IDENTIFICATION OF FLAVOR COMPONENTS
OF LOGANBERRY ESSENCE

by

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INTRODUCTION

The loganberry (Rubus ursinus var loganobaccus) is a
hybrid resulting from a cross between the Red Antwerp, a
variety of the European raspberry, and the California dew
berry. It was developed by J. H. Logan of Santa Cruz,
California. The flavor is more typical of the blackberry,
probably because of its high acidity (84, p. 630-632).

The production of loganberries in Oregon during 1960
was 950 tons. In dollar value per acre, it is third only
to tame blackberries and red raspberries. The total value
of the 1960 crop was approximately one quarter of a million
dollars (43).

The loganberry exhibits very desirable flavor char-
acteristics and because of its monetary value and sig-
nificance to the berry growers of the state, an examina-
tion of its flavor appeared desirable. Chemical definition
of the flavor could have useful applications in several
respects. First, knowledge of the chemical composition
of the highly acceptable flavor would be valuable to the
plant breeder when developing more productive varieties
by allowing him to select breeding stock on the basis of
flavor producing capacity as well as other desirable

characteristics. Second, the information on flavor composition could ultimately lead to more realistic quality control procedures for food processors. A knowledge of the types of compounds comprising the flavor, their stability to environmental factors, and their relative concentrations in the flavor would be of value when attempting to market a uniformly high quality product.

A third justification for the investigation was concerned with the curiosity of the candidate to learn what is involved in the study of flavor composition. The purpose of the investigation, therefore, was to attempt to identify the compounds contained in the flavor extract of loganberry essence. The study consisted of the evaluation of isolation procedures, the study of the carbonyl compounds as 2,4-dinitrophenylhydrazine derivatives, and the fractionation of the flavor by means of gas-liquid chromatography. Attempts were made to establish identification of compounds wherever possible.

REVIEW OF LITERATURE

Flavor Isolation Methods

There have been no reports, in the literature,
specifically dealing with the isolation of the flavor
components of loganberries. Techniques developed for
other fruits and essential oils, however, are applicable
to loganberries. The methods vary depending upon the raw
material and the delicacy of the flavor being isolated.
To appreciate the problems relevant to flavor isolation,
a review of the more common techniques is presented.

The major objective of isolation procedures is to
obtain a representative sample in the highest yield
possible. In most cases, some sacrifice in yield is taken
in order to avoid contamination or alteration of flavors
induced by the more rigorous treatments. Such is the case
with the enfleurage or cold fat extraction technique
developed in the French perfumery industry. This tech-
nique, while used almost exclusively for isolation of
perfume constituents, could have application in certain
flavor problems. It consists of laying flowers, which
have the ability to produce their perfume for many hours
after they are picked, on specially prepared fat. The
flowers are allowed to stay in contact with the fat only
as long as they continue to produce perfume. After the
harvest season the fat, which is never renewed, is

saturated with flower oil. This oil is extracted with alcohol and the extract is called absolute of enfleurage (42, p. 189-198). The flowers, which have been removed from the fat, are extracted with alcohol and the extract is used for perfume blends. The enfleurage procedure is limited to flowers which can produce perfume after they are picked. The main disadvantage lies in the extract, which contains about 1% fat. The fat can lead to off odor production.

Volatile solvent extraction techniques have been used extensively for the isolation of essential oils. They are important for extraction of perfume components of flowers where the components are heat labile. They also are used to extract flavors from concentrated essences (5, p. 303; 20, p. 675-680; 21, p. 680-685; 47, p. 587-590; 60, p. 353-355).

The method was first employed by Robiquet in 1835 to extract perfume from flowers. The technique has been refined over the years so that current workers employ rather elaborate, continuous liquid-liquid extractors. An example is the work of Coppens and Hoejenbos (20, p. 675-680; 21, p. 680-685) who used an extractor with diethyl ether as a solvent to isolate the essential oils from raspberries and strawberries. The most common solvents employed are diethyl ether and low boiling hydrocarbons. Tsuda (79,

p. 10-13) has found liquid Freon very useful for perfume extractions.

The main advantage of the solvent extraction technique is that it is a relatively mild treatment, and it avoids destruction or alteration of flavor components. Solvent extraction has been reported to be superior to steam distillation in yielding a more true to nature odor (42, p. 200). In addition, some perfumes and flavors do not lend themselves to distillation. Flowers such as jasmine, acacia, hyacinth, and violet do not yield their volatile oils on steam distillation; therefore, they must be isolated by some other means (42, p. 201).

Solvent extraction procedures have certain disadvantages, two of which are the presence of pro-oxidant peroxides in ethers and the problem of isolating unwanted pigments and waxes along with the volatile flavor material (5, p. 303).

Probably the most commonly used method to concentrate fruit flavors is steam distillation (42, p. 88-188). Steam distillation is based upon the principle that a mixture of liquids, immiscible with water, boils at a temperature lower than that of any one component alone. The boiling temperature for any two-phase liquid will always be lower than the boiling point of either of the pure liquids at the same total pressure. Advantage is taken

of this fact in isolation of many flavors that would boil
at higher temperatures or that tend to decompose when
heated to their normal boiling point. Regular steam dis-
tillation is not in itself very satisfactory because the
flavor components remain in high dilution in the distillate.
Considerable effort, therefore, has been devoted to effect
concentration of the flavor material. Milleville (61, p.
1-14) in 1944 devised a flavor concentrator which was used
for collection of apple volatiles. The volatile flavor
constituents were concentrated up to 150 fold. The pro-
cedure involved preheating the apple juice to 320°F. under
75 psi (gauge) followed by quick release of pressure which
caused instant vaporization of the juice. This method was
used later by Griffin (41, p. 1545-1547, 1694, 1696) to
concentrate flavors from grape, strawberry, blackberry,
youngberry, huckleberry, peak, and rhubarb.

The relative high heat treatments employed in the
above procedure were undesirable in that it was difficult
to reproduce the fresh flavor of the fruit. To overcome
this Dimick and Makower (28, p. 517-520) developed a low-
pressure low-temperature distillation unit for isolation
of strawberry flavor. The apparatus was operated at 5 mm
of mercury and at 35° to 40°C. The laboratory scale model
was capable of handling 100 kilograms of strawberry juice
per day. Winter (83, p. 250-255) used a similar unit but

flooded the system with an inert gas to inhibit oxidation
of flavor components. Units similar in principle to that
of Dimick and Makower are in current use for commercial
preparation of fruit flavor essence. This type of system
offers many advantages to flavor chemists since relatively
large quantities of raw materials can be handled. Inherent
in the process is the danger of losing highly volatile
compounds that may be critical to the complete flavor. An
additional disadvantage is that the formation of azeotropes
limits the efficiency of the distillation process. To
overcome these problems in distillation procedures, some
workers have used solvent extraction in conjunction with
distillation columns to concentrate the flavor (47, p.
587-590; 5, p. 305)

For the purpose of studying flavor composition, water
insoluble derivatives of flavor compounds has proven to be
a quick and efficient method to separate whole families of
compounds from a dilute concentration of fruit essence.
Relatively small amounts of unknown compounds, in some
cases parts per million, have been successfully identified
by this method (29, p. 73-75; 83, p. 250-255). Probably
the best example of this technique is the formation of
2,4-dinitrophenylhydrazine[1] derivatives of carbonyl

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[1] 2,4-dinitrophenyl is hereinafter abbreviated DNP.

compounds. The first to use this method was Curtis and Dedichen in 1894 (23, p. 241-247). Later Bulow (15, p. 344) used DNP-hydrazine reagent to detect small amounts of acetone.

Through the years the DNP-hydrazine-carbonyl reaction conditions have undergone many revisions. The use of an acidified-alcoholic medium to prepare derivatives has been very popular. Some of the acids used were hydrochloric (3, p. 2955-2959), sulfuric (10, p. 756-759), and phosphoric (49, p. 5888-5889). A methanol-phosphoric acid medium was used by Ferrante and Bloom (35, p. 381-384). Some DNP-hydraxones were prepared in refluxing pyridine (12, p. 3131-3138), but because of the instability of the DNP-hydrazones in basic solutions, the reaction products had to be purified by chromatography.

Collatz and Neuberg (19, p. 27-37) were the first to use a saturated solution of DNP-hydrazine reagent in 2 N hydrochloric acid. Later, Torres and Brosa (78, p. 34-36) suggested using aqueous sulfuric acid in place of hydrochloric acid. Their reagent was later used by Houghton (44, p. 62-64) and by Perkins and Edwards (63, p. 208-211) for the quantitative determination of ketones and aldehydes.

Some undesirable side reactions have been reported for the DNP-hydrazine-carbonyl reaction. Derivatives prepared in chloroform-acetic acid have led to the

formation of a DNP-hydrazone of monoacetate (76, p. 758-761; 67, p. 822-832). In some cases the reaction conditions have led to the esterification of hydroxyl groups (67, p. 822-832) and acid groups (4, p. 222-226; 76, p. 758-761) present in the carbonyl compounds.

In the reaction of carbonyls with DNP-hydrazine, the starting material is sometimes altered leading to false interpretation of results (74, p. 43-48; 1, p. 3836; 66, p. 4331-4334; 30, p. 1003-1010). There is also the possibility of the formation of hydrazides (17, p. 1090-1099) if esters are present in the reaction medium.

Separation and Purification of Flavor Compounds

In general, the material giving rise to a specific flavor is a mixture of different families of compounds as well as different classes in a specific family. Coupled with the task of separating the multiple-family, multiple-class mixture is the problem of handling micro-quantities of material. It was not until the advent of chromatography, therefore, that suitable tools became available to give the needed impetus to flavor research. The chromatographic process is ideally suited in that high resolution of mixtures is possible when employing relatively small quantities of sample.

Chromatography is a separation process applicable to

molecular mixtures which depends on the distribution of the mixture between two phases: a thin phase and a bulk phase which are brought together in a differential counter-current manner. The two types of chromatographic processes of major concern to the flavor chemists are adsorption and partition.

Adsorption Chromatography: This method, often referred to as the classical method, was first used by M. Tswett at the turn of the century. The separation occurs between a liquid mobile phase and a stationary adsorbed interfacial phase. Whether the process is true adsorption or the partitioning of solute between free and adsorbed mobile solvent is not important. Separation by this procedure seems to be effected primarily by affinity of adsorptive sites of the solute molecules for the adsorbent. Usually the more polar the solvent, the greater its eluting power (16, p. 220-225). The most popular adsorbents are alumina (36, p. 584-586) and magnesia (70, p. 1-7). Charcoals, clays, and bentonite have also been used (16, p. 220-231).

Adsorption column chromatography has found wide application in flavor chemistry for separation of acids (29, p. 73-75; 83, p. 250-255), alcohols (9, p. 1162-1164), and carbonyl derivatives (36, p. 584-586; 70, p. 1-7). The method has been particularly useful for separation of

DNP-hydrazones into classes.

Most liquid partition systems will separate the DNP-
hydrazones of aliphatic monocarbonyls into fractions con-
taining alkan-2-ones, alkanals, alk-2-enals, and alk-2,4-
dienals with chain lengths of C_n, C_{n+1}, C_{n+2}, and C_{n+4}
respectively. Adsorption chromatography procedures,
developed by Schwartz et al. (70, p. 1-7), in which mag-
nesium oxide and ethylene chloride are used, enable
separation of the above classes. The efficiency of
separation seems to be dependent upon the acid strength
of the DNP-hydrazones. The acid strength of a number of
hydrazones has been measured by Timmons (77, p. 2613-
2623). The mechanism of ionization in alkali is asso-
ciated with formation of a colored quinoidal compound and
has been discussed by Timmons (77, p. 2613-2623), Braude
and Jones (11, p. 498-503), and Jones et al. (51, p. 105-
107). The ionization of m-dinitro compounds in general
has been discussed by Porter (64, p. 805-807).

Strain (76, p. 758-761), in 1935, working with ad-
sorption columns, found that basic adsorbents such as
magnesium oxide caused decomposition of DNP-hydrazones.
However, Schwartz et al. (70, p. 1-7) using the same
adsorbent, have successfully separated DNP-hydrazones
with no appreciable loss of derivatives. Other adsorbents
which have been used with notable success have been

magnesium sulfate (13, p. 238-240; 73, p. 3583-3586), zinc
carbonate (80, p. 57-58), calcium sulfate (72, p. 55-56),
alumina (81, p. 61-65; 50, p. 91; 2, p. 3051-3055), and
silicic acid (57, p. 980-983).

Liquid-Liquid Partition Chromatography: Liquid-
liquid partition, more commonly called partition chroma-
tography, has been a valuable tool in flavor research.
Two liquid phases are employed: the mobile phase and a
thin layer stationary phase supported by an inert solid.
The components of the solute are partitioned between the
two phases, the affinity of each compound for a particular
phase being dependent upon molecular interactions of the
compound with the two phases. A treatment of the theory
of liquid-liquid partitioning is given elsewhere (16, p.
107).

Column partition chromatography has been used exten-
sively by flavor chemists to separate derivatives of car-
bonyl compounds (26, p. 463-474; 27, p. 585-597; 71, p.
1450-1462). The partition column using hexane equilibrated
with nitromethane as the mobile phase, and nitromethane
coated on Celite as the stationary phase as described by
Day et al. (26, p. 463-474) has been proven to be an
excellent method for separation of complex mixtures of
DNP-hydrazones of monocarbonyl compounds (27, p. 585-597;

62, p. 49-55). Corbin et al. (22, p. 322-329) has developed a liquid-liquid partitioning system for DNP-hydrazones which employs hexane equilibrated with acetonitrile as the mobile phase and acetonitrile coated on Celite as the stationary phase. By using a fraction collector, aliquots of the effluent are collected and their absorbance determined with an ultra-violet spectrophotometer. Their procedure enables detection of trace amounts of derivatives, which ordinarily are not visible on the column.

Paper partition chromatography has also been used as a means to separate complex mixtures of DNP-hydrazones (52, p. 356-358; 39, p. 392-400; 86, p. 192-197). Paper partition chromatography differs from column partition only in physical means. In paper chromatography there are no walls to retain the phases mechanically; the outer limit is set by the surface tension of the mobile phase rather than by the walls of a column (16, p. 133).

Gaddis and Ellis have used paper chromatography to separate DNP-hydrazones of monocarbonyls into classes much in the same way as was done by adsorption columns (37, p. 283-297). The classes were then separated by chain length on paper using different mobile and stationary phases (38, p. 870-875). These procedures have been used by Yu et al. (86, p. 192-197) for the separation of DNP-hydrazones of volatile monocarbonyls from autoxidized

salmon oil.

Gas-Liquid Chromatography: With the advent of gas
chromatography by James and Martin (46, p. 679-690) in
1950, analysis of minute quantities of organic compounds
was made possible. As the equipment was refined, the
sensitivity of the method was vastly improved until now
it is common to detect compounds at 10^{-12} molar concentra-
tion. The principle of gas liquid chromatography (GLC) is
comparable to liquid-liquid chromatography except a gas
is used as the mobile phase instead of a liquid. The sta-
tionary phase consists of a high molecular weight liquid
substance which has been coated on an inert solid support
such as fine mesh Celite or Firebrick. The stationary
phase, coated on a solid support, is packed into a small
diameter column and the mobile phase is supplied as a
small flow of gas through the column. If a sample is in-
jected, the gas will sweep it into the column where the
organic compounds of the sample will be separated according
to their vapor pressure in the stationary phase. Those
with the highest vapor pressure will travel through the
column at a much faster rate. As the individual fractions
are eluted from the column, they are sensed by a detector
which sends an electrical impulse to a recorder. This
impulse, in the form of voltage, drives a pen which records

the fraction as a peak on a moving chart. Usually, the
size of the peak will depend on the quantity of the frac-
tion being detected. After all of the fractions has been
eluted from the column, a chromatogram will have been drawn
on the chart which will depict the number of fractions the
sample was separated into as well as their relative concen-
trations. The chromatogram will also show the amount of
time which is needed to elute a particular fraction or
component from the column.

Small sample size and quick separation of compounds
are but two of the many advantages of gas chromatography.
The number of theoretical plates which can be attained by
using a gas chromatographic column are appreciably higher
than the best distilling columns. Compounds which have
only one degree Centigrade difference in boiling points
can be efficiently separated using appropriate columns
(14, p. 155-166).

There are a number of methods for identification of
the fractions eluted from a GLC column. One of the methods
is based on the amount of gas that is needed to push one
half of a particular fraction off of the column. This is
called retention volume (V_R). If all of the instrument
parameters remain the same, the retention volume of a
particular fraction will also be held constant. This then
can be used as a means of tentatively identifying

components found in citrus fruits (6, p. 213-216). Additional credence can be given to this method if another column, packed with a different stationary phase, is used in conjunction with the first. This is analogous to the use of two dimensional paper chromatography for establishing identification.

Another method of emplementing the identification of eluted fractions from a GLC column has been suggested by Walsh and Merritt (82, p. 1378-1381). They collected the effluents into different reagents which produced color reactions depending on the functional group. This procedure proved quite successful when large fractions were eluted from the chromatograph. Infrared spectrophotometry also has found application for establishing the identity of functional groups in fractions and in some cases it has served to establish conclusive identification (6, p. 213-216; 48, p. 499-507).

Gas chromatography has been instrumental in obtaining important information on the chemical make-up of flavors in such products as citrus fruits (6, p. 213-216; 7, p. 401-411; 18, p. 731-738; 74, p. 43-48), strawberries (29, p. 73-75), coffee (68, p. 254-261), blackpepper (48, p. 499-507), milk (25, p. 932-941), and pears (47, p. 587-590) to name a few.

Thin Layer Chromatography: A new chromatography method which has very good potential is a thin layer technique (75, p. 378). The method involves the spreading of a thin layer of silicic acid on a piece of glass and the layer then acts as the adsorbent or support for a stationary phase. Essentially, this is an open column. Removal of specific fractions can be accomplished quite easily without the disruption of other fractions. It is also a much more rapid chromatography procedure than either paper or column and has been used successfully to separate terpenes (53, p. 420-425), fats, oils, and waxes (58, p. 383-385).

After the organic compounds have been separated by appropriate chromatographic methods, they can be conclusively identified by infrared spectrophotometry (9, p. 1162-1164; 54, p. 1295; 69, p. 1288-1303), X-ray diffraction patterns (29, p. 73-75), melting points (27, p. 585-597), and nuclear magnetic resonance (59, p. 691-693).

It is evident from this review that no single analytical method will give conclusive identification of flavor compounds. Only by using a combination of methods does identification become possible.

Flavor Components of Loganberry and Related Fruits

Research on the flavor composition of the loganberry
has been limited to the analysis of certain acids by
Daughters (24, p. 30). Large quantities of citric acid
and trace amounts of formic, acetic, and malic acids were
reported. Since no other information is available on the
loganberry, a review of the literature on raspberries is
appropriate. Raspberry flavor is quite distinct from
loganberry but similarities in types of compounds should
occur since both are members of the same botanical family.

In 1929, Elze (32, p. 72) reported anisaldehyde as
the semicarbazone, irone as the p-bromophenylhydrazone,
nerol, and an unidentified paraffin wax melting at 43-44°C.
from the steam distillate of raspberries. All of the
above compounds make up part of the formula for imitation
raspberry flavor which was used prior to 1929. Subsequent
evidence suggests that Elze was working on a raspberry
flavor preparation adulterated with artificial flavor.

Coppens and Hoejenbos (20, p. 675-680) were the first
workers to intensively investigate the flavor components
of raspberries. In 1939, using ethyl ether to isolate the
flavor, they reported the following compounds: acetic,
n-caproic, and benzoic acids; ethyl, isoamyl, benzyl, and
phenylethyl alcohols; diacetyl, menthone, benzaldehyde,
and ethyl acetate. There also was some evidence to support

the existence of acetoin (3-hydroxy-2-butanone), coumarin, and a salicylate in raspberry flavor (20, p. 675-680).
Later Bohnsack (8, p. 72-74), in 1942, discovered leaf alcohol and isobutyl alcohol in raspberry flavor.

McGlumphy (60, p. 353-355) repeated much of the work of Coppens and Hoejenbos in 1951 and he identified some additional compounds that had not been reported in raspberry flavor previously. They were as follows: formic and hydrocinnamic acids; n-butanol, menthol, acetone, acetaldehyde, iso-butanal, ethyl acrylate, ethyl salicylate, and catechol. n-Caproic acid, phenylethyl alcohol, and coumarin were not found. Furthermore, none of the compounds identified by Elze were found by McGlumphy.

Glichitch (40, p. 241-243), in 1937, reported the relative concentrations of certain classes of compounds in the essential oil of black current buds. The terpenes represented 85% of the essential oil and consisted of β-pinene, sabinene, caryophyllene, and d-cadinene. Terpene alcohols, probably sabinol and terpineols, accounted for 6% and phenol plus beta naphthol were reported as 0.25%. Traces of aliphatic esters also were found.

EXPERIMENTAL PROCEDURE

Source of Loganberry Essence

The loganberry essence was obtained from Smucker's Company of Oregon City. The actual process used in concentration of the essence was not available from the company. Information on comparable fruit concentrators would indicate an operation temperature of approximately 35°C. at 5 mm mercury. The capacity of such concentrators is approximately 33,000 gallons of juice per day. The juice is concentrated about 3 fold. For example, from one hundred and eighty gallons of loganberry juice, sixty gallons of concentrate is recovered. This leaves one hundred and twenty gallons of condensate which is further concentrated to give approximately 0.77 gallons of essence. The loganberry essence is concentrated approximately 150 times. The recovered essence has no color and it has the characteristic loganberry odor.

Analysis of Carbonyl Compounds

A preliminary investigation of the flavor components of loganberry essence using DNP-hydrazine indicated a relatively large amount of carbonyl compounds. The amount of precipitate formed warranted further investigation as to the identity and concentration of carbonyl material present in loganberry essence.

Preparation of DNP-hydrazine Derivatives: Three
hundred milliliters of loganberry essence was reacted with
300 ml of 5N hydrochloric acid saturated with DNP-
hydrazine. The mixture was allowed to react overnight
at room temperature in a closed one liter flask.

The DNP-hydrazones were removed from the reaction
mixture by extracting five times with 50 ml portions of
chloroform. The chloroform extracts were combined and
the chloroform was removed at reduced pressure. Excess
reagent was removed from the DNP-hydrazones with the aid
of a short Dowex 50 column. Advantage was taken of the
fact that DNP-hydrazine is sufficiently basic to be taken
up by a strong cation exchange resin whereas the DNP-
hydrazones exhibit weakly acidic properties and will not
be held by the resin (77, p. 2613-2623). The dry DNP-
hydrazone-hydrazine mixture was made up to 50 ml with
methanol-benzene (1:1). The mixture was added to a column
containing 15 grams of Dowex 50 resin. The flow rate was
controlled by a restriction placed at the outlet of the
column. The resulting effluent, freed of DNP-hydrazine
reagent, was then dried under reduced pressure and was
subsequently subjected to chromatographic analysis.

Chromatographic Separation of DNP-hydrazones

Adsorption columns, using magnesium oxide, enable
separation of mixtures of carbonyl compounds into classes

such as alk-2-ones, alkanals, alk-2-enals, and alk-2,4-dienals (70, p. 1-7). By using partition columns, which separate carbonyls on the basis of chain length (22, p. 322-329), in conjunction with adsorption columns, complete separation of the carbonyl mixture can be achieved. The paper chromatography methods of Gaddis and Ellis (38, p. 870-875) and Ellis et al. (31, p. 475-478) can be used to determine purity of fractions obtained from the aforementioned columns.

The above chromatographic procedures were employed to facilitate separation and identification of the DNP-hydrazone mixture obtained from loganberry essence. The scheme is shown in Figure 1 and is described in detail below.

Class Separation of DNP-hydrazones: Eight adsorption columns were required to handle the quantity of derivatives present in the loganberry essence. The adsorption columns were prepared by mixing magnesium oxide (dried at 380°C. for 48 hours) and Celite 545 (dried at 160°C. for 24 hours) in a ratio of 1:2. Each column contained 37.5 grams of the adsorbent mixture. Ethylene chloride was added to the mixture to produce a slurry. A mechanical stirrer was used to mix the slurry. The slurry was poured into a 25 mm I.D. glass column and the column was packed

Figure 1

Chromatographic Scheme for Separation of the
DNP-hydrazone Mixture

LOGANBERRY ESSENCE (300 ml.)

↓ Excess acidified DNP-
hydrazine reagent

REACTION MIXTURE

↓ Extracted with chloroform

CHLOROFORM EXTRACT (chloroform removed)

↓

DOWEX 50 COLUMN

DNP-HYDRAZINE   DNP-HYDRAZONES

ADSORPTION COLUMNS

FRACTION 1   FRACTION 2   FRACTION 3   FRACTION 4
(column 1)   (column 2)   (column 1)   (column 3)

C_2 al C_3-C_{13} al C_5-C_7 enals   unknowns   unknowns

(column 2)

C_2-C_{13} al

C_3 and C_8 one

PAPER CHROMATOGRAPHY

Column 1   hexane-nitromethane-Celite

Column 2   hexane-acetonitrile-Celite

Column 3   silicic acid-Celite

using air pressure. The top of the packing was pressed down with the aid of a perforated disk attached to a rod. A piece of filter paper, slightly smaller in diameter than the glass column, was placed on top of the packing material to prevent disruption of adsorbent when the sample was introduced.

The DNP-hydrazone residue, representing the carbonyls from 300 ml of loganberry essence, was made up to a 50 ml volume using ethylene chloride. Five ml of this solution was added to each adsorption column. After the solution of derivatives had passed onto the column, ethylene chloride was added and the column was allowed to develop. The adsorption columns resolved the DNP-hydrazone mixture into four major fractions (Table 1). As each fraction was eluted from the column it was collected in a 250 ml. ground glass flask. The ethylene chloride was removed under reduced pressure. Identical fractions from each of the eight columns were pooled for subsequent studies.

The data presented in Table 1 indicated that Fraction 1 contained DNP-hydrazones of saturated aldehydes. The light red color of Fraction 2 coupled with the observed absorption maxima at 372 mu suggested that this fraction largely contained alk-2-enals. A red band was consistently observed which tailed Fraction 2. Complete separation of

Table 1

Class Separation of DNP-hydrazones
on Magnesium Oxide Adsorption Columns

Fraction	Color	λMax. in Chloroform
1	dark brown	355 mu
2	light red to red	372-380 mu
3	several red bands	366-383 mu
4	dark blue	--------

this band from Fraction 2 could not be accomplished on the
adsorption column and was subsequently collected as a part
of Fraction 2. A number of faint red bands made up Frac-
tion 3. The color of Fraction 4 suggested the presence of
dicarbonyls. Previous work by Schwartz et al. (70, p.
1-7) has shown that bis DNP-hydrazones move very slowly
or not at all on the adsorption column. The bis DNP-
hydrazones were eluted from the column with 25% nitro-
methane in ethylene chloride as described by Schwartz et
al. (70, p. 1-7).

Analysis of DNP-hydrazone Classes: Each of the four
fractions that were obtained from the magnesium oxide
column were further fractionated by partition chromatogra-
phy in order to ascertain identity of individual compounds
in each fraction and to establish their concentrations.

The manipulation of each fraction is presented below.

Fraction 1. Preliminary examination of Fraction 1,
which represented the alkanals, revealed that the DNP-
hydrazone of acetaldehyde comprised the major portion of
the mixture. The concentration of this compound was so
great that it was impossible to analyze for other DNP-
hydrazones because of dilution. It was necessary, there-
fore, to separate the acetaldehyde derivative from the
remainder of the DNP-hydrazones of Fraction 1. This was
accomplished by means of the hexane-nitromethane-Celite
partition column of Day et al. (26, p. 463-474). Short
columns were used (6 grams in 5 mm I.D. glass tubes) which
enabled rapid separation of the acetaldehyde from the
other DNP-hydrazones. All fractions preceding the acetal-
dehyde band were collected in a 250 ml ground glass flask
and the hexane removed at reduced pressure. The acetalde-
hyde, which was the last fraction to be eluted from the
columns, was collected in a separate flask. The hydra-
zones preceding acetaldehyde on the columns were dissolved
in chloroform and the moles of carbonyl determined by
reading the absorbency of the mixture at 358 mu and using
the formula:

Moles of carbonyl $= \dfrac{A}{\mathcal{E} \cdot \dfrac{1000}{x}}$

where:

A = absorbency
$\mathcal{E}$ = molar absorptivity
x = volume of DNP-hydrazone-chloroform solution

The molar absorptivity of 2.1×10^4 was used for the DNP-hydrazones of alkanals.

After determination of the moles of carbonyl in the mixture the chloroform was evaporated and the DNP-hydrazones were dissolved in eleven ml of hexane equilibrated with acetonitrile. Ten ml of the solution were chromatographed on the hexane-acetonitrile-Celite column (22, p. 322-329). The eluate was collected in ten ml fractions by means of an automatic fraction collector. The absorbance of the fractions was read at 335 mu and the resulting data were plotted on graph paper in the manner described by Corbin et al. (22, p. 322-329). By measuring the relative areas under each chromatographic peak with a planimeter and using the moles of carbonyls determined for the mixture, the quantities of each compound were calculated. The same procedure was used for acetaldehyde. The quantity of each saturated monocarbonyl found in Fraction 1 are given in Table 3.

Data to substantiate identification of the components of Fraction 1 are given in Table 2. These included

agreement of peak volumes of authentic compounds with the peak volumes of the chromatographic bands of Fraction 1. The validity of this procedure for identification of compounds in a specific class of carbonyls is discussed by Corbin et al. (22, p. 322-329). Additional evidence to establish identity of the compounds included determination of absorption maxima of chloroform solutions and agreement in chromatographic behavior of the unknowns with authentic derivatives when paper chromatographed by the procedure of Ellis et al. (31, p. 475-478).

The identification of acetaldehyde also was confirmed by melting point analysis. It was not possible to obtain melting points on the other compounds shown in Table 2 because of a lack of sufficient material.

Band 12 of Table 2 contained DNP-hydrazones of acetone and propanal. The mixture was separated on a small diameter (12 mm O.D.) magnesium oxide column and the identity of each compound was established by the above procedures.

Fraction 2. The fraction represented the alk-2-enals and it was analyzed by the same procedures described for Fraction 1. Qualitative and quantitative results are given in Tables 4 and 5 respectively. A portion of Band 4 from Fraction 2 was prepared as a micropellet with potassium

bromide and an infrared spectrum was obtained. The spectrum is shown in Figure 2. The limited quantities of DNP-hydrazones in Fraction 2 prohibited melting point analysis.

Fraction 3. The DNP-hydrazones of Fraction 3 were separated on the hexane-nitromethane-Celite columns described by Day et al. (26, p. 463-471). The mixture was separated into seven chromatographic bands. All components in Fraction 3 were present in very low concentrations and it was impossible to obtain sufficient information to ascertain identification. The adsorption spectra of the chromatographic bands were determined and the maxima are shown in Table 6. Sufficient amounts of Band 5 were available to obtain a weak infrared spectrum in a potassium bromide micro-pellet. The spectrum is presented in Figure 3.

Fraction 4. This fraction was eluted off of the magnesium oxide column using a mixture of 25% nitromethane in ethylene chloride as described by Schwartz et al. (70, p. 1-7). The fraction was taken to dryness under reduced pressure. A 70 gram silicic acid adsorption column was prepared according to the procedure of Wolfrom and Arsenalt (85, p. 693-695). The bis-DNP-hydrazones were dissolved in a benzene solution containing 20% nitrobenzene.

Two ml of the solution were added carefully to the column
and the column was allowed to develop with benzene. Two
fractions were collected in ground glass flasks and the
benzene removed under reduced pressure. Ultra-violet
absorption analyses were carried out on the fractions
using chloroform and ethanolic-potassium hydroxide as
solvents. The results are shown in Table 7.

Gas Chromatographic Analysis of Loganberry Flavor

Extraction of Flavor from the Essence: Several
different solvents were evaluated for isolation of flavor
from the essence. Ethyl ether gave satisfactory results,
but it was decided that ethyl chloride would be a better
solvent to use because of its lower boiling point (12.2°C.).
This facilitated its removal from the extract at room
temperature and the ethyl chloride, upon evaporation,
left no odorous residue to interfere with subsequent
sensory evaluations.

A 100 ml portion of essence was saturated with sodium
chloride and extracted five times with 25 ml aliquots of
ethyl chloride in a 250 ml separatory funnel. The extrac-
tion procedure was carried out at approximately 5°C. The
ethyl chloride extract, which contained the flavor com-
pounds, was dried over anhydrous sodium sulfate, and stored
in a refrigerator overnight. The ethyl chloride extract

was decanted from the sodium sulfate into a 50 ml pear shaped distilling flask equipped with a short water cooled condensor. Evaporation was carried out at approximately 21°C.

After removal of the ethyl chloride, the flavor concentrate was transferred to a five ml centrifuge tube, capped with a rubber stopper and stored at 0°C. in the refrigerator until used for analysis.

Evaluation of the extraction efficiency was carried out by diluting the extract to various volumes with distilled water and comparing the intensity of odor with the original essence. No appreciable difference could be detected between the extract and the original essence when the extract was diluted up to 75 % of the original volume of the essence from which it was isolated.

Chromatography Instruments: A Barber-Colman Model 20 gas chromatograph, equipped with a beta-ionization detector, was used in establishing retention volumes of the fractions in loganberry extract. The β-ionization detector was found to be most desirable because of its high sensitivity. The remainder of the gas chromatography analyses were conducted on an Aerograph Model A-100 gas chromatograph. This instrument contained a thermal conductivity detector. Although the sensitivity of the

thermal conductivity detector was less than the

β-ionization cell, larger samples could be injected into

the column. The large chromatographic fractions eluted

from the column facilitated both their objective and sub-

jective analysis.

Column Preparation: The two stationary phases, polar

and non-polar, that were used during the investigation

were diethylene glycol succinate (DEGS) and Apiezon M.

Size graded Celite 545, treated as described by Farquhar

et al. (33), was coated with 20 per cent by weight of

stationary phase, and packed into 9 foot columns. One-

eighth inch O.D. columns were used in the Barber-Colman

instrument, and 1/4 inch O.D. columns were used in the

Aerograph instrument. The columns were conditioned three

days by heating to 175°C. and flushing with nitrogen at a

flow of 10 ml per minute.

Aroma Evaluation of Gas Chromatographic Fraction of

Loganberry Flavor Extract: The Aerograph gas chromato-

graph equipped with a 9 foot by 1/4 inch O.D. DEGS column

was used to separate the loganberry extract. A 15 micro-

liter sample of the loganberry extract was used per

analysis. Individual fractions and combinations thereof

were collected by bubbling the fractions from the gas

chromatograph through five ml of cold distilled water in
25 ml erlenmeyer flasks. The flasks were stoppered and
allowed to come to room temperature for aroma evaluation.
The results are shown in Table 8. Refer to Figure 4 for
peak number designations.

Chemical Evaluation of Gas Chromatographic Fractions
of Loganberry Flavor Extract: The method described by
Walsh and Merritt (82, p. 1378-1381) was used to analyze
the effluents from loganberry extract for certain func-
tional groups. Only three functional group tests were
used, namely, alcohol, ester, and carbonyl. The reagents
used were as follows:

Carbonyls: DNP-hydrazine reagent

Alcohols: Vandium oxinate reagent (34, p. 172-173)

Esters: Sodium and 1,2-nitrobenzene (34, p. 241-
243).

One milliliter quantities of the above reagents were
put into small 5 ml capacity test tubes. Twenty-five
tubes of each reagent were prepared for the trapping of
individual loganberry fractions. A 15 microliter sample of
the loganberry extract was needed for each of the three
tests to insure efficient quantity of eluted fractions for
functional group analysis. The fractions were trapped in
the small test tubes by immersing the outlet of the gas

chromatograph in the reagent. This procedure was carried out with all three reagents. The results are shown in Table 9.

Relative Corrected Retention Volumes of Chromatographic Fractions of Loganberry Flavor Extract: The Barber-Colman Model 20 gas chromatograph, equipped with a β-ionization detector, was used to determine the relative retention volumes of the chromatographic fractions of the loganberry extract and of a number of known compounds. Analytical columns of 1/8 inch O.D. packed either with DEGS or Apiezon M stationary phases were used for the analyses. Instrument parameters as well as sample size are listed on the chromatograms (Figures 4 and 5).

The identification of the peaks found in Figures 4 and 5 was accomplished by comparing their relative corrected retention volumes with those of known compounds (Tables 10 and 11). The formulae used for calculation of corrected retention volumes (V_R^o) were as follows:

$$V_R^o = f \times Fc \times Tr$$

where:

f = pressure gradient through the column

$$= \frac{3\ (Pi/Po)^2 - 1}{2\ (Pi/Po)^3 - 1}$$

Fc = corrected gas flow

$$= \frac{273 + Tc}{273 + Ta} \times Fa$$

where:

Pi = inlet pressure

Po = outlet pressure

Fa = measured gas flow at ambient temperature

Tc = column temperature

Ta = ambient temperature

An internal standard, linalool ($V_R^o / V_R^o = 1$) was used to calculate the relative retention volumes of the loganberry fractions.

Infrared Analysis of Gas Chromatographic Fractions of Loganberry Flavor Extract: A Beckman IR-5 double beam spectrophotometer was used to analyze the chromatographic fractions of loganberry obtained from the Aerograph gas chromatograph. Only fractions which were in high concentration and exhibited high boiling points were analyzed.

Analytical grade potassium bromide, ground to 300 mesh and dried at 100°C. for 24 hours, was used to prepare crystals for analysis of the GLC fractions in thin films. Thin rectangular crystals of 0.062 inch thickness were prepared by pressing the KBr for 10 minutes at 8,000 psi. The crystals were stored in an evacuated desiccator containing phosphorous pentoxide until needed. A 15 microliter sample of the loganberry extract was injected into the gas chromatograph. Peaks 20, 22, 23, and 24 (Figure 4)

were allowed to condense on a KBr crystal held at the
outlet of the gas chromatograph. Another KBr crystal was
placed over the condensate (sandwich technique) to give a
thin film. The two crystals, with the thin film of con-
densate trapped between, were placed in the beam of the
infrared spectrophotometer. A duplicate set of KBr
crystals were used to balance out the double beam.
Spectra of the collected chromatographic peaks was then
taken over the range of 2-16 microns. Only the spectrum
of gas chromatography peak 22 (Figure 4) was of sufficient
quality to be included herein (Figure 6).

RESULTS AND DISCUSSION

Analysis of DNP-hydrazones of Carbonyl Compounds

Fraction 1, Saturated Monocarbonyls: The qualitative analysis of the saturated monocarbonyls, Fraction 1, are presented in Table 2. The tentative identification of the saturated monocarbonyls was ascertained by using paper and column chromatography and ultraviolet light absorption (Table 2). Acetaldehyde was conclusively identified by melting point analysis.

The unknown derivative found in Band 1 (Table 2) had an absorption maximum in chloroform of 363 mu. This corresponds to the absorption maximum of alkan-2-ones. The reverse phase chromatographic method of Ellis et al. (31, p. 475-478) was used to study the chain length of the unknown. A number of DNP-hydrazones of known ketones containing up to 17 carbons were used as references. All of the paper chromatograms were allowed to develop until either the unknown or the reference ketone moved on the paper strip. No partitioning of the unknown was observed even though the authentic C_{17} ketone derivative traveled an appreciable distance on the paper strip. All available information indicated that the unknown derivative was a long chain compound, but this point could not be confirmed. Additional investigations of the compound could not be

TABLE 2

Identification of DNP-hydrazones in Fraction 1

Band No.	DNP-hydrazone	λ max. (chloroform)	Identification Methods
1	Unknown	363	A B
2	Tridecanal	357	A B
3	Dodecanal	358	A B
4	Undecanal	357	A B
5	n-Decanal	357	A B
6	n-Nonanal	358	A B
7	2-Heptanone	362	A B
8	n-Heptanal	358	A B
9	n-Hexanal	358	A B
10	n-Pentanal	357	A B
11	n-Butanal	358	A B
12	Acetone	362	A B
	Propanal	358	A B
13	Unknown	351	A B
14	Ethanal	355	A B C

A Hexane-acetonitrile-Celite partition column

B Paper chromatography methods

C Melting point determination

carried out because of the limited quantity available.

The unknown compound found in Band 13 (Table 2) of Fraction 1 presented an equally puzzling problem. The ultraviolet spectrum of this compound in chloroform, revealed a maximum at 351 mu. The absorption maximum does not agree with any of the known saturated monocarbonyls. The paper chromatography methods of Gaddis and Ellis (38, p. 870-875) and Ellis et al. (31, p. 475-478) revealed that the unknown behaved like an alkanal and partitioned between ethanal and propanal. Infrared analysis and a melting point determination proved futile because of impurities present in the fraction.

The quantities of the various compounds contained in Fraction 1 are shown in Table 3. Over 63% of the total concentration was found to be acetaldehyde. This would amount to 1333 parts per billion in reconstituted loganberry juice. Most of the remaining 36% of the saturated monocarbonyls consisted of acetone (160 ppb), and an unknown component. In somewhat lesser concentration, the following aldehydes and ketones were tentatively identified in the loganberry essence: propanal (6 ppb); n-butanal (37 ppb); n-pentanal (37 ppb); n-hexanal (29 ppb); n-heptanal (19 ppb); 2-heptanone (27 ppb); n-nonanal (22 ppb); n-decanal (20 ppb); n-undecanal (13 ppb); n-dodecanal (19 ppb); n-tridecanal (19 ppb), and an unknown

TABLE 3

Quantitative Determination of DNP-hydrazones in Fraction 1

Compound	Rel. Per Cent Concentration	uM/100 ml (1)	mg/100 ml (1)	ppb (2)
Unknown	0.70	5.0	----	----
Tridecanal	0.19	1.4	0.28	19
Dodecanal	0.21	1.5	0.28	19
Undecanal	0.17	1.2	0.20	13
n-Decanal	0.21	1.5	0.30	20
n-Nonanal	0.32	2.3	0.33	22
2-Heptanone	0.50	3.6	0.41	27
n-Heptanal	0.35	2.5	0.29	19
n-Hexanal	0.60	4.3	0.43	29
n-Pentanal	0.90	6.5	0.56	37
n-Butanal	1.00	7.2	0.52	37
Acetone	5.80	41.8	2.40	160
Propanal	0.23	1.7	0.09	6
Unknown	25.10	180.9	----	----
Ethanal	63.72	459.6	20.20	1333

(1) uM per 100 ml of 150 fold loganberry essence

(2) Concentration of carbonyls in loganberry juice

ketone.

Fraction 2, α -β-Unsaturated Monocarbonyls: Three
alk-2-enals were tentatively identified in Fraction 2 and
their quantities determined (Tables 4 and 5). They were
pent-2-enal (2 ppb), hex-2-enal (15 ppb), and hept-2-enal
(2 ppb). By far the major component found in Fraction 2
was an unknown which traveled in Band 4 on the hexane-
acetonitrile-Celite column (Table 4). This component
comprised approximately 72 per cent of the total DNP-
hydrazones in Fraction 2. The material gave an absorption
maximum in chloroform at 380 mu which does not correspond
to a specific class of monocarbonyls. The material
traveled at a rate, intermediate between the alk-2-enal
and alk-2,4-dienal classes when developed on paper
chromatograms following the procedure of Gaddis and Ellis
(38, p. 870-875). The mobility of the unknown was com-
parable to but-2-enal on the hexane-acetonitrile-Celite
column. Infrared analysis of KBr micropellets, Figure 2,
was of no assistance in revealing the identity of the
material.

Some of the properties of Band 4 (Table 4), suggested
the presence of a DNP-hydrazone of a dicarbonyl. To fur-
ther investigate this point, a portion of Band 4 was
reacted with an acidified DNP-hydrazine solution. The

TABLE 4

Identification of DNP-hydrazones in Fraction 2

Band No.	DNP-hydrazone	λ max. (chloroform)	Identification Methods
1	Hept-2-enal	372	A B
2	Hex-2-enal	372	A B
3	Pent-2-enal	372	A B
4	Unknown	380	A B C

A - Hexane-acetonitrile-Celite partition column

B - Paper chromatography methods

C - Infrared analysis

TABLE 5

Quantitative Determination of DNP-hydrazones in Fraction 2

Compound	Rel. Per Cent Concentration	uM/100 ml (1)	mg/100 ml (1)	ppb (2)
Hept-2-enal	2.41	0.24	0.63	2
Hex-2-enal	22.62	2.30	0.23	15
Pent-2-enal	3.19	0.33	0.03	2
Unknown	71.78	7.30	----	--

(1) uM per 100 ml of 150 fold loganberry essence

(2) Concentration of carbonyls in loganberry juice
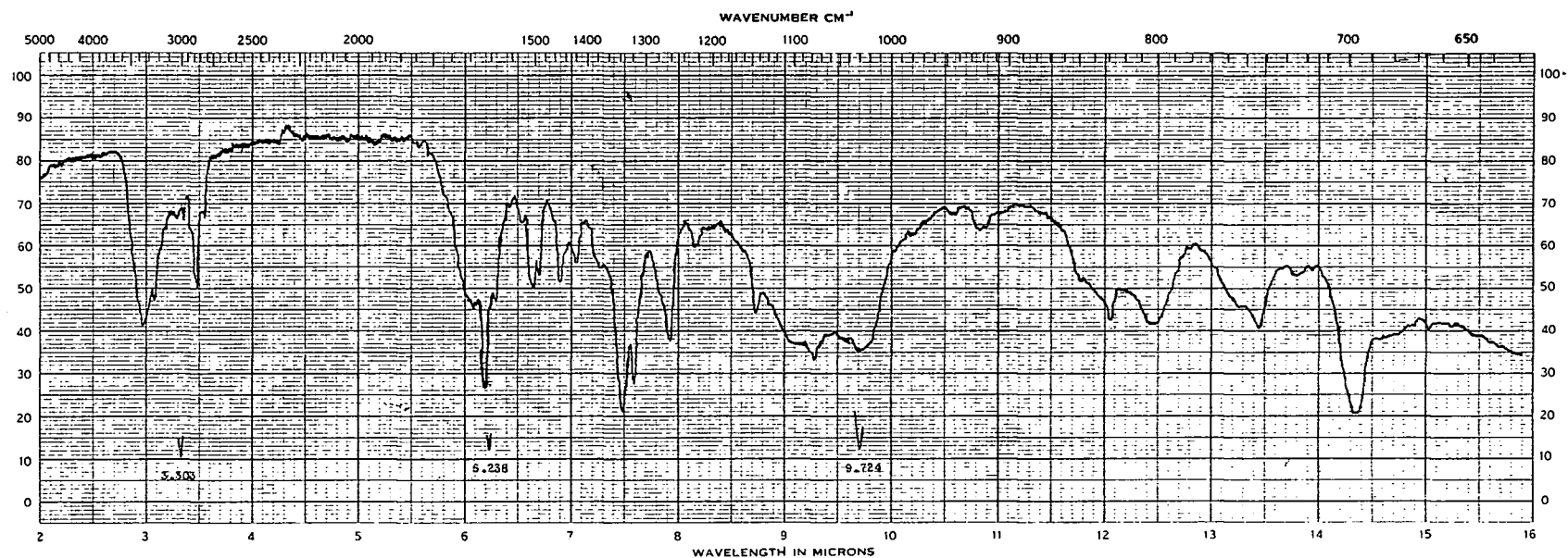
Figure 2.   Infrared Spectrum of a KBr Pellet of Band 4, Table 4.

reaction mixture was extracted with chloroform and the chloroform subsequently evaporated from the extract. The extract was dissolved in ethylene chloride and chromatographed on a small magnesium oxide column. A dark blue band developed on the column which indicated the presence of a bis-DNP-hydrazone of an α-dicarbonyl. As additional ethylene chloride was passed through the column a faint red band was observed to develop in front of the blue band. The red band is indicative of a DNP-hydrazone of a dicarbonyl, and in this case, it could be attributed to the un-reacted mono-derivative of a dicarbonyl. The characteristic colors of the dicarbonyl derivatives on magnesium oxide and the difficulty of obtaining complete bis-DNP-hydrazones from dicarbonyls has been reported by Lindsay et al. (55). While the evidence was inconclusive, the data tend to indicate that Band 4 of Fraction 2 (Table 4), was a DNP-hydrazone of an α-dicarbonyl.

Hex-2-enal, Band 2, Table 4, has been found in a number of fruits. Winter (83, p. 250-255) has demonstrated that the presence of oxygen during crushing of strawberries influences the amount of hex-2-enal in the fruit. Dimick (29, p. 73-75) stated that freezing or heating the berries prior to making them into a puree resulted in a decided increase in hex-2-enal concentration.

Fraction 3: Because of the similarity in color of the components of Fraction 3 with that developed by Band 4 of Fraction 2 and the peculiar absorption maximum found for the chromatographic bands (Table 6), a portion of each band was reacted with additional DNP-hydrazine. Small magnesium oxide columns were prepared and the resulting products, dissolved in ethylene chloride, were chromatographed. The color of the developed bands was the same as that observed in the case of Band 4 (Table 4). The evidence again suggested that Fraction 3 was composed of DNP-hydrazones of α-dicarbonyls. Further investigation of the components of Fraction 3 was not continued due to the lack of sufficient derivatives.

Fraction 4: Fraction 4, from the magnesium oxide column, exhibited all of the properties of bis-DNP-hydrazones of dicarbonyls. The blue to violet color developed in alcoholic KOH, and the color of the fraction on the magnesium oxide column were indicative of α-dicarbonyl derivatives. When a small portion of Fraction 4 was chromatographed on a silicic acid column (85, p. 693-695), two bands were observed. Both of the bands gave absorption maxima in the regions of 390 and 430 mu in chloroform, which is indicative of bis-DNP-hydrazones of α-dicarbonyls. The absorption maximum of Band 1 in

TABLE 6

Ultra-violet Light Absorption of Fraction 3 Constituents

Band No.	λ Max. (chloroform)	λ Max. (alcoholic-KOH)
1	----	475
2	----	---
3	375 mu	460-463 mu
4	376 mu	470 mu
5	376 mu	462 mu
6	366 mu	463 mu
7	383 mu	---

TABLE 7

Ultra-violet Light Absorption of Fraction 4 Constituents

Band No.	λ Max. (chloroform)	λ Max. (alcoholic-KOH)
1	393; 430 mu	555-560 mu
2	388; 436 mu	-----------
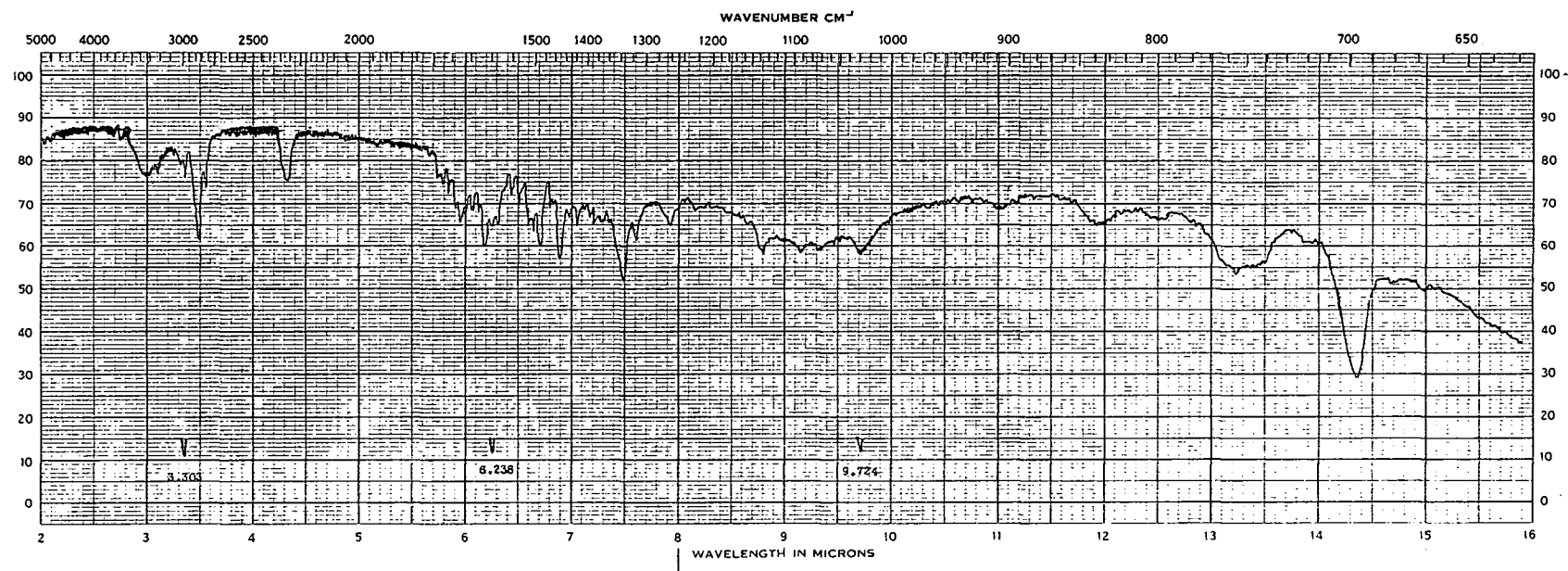
Figure 3. Infrared Spectrum of a KBr Pellet of Band 5, Table 6.

ethanolic-KOH also agreed with that of authentic bis-DNP-
hydrazones of α-dicarbonyls (55).

Gas Chromatographic Analysis of Loganberry Flavor

The odoriferous character of different fractions of
the chromatographed loganberry extract proved to be very
useful, in some cases, in helping to establish identifica-
tion of compounds in the GLC peaks. The reliability of
the odor evaluation was impaired, however, because of small
concentrations of individual fractions and the partial
overlapping of some fractions with each other. This over-
lapping was caused by the close proximity of one fraction
to another and the ability of some fractions to condense
in the gas outlet port of the GLC instrument, which caused
mixing with subsequent fractions. The first problem could
be alleviated by temperature programming. By starting at
a low temperature (70°C.) and increasing the column tem-
perature slowly, the first few peaks could have been
spread out or separated from each other so as to facilitate
collection of each individual fraction.

The problem of the fractions condensing in the gas
outlet port was of a more serious nature. It was found
that after a sample of loganberry extract had been
chromatographed, the gas outlet port had a persistent
loganberry odor coming from it. This suggested partial

condensation of eluted fractions. To remedy this, the
detector and the gas outlet port were washed repeatedly
with acetone before each analysis. A heating tape was
wrapped around the gas outlet port to inhibit condensation
of eluted fractions in the line. Collection needles
attached to the gas outlet port were renewed after each
fraction had been collected.

Sensory Evaluation of Chromatographed Fractions:
After the above precautions were taken, an attempt was
made to collect the fractions of a sample of chromato-
graphed loganberry extract. The results are shown in
Table 8. As shown in Table 8, there was still evidence
of fractions overlapping with each other. The odor of
Peak 1 was prevalent in Peaks 2, 3, and 4. This was also
the case in Peaks 12 and 13. There was a noticeable odor
difference between Peaks 18 and 19 which was not found in
any of the preceding fractions. Both peaks exhibited
reasonably strong aromatic odors. Peaks 20 and 22 were
very reminiscent of loganberries. Because of the size of
Peak 20 it was difficult to believe that its odor could
be influenced by any of the preceding fractions. However,
Peak 20 was still easily detected by the nose when Peak 22
was being eluted. This may have caused a significant
difference in the true odor of Peak 22. Because of the

TABLE 8
Aroma Evaluation of Fractions of Chromatographed
Loganberry Flavor Extract

PEAK*	AROMA CHARACTERISTICS
1	weak, terpene odor
2	similar to 1
3-4	similar to 1
5-7	pleasant flowery odor
8-10	weak, minty odor
11	weak terpene, aromatic
12	minty
13	similar to peak 12
14	similar to peak 11
15-16	weak terpene odor
17-18	pleasant terpene
19	pleasant terpene
20	strong, pleasant, terpene, loganberry character
22	strong, pleasant, aromatic odor, loganberry character
23	weak, unpleasant odor
24	pleasant minty odor, aromatic
1-19	pleasant, minty
20-24	strong pleasant odor, very reminiscent of loganberry

Instrument: Aerograph Model A-100; Column: Nine feet by
1/4 inch O.D. packed with 20% DEGS on Celite 545; Column
temperature: 100°C.; Helium flow: 50 ml/min

* Refer to Figure 4 for Peak No. designations.

rather putrid odor of Peak 23, it was easily differentiated from previous fractions. By the time Peak 24 was eluted from the GLC instrument, the gas outlet port was saturated with loganberry odor.

The combination of Fractions 1 through 19 and 20 through 24 revealed that the majority of the flavor character of loganberries was contained in the latter fractions and consisted of relatively high boiling compounds. A pleasant fragrance was detected in Fractions 1 through 19.

Functional Group Analysis of Chromatographic Fractions: The same problems which were encountered in the preceding work were evident in the functional group analysis of fractions of chromatographed loganberry flavor extract. An additional drawback was the relative low concentrations of some of the fractions. Even though micromethods were used, substantial color development was observed in only 9 of the 72 functional group tests attempted. In 7 other cases, questionable results were obtained (Table 9). The tests suggested that Peaks 13, 14, 16, 20, 22, and 24 contain compounds with alcoholic groups. Positive ester tests were observed for Peaks 11, 12, 13, 16, 18, 19, 20, and 23. Only two peaks, 14 and 16, gave positive carbonyls tests.

TABLE 9

Functional Group Analysis of Fractions of Chromatographed
Loganberry Flavor Extract

PEAK[*]	FUNCTIONAL GROUP TESTS		
	ALCOHOL	ESTER	CARBONYL
1-10	-	-	-
11	-	++	-
12	-	+	-
13	++	+	-
14	+	-	+++
15	-	-	-
16	++	++	+
17	-	-	-
18	-	+++	-
19	-	+++	-
20	++	+	-
21	-	-	-
22	++	-	-
23	-	+	-
24	+	-	-

Instrument: Aerograph Model A-100; Column: Nine foot
by 1/4 inch O.D. packed with 20% DEGS on Celite 545;
Column temperature: 100°C.; Helium flow: 50 ml/min

[*]Refer to Figure 4 for Peak No. designations.

From the preceding data it can be seen that some fractions gave positive tests for two and sometimes three functional groups. Positive ester and alcohol color reactions were evident in Peaks 13 and 20. A very positive carbonyl precipitate was formed along with a rather weak alcohol test from the constituents in Peak 14. The precipitate which was formed on reaction of Peak 14 with DNP-hydrazine was transferred to an alcoholic-KOH solution. The violet color produced was indicative of a dicarbonyl. Peak 16 gave positive tests for alcohol, ester, and carbonyl, although the carbonyl test was of questionable value.

Evaluation of the Relative Retention Data for the GLC Fractions of Loganberry Flavor Extract: The relative retention volumes (V_R^0 / V_R^0) of the chromatographed fractions of loganberry flavor extract are given for the diethylene glycol succinate column in Table 10. Peak designations, as shown in Table 10, are from the chromatogram shown in Figure 4. The V_R^0 / V_R^0 for authentic compounds also are given in the table. The relative retention volumes of the authentic compounds are all within 3% of the corresponding values for the unknown peaks. According to James (45, p. 1564-1570) it is experimentally impossible to discriminate between two or more compounds whose relative retention volumes differ by approximately 7 per cent

TABLE 10

Relative Retention Volumes of Compounds Tentatively
Identified in Loganberry Flavor Extract Using a DEGS
Column

PEAK NO.*	V_R^0 / V_R^0 UNKNOWN	KNOWN	COMPOUND
1	0.032	0.032	ether
2	0.072	-----	-----
3	0.104	0.104	propyl propionate
		0.104	ethyl isovalerate
		0.101	2,2-dimethyl-1-pentanol
4	0.112	-----	-----
5	0.135	0.131	3-methyl-3-pentanol
6	0.143	0.143	ethyl valerate
		0.140	n-butanol
7	0.155	-----	-----
8	0.187	0.188	methyl hexanoate
		0.180	2-methyl-1-butanol
9	0.207	-----	-----
10	0.223	0.223	amyl alcohol
11	0.267	0.271	hexyl acetate
12	0.291	0.288	methyl heptanoate
13	0.347	0.343	n-hexanol
14	0.378	-----	-----
15	0.466	0.452	methyl octanoate
		0.452	octan-2-ol
16	0.502	0.502	ethyl octanoate
17	0.645	-----	-----
18	0.741	-----	-----
19	0.884	-----	-----
20	1.00	1.00	linalool
21	2.08	-----	-----
22	2.35	2.35	-terpineol
23	3.18	3.17	hexanoic acid
24	3.38	-----	-----

Instrument: Barber-Colman Model 20 with -ionization
detector; Column: 9 foot by 1/8 inch O.D. packed with
20% DEGS on Celite 545; Column Temperature: 100°C.
Argon flow: 28 ml/min

* Refer to Figure 4 for Peak No. designations.

LOGANBERRY FLAVOR EXTRACT

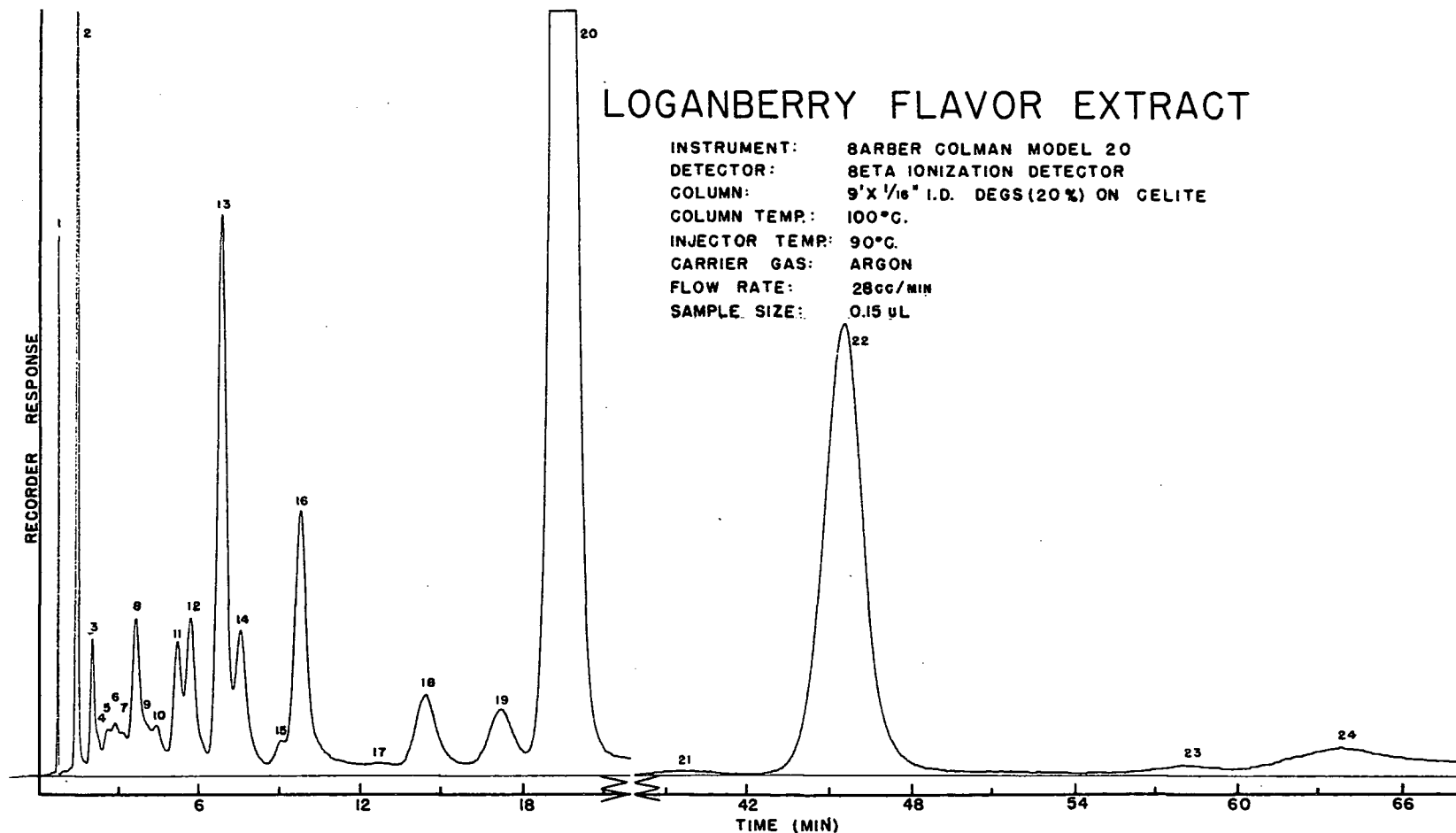| INSTRUMENT: | BARBER COLMAN MODEL 20 |
DETECTOR:	BETA IONIZATION DETECTOR
COLUMN:	9'X 1/16" I.D. DEGS(20%) ON CELITE
COLUMN TEMP.:	100°C.
INJECTOR TEMP.:	90°C.
CARRIER GAS:	ARGON
FLOW RATE:	28cc/MIN
SAMPLE SIZE:	0.15 µL

Figure 4. Gas Chromatogram of Loganberry Flavor Extract on a DEGS Column.

or less.

Tentative identification of alcohols was found in Peaks 3, 5, 6, 8, 10, 13, 15, 20, and 22. These data are in close agreement with the results obtained from the functional group tests (Table 9). Peaks 13, 20, and 22 gave strong alcohol tests and because of the very small relative concentrations of Peaks 10 and 15, positive alcohol tests probably could not be expected. Possible explanation of the positive alcohol test observed in Peak 14 could be its contamination by Peak 13.

A sample of commercially available geraniol, when separated by GLC, gave three peaks of about equal concentration. The last of these peaks had a relative retention volume corresponding to Peak 24 (Figure 4). The composition of the geraniol sample could not be determined by infrared spectroscopy. There was, however, a distinct odor similarity between the third peak in the geraniol sample and Peak 24 of chromatographed loganberry flavor extract, Figure 4.

Peak 22 (Figure 4) was conclusively identified by comparing its infrared spectrum with that of a known sample of α-terpineol. The two spectra are shown in Figures 6 and 7.

The infrared spectrum of Peak 20 was of such poor quality that it was not included in this manuscript.

LOGANBERRY FLAVOR EXTRACT

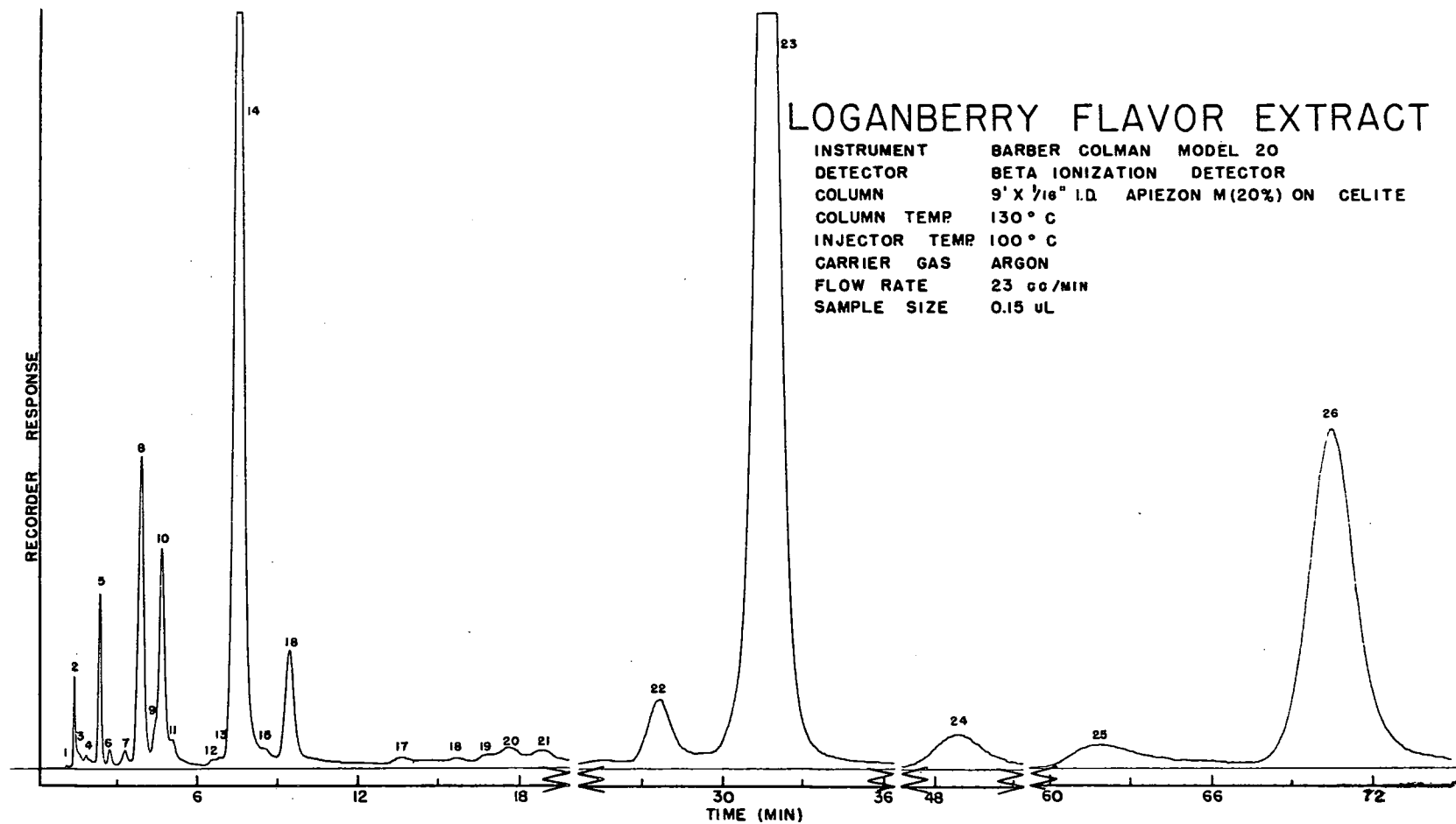| INSTRUMENT | BARBER COLMAN MODEL 20 |
DETECTOR	BETA IONIZATION DETECTOR
COLUMN	9' X 1/16" I.D. APIEZON M(20%) ON CELITE
COLUMN TEMP.	130° C
INJECTOR TEMP.	100° C
CARRIER GAS	ARGON
FLOW RATE	23 cc/MIN
SAMPLE SIZE	0.15 uL

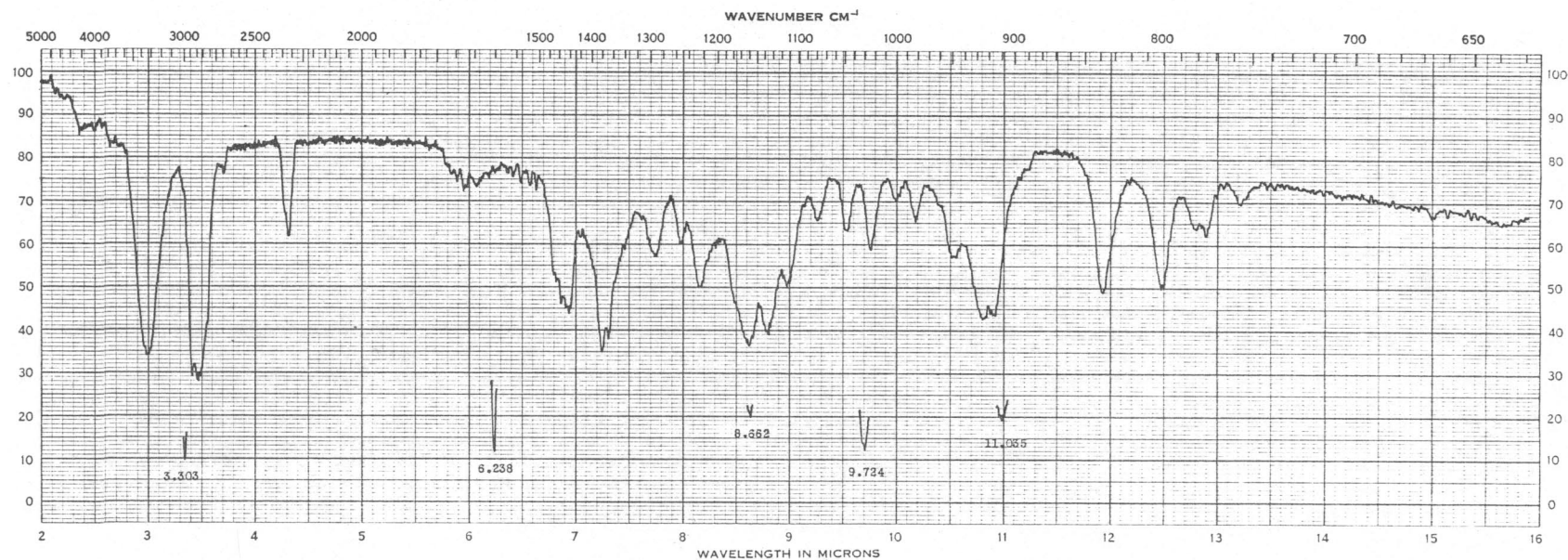Figure 5.   Gas Chromatogram of Loganberry Flavor Extract on an Apiezon M Column.

Figure 6. Infrared Spectrum of a Thin Film from Peak 22 of Figure 4,
Obtained from Gas Chromatographed Loganberry Flavor Extract.
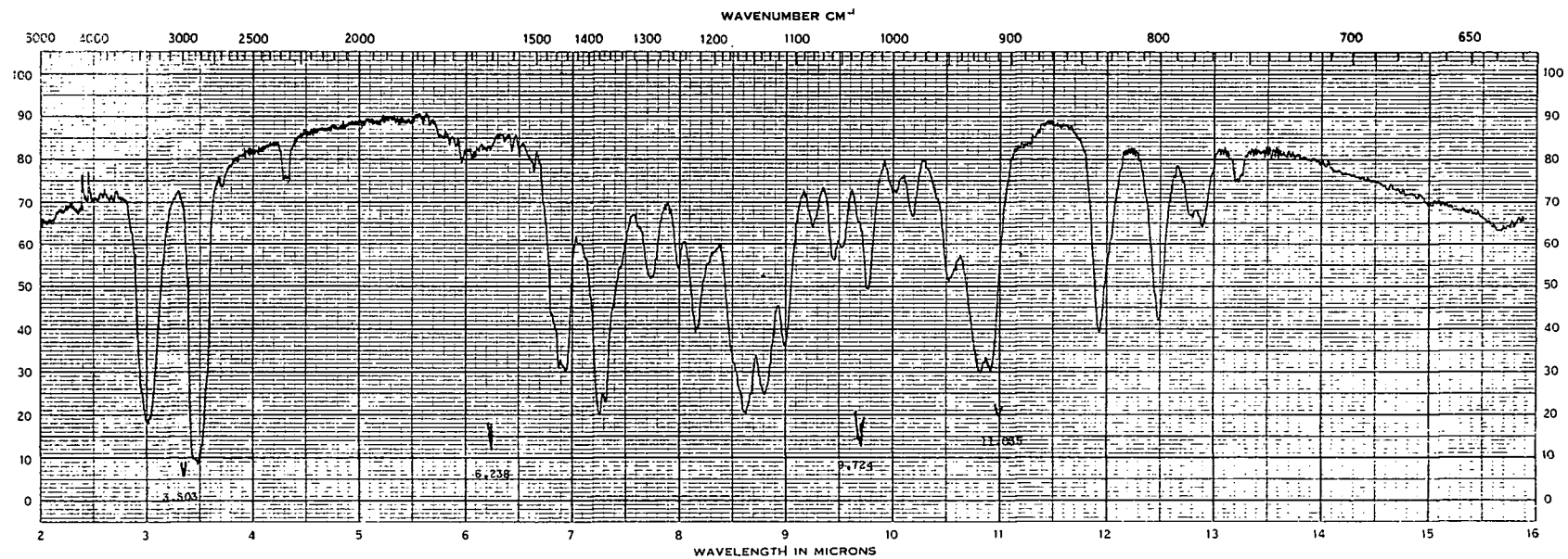
Figure 7. Infrared spectrum of a thin film of authentic ⍺-terpineol.

There was, however, some similarity of the spectrum with that of linalool. The odors of linalool and Peak 20 were identical as were their relative retention volumes.

Data for the tentative identification of esters in Peaks 3, 6, 8, 11, 12, 15, and 16 (Figure 4) are shown in Table 10. Peaks 11, 12, and 16 gave positive ester color reactions when reacted with sodium and 1,2-dinitrobenzene reagent. The limited amounts of Peaks 3, 6, 8, and 15 could explain the absence of a functional group color reaction for the esters in these peaks.

Peaks 18 and 19 gave the most reliable ester tests of the chromatographic fractions. None of the available authentic esters were found which gave relative retention volumes corresponding to Peaks 18 and 19. It was observed, howeveb, that when an authentic sample of linalool was chromatographed, two peaks were evident on the chromatogram. By far the highest concentration was the peak which had a relative retention volume corresponding to Peak 20. A smaller, but significant peak was observed which had the same relative retention volume as that of Peak 19. This smaller peak, an impurity of the linalool sample, could not be identified. From the functional group tests it is suggested that it may be an ester of linalool. The odor of the linalool contaminant was similar to that of Peak 19.

An authentic sample of hexanoic acid was observed to have a corresponding relative retention volume in agreement with that of Peak 23. The odor of Peak 23, although similar to hexanoic acid, was undoubtedly masked somewhat by contamination of other fractions which condensed on the gas outlet port. Infrared analysis of Peak 23 proved futile.

An attempt was made to confirm the results of functional group analysis indicating the presence of a dicarbonyl compound in Peak 14 (Figure 4). However, none of the available authentic compounds gave comparable retention data.

Further evidence to support the assignment of tentative identities to the various chromatographic peaks in Figure 4 was obtained by GLC analysis of the loganberry flavor extract on the non-polar, Apiezon M column. The resulting chromatogram is depicted in Figure 5 and the relative retention data are given in Table 11. The data substantiate the tentative assignment of the peak identities obtained on the polar-type, DEGS column.

It was shown earlier in this thesis that carbonyl compounds were present in loganberry essence. This leads to the question, why only one carbonyl was detected by functional group tests, and none were found to have

TABLE 11

Relative Retention Volumes of Compounds Tentatively
Identified in Loganberry Flavor Extract Using an
Apiezon M Column

PEAK NO.[*]	V_R^Q / V_R^Q		COMPOUND
	UNKNOWN	KNOWN	
1	0.032	-----	-----
2	0.041	-----	-----
3	0.048	0.048	ether
4	0.056	-----	-----
5	0.072	-----	-----
6	0.081	0.081	n-butanol
7	0.100	-----	-----
8	0.119	0.119	2-methyl-1-butanol
9	0.138	0.134	amyl alcohol
10	0.143	0.143	3-methyl-3-pentanol
11	0.158	0.153	propyl propionate
12	0.205	0.201	ethyl isovalerate
13	0.215	-----	-----
14	0.237	0.234	n-hexanol
15	0.268	0.263	ethyl valerate
		0.272	2,2-dimethyl-1-pentanol
16	0.296	0.305	methyl hexanoate
17	0.430	-----	-----
18	0.497	0.501	hexyl acetate
19	0.535	0.539	octan-2-ol
20	0.561	0.561	methyl heptanoate
		0.561	hexanoic acid
21	0.592	-----	-----
22	0.869	-----	-----
23	1.00	1.00	linalool
		1.03	methyl octanoate
24	1.54	1.57	ethyl octanoate
25	1.96	-----	-----
26	2.24	2.24	-terpineol

Instrument: Barber-Colman Model 20 with -ionization
detector   Column: Nine foot by 1/8 inch O.D. packed with
20% Apiezon M on Celite 545   Column temperature: 130°C.
Argon flow: 23 ml/min

* Refer to Figure 5 for Peak No. designations.

similar retention volumes to peaks found in chromatographed loganberry flavor extract. This can be explained by the relatively small amounts of carbonyls, excluding acetaldehyde, which are present in loganberry essence. The highest sensitivity which is attained by the β-ionization detector cell of the Model 20 Barber-Colman GLC instrument is 10^{-9} moles. For ease of operation the instrument was accentuated to 1/100 of its sensitivity, or 10^{-7} moles. The highest concentration of any one carbonyl found in loganberry essence, excluding acetaldehyde and an unknown, was acetone. If complete separation of acetone from the water in the essence was achieved and conditions were such that evaporation of it was negligible, only 10^{-7} moles would be present in the extract. The probability of satisfying all of the above conditions is unlikely. The absence of acetaldehyde in the GLC data may be explained by its loss during the extraction of flavor from the essence. Most of the acetaldehyde is lost during evaporation of ethyl chloride from the extract.

Decomposition of Oxygenated Terpenes in the Injection Heater of the Gas Chromatograph: The feasibility of achieving plug flow during sample charging of the gas chromatograph has been pointed out by Porter et al. (65, p. 2999). To facilitate plug flow, most of the newer gas

chromatographs contain separately heated injection ports enabling operation at temperatures sufficient to provide instant vaporization of charged samples. This feature, while highly desirable in approaching a plug flow pattern, can present serious problems when the sample contains compounds of questionable stability. The problem was encountered during the analysis of the loganberry extract. Considerable effort had been devoted to the analysis of thermal artifacts prior to the accidental observation that chromatographic patterns of the loganberry were completely different where the flask heater temperature was varied.

Gas chromatographic separation of loganberry flavor extract initially was carried out at a column temperature of 100°C. (DEGS) or 130°C. (Apiezon M) and an injector temperature of 205°C. The chromatograms obtained with DEGS and Apiezon M columns are shown in Figures 8 and 9 respectively. Quite by accident the loganberry flavor extract was chromatographed when the injector temperature was lower and the chromatograms obtained (Figures 5 and 6) were different as compared to Figures 9 and 10. This suggested the possible decomposition of some compounds in the loganberry flavor extract by the injector temperature. When α-terpineol, later conclusively identified by infrared spectrometry as Peak 26 (Figure 5) in chromatographed

LOGANBERRY FLAVOR EXTRACT

INSTRUMENT	BARBER COLMAN MODEL 20
DETECTOR	BETA IONIZATION DETECTOR
COLUMN	9'x 1/16" I.D. DEGS(20%)ON CELITE
COLUMN TEMP.	100° C.
INJECTOR TEMP.	205° C.
CARRIER GAS	ARGON
FLOW RATE	27 cc/min
SAMPLE SIZE	0.35 uL

X10

X3.3

RECORDER RESPONSE

TIME (MIN)
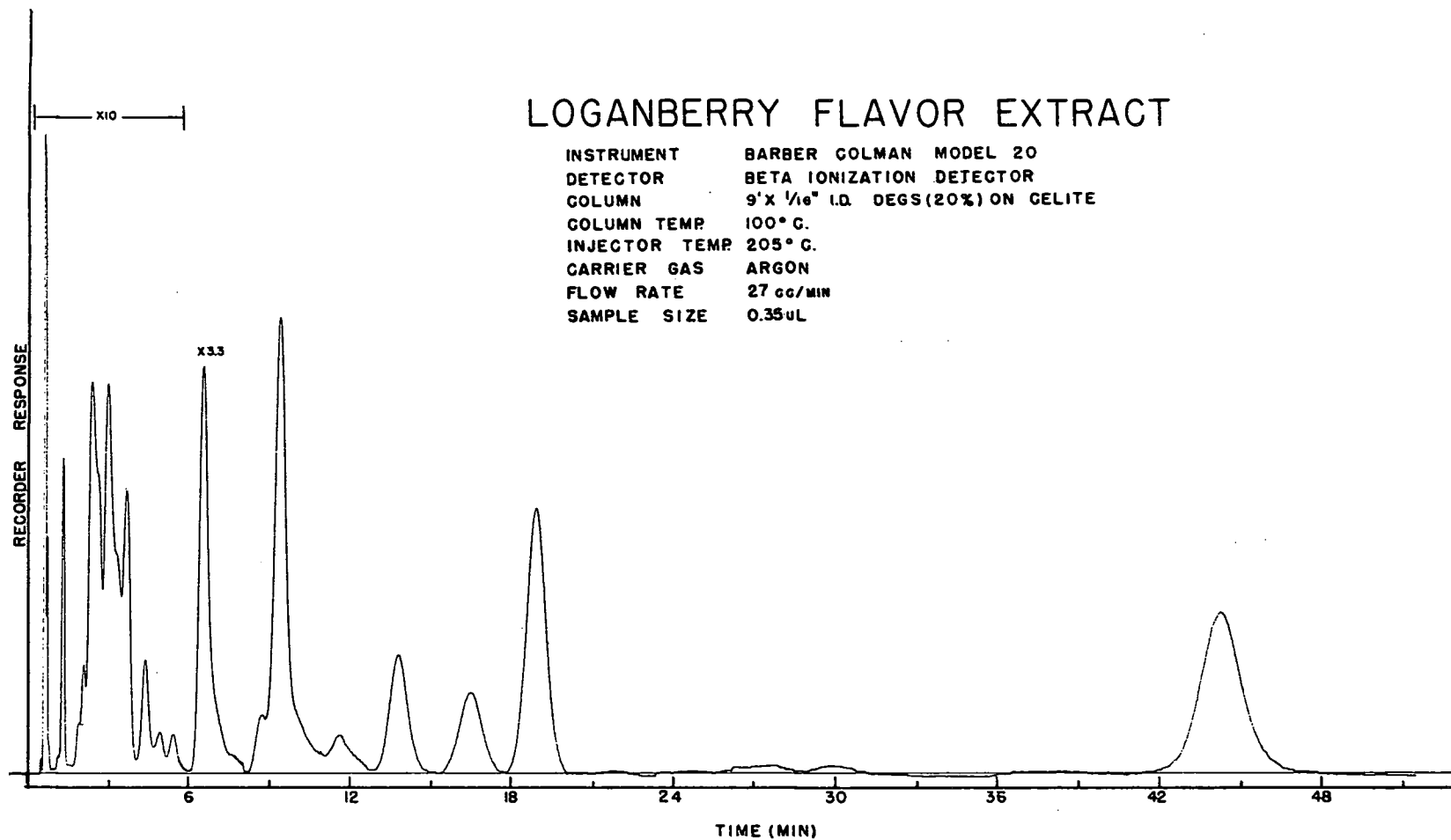
Figure 8.   Gas Chromatogram of Loganberry Flavor Extract on a DEGS Column
with the Injector Temperature at 205°C.

LOGANBERRY FLAVOR EXTRACT

INSTRUMENT	BARBER COLMAN MODEL 20
DETECTOR	BETA IONIZATION DETECTOR
COLUMN	9'X¹⁄₁₆" I.D. APIEZON M(20%) ON CELITE
COLUMN TEMP	130°C.
INJECTOR TEMP	205°C.
CARRIER GAS	ARGON
FLOW RATE	29cc/min
SAMPLE SIZE	0.15 uL

RECORDER RESPONSE

TIME (MIN)
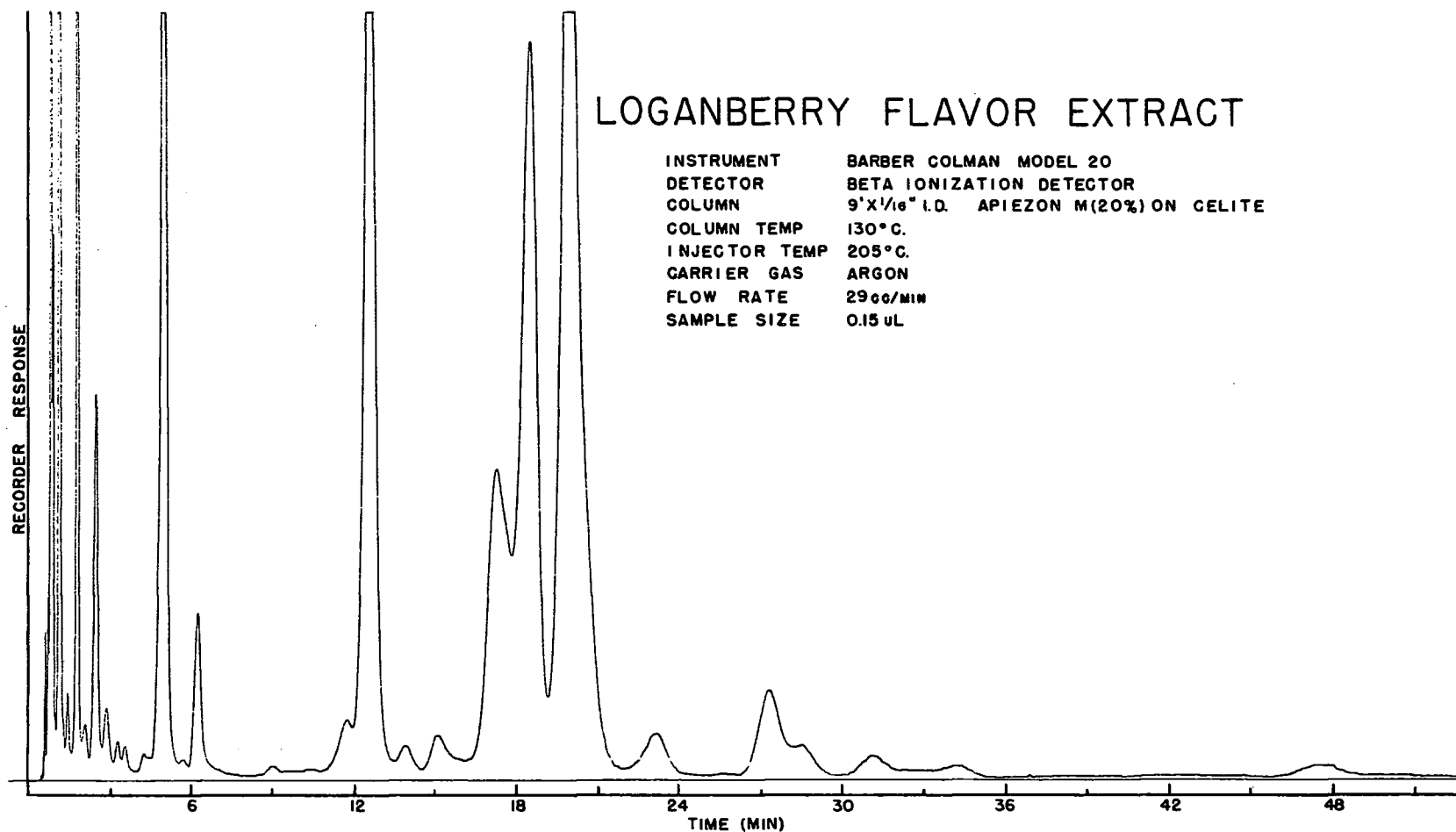
Figure 9.   Gas Chromatogram of Loganberry Flavor Extract on an Apiezon M Column with the Injector Temperature at 205°C.

RECORDER RESPONSE

SAMPLE   ALPHA TERPINEOL
INSTRUMENT   BARBER COLMAN MODEL 20
DETECTOR   BETA IONIZATION DETECTOR
COLUMN   9' X 1/16" I.D.   APIEZON M(20%) ON CELITE
COLUMN TEMP.   130° C
INJECTOR TEMP. 205° C
CARRIER GAS   ARGON
FLOW RATE   29 cc/min
SAMPLE SIZE   0.05 uL

6   12   18   24   30   36   42   48
TIME (MIN)

Figure 10.   Gas chromatogram of ∝-terpineol with injector
temperature at 205°C.

loganberry flavor extract, was chromatographed using an
injector temperature of 205°C. a number of peaks were ob-
served (Figure 10). When α-terpineol was chromatographed
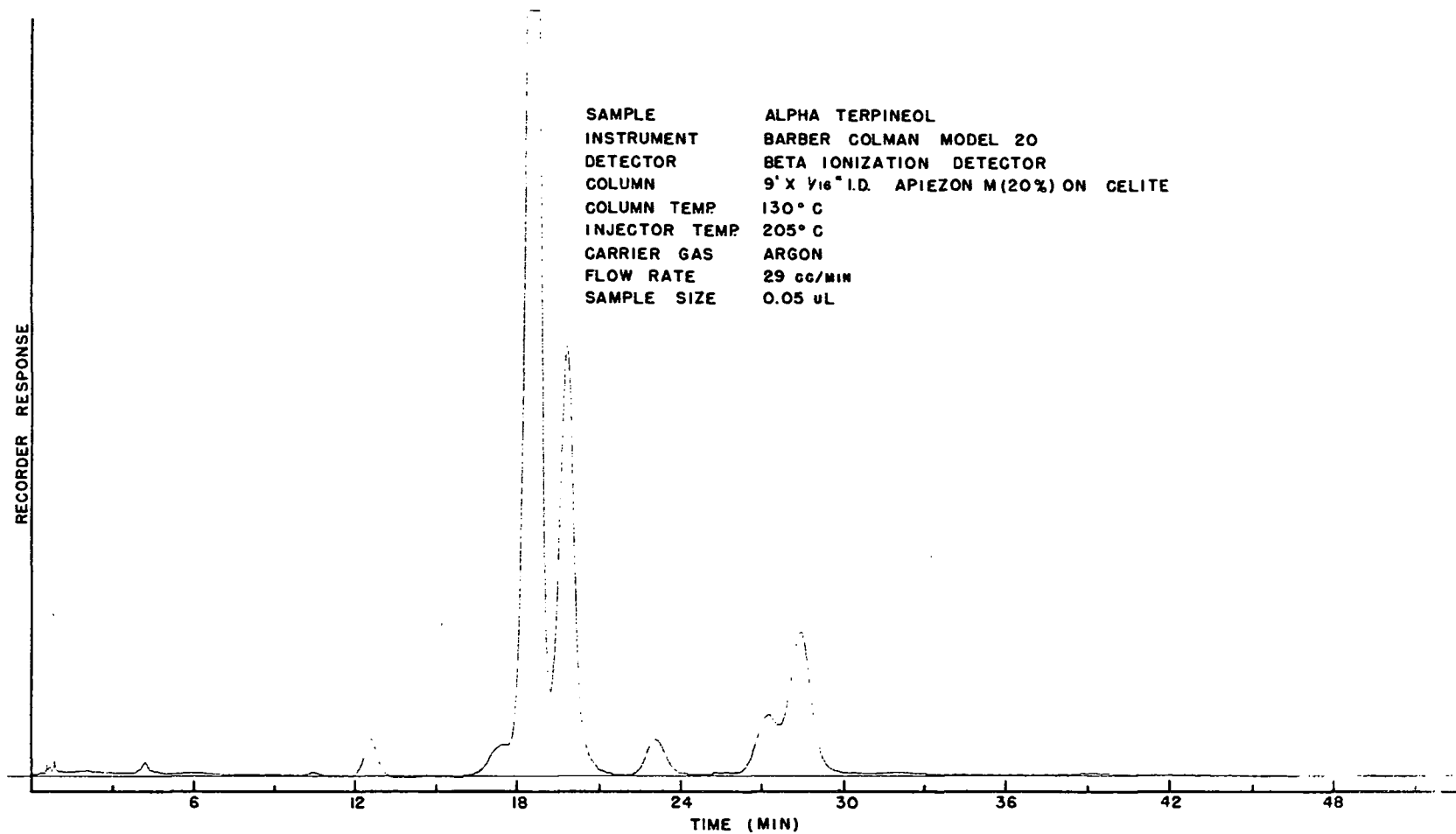with the injector temperature at 100°C. a completely
different chromatogram was obtained (Figure 11).

Comparison of chromatograms of Figures 10 and 11 show
that α-terpineol is completely destroyed in Figure 10,
whereas only slight decomposition was noted in Figure 11.
The only difference in operation conditions for the two
chromatograms was the flash heater temperature. That some
decomposition was still evident, even at 100°C. emphasizes
the lability of the compound. Similar results were ob-
served for linalool. Oxygenated terpenes are not peculiar
in this respect, and like many compounds, they present a
problem in achieving plug flow due to their relatively
high boiling points. Evaluation of flash heater tempera-
tures for unknown mixtures, to avoid decomposition, may
prove desirable in most cases. While low flash heater
temperatures tend to give undesirable skewed peaks (Figure
4), it is more expedient than to devote time to analyzing
thermal artifacts.

SAMPLE   ALPHA TERPINEOL
INSTRUMENT   BARBER COLMAN MODEL 20
DETECTOR   BETA IONIZATION DETECTOR
COLUMN   9' X 1/16" I.D. APIEZON M (20%) ON CELITE
COLUMN   TEMP. 130° C
INJECTOR   TEMP. 100° C
CARRIER   GAS   ARGON
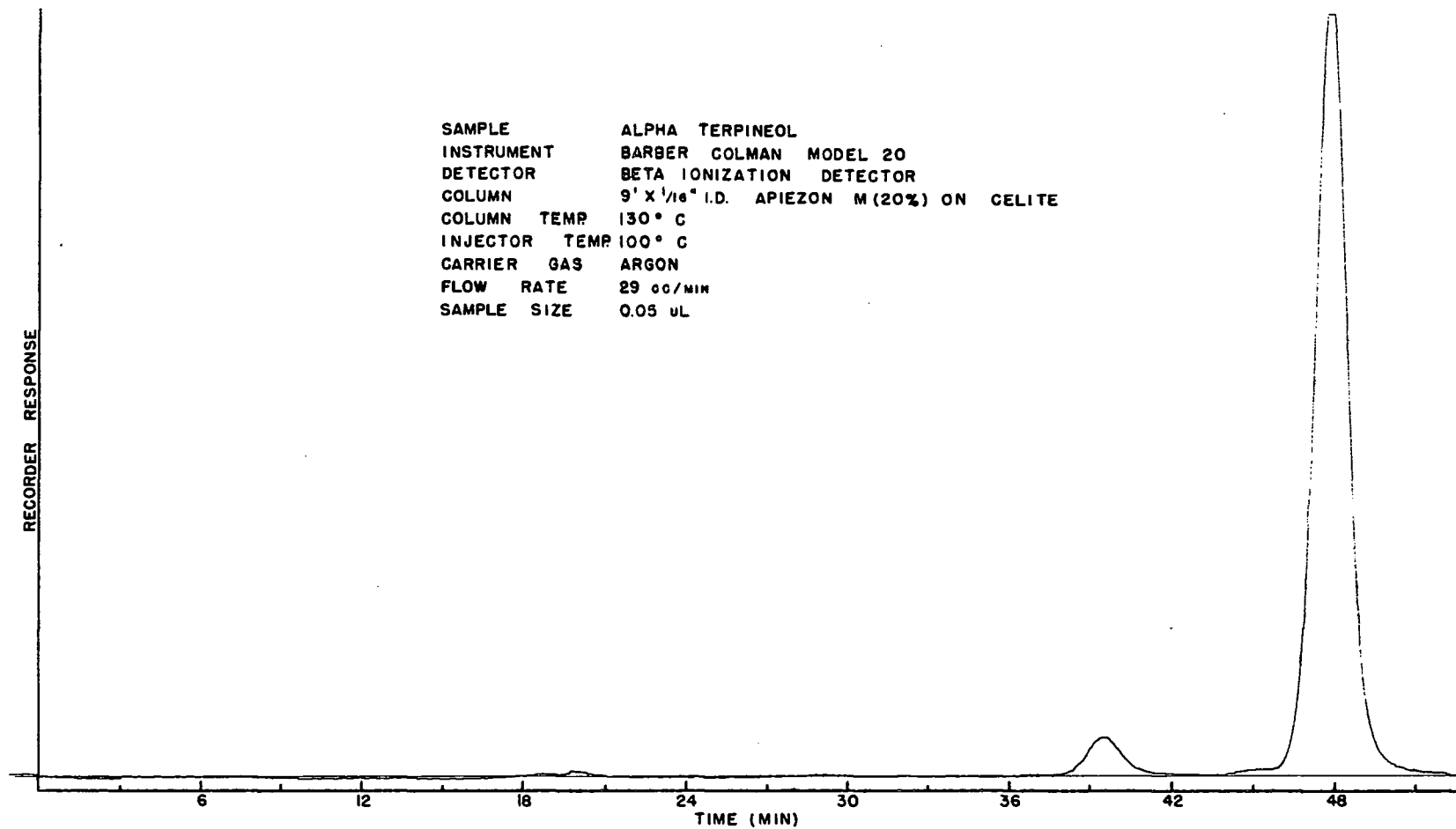FLOW   RATE   29 cc/min
SAMPLE   SIZE   0.05 uL

Figure 11. Gas chromatogram of α-terpineol with injector temperature at 100°C.

SUMMARY AND CONCLUSIONS

1.   Loganberry essence, representing a 150 fold concen-
tration of the flavor, was found to exhibit the typical
loganberry odor.   There was no evidence of a "heated
flavor."

2.   The carbonyl compounds in the essence were isolated
as DNP-hydrazones.   The DNP-hydrazones were separated into
classes by magnesium oxide adsorption columns and each
class was subsequently separated into single component
fractions by column partition chromatography.   Evidence
to establish identification of the DNP-hydrazones included
a comparison of the chromatographic behavior of authentic
and unknowns on adsorption and partition columns as well
as on paper chromatograms.   In addition, absorption proper-
ties of chloroform solutions in the ultraviolet region was
used for identification.

The saturated monocarbonyl derivatives were separated
into 14 fractions.   Acetaldehyde was conclusively identi-
fied and tentative identification was obtained for eleven
of the remaining fractions.   These were propanal, acetone,
n-butanal, n-pentanal, n-hexanal, n-heptanal, 2-heptanone,
n-nonanal, n-decanal, n-undecanal, n-dodecanal, and
n-tridecanal.   Quantitative data revealed that acetaldehyde
comprised 63% of this fraction.

Three compounds were tentatively identified in the alk-2-enal fraction. They were pent-2-enal, hex-2-enal, and hept-2-enal. Hex-2-enal was in the highest concentration. Evidence is presented which indicates that an unknown component in the alk-2-enal fraction was in fact a DNP-hydrazone of an α-dicarbonyl compound.

Two other major fractions, isolated by the magnesium oxide column, were found to contain dicarbonyl compounds. Fraction 3, which moved slower than the alk-2-enal fraction was found to contain DNP-hydrazones of dicarbonyls. Fraction 4, from the magnesium oxide column contained bis-DNP-hydrazones of α-dicarbonyls and it was separated into two fractions by silicic acid column chromatography. None of the dicarbonyl derivatives were identified.

3. The odor properties of gas chromatographic fractions of loganberry flavor were determined. The most characteristic odor was contained in the lower vapor pressure fractions.

4. Tentative identification for 17 chromatographic fractions of the flavor was obtained by chromatographing the flavor on polar (diethylene glycol succinate) and non-polar (Apiezon M) columns and comparing the relative retention volumes (V_R^0 / V_R^0 of linalool = 1) of the unknown peaks with values for authentic compounds. Functional group

analysis of chromatographic peaks gave credence to the relative retention data in establishing identities of compounds. Compounds tentatively identified were propyl propionate, ethyl isovalerate, 2,2-dimethyl-1-pentanol, 3-methyl-3-pentanol, ethyl valerate, n-butanol, methyl hexanoate, 2-methyl-1-butanol, amyl alcohol, hexyl acetate, methyl heptanoate, n-hexanol, methyl octanoate, octan-2-ol, ethyl octanoate, linalool, and hexanoic acid. α-terpineol, a major component of loganberry flavor, was conclusively identified by its infrared spectrum.

5. While evidence obtained in this investigation was not conclusive, it would appear that the major flavor components of loganberry are α-terpineol, linalool, geraniol, and an ester of linalool. The many other flavor components may be essential but of less importance.

6. It was found that excessively high injector temperatures caused thermal decomposition of some compounds found in the loganberry flavor extract, notably the oxygenated terpenes. It was observed that completely different chromatograms were obtained when the injector temperature was changed from 100°C. to 205°C. It was concluded that careful consideration should be given to the injector temperature which is to be used when mixtures of unknown

compounds are to be chromatographed to prevent the formation of thermal artifacts.

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