Characterization of Some New C_{16} and C_{17} Unsaturated Fatty Aldehydes

THOMAS R. KEMP, Department of Horticulture, University of Kentucky, Lexington, Kentucky 40506

ABSTRACT

The following six volatile fatty aldehydes, including five new compounds have been characterized by spectral analyses (mass spectra presented) and ozonolysis: *cis*-7-hexadecenal, *cis*-8-heptadecenal (previously known), *cis,cis*-7,10-hexadecadienal, *cis,cis*-8,11-heptadecadienal, *cis,cis,cis*-7,10,13-hexadecatrienal, *cis,cis,cis*-8,11,14-heptadecatrienal. Compounds were separated (gas liquid chromatography) from a volatile concentrate prepared by reduced pressure steam distillation of an aqueous cucumber homogenate. These aldehydes were not detected by our methods when the tissue was extracted with $CHCl_3$-MeOH.

INTRODUCTION

Previous work in this laboratory has involved qualitative analysis of volatile concentrates prepared by reduced pressure steam distillation of plant tissues (1,2). Recently, a group of unusual C_9 aldehydes and their corresponding alcohols were isolated and characterized in a concentrate prepared from cucumber homogenate (3). Included among the compounds identified were nonanal, 2-nonenal, 3-nonenal, 6-nonenal, 2,6-nonadienal and 3,6-nonadienal. Further analysis of this volatile concentrate has now resulted in the characterization of a group of C_{16} and C_{17} unsaturated fatty aldehydes. To our knowledge these aldehydes, with one exception, have not been previously reported in the literature.

MATERIALS AND METHODS

Volatile fatty aldehydes were isolated from cucumber homogenate in the following way. 1.6 kg fresh cucumber (peeled) was homogenized with 2 liter distilled water in a Waring Blendor. The homogenate was transferred to a 12 liter flask of a water recycling apparatus (Clevenger) (4) which was adapted for vacuum steam distillation. Four ml redistilled hexane were placed on top of the water layer in the side arm of the apparatus. The pressure in the system was reduced, and steam distillation was carried out at 60-70 C for 3 hr. Hexane layers from several runs were combined and dried over Na_2SO_4, and the solvent was removed under a stream of N_2 to give a concentrate of volatile compounds.

Gas liquid chromatography (GLC) was carried out using a Barber-Coleman Series 5000 instrument equipped with a thermal conductivity detector. The volatile concentrate was initially separated on a 1.8 m x 6 mm outside diameter stainless steel column packed with 20% SE-30 coated on 60-80 mesh, acid washed, silanized Chromosorb W. The column temperature was programed from 100-180 C at 1 C/min. Fractions were collected in U-shaped glass tubes, cooled in dry ice-actone and the tubes were immediately sealed (70% recovery pentadecanal). Rechromatography was accomplished on a 1.8 m x 6 mm outside diameter stainless steel column packed with 10% diethylene glycol succinate (DEGS) coated on 60-80 mesh, acid washed, silanized Chromosorb W. Resulting components were collected and subjected to spectral analyses and ozonolysis.

Mass spectra were recorded on a Hitachi RMU-6E double focusing instrument using an ionizing energy of 70 eV; IR spectra on a Beckman IR-8 equipped with a mirror beam condenser using CS_2 as solvent; and NMR spectra on a Varian HA 60 instrument (with an internal field lock and equipped with a 1024-channel CAT) using CCl_4 as solvent and tetramethylsilane as internal standard.

Ozonolysis was carried out using amicro-ozonizer according to the procedure of Beroza and Bierl (5). Identification of ozonolysis products was based on comparison of GLC retention times of the products with those of aldehyde standards.

After chemical characterizations were completed, cucumbers were submitted to solvent extraction. 740 g peeled cucumbers (26.6 g dry wt) were homogenized with $CHCl_3$-MeOH (2:1) and lipids were extracted according to the procedure of Folch, et al., (6). After solvent evaporation, the lipid residue (419 mg) was diluted with n-hexane and applied to a 15 g silicic acid column (n-hexane equilibrated). Lipids were eluted with solvent mixtures described in the procedure of Gilbertson, et al., (7) except n-heptane was replaced with n-hexane. Pentadecanal (standard) was eluted in the n-hexane-1% ether and n-hexane-4% ether fractions as shown by GLC. GLC of the fractions was carried out using the SE-30 column and temperature program described above. A 12 cm pre-column was attached to the gas chromatograph to prevent accumulation of nonvolatile lipids.

RESULTS AND DISCUSSION

The volatile concentrate obtained by steam distillation was separated by GLC on a SE-30 column and fractions corresponding to the two major peaks following pentadecanal (identified earlier) were collected. (The combined wt of the two fractions obtained from 6.40 kg fresh cucumbers was 4.7 mg). The two fractions were individually rechromatographed on a DEGS column, and in each case, 3 components were characterized by spectral analyses and ozonolysis. Mass spectra of the compounds characterized are presented as Figures 1 and 2. Table I lists relative retention times and area percentage data.

The second fraction to elute from the SE-30 column was larger, and the components obtained from it will be discussed below. These compounds are designated I, II, and III.

Compound I was the principal component of the larger fraction (Table I) and yielded a mass spectral molecular ion (M^+) peak at m/e 248 and major peaks at m/e 79, 67, 41, 95 and 93 (Fig. 1). IR analyses revealed absorption bands at 2700 (-CHO), 1730 (C=O) and 720 cm^{-1} (-CH=CH-,*cis*), but no band in the 960-970 cm^{-1} region (-CH=CH-,*trans*), indicating an aliphatic aldehyde with *cis* unsaturation. The mass and IR spectra were consistent with a C_{17} aldehyde containing 3 double bonds. NMR spectrum yielded diagnostic resonances at 9.70 (IH,t,-CHO), 5.33 (6H,m,-CH=CH-) and 2.81δ (4H,t,=CH-CH_2-CH=), the latter resonance indicating that double bonds are separated by a methylene group. Ozonolysis of the compound gave propanal which established the position of the terminal double bond at carbon 14. Because the double bonds are separated by a methylene group, the remaining double bonds are located at carbons 11 and 8. Therefore, based on spectral and ozonolysis data, compound I was identified as *cis,cis,cis*-8,11,14-heptadecatrienal.

Compound II eluted prior to I from DEGS (Table I) and

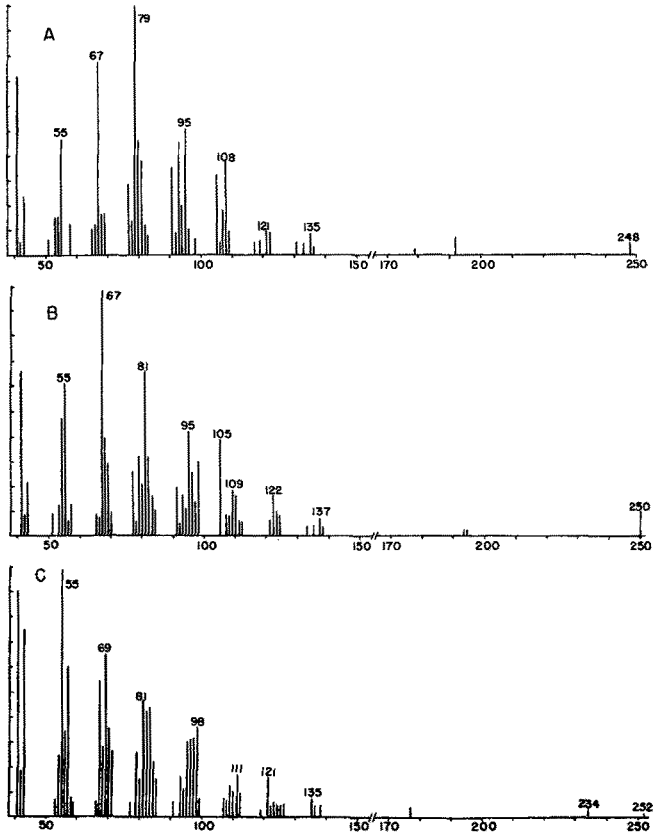

FIG. 1. Mass spectra of (A) *cis,cis,cis*-8,11,14-heptadecatrienal, (B) *cis,cis*-8,11-heptadecadienal, and (C) *cis*-8-heptadecenal.

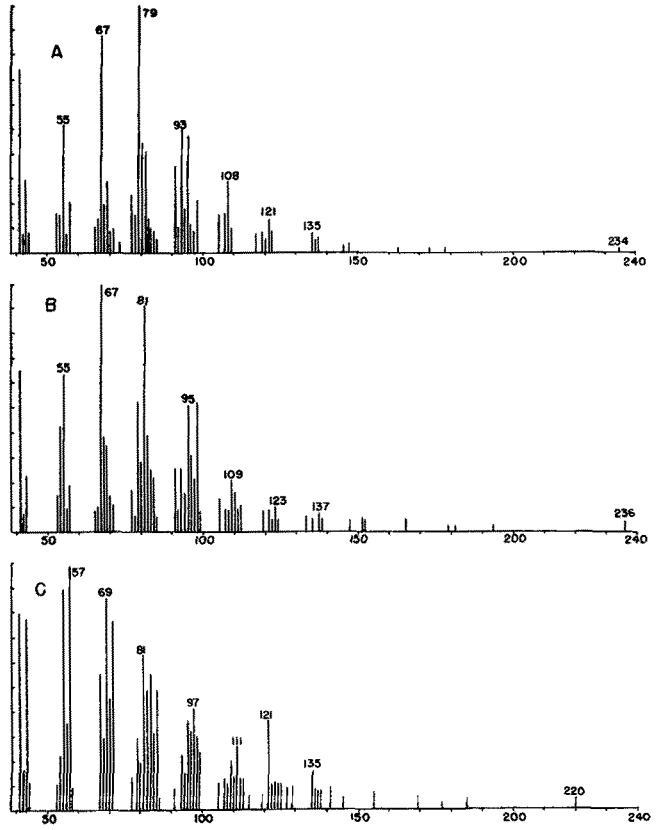

FIG. 2. Mass spectra of (A) *cis,cis,cis*-7,10,13-hexadecatrienal, (B) *cis,cis*-7,10-hexadecadienal, and (C) *cis*-7-hexadecenal.

yielded a mass spectral M^+ peak at m/e 250 and major peaks at m/e 67, 81, 41, 55 and 54 (Fig.). An IR spectrum showed bands at 2700, 1730 and 720 cm^{-1}, as in the previous case, indicating an aliphatic aldehyde with *cis* unsaturation. The mass and IR spectra were consistent with a C_{17} aldehyde containing two double bonds. An NMR spectrum was obtained with resonances at 9.70 (t,-CHO), 5.30 (m,-CH=CH-) and 2.71δ (t,=CH-CH_2-CH=), the latter indicating that the double bonds are separated by a methylene group (analogous to results obtained with I). Ozonolysis of the compound yielded hexanal which permitted the assignment of the terminal double bond at carbon 11. Because the double bonds are separated by a methylene group, the second bond is located at carbon 8. Therefore, compound II was identified as *cis,cis*-8,11-heptadecadienal.

Compound III was a minor component and eluted prior to II from the DEGS column (Table I). Compound III yielded a mass spectral M^+ peak at 252 and major peaks at m/e 55, 41, 43, 69 and 57 (Fig. 1). An IR spectrum showed bands at 2700, 1730 and 720 cm^{-1}, indicating a *cis* unsaturated aliphatic aldehyde. The mass and IR spectra were consistent with a C_{17} aldehyde containing one double bond. Ozonolysis yielded nonanal, which placed the double bond at carbon 8. Therefore, compound III was identified as *cis*-8-heptadecenal.

A shoulder was observed on the GLC peak representing compound II and also on the peak representing compound III; in each case, a mass spectrum of the component comprising the shoulder was nearly identical to that of the original compound indicating the presence of an isomer.

The three components which were separated from the smaller SE-30 fraction were designated IV, V, and VI, and are discussed below.

Compound IV was the principal component (Table I) and yielded a mass spectral M^+ peak at m/e 234 and major peaks at m/e 79, 67, 41, 55 and 93 (Fig. 2). The spectral pattern of IV was very similar to that of I except that the mol wt was 14 mass units less than that of I. The IR spectrum (bands at 2700, 1730 and 720 cm^{-1}) matched that of I, and ozonolysis yielded propanal. Hence, compound IV was identified as *cis,cis,cis*-7,10,13-hexadecatrienal (C_{16} homolog of I).

TABLE I

Gas Liquid Chromatographic Analysis of Fatty Aldehydes Isolated from Cucumber Homogenate

Compound number	Aldehyde	Relative retention time (15:0)[a]	Area percentage
I	17:3	4.31	64.0
II	17:2	3.12	23.6
III	17:1	2.47	2.5
IV	16:3	2.95	6.7
V	16:2	2.11	2.7
VI	16:1	1.65	0.4

[a]Retention time relative to pentadecanal (1.00) on diethylene glycol succinate (DEGS) column.

Compound V eluted prior to compound IV on DEGS (Table I) and yielded a mass spectral M^+ peak at m/e 236 and major peaks at m/e 67, 81, 41, 55 and 79 (Fig. 2). The spectral pattern was very similar to that of compound II except that the mol wt was found to be 14 mass units less than that of compound II. In a manner analogous to that of the previous case, an IR spectrum of compound V (bands at 2700, 1730 and 720 cm^{-1}) matched that of compound II, and ozonolysis yielded hexanal. Hence, compound V was identified as *cis,cis*-7,10-hexadecadienal (C_{16} homolog of compound II).

Compound VI was a minor component and eluted prior to compound V on DEGS (Table I). Compound VI yielded a mass spectral peak at m/e 220 (M^+-H_2O) and major peaks at m/e 57, 55, 69, 41 and 43 (Fig. 2); the spectrum was similar to that of compound III. Also, an IR spectrum

(bands at 2700, 1730 and 720 cm^{-1}) matched that of compound III and ozonolysis yielded nonanal. Hence, compound VI was identified as *cis*-7-hexadecenal (C_{16} homolog of III).

Although the primary objective of our work was characterization of the previously unknown fatty aldehydes encountered, an experiment was run to gain some insight into whether the aldehydes were formed in the aqueous homogenate or were present in the intact tissue. Cucumbers were extracted with $CHCl_3$-MeOH and the resultant lipids were fractionated by column chromatography and analyzed by GLC. GLC peaks corresponding to the fatty aldehydes characterized above were not observed suggesting that these compounds are formed in the homogenate. This is in agreement with the work of Fleming, et al., (8) who showed that several short chain (C_6 and C_9) aldehydes, including flavor constituents, were formed when cucumber tissue was ruptured or macerated, but were not present in significant amounts in the intact tissue.

The six aldehydes identified here bear a structural relationship to the common C_{18} unsaturated fatty acids-oleic, linoleic and linolenic. 8-Heptadecenal and 7-hexadecenal are aldehydes which would result from the removal of one and two carbon atoms, respectively, from the carboxyl end of oleic acid. In a like manner, 8,11-heptadecadienal and 7,10-hexadecadienal are related to linoleic acid while 8,11,14-heptadecatrienal and 7,10,13-hexadecatrienal are related to linolenic acid. 8-Heptadecenal has been reported in the literature previously by Hitchcock and James (9). This compound was shown to be an intermediate in the α-oxidation of oleic to 8-heptadecenoic acid in higher plants.

ACKNOWLEDGMENTS

C. Morris provided technical assistance; M. Wassef gave advice and supervised the solvent extraction. The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published with the approval of the Director.

REFERENCES

1. Kemp, T.R., L.P. Stoltz, and D.E. Knavel, J. Agr. Food Chem. 20:196 (1972).
2. Kemp, T.R., D.E. Knavel, L.P. Stoltz, and R.E. Lundin, Phytochemistry 13:1167 (1974).
3. Kemp, T.R., D.E. Knavel, and L.P. Stoltz, J. Agr. Food Chem. 22:717 (1974).
4. Kemp, T.R., L.P. Stoltz, W.T. Smith, and C.E. Chaplin, Proc. Amer. Soc. Hort. Sci. 93:334 (1968).
5. Beroza, M., and B.A. Bierl, Anal. Chem. 39:1131 (1967).
6. Folch, J., M. Lees, and G.H. Sloane Stanley, J. Biol. Chem. 226:497 (1957).
7. Gilbertson, J.R., R.C. Johnson, R.A. Gelman, and C. Buffenmyer, J. Lipid Res. 13:491 (1972).
8. Fleming, H.P., W.Y. Cobb, J.L. Etchells, and T.A. Bell, J. Food Sci. 33:572 (1968).
9. Hitchcock, C., and A.T. James, J. Lipid Res. 5:593 (1964).

[Received April 22, 1975]