectral charts were taken on the two small peaks preceding the last major peak. The first chart shows mass spectral features of both 3-hexen-1-yl caproate (146) and ethyl decanoate (147). Although, in the mixture, 3-hexen-1-yl caproate shows only three characteristic peaks (masses 99, 82 and 67), their ratios match the spectrum of the authentic compound well enough to give firm identification. In the next chart, the spectrum of component 146 is essentially absent and that of ethyl decanoate predominates. Note also the trace component, 148, evidenced at masses 136 and 121. These mass peaks are definite indications of a C$_{10}$ terpene (presumably an alcohol or aldehyde) but further structural identification is not feasible.

The next mass spectral chart was taken at the start of the larger chromatographic peak. Two components are observed. First, the features of 3-hexen-1-yl caproate are again evidenced. It is concluded that this must be *trans* and the previous one *cis*. A chromatographic retention-time check with *cis*-3-hexen-1-yl caproate confirmed this. The other component is identified as benzyl acetate by comparison of this spectrum with other mass 150 compounds catalogued in the mass spectral literature. (The features of this spectrum give an interesting contrast with those of ethyl benzoate, component 142.) A few seconds later, 3-hexen-1-yl caproate was absent, but another component, 151, was observed with the benzyl acetate. The mass peaks at 136, 121 and 93 immediately suggest a C$_{10}$ terpene and the peak at mass 59 [the ion fragment (CH$_3$)$_2$COH$^+$] indicates possible α-terpineol. Thus, identification by comparison with the mass spectrum of authentic α-terpineol and by GC was confirmed. Still on this same peak, the next mass spectral chart showed no benzyl acetate; α-terpineol is still present but in addition, another component, identified as 2-hexen-1-yl caproate, is recorded. Unlike the 3-hexen-1-yl caproates (components 146 and 149), this isomer gives significant parent ionization at mass 198 so that the identification is simplified.

As a final illustration, the chart taken on the small shoulder peak (component 153) is shown. The α-terpineol and 2-hexen-1-yl caproate are essentially absent and a reasonably clear spectrum corresponding to a pentyl octanoate is obtained. This is quickly identified by the characteristic ester peaks (C$_5$H$_{10}$$^+$ from the pentyl, C$_7$H$_{10}$CO$^+$ from the octanoate, etc.) but isomer information is again not given.

This section of the chromatogram was chosen for illustration for two reasons. First, it shows that even components of relatively high boiling points can be quickly pumped from a mass spectrometer. Second, the peaks in this section have several components which, fortuitously, can be easily identified. It is noted, however, that

only about one-half the components of the total oil have been reasonably identified but, as would be expected, almost all the major peaks are determined.

Curiously, all the more abundant peaks that have not been identified are in section C, notably components 82, 93, 110, 111, 116 and 118. Even though the mass spectra of these unknowns are fairly intense, only crude speculation is possible regarding their identity. Some firm conclusions can still be made, however. For example, it can be stated that they are not aliphatic esters, acetals, ketones, common aromatic esters, etc. In two cases (components 111 and 116), nearly identical mass spectra were obtained, so it may be concluded that these are *cis* and *trans* isomers. The further speculation that they are methyl ethers of terpene alcohols requires substantiation before they could be listed in Table I.

Identification of the unknown components may become possible as the number of compounds catalogued in the mass spectral literature increases. Separation by use of several different packed columns would be extremely difficult. Possibly, the identity of some will be clearer if high resolution mass spectra can be obtained, thus revealing the true elemental composition of each ion fragment. The technique for obtaining such data has been recently demonstrated[18].
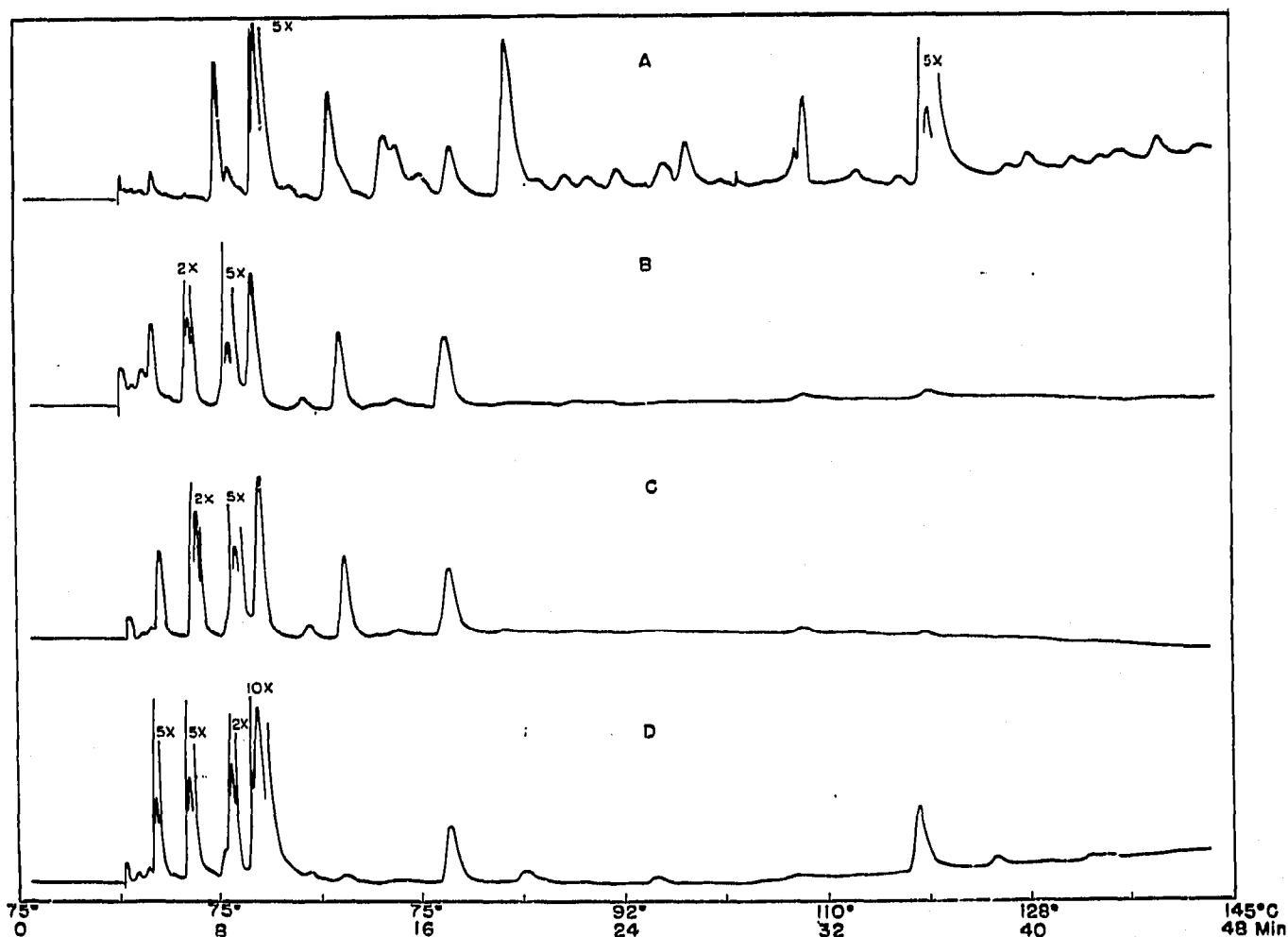


Fig. 3. Chromatograms of strawberry vapors (aromagrams) : A, vapor of oil used for mass spectral analysis; B and C, duplicate samples of vapor above whole strawberry; D, vapor above a whole strawberry of different variety.

Many of the identifications made in this work have been previously reported[19-23]. However, determination of the many ester isomers had not been previously possible. The existence of acetals was not realized prior to Part I[4] of this series. Some of the aromatic compounds had been suggested but the confirmation here by mass spectrometry seems desirable. Furfural had been reported but the furfural derivatives are new identifications. Of the terpenes, α-terpineol had been previously established but linaloöl had not, even though it is one of the more prominent components (component 124). Borneol, reported in previous work, was not detected and, from mass spectral evidence, it could not be any of the three or four trace terpenes reported here. However, such differences could be due to use of different strawberry varieties.

The present work demonstrates significant progress in the investigation of volatiles from fruits and vegetables. The ability to analyze microgram quantities existing in complicated mixtures facilitates the correlation of chemical structure with organoleptic evaluation, and will make direct vapor analysis by gas chromatography (aromagrams[24]) more meaningful and permit chemical identification of aroma quality.

The four aromagrams shown in Fig. 3 illustrate how this study will be useful in flavor and odor studies of fresh fruits. Chromatogram A is a vapor sample from the strawberry oil used in this study. Chromatograms B and C are from duplicate vapor samples from a whole strawberry of one variety and chromatogram D is from a whole strawberry of another variety. Although more components are observed in the vapor from the strawberry oil, it is possible to correlate the components of the vapors from a fresh berry with the constituents identified in the isolated oil. Also, it can be seen that differences between fruits of different varieties can be chemically determined as is indicated by the differences between D and the duplicates B and C. Differences due to different storage conditions have also been reported[24].

Correlations of this type will be easier and better as columns are developed to separate the components of vapor samples as efficiently as the small-bore capillaries can separate liquid samples.

## SUMMARY

The technique of combined mass spectrometry and gas chromatography has been applied to the analysis of a complex oil of strawberry volatiles. Capillary gas chromatography indicated over 150 components. Most of the major components have been identified. These include alcohols, esters, acetals, aldehydes, furfural, methyl furfural, aromatic aldehydes, ketones, and esters, a few terpenes, and a few aromatic hydrocarbons. The strength of this technique is shown by the determination of several compounds in one sharp chromatographic peak. On the other hand certain prominent species could not be identified by the low resolution (resolution about 200) mass spectral data obtained.

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