Volatile Constituents of Banana (*M. cavendishii*, Variety Valery)

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Volatile constituents of banana fruit were identified by investigation of the neutral and acidic fractions obtained by hydrolysis of odor concentrates. Hydrogenation of the neutral alcohol fraction indicated the presence of 11 unsaturated components. Combined gas chromatographic–mass spectrometric analysis resulted in identification of *trans*- and *cis*-4-hexen-1-ol, two octen-1-ols, the C_1 through C_6 alkanols, 2-methylpropanol, 3-methylbutanol, 2-pentanol, 2-heptanol, 2-pentanone, and 2-heptanone. Methylation of the acidic fraction followed by GC-MS analysis showed the presence of acetate, butyrate, 2-methylpropanoate, hexanoate, 3-hexenoate, 2-hexenoate, and octanoate. Propanoate was tentatively identified.

The banana is one of the few fresh fruits available year-round to consumers in many parts of the world. The fruit is grown in the tropics, picked in a mature but unripe state, shipped to a point close to that of retail sale, and ripened under controlled conditions. Banana puree and dehydrated banana are produced primarily for incorporation in other prepared food products. In general, processed banana products undergo undesirable flavor and texture changes which make them unsatisfactory for use in products other than baked goods.

Since it is possible to ripen bananas in the laboratory under conditions identical to those used in commercial practice, this fruit is an ideal medium for fundamental studies of the metabolic processes involved in the production of volatile compounds which contribute to aroma. A better understanding of the nature and mechanism of production of these compounds could lead to the development of fruit varieties and methods of processing to yield products, the flavor of which more closely resembles that of fresh fruit. Such products could reduce the cost of transporting the fragile fresh fruit and eliminate the economic loss resulting from fruit which has been allowed to overripen.

This paper describes results of continued research on the isolation and identification of volatile constituents of ripe banana fruit. The increase in quantity of these constituents observed as the fruit ripens and its flavor develops, suggests a fundamental interrelationship between these substances and biochemical processes occurring in the fruit. Results of a study of changes during ripening in certain banana fruit components which may be precursors to the volatile constituents are presented elsewhere (Goldstein, 1966; Myers, 1968).

Previously reported investigations (Hultin and Proctor, 1961; Issenberg and Wick, 1963; Issenberg *et al.*, 1964; McCarthy *et al.*, 1964; Wick *et al.* 1966) resulted in the identification of about 39 volatile constituents (Table I). Identifications made in this laboratory were based on comparison of retention data and infrared spectra of banana components trapped from selected gas chromatographic columns, with retention data and spectra of authentic reference compounds purified in the same manner (Issenberg and Wick, 1963; Myers, 1968; Wick *et al.*, 1966). Initial attempts to use coupled gas chromatography–mass spectrometry to characterize substances present in amounts too small to permit infrared analyses were unsuccessful because of inadequate resolving power of the chromatographic columns employed. More than 70 components were detected, but the mass spectra could not be reliably interpreted even though tentative structures for many chromatographic peaks might have been proposed. Most of the unknown substances appeared to be esters. Successful identification of components obviously required use of highly efficient chromatographic columns.

The best separation achieved thus far shows the presence of about 200 peaks. Some peaks may be artifacts accumulated during isolative procedures. Despite rather good separation, one cannot expect to accomplish definitive identification of unknown components by direct GC-MS analysis of a total banana odor concentrate. "Single" peaks still, in all probability, contain more than one compound. Some means for simplification of the very complex mixture was required. Since previous evidence (Wick *et al.*, 1966) had indicated that many constituents were esters, the odor concentrate was hydrolyzed and the resulting components of the less complex "alcohol" and "acid" fractions were isolated and characterized. This knowledge, together with information gained by direct study of constituents of aroma concentrate, has given additional understanding of the identity of the volatile fraction of ripe banana fruit.

Table I. Previously Identified Banana Constituents

Alcohols	Esters
Methanol	Methyl acetate
Ethanol	Ethyl acetate
1-Propanol	*n*-Butyl acetate
2-Propanol	2-Methyl propyl acetate
1-Butanol	1-Pentyl acetate
2-Methylpropanol	2-Pentyl acetate
1-Pentanol	3-Methylbutyl acetate
2-Pentanol	1-Hexyl acetate
3-Methylbutanol	1-Propyl propionate
1-Hexanol	1-Pentyl propionate
2,3-Butylene glycol	Ethyl butyrate
Acetoin	1-Butyl butyrate
Aldehydes	2-Methylpropyl butyrate
Acetaldehyde	1-Pentyl butyrate
trans-2-Hexenal	2-Pentyl butyrate
Ketones	3-Methylbutyl butyrate
2-Butanone	Aromatic compounds
2-Pentanone	Eugenol
2-Octanone	Methyl eugenol
Acids	Elimicin (1-allyl-3,4,5-trimethoxybenzene)
Formic	
Acetic	

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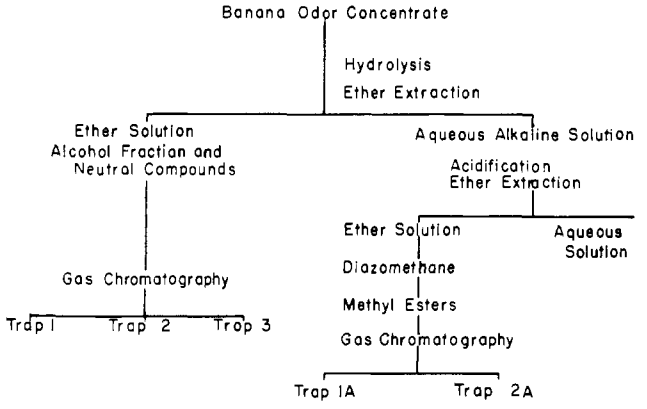

Figure 1. Hydrolysis of banana odor concentrate and isolation of alcohol and methyl ester fractions

EXPERIMENTAL PROCEDURES

Banana odor concentrates in yields ranging from 150 to 338 p.p.m. were obtained as previously described (Wick *et al.*, 1966) by lyophilization of ripe banana pulp (*M. cavendishii*, variety Valery). All concentrates exhibited typical ripe banana aroma.

Hydrolysis Method. The separation scheme is given in Figure 1. Banana concentrate [0.2 ml. (about 150 mg.)] in 8 ml. of 5% aqueous potassium hydroxide was heated in a sealed tube at 100° to 150° C. for 1.5 hours. The initial turbid mixture became a clear yellow solution during heating. It was allowed to come to room temperature, rinsed into a separatory funnel with 5 ml. of water, and extracted with three 3-ml. portions of diethyl ether. The resulting ether layer was washed twice with aqueous saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated by careful distillation and evaporation under nitrogen, to yield the "alcohol" fraction, an isolate which contained neutral components.

The aqueous alkaline layer was brought to pH 1 or 2 with about 0.7 ml. of 12*M* hydrochloric acid and extracted with ether. The ether extract, which contained acidic components of the banana odor concentrate, was dried over anhydrous sodium sulfate and then treated drop by drop with an ether solution of diazomethane (Blatt, 1943). Addition was stopped when a deepened yellow color persisted. The solution was allowed to stand at room temperature 15 minutes, then dried over anhydrous sodium sulfate and concentrated as described above to minimum volume. This isolate contained methyl esters of acids obtained from hydrolysis of esters in banana odor concentrate and methyl esters of free acid components of the concentrate.

Gas Chromatography. Analytical separations were carried out under the conditions given in Figures 2 through 6.

Preparative Separations. ALCOHOL AND NEUTRAL FRACTION. 6-foot × 0.25-inch i.d., 20% Carbowax 4000 on Chromosorb W (60- to 80-mesh) column. Helium flow, 70 ml. per minute. Split ratio at flame ionization detector 1 to 5. Trap 1 was collected from 0 to 22 minutes at 60° C. Trap 2 was collected from 22 to 89 minutes, while column temperature was increased at 1° per minute. Trap 3 was collected from 89 to 120 minutes, while column temperature was increased at 2° per minute.

METHYL ESTER FRACTION. 10 foot × 1/8-inch o.d. 10% DEGS on Chromosorb W (35- to 80-mesh) column. Helium flow, 20 ml. per minute. Split ratio at flame ionization detector 1 to 5. Temperature program, 10 minutes at 40° C., 10° per minute to 175°, then isothermal. Trap 1A was collected from 0 to 15 minutes. Trap 2A was collected during the rest of the chromatogram.

Removal of Alcohols by Auxiliary Boric Acid Column. A 5 × 1/8 inch tube packed with 15% boric acid–3% SE 30 on Chromosorb G (100- to 120-mesh) was attached between the end of the 500-foot × 0.02-inch SF 96 (50) open-tubular column and the flame ionization detector. The auxiliary

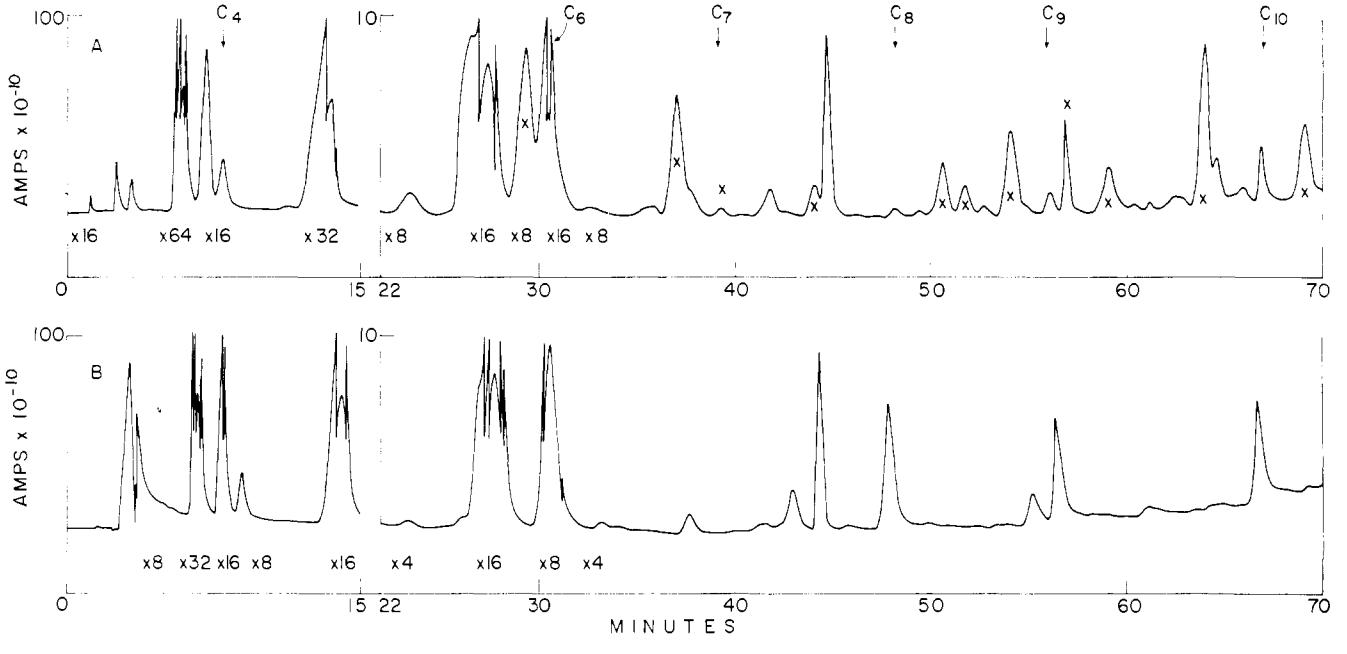

Figure 2. Hydrogenation of alcohol and neutral fraction

A. Before hydrogenation. Carbon number markers based on *n*-alkanols
B. After hydrogenation
Column. 10-foot × 1/8-inch, o.d., 5% Carbowax 4000 on Chromosorb W (80- to 100-mesh)
Helium flow. 23 ml. per minute
Temperature program. 22 minutes at 60° C., 2° per minute for 63 minutes

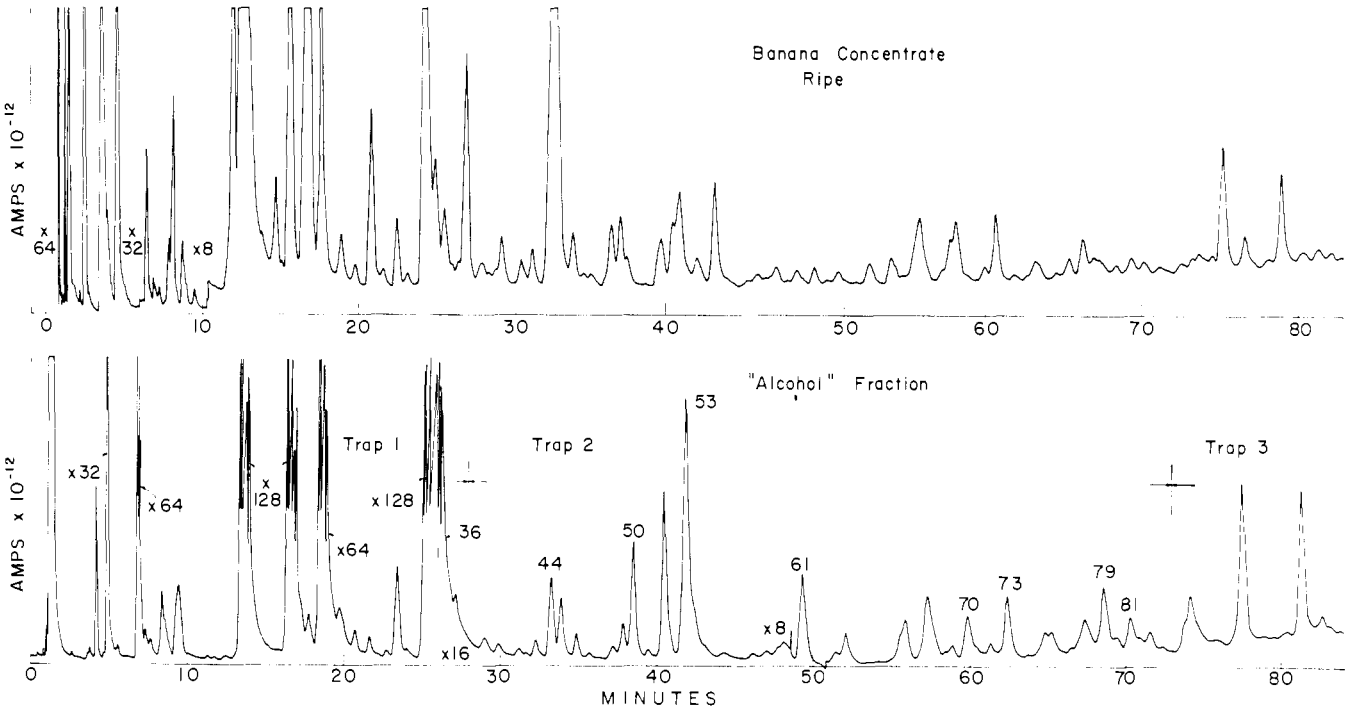

Figure 3. Effect of hydrolysis

A. Banana concentrate
B. Alcohol fraction
Column. 50-foot × 0.02-inch i.d. Carbowax 1540 support-coated open-tubular (Perkin-Elmer Corp.)
Helium flow. 4 ml. per minute
Temperature program. 10 minutes at 60° C. to 142° at 1° per minute; isothermal at 142° C.
Sample size range. 0.2 to 0.5 μl.

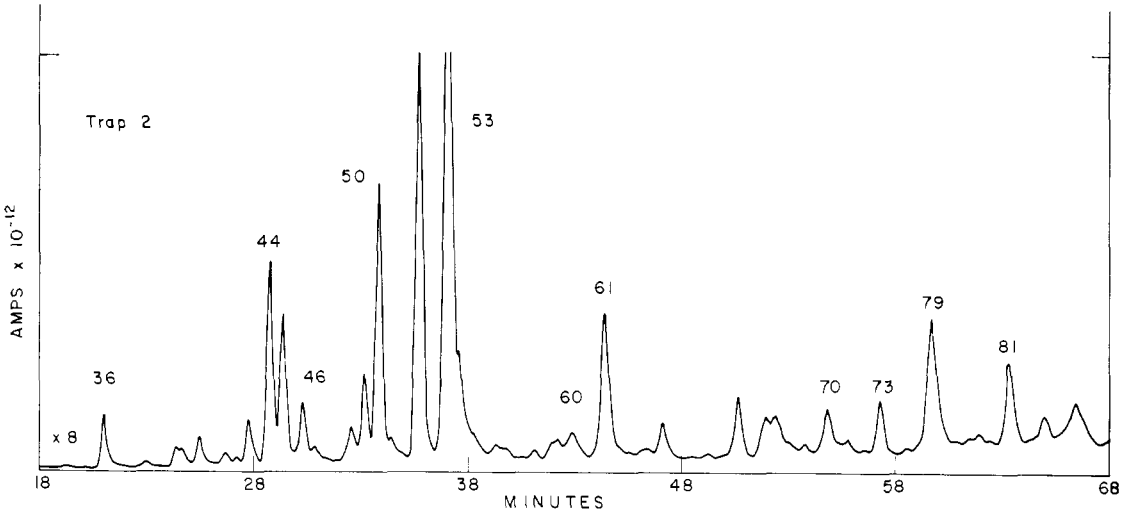

Figure 4. Components in trap 2
Alcohol fraction. Column and conditions as in Figure 3

column was conditioned at 225° C. overnight before use as suggested by Ikeda *et al.* (1964).

Mass Spectrometry. The support-coated open-tubular columns (DEGS for methyl ester separation and CW 1540 for neutral component separation) were used in the mass spectrometer inlet chromatograph (Model 204-C, Varian-Aerograph, Walnut Creek, Calif.). Column effluent passed through a stainless steel capillary restriction (1.9 meters × 0.50 mm. i.d.) and fritted glass enricher (Watson and Biemann, 1965), both maintained at 200° C., into the ion source of a double-focusing mass spectrometer (Hitachi-Perkin-Elmer, RMU-7). Chromatograms were recorded from the total ion-current monitor located between the electrostatic and magnetic analyzers. Helium carrier-gas flow rate was 6 ml. per minute. Injector temperature was 225° C., and the chromatograph detector oven was maintained at 235° C. Mass spectra were scanned magnetically over the range *m/e* 4 to 400 in 6 seconds. Electron current, 100 μa. Ionizing energy, 70 e.v. Accelerating voltage, 2500 volts. Ion-source temperature, 250° C.

Hydrogenation of Alcohol and Neutral Fraction. Adams catalyst (platinum oxide, about 10 mg.) was suspended in 50 μl. of absolute ethanol. The mixture was shaken at room temperature in an atmosphere of hydrogen for 0.5 hour.

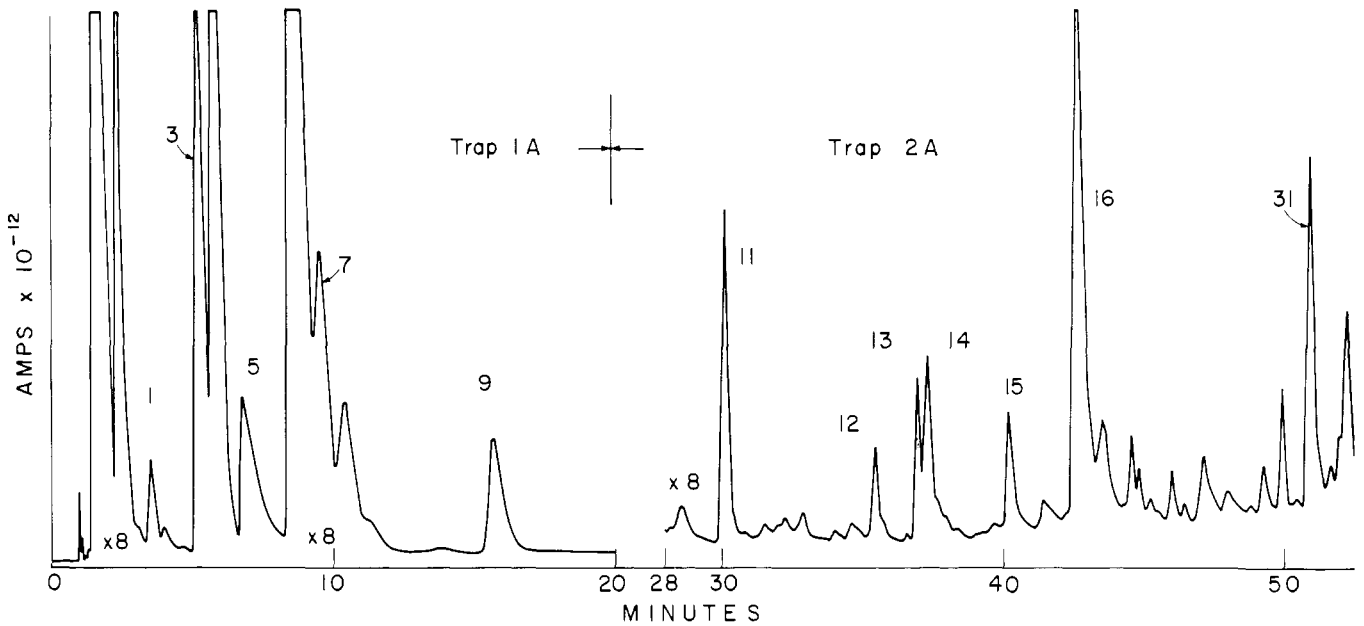

Figure 5. Investigation of methyl ester fraction

Traps 1A and 2A
Column. 50-foot × 0.02-inch i.d. diethylene glycol succinate (DEGS) support-coated open-tubular (Perkin-Elmer Corp.)
Helium flow. 4 ml. per minute
Temperature program. 10 minutes at 25° C., 2° per minute to 100° when column bleed became unacceptable

Alcohol and neutral fraction (10 μl.) and 50 μl. of absolute ethanol were added and hydrogenation was continued for 4.5 hours. Catalyst was removed by filtration, and washed with 0.5 μl. of ethanol. The filtrate (about 90 μl.) contained approximately 10% of the reduced fraction. Figure 2 illustrates the change in composition brought about by hydrogenation.

MATERIALS

Compounds used as reference samples were either obtained from commercial sources or synthesized according to the procedures described below. The compounds were purified by gas chromatography and their infrared and mass spectra recorded for comparison with the chromatographic and spectral properties of substances obtained from banana fruit.

2-HEPTANOL was obtained by lithium aluminum hydride reduction of 2-heptanone.

trans-4-HEXEN-1-ol. The tetrahydropyranyl ether of 4-pentyn-1-ol was prepared by reaction of acetylene and tetrahydropyranyl-3-bromopropanyl ether in the presence of liquid ammonia. It was treated with methyl iodide in the presence of sodamide and liquid ammonia to yield the tetrahydropyranyl ether of 4-hexyn-1-ol. The free hexynol was obtained by heating in the presence of phosphoric acid. Reduction in the presence of sodium and liquid ammonia (Crombie and Harper, 1950) yielded 4-hexen-1-ol.

cis-4-HEXEN-1-ol was prepared by hydrogenation of 4-hexyn-1-ol in the presence of Lindlar's catalyst (Lindlar, 1952).

trans-2-OCTEN-1-ol was obtained by lithium aluminum hydride reduction of methyl *trans*-2-octenoate.

METHYL *trans*-2-HEXENOATE. Condensation of 1-butanol with malonic acid in the presence of pyridine yielded *trans*-2-hexenoic acid, which was methylated with diazomethane.

METHYL *trans*-3-HEXENOATE resulted from diazomethane treatment of *trans*-3-hexenoic acid prepared by condensation of 1-butanol and malonic acid in the presence of triethanolamine.

RESULTS AND DISCUSSION

The nature of the esters in the multicomponent banana odor concentrates (their complexity is indicated by chromatograms in Figures 3, 6, 7, and 8) was investigated by hydrolysis of the concentrates and examination of the resulting "alcohol" and acid fractions. The possibility that alkaline hydrolysis might cause isomerization of double bonds and polymerization of carbonyl components was recognized. To learn how damaging the conditions would be to unsaturated alcohols existing free in the neutral fraction or formed by the hydrolysis, authentic samples of *cis*-3-hexen-1-ol and *trans*-2-hexen-1-ol were subjected to the same treatment as that used for hydrolysis of banana odor concentrates. The products were isolated and their composition was compared gas chromatographically with the composition of the initial alcohols. No difference was found between untreated *cis*-3-hexen-1-ol and "hydrolyzed" *cis*-3-hexen-1-ol or between chromatograms of "hydrolyzed" and untreated *trans*-2-hexen-1-ol. It was, therefore, appropriate to investigate the identity of components in the alcoholic and neutral fraction obtained by alkaline hydrolysis of banana odor concentrate.

Separation of the alcohol fraction on a packed column is illustrated in Figure 2, *A*. Liquid-phase hydrogenation in the presence of Adam's catalyst caused the 11 peaks marked X in Figure 2, *A*, to disappear. The resulting reduced alcohol fraction had the composition shown in Figure 2, *B*. Using only the retention behavior of known *n*-alkanols as a general guide, it appeared that *n*-C_8, C_9, and C_{10} alcohols may have been major hydrogenation products, and that the 11 unknown unsaturated peaks in Figure 2, *A*, probably ranged in chain length from 6 to 10 carbon atoms. Review of already known volatile banana constituents (Table I) indicated that the C_1 through C_6 *n*-alkanols, and iso-C_3 through C_5 alcohols, and 2-pentanol must also be present. Based on retention data this, in fact, appeared to be the case. In any event, hydrogenation had provided tentative evidence that the alcohol fraction contained numerous unsaturated com-

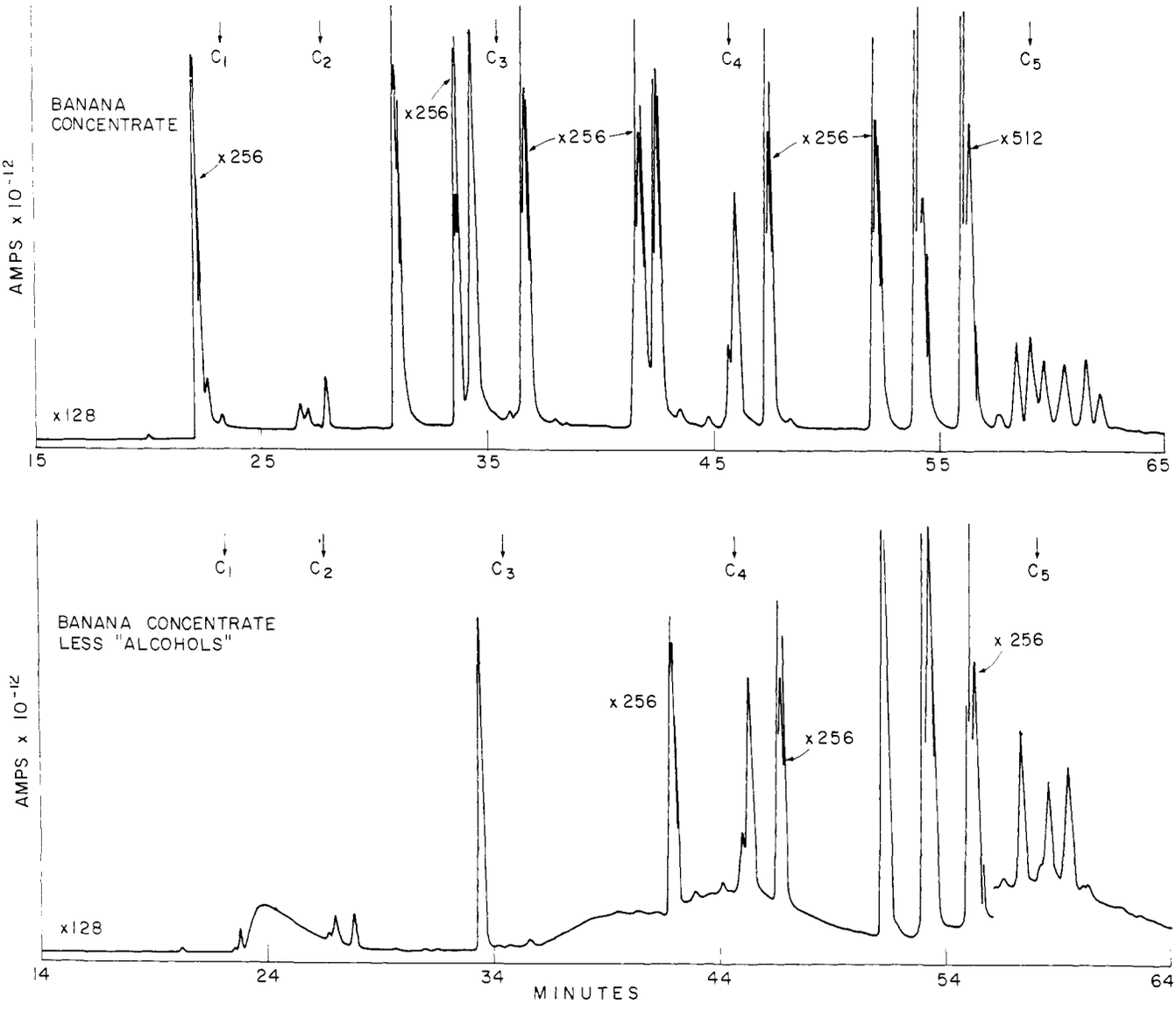

Figure 6. Separation of total banana concentrate

Top. Banana odor concentrate
Bottom. Banana concentration through auxiliary boric acid column
Carbon number markers represent acid moiety in ethyl esters
Column. 500-foot × 0.02-inch i.d. stainless steel coated with GE SF-96 (50) + 5% Igepal CO-880 (Teranishi, 1964)
Helium flow. 4 ml. per minute
Temperature program. 75° to 175° C. at 1° per minute
Sample size. 0.3 μl.

ponents. If shown ultimately to be alcohols, they could have been derived from many different esters in the total odor concentrate.

The unknown unsaturated substances were present in quantities too small to permit investigation by infrared spectrophotometry. Mass spectrometry provided the sensitivity required for characterization of the small quantities of material which could be separated on high efficiency gas chromatographic columns. Separations of total banana odor concentrate and of the alcohol and neutral fraction on a SCOT Carbowax 1540 column are shown in Figure 3. At least 96 peaks were detected in the alcohol isolate (curve *B*) in contrast to approximately 42 components shown by the packed column (Figure 2, *A*). About 111 peaks were found in the total odor concentrate (curve *A*). Essentially all the 38 previously identified banana constituents listed in Table I were eluted in the first 38 minutes of this chromatogram. They are by far the major components. Ultimate use of a 500-foot open-tubular Carbowax 4000 capillary column prepared according to Teranishi and Mon (1964) separated the same odor concentrate into about 200 components (Figure 6, *A*).

Careful comparison of the chromatograms in Figure 3 showed that numerous peaks in the concentrate were removed by hydrolysis. In turn, a number of peaks not present in the concentrate were found in the alcohol fraction. Components of the alcohol fraction were collected in three traps (Figure 3, lower chromatogram). Most known alcohol constituents of banana, and alcohols expected to be released from known esters by hydrolysis, were (based on retention data) in trap 1. The constituents of traps 2 and 3 were thus of particular interest. Repeated separations of the concentrate on a preparative scale (20% CW 4000) column allowed accumulation of traps 1, 2, and 3 for rechromatography and mass spectrometry. Trap 2, which was expected to contain the

unknown, unsaturated components indicated (Figure 3) by hydrogenation to be present, exhibited a pleasant banana-like aroma. This was particularly interesting, since it implied that free alcohols or other neutral substances not affected by hydrolysis were important odor components. Trap 1 smelled like 3-methylbutanol and contained by far the largest quantity of material. Trap 3 had an unpleasant, sharp odor and, like trap 2, contained minor constituents of the "alcohol" fraction. Its contents are under current investigation.

Components in Trap 1 (Figure 3). Based on comparison of mass spectra and retention data of authentic reference samples with those of components in trap 1, the following compounds were identified. They are the major components of the trap and account for 9 of the approximately 38 substances present. The six strongest mass spectral peaks (*m/e*) and the molecular ion peak (when present) are listed for each compound. Their intensities relative to that of the base peak (100%) are given in parentheses.

METHANOL. Retention data. Mass spectrum too weak.
ETHANOL. Retention data. Mass spectrum too weak.
2-PENTANONE. *m/e* 43 (100%), 27 (30%), 41 (27%), 86 (molecular ion 27%), 71 (18%), 58 (17%), 39 (17%).
2-METHYLPROPANOL. *m/e* 43 (100%), 41 (68%), 42 (63%), 31 (63%), 33 (57%), 27 (42%), 74 (molecular ion 8%).
2-PENTANOL. *m/e* 45 (100%), 29 (60%), 43 (48%), 41 (34%), 42 (33%), 31 (29%), 70 (molecular ion-18, 4%).
1-Butanol. *m/e* 45 (100%), 43 (96%), 41 (82%), 31 (64%), 42 (55%), 27 (39%), 74 (molecular ion 10%).
2-HEPTANONE. *m/e* 43 (100%), 58 (64%), 28 (58%), 59 (14%), 15 (9%), 44 (8%), 29 (8%).
3-METHYLBUTANOL. *m/e* 55 (100%), 70 (molecular ion-18, 88%), 42 (80%), 41 (78%), 43 (65%), 57 (53%), 29 (42%).
1-PENTANOL. *m/e* 42 (100%), 55 (93%), 41 (92%), 70 (molecular ion-18 91%), 29 (90%), 43 (89%).

With the exception of 2-heptanone, all the compounds had been previously identified as banana constituents.

Components in Trap 2 (Figures 3 and 4). Separation of trap 2 is shown in Figure 4. Comparison of retention data and mass spectra of authentic reference compounds with those of components of trap 2 resulted in identification of the following alcohols.

PEAK 36. 3-Methylbutanol (residue from trap 1).
PEAK 50. 2-Heptanol. *m/e* 45 (100%), 55 (32%), 98 (molecular ion-18, 19%), 83 (18%), 56 (17%), 41 (16%), 70 (10%).
PEAK 53. 1-Hexanol. *m/e* 56 (100%), 55 (57%), 43 (40%), 41 (39%), 69 (35%), 42 (33%), 84 (molecular ion-18, 15%).
PEAK 60. *trans*-4-Hexen-1-ol. *m/e* 67 (100%), 41 (66%), 55 (45%), 82 (43%), 39 (31%), 57 (28%), 100 (molecular ion 6%).
PEAK 61. *cis*-4-Hexen-1-ol. *m/e* 67 (100%), 41 (95%), 55 (82%), 39 (78%), 82 (63%), 57 (46%), 100 (molecular ion 6%).

The mass spectra of peaks 60 and 61 allowed their straightforward identification as 4-hexen-1-ols but not assignment of trans or cis configurations. Determination that peak 60 was *trans*-4-hexen-1-ol and peak 61 the cis isomer was based on comparison of the retention characteristics shown in Table II for a known mixture of authentic 1-hexen-1-ols and 1-hexanol. The mass spectra of both the reference compounds and the banana components were in agreement with those of Honkanen *et al.* (1963). No evidence was obtained that cis- and trans-isomers of either 3-hexen-1-ol or 2-hexen-1-ol were present in detectable amounts.

Peaks 79 and 81 in trap 2 were identified as octen-1-ols. Their mass spectra contained the same major ion fragments as the *cis*- and *trans*-3-octen-1-ols and *trans*-2-octen-1-ols. However, the relative abundance of the peaks differed from those observed in the reference compounds. Retention data suggested that peak 79 was *cis*-3-octen-1-ol and peak 81 *trans*-2-octen-1-ol (Table II). No conclusion could be reached however, because mass spectra and retention data for the other isomers possible (the 4-, 5-, 6-, and 7-octen-1-ols) were not available. The presence of one or more of these compounds could not be ruled out. The presence of octen-1-ols in trap 2 was further supported by Figure 2, *B*, which showed that hydrogenation of the alcohol fraction caused formation of a peak having the retention time of 1-octan-1-ol. The major ion fragments in the mass spectra of peaks 79 and 81 (Figures 3 and 4) were the following:

PEAK 79. *m/e* 81 (100%), 55 (77%), 41 (63%), 68 (60%), 67 (59%), 82 (42%), 110 (molecular ion-18, 23%).
PEAK 81. *m/e* 67 (100%), 81 (96%), 41 (95%), 55 (92%), 82 (72%), 68 (69%), 110 (molecular ion-18, 32%).

The mass spectra of the reference samples were:

cis-3-OCTEN-1-OL. *m/e* 55 (100%), 81 (63%), 68 (50%), 41 (39%), 67 (38%), 56 (24%), 110 (molecular ion-18, 20%).
trans-2-Octen-1-OL. *m/e* 55 (100%), 67 (76%), 110 (molecular ion-18, 63%), 39 (58%), 81 (48%), 41 (47%), 68 (43%).

Peaks 43, 44, 45, and 46 in trap 2 (Figures 3 and 4) are believed to be isomeric 2-heptanols ($M^+ = 116$). The base peak in each is at *m/e* 45, characteristic of $[CH_2CHOH]^+$. None had a significant peak at *m/e* 31, thus ruling out primary alcohols $[CH_2OH]^+$. All had a peak at *m/e* 101 typical of the loss of CH_3^+ ($M^+ = 15$). Variations in the spectra were related to the $C_5H_{11}^+$ fragment and were probably due to different chain branching in each compound. The presence of branched-chain 2-heptanols in these peaks was compatible with their retention behavior, since they were eluted before 2-heptanol, the straight-chain isomer.

Peaks 49 and 52 appeared to be isomers of 1-hepten-2-ol ($M^+ = 114$). The base peak in both was *m/e* 70 $[C_5H_{10}]^+$ with *m/e* 45 $[CH_3CHOH]^+$ next most abundant. Further evidence that peak 52 was unsaturated was provided by Figure 2. Hydrogenation of the alcohol fraction caused removal of a peak in Figure 2, *A*, at about 30 minutes just before 1-hexanol. This peak corresponds to peak 52 in trap 2 (Figure 4). Its hydrogenation supported the probability that peak 52 is an hepten-2-ol isomer.

Investigation of Methyl Ester Fraction. Separation of the

Table II. Retention Data for Components of Trap 2 and Authentic Reference Samples (Hexenols and Octenols)

Reference Compounds	Rel_{tR}
1-Hexanol	1.00[a]
trans-3-Hexen-1-ol	1.02
cis-3-Hexen-1-ol	1.07
trans-2-Hexen-1-ol	1.12
trans-4-Hexen-1-ol	1.14
cis-4-Hexen-1-ol	1.18
1-Octanol	1.56
trans-3-Octen-1-ol	1.58
cis-3-Octen-1-ol	1.62
trans-2-Octen-1-ol	1.68
Trap 2	
Peak 53	1.00
Peak 60	1.15
Peak 61	1.18
Peak 79	1.64
Peak 81	1.68

[a] Absolute retention time about 41 minutes. 50-foot SCOT CW 1540 column at 100° C.

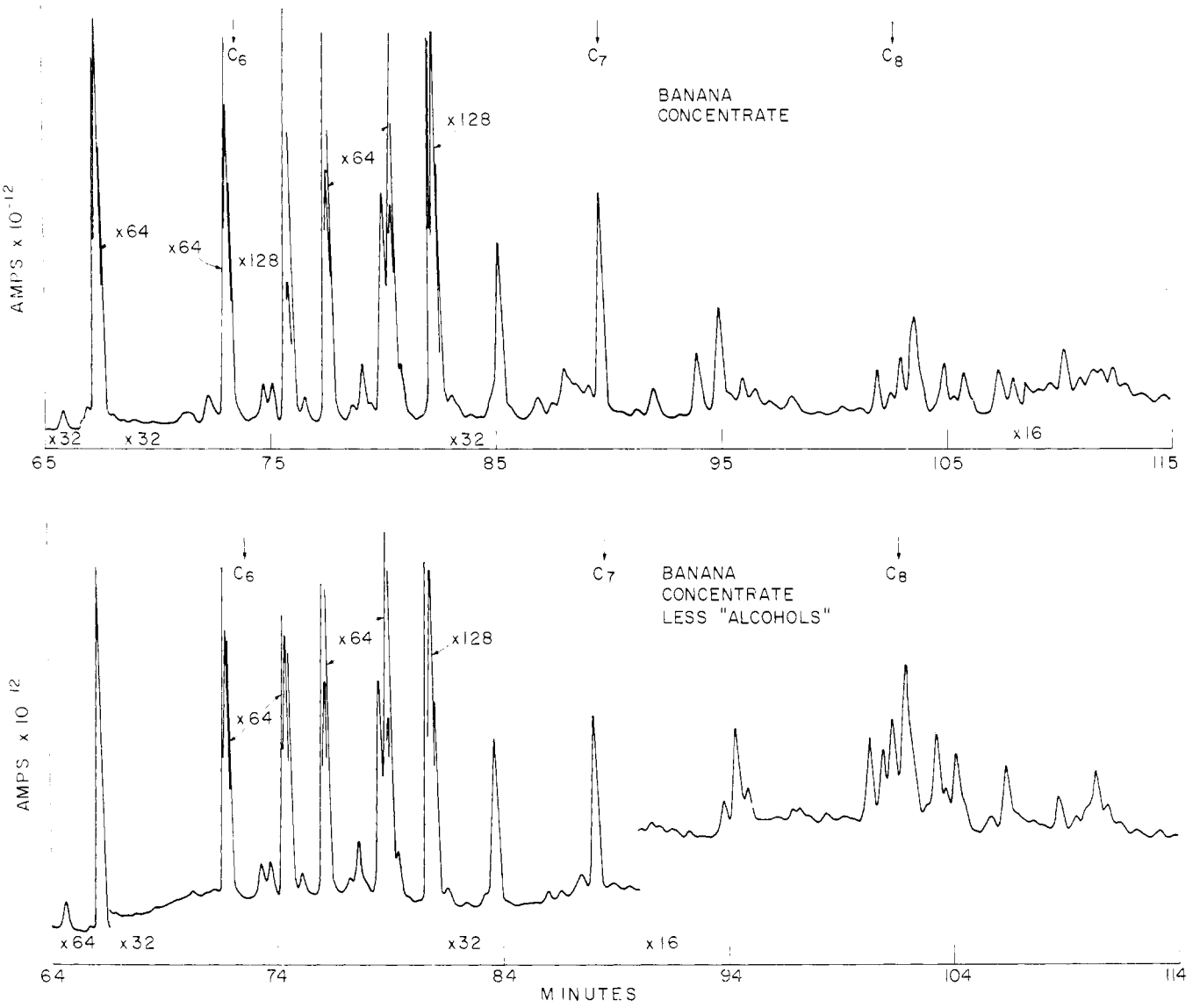

Figure 7. Separation of total banana concentrate

Conditions same as in Figure 6

esters prepared by methylation of acids formed during hydrolysis of the alcohols and neutral fraction is shown in Figure 5. The chromatogram shows results of the rechromatography of traps 1A and 2A (Figure 1) accumulated from the total methyl ester fraction. Mass spectra were recorded from each peak. Comparison of the spectra and retention behavior of each component with those of reference samples showed that only peaks 4, 7, 8, and 9 in trap 1A contained methyl esters. The other peaks represented residual components of the alcohol fraction—for example, peak 6 was 3-methylbutanol. The following methyl esters were identified in trap 1A.

Peak 4. Methyl acetate. The spectrum differed somewhat from that of the authentic sample in the relative abundance of the major ion fragments. The differences resulted because methyl acetate was not completely resolved from residual alcohol components. Its spectrum was *m/e* 43 (100%), 74 (molecular ion 24%), 42 (10%), 44 (10%), 15 (8%), 29 (7%), 59 (7%). The spectrum of known methyl acetate was: *m/e* 43 (100%), 74 (molecular ion 50%), 42 (22%), 59 (18%), 29 (13%), 15 (9%), 44 (7%).

Peak 8. Methyl 2-methylpropionate. Although peak 8 was a mixture, its spectrum and retention time were in general agreement with those of a reference sample. Its spectrum was *m/e* 43 (100%), 71 (75%), 87 (33%), 59 (26%). The spectrum of authentic methyl 2-methylpropionate was *m/e* 43 (100%), 71 (43%), 41 (31%), 59 (29%), 87 (22%), 27 (17%), 102 (molecular ion 14%).

Peak 9. Methyl butyrate *m/e* 43 (100%), 71 (94%), 74 (89%), 59 (43%), 41 (39%), 87 (29%), 102 (molecular ion 5%).

The presence of methyl acetate and methyl butyrate was expected, since many acetate and butyrates are known constituents of banana fruit (Table I). The presence of methyl propionate was not confirmed. Based on retention data it may have been in peak 7. However, the mass spectrum was not definitive.

Peak 11. Methyl hexanoate. *m/e* 74 (100%), 43 (44%), 41 (26%), 87 (23%), 59 (22%), 27 (17%), 130 (molecular ion 2%).

Peak 13. Methyl 3-hexenoate. *m/e* 41 (100%), 74 (65%), 68 (64%), 69 (53%), 59 (41%), 39 (29%), 128 (molecular ion 5%).

Peak 14. Methyl 2-hexenoate. *m/e* 55 (100%), 41 (70%), 97 (54%), 87 (49%), 68 (37%), 74 (36%), 128 (molecular ion 3%).

The mass spectra of the hexenoates were the same as those of the reference compounds but they differed from published

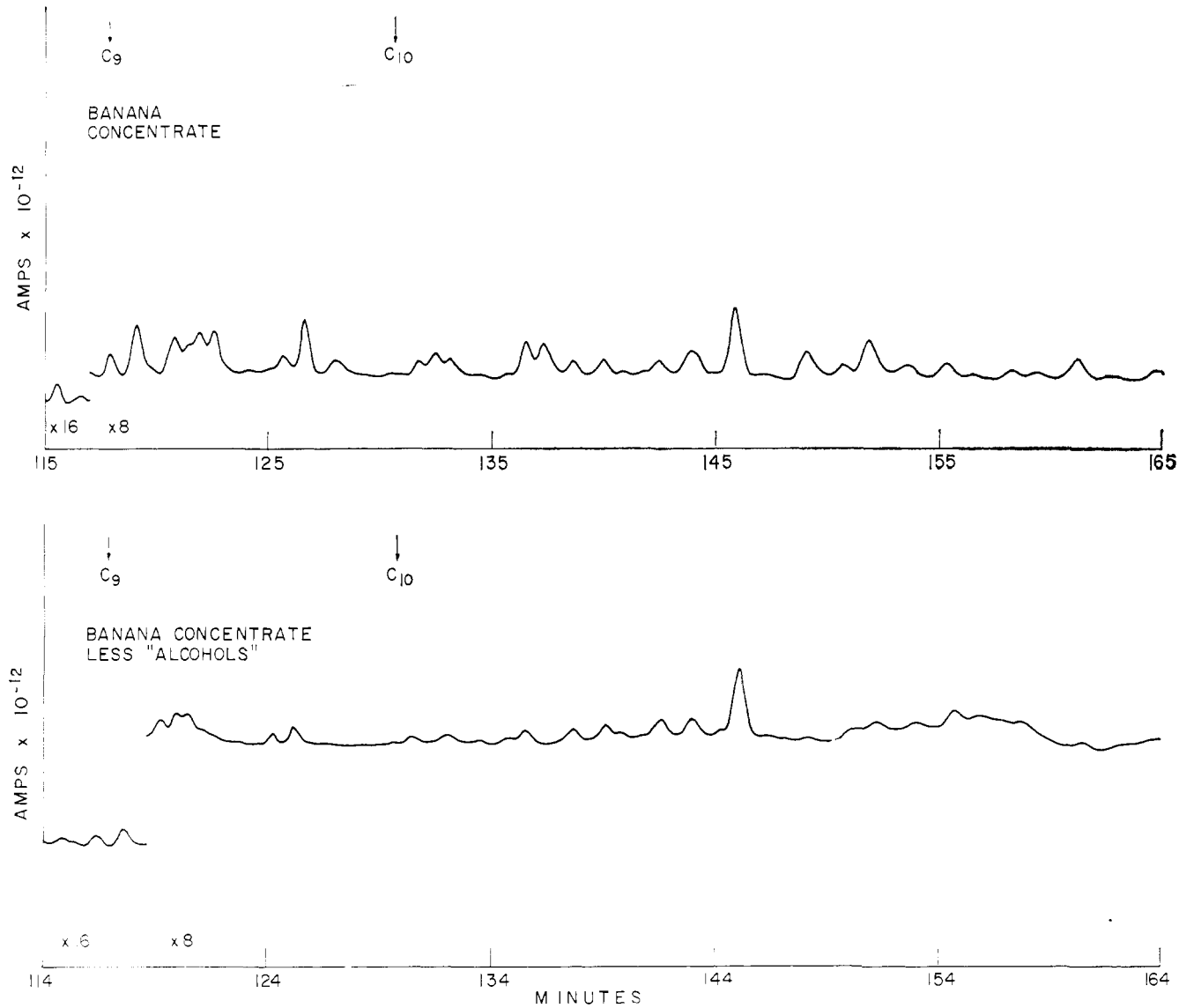

Figure 8. Separation of total banana concentrate

Conditions same as in Figure 6

spectra in the relative abundance of ion fragments. Since mass spectra of cis- and trans- forms do not allow differentiation, it is not known which isomer or isomers were present in peaks 13 and 14.

PEAK 16. Methyl octanoate. *m/e* 74 (100%), 43 (50%), 87 (47%), 59 (35%), 41 (30%), 55 (28%), 158 (molecular ion 5%).

CONCLUSIONS

Based on investigation of the acidic and neutral fractions resulting from hydrolysis of banana odor concentrate, the compounds given in Table III were identified. The C_1 through C_6 saturated alcohols were already known to be present in both free and esterified forms in banana concentrates but it was not clear whether the higher alcohols also were present in nonesterified form. Comparison of the pattern of constituents in the total concentrate and in the alcohol fraction in Figure 3 suggested that most alcohols existed primarily in the ester form. However, the separation of compounds was really not adequate to allow a conclusion to be reached. The absence of 3-hexen-1-ols and 2-hexen-1-ols in the banana alcohol fraction was surprising, since these substances are commonly found in nature. If it had not been determined with reference samples that these hexen-1-ols were not destroyed by the conditions used for hydrolysis, their absence would be seriously in doubt. Under the circumstances they, in fact, appeared not to be present.

Table III. Compounds Identified after Hydrolysis of Banana Odor Concentrate

Alcohols, Existing Free or in Esters	
Methanol	2-Heptanol
Ethanol	*trans*-4-Hexen-1-ol
1-Butanol	*cis*-4-Hexen-1-ol
2-Methylpropanol	*trans*-2-Octen-1-ol (?)
1-Pentanol	*cis*-3-Octen-1-ol (?)
2-Pentanol	Four branched-chain 2-heptanol isomers
3-Methylbutanol	Two 2-heptenol isomers
1-Hexanol	
Acid Moieties in Esters	
Acetic	*n*-Hexanoic
Propionic (?)	*n*-Hex-3-enoic
2-Methylpropionic (?)	*n*-Hex-2-enoic
1-Butanoic	1-Octanoic

During final stages of this investigation, Murray *et al.* (1968) reported the composition of the volatile alcohol fraction of ripe bananas. Ethanol, 1-propanol, 1-butanol, 2-methylpropanol, 2-pentanol, 2-methylbutanol, 3-methylbutanol, 1-hexanol, 2-heptanol, *cis*- and *trans*-3-hexen-1-ol, *cis*- and *trans*-4-hexen-1-ol, and *cis*-2-penten-1-ol (tentative) were identified. The differences between their results and those of this investigation may be due to varietal differences in the fruit examined.

The best separation achieved to date of total banana concentrate was obtained on a 500-foot nonpolar [SF96(50)] capillary column (Figures 6, 7, and 8). Known banana constituents among the approximately 200 peaks were located by use of Kovats retention indices (Kovats, 1958) based on a homologous series of ethyl esters as reference standard (Kratz and Mosciano, 1966). Peaks corresponding to the alcohols identified in trap 2 (Figure 4) are eluted between 57 and 64 minutes in Figure 6 (top curve). These same peaks were adsorbed (lower curve) when a small auxiliary boric acid column was attached to the capillary column, suggesting (Ikeda *et al.*, 1964) that they were indeed alcohols. As shown in the lower chromatograms in Figures 6, 7, and 8, a number of the nearly 200 banana constituents were adsorbed by the boric acid column. They may be free alcohols. Based on the results of this investigation, the esters among the substances remaining in the lower chromatogram of Figures 6, 7, and 8 are made up of, at the least, the alcohols and acids listed in Table III. More diversity was found among the alcohols than among the acids, in contrast to that observed in pear esters (Jennings, 1961), where the acid moieties were much more varied in nature than the alcohols. The identification of methyl esters of 2-methylpropanoic, 3-hexenoic, 2-hexenoic, hexanoic, and octanoic acids provided initial evidence that esters of these acids were volatile components of banana fruit. Future work must elucidate whether both 3-hexenoates and 2-hexenoates are, in fact, natural banana constituents or whether one of these isomers resulted from double bond rearrangement during the isolative procedures.

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