cis-Vaccenic Acid in Pulp Lipids of Commonly Available Fruits

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Fatty acids of commonly available fruit pulps have been analyzed by capillary gas chromatography of their methyl esters and by gas chromatography-mass spectrometry as their dimethyl disulfide adducts. The gas chromatographic and mass spectrometric data proved that these fruits always contained *cis*-vaccenic (*cis*-11-octadecenoic) acid as a component fatty acid of their pulp lipids. The concentration of *cis*-vaccenic acid in total octadecenoic acids ranged from 1.9% to 95.1% in the fruit pulps examined. The highest concentration of this acid was detected in pulp lipids of Japanese persimmon (*Diospyros kaki*). In fruit pulp lipids, *cis*-vaccenic acid was a common octadecenoic acid as well as oleic acid.

Many studies have shown that *cis*-vaccenic (1) (*cis*-11-octadecenoic) acid is a component fatty acid of microorganisms, animal tissues and human tissues. The metabolism, distribution and origin of *cis*-vaccenic acid in these materials have been well investigated (2-6). On the contrary, less attention has been given to *cis*-vaccenic acid in higher plant lipids because of its low concentration (i.e., 0.5-2%) in common vegetable seed oils. The biosynthetic pathway of *cis*-vaccenic acid has not yet been confirmed in higher plants (7,8). We revealed the content of *cis*-vaccenic acid (as pyrrolidide) in triacylglycerols of seven kinds of common vegetable seed oils by gas chromatography-mass spectrometry (GC-MS) (9). Recently, we devised a convenient procedure for preparation of dimethyl disulfide adducts of fatty acid positional isomers (10), and developed a rapid and simple method for determination of *cis*-vaccenic acid content by GC-MS of the adducts (11). By use of a combination of these GC-MS systems, a convenient capillary GC, and other chromatographic, spectrometric and chemical methods, *cis*-vaccenic acid has been proved to be one of the major fatty acids (35-50% of total octadecenoic acids) in pulp lipids of mango (*Mangifera indica*) (12).

In this paper, we report the presence of *cis*-vaccenic acid in fruit pulp lipids and describe analytical methods suitable for determination of monoenoic fatty acid positional isomers. No paper has hitherto been published on *cis*-vaccenic acid in fruit pulp lipids except for our previous paper on mango pulp lipids (12).

EXPERIMENTAL PROCEDURES

Materials and extraction of total lipids. All fruit samples (Table 1) were purchased from local markets in Kobe in 1985-1986. The pulp parts of fully ripe samples (3 to 7 pieces), being uniform in weight and color, were immersed in boiling water for 5 min to inactivate enzymes (13) and extracted with chloroform/methanol (2:1, v/v) in a Waring blender. The homogenate was filtered and the residue was extracted twice with the same solvent mixture. The combined extracts were washed with water and dried over anhydrous Na_2SO_4. After removal of Na_2SO_4 and insoluble nonlipid contaminants by filtration, the solvent was evaporated nearly to dryness by a rotary evaporator below 35 C. The total lipids thus obtained were dissolved in chloroform and stored under an atmosphere of N_2 at −20 C until analysis.

Separation of total lipids. An aliquot of total lipids in chloroform was applied on commercially precoated silica gel plate (No. 11845, E. Merck, Darmstadt, West Germany), developed with benzene/ether/methanol (32:7:1, v/v/v), and separated into polar lipids (R_f 0.0-0.3) and nonpolar lipids (R_f 0.35-1.0). The R_f value of monoacylglycerols was 0.35 under these conditions.

Derivatization. Fatty acid methyl esters (FAME) of total lipids and nonpolar lipids containing free fatty acids were prepared by methanolysis (0.5 N KOH/methanol) and successive methylation (BF_3/methanol) (12). Polar lipids were converted to FAME by methanolysis with CH_3ONa reagent (12). The formed FAME were purified by thin layer chromatography (TLC) with hexane/ether/acetic acid (90:10:1, v/v/v) as a developing solvent. Dimethyl disulfide adducts of FAME were

TABLE 1

Fruit Samples Examined

Plant name	Common name	Source
Bombacaceae		
Durio zibethinus	Durian	Thailand
Caricaceae		
Carica papaya	Papaya	USA
Cucurbitaceae		
Citrullus lanatus	Watermelon	Japan
Cucumis melo var. *reticulatus*	Muskmelon	Japan
C. melo cv. "Prince"	Princemelon	Japan
Ebenaceae		
Diospyros kaki	Japanese persimmon	Japan
Guttiferae		
Garcinia mangostana	Mangosteen	Thailand
Rosaceae		
Malus pumila	Apple	Japan
Prunus avium	Cherry	USA
P. persica	Peach	Japan
Pyrus pyrifolia	Japanese pear	Japan
Rutaceae		
Citrus limon	Lemon	USA
C. paradisi	Grapefruit	USA
C. sinensis	Sweet orange	USA
C. unshiu	Japanese mandarin	Japan
Sapindaceae		
Litchi chinensis	Litchi	Taiwan
Vitaceae		
Vitis vinifera	Grape (cv. Campbell)	Japan
	Grape (cv. Delaware)	Japan
	Grape (cv. Muscat)	Japan

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prepared according to the method previously described (10,12).

GC and GC-MS. Capillary GC was carried out with a Hitachi 263-30 gas chromatograph equipped with a Hitachi Chromatointegrator D-2000, a flame ionization detector, a glass-lined splitter (split ratio: 1/10) and a 50-m × 0.24-mm i.d. SS-10 (medium polarity, chemically bonded type) fused silica capillary column (Shinwakako Co. Ltd., Kyoto, Japan). Carrier gas was He (1.0 ml/min), and column temperature was programmed from 100 C to 185 C at 4 C/min. GC-MS analysis was performed on a Hitachi 663-30 gas chromatograph coupled to a Hitachi M-80A double focusing mass spectrometer with an M-003 minicomputer on-line system. Column temperature was maintained at 240 C, and other operating conditions were the same as previously described (12).

RESULTS AND DISCUSSION

The 19 varieties of plants (9 families) listed in Table 1, popular fruits in Japan and elsewhere in the world, were analyzed for the presence of *cis*-vaccenic acid in their pulp lipids. Table 2 shows the fatty acid compositions and *cis*-vaccenic acid contents in total lipids of the 19 fruit pulps. These fruits always contained *cis*-vaccenic acid as a component fatty acid of their pulp lipids, though the content varied (1.9-95.1% of octadecenoic acids and 0.8-29.0% of total fatty acids). These results indicated that *cis*-vaccenic acid was a common octadecenoic acid in the fruit pulp lipids, as was oleic acid. The lipid contents of all the fruit pulps examined were very low (less than 1 wt% of wet pulps).

The profiles of fruit pulp fatty acids were generally complex and clearly different from those of common vegetable seed oils (soybean, safflower, etc.) with respect to the variety of fatty acids, especially their monoenoic acid positional isomers. In general, 30 or more species of fatty acids were observed in capillary GC run of FAME from fruit pulp lipids. Figure 1 shows typical gas chromatograms of FAME taken in the course of *cis*-vaccenic acid screening. Japanese persimmon (Fig. 1A) was given as the richest source of *cis*-vaccenic acid, and sweet orange (Fig. 1B) as one of the citrus fruits found rich in *cis*-vaccenic acid. Essential oils in the citrus fruits were thought to disturb the GC analysis; however, preliminary purification of FAME using silica gel TLC provided a clear-cut gas chromatogram (Fig. 1B). The identification of the FAME by capillary GC depended on the coincidence of their retention times with those of reference substances (12) and/or the FAME mixtures prepared from plant lipids (9-12). More than two independent analytical systems should be employed for accurate qualitative and quantitative analyses of mo-

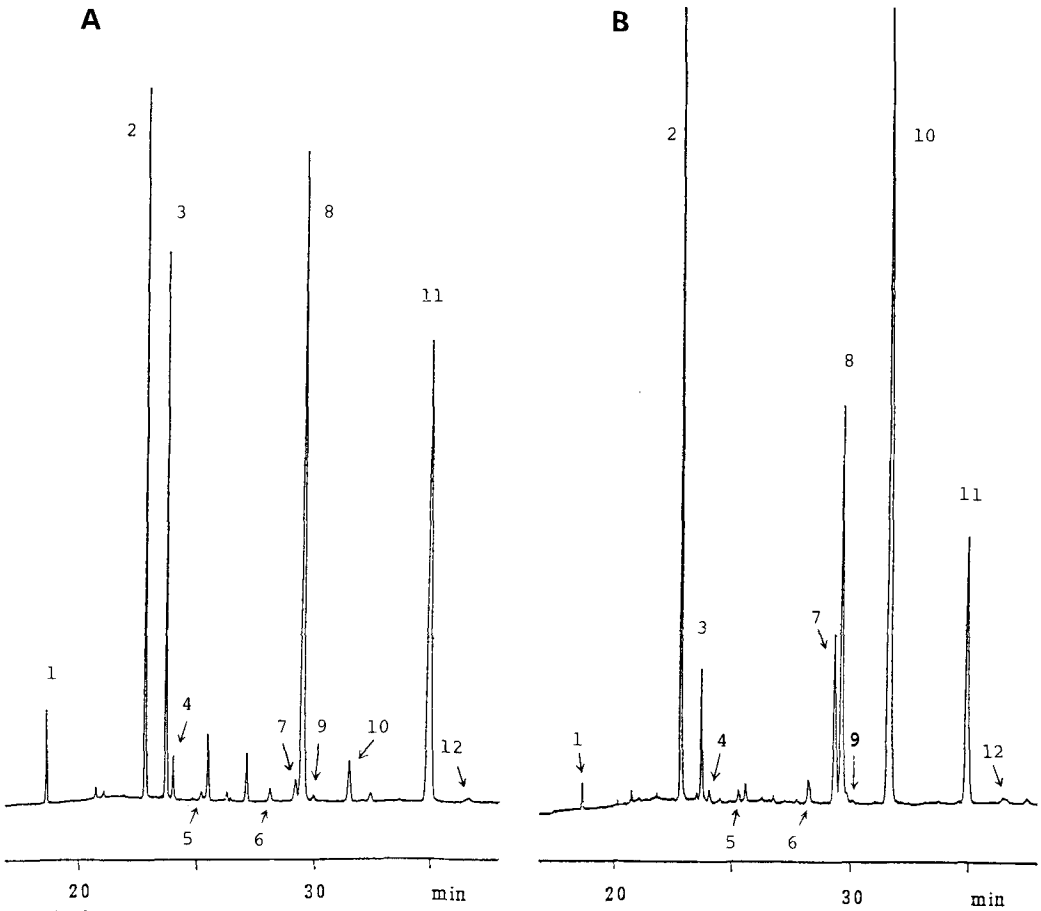

FIG. 1. Capillary gas chromatograms of fatty acid metyl esters from pulp total lipids of Japanese persimmon (A) and sweet orange (B). 1=14:0; 2=16:0; 3=16:1 (n-7); 4=16:1 (n-5); 5=17:0; 6=18:0; 7=18:1 (n-9); 8=18:1 (n-7); 9=18:1 (n-5); 10=18:2 (9,12); 11=18:3 (9,12,15); 12=20:0.

noenoic acid positional isomers. Thus, the identification and estimation of fatty acids by capillary GC were followed by GC-MS analysis as their dimethyl disulfide adducts (11,12). Their mass chromatographic data on molecular ion and characteristic key fragment ions showing the original double bond positions in the starting esters supported the capillary GC data. Mass spectra taken at the positions of mass chromatographic responses revealed the structure of individual isomers more accurately. The proportion of *cis*-vaccenic acid in pulp total lipids of Japanese persimmon (Fig. 1A) was estimated as 96.0% by a calibration curve based on peak areas of m/z 145 for the dimethyl disulfide adduct of methyl *cis*-vaccenate and m/z 173 for that of methyl oleate (11,12). This proportion (96.0%) by mass chromatography was almost equal to the calculated proportion (95.1%) by capillary GC (Table 2). Although *cis*-13-octadecenoic acid was detected in some samples by capillary GC (cf. Fig. 1) and GC-MS analyses, the content of this acid (0.0–0.9%) was much lower than that of *cis*-vaccenic or oleic acid. Therefore, in the equation used to calculate the content of *cis*-vaccenic acid (Table 2), the denominator "*cis*-vaccenic + oleic" might be regarded as "total octadecenoic". Thus these methods, a combination of capillary GC and GC-MS analyses, have made it possible to detect and estimate *cis*-vaccenic acid together with other monoenoic acid positional isomers in a short time.

Methyl *cis*-vaccenate cannot be separated from *trans*-13-octadecenoate (also methyl oleate from *trans*-vaccenate) on the capillary column used in this study. In our early experiment (10), GC-MS analysis of dimethyl disulfide adducts of a standard mixture (methyl oleate, elaidate, *cis*-vaccenate and *trans*-vaccenate) exhibited that the adducts of *trans*-isomers eluted just after that of the corresponding *cis*-isomers with partial resolutions even on the nonpolar, short packed column. Mass chromatography of the adducts of FAME from all the fruit pulp lipids examined in this study showed the absence of *trans*-monoenoates. The absence of the *trans*-monoenoates in these lipids was further confirmed by argentation TLC analysis and infrared spectrometry as previously described (12). For simplified, simultaneous separation of both positional and geometric isomers, an improvement of capillary GC-MS analysis of their dimethyl disulfide adducts is under way in our laboratory.

The distribution of *cis*-vaccenic acid among lipid classes of fruit pulps is not clear except for the case of mango pulp lipids (12). The fatty acids of nonpolar and polar lipids from the 19 samples were analyzed by capillary GC and GC-MS in the same manner described above. *cis*-Vaccenic acid occurred in both the nonpolar and polar lipids of all the fruit pulps, as illustrated in Figure 2. The proportions of *cis*-vaccenic acid to oleic acid and of octadecenoic acids to total fatty acids in nonpolar lipids of a pulp were similar to those in polar lipids of the pulp. Polar lipids are known to be an important constituent of membrane lipids (15). The presence of *cis*-vaccenic acid in pulp polar lipids of the

TABLE 2

Major Fatty Acid Composition and *cis*-Vaccenic Acid Content in Total Lipids of Various Fruit Pulps[a]

Plant	Fatty acid (wt %)									
	14:0	16:0	16:1 (n-7)	18:0	18:1 (n-9)	18:1 (n-7)	18:2 (9, 12)	18:3 (9,12,15)	Others[b]	*cis*-Vaccenic + Oleic[c] (wt %)
Durian	0.6	31.3	3.4	2.1	47.1	4.3	1.3	7.1	2.8	8.4
Papaya	7.2	24.9	14.9	1.6	11.8	3.4	4.2	19.2	12.8	22.4
Watermelon	Tr	14.1	0.3	3.5	22.1	1.2	43.8	10.7	4.3	5.2
Muskmelon	0.6	23.0	11.9	1.1	1.9	7.7	24.0	23.4	6.4	80.2
Princemelon	0.4	26.7	22.6	1.3	2.6	6.0	15.2	19.0	6.2	69.8
Japanese persimmon	1.9	18.3	13.7	0.6	1.5	29.0	1.8	26.5	6.7	95.1
Mangosteen	1.4	22.7	0.6	4.4	51.9	1.0	0.9	4.7	12.4	1.9
Apple	0.7	31.9	0.2	4.6	4.2	0.8	46.2	4.9	6.5	16.0
Cherry	Tr	14.9	0.3	5.4	43.3	1.2	12.6	18.2	4.1	2.7
Peach	0.2	24.1	0.2	3.7	1.3	3.3	51.9	11.8	3.5	71.7
Japanese pear	0.2	33.1	0.2	2.5	10.5	1.5	46.5	1.3	4.2	12.5
Lemon	0.4	21.5	0.8	1.7	5.8	3.9	36.2	16.0	13.7	40.2
Grapefruit	0.3	20.9	4.6	1.4	6.6	19.4	34.9	7.2	4.7	74.6
Sweet orange	Tr	19.7	3.1	1.0	3.9	16.9	36.0	13.6	5.8	81.3
Japanese mandarin	Tr	11.7	2.0	1.3	27.5	22.3	18.0	7.7	9.5	44.8
Litchi	0.3	22.2	0.9	1.8	33.9	3.5	18.0	18.2	1.2	9.4
Grape (cv. Campbell)	0.9	29.3	0.2	3.3	9.1	1.1	40.2	8.6	7.3	10.8
Grape (cv. Delaware)	0.5	30.6	0.3	4.0	4.1	1.0	39.8	9.8	9.9	19.6
Grape (cv. Muscat)	0.5	22.7	0.8	2.3	14.2	1.5	37.0	13.0	8.0	9.6

[a]Determined by capillary GC. Each value is an average of three determinations.

[b]Including saturated acids (except for 14:0, 16:0 and 18:0), minor monoenoic and polyenoic acids, and unidentified acids.

[c]Calculated from the data by capillary GC, followed by confirming the values by mass chromatography as their dimethyl disulfide adducts.

Tr, trace (below 0.2 wt%).

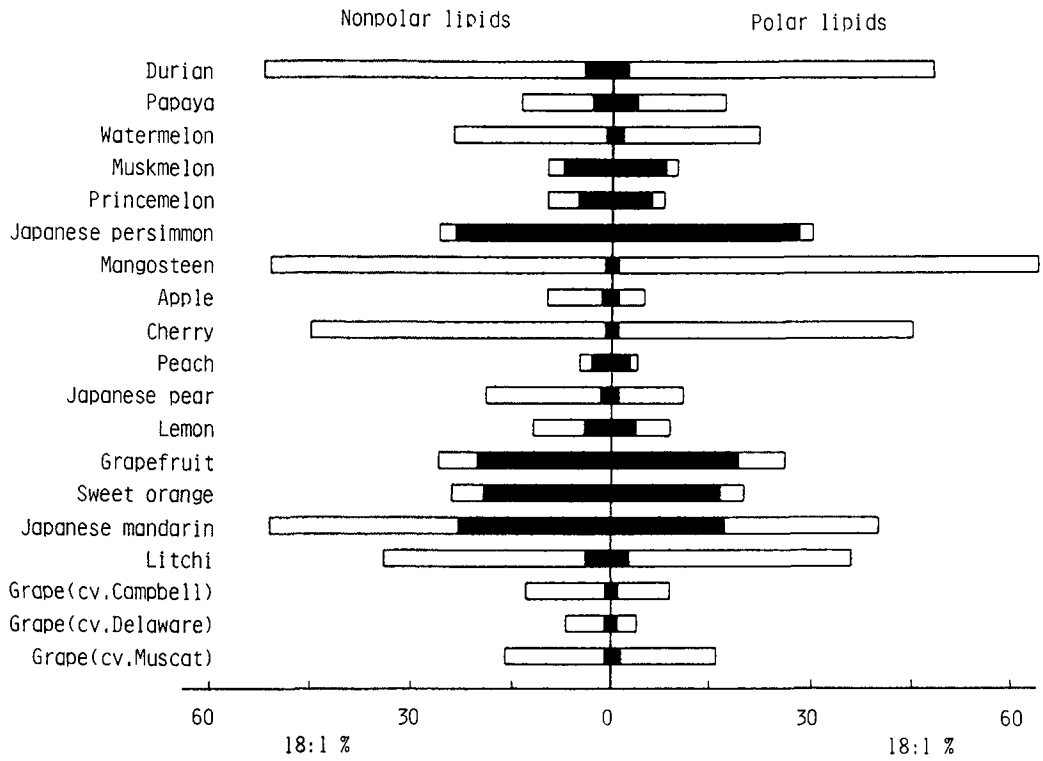

FIG. 2. *cis*-Vaccenic acid content in total fatty acids of nonpolar lipids and polar lipids from various fruit pulps. ■, *cis*-vaccenic acid; □, oleic acid.

19 samples supports our previous proposal (12) that either *cis*-vaccenic acid has some unknown physiological or biochemical functions due to its n-7 structure in membrane lipids or it acts merely as a hydrophobic material of cell membranes of fruit pulps.

Erucic acid of rapeseed (16-18) and petroselinic acid of parsley seed (19) were localized on nonpolar lipid fractions. The fatty acid compositions of the nonpolar and polar lipids in these seeds were clearly different (16-19). These seeds contained large amounts of triacylglycerols and small amounts of polar lipids (16-19). In peach pulp, however, the fatty acid composition of polar lipids was almost the same as that of both nonpolar and total lipids (Table 2). The other fruit pulps had a similar tendency in their fatty acid profiles. These results agreed with the previous observations on mango (12), papaya (20) and Concord grape (*Vitis labrusca* var. Concord) (21) pulps. From these facts, the acylation in fruit pulps seems to proceed nonspecifically with regard to the structures of acyl and glyceryl moieties. As to lipid class compositions, no marked difference in the proportion was observed between polar and nonpolar lipids in the 19 fruit pulps and other pulps (12,20,21).

In addition to commonly available fruits, we analyzed a number of minor fruits and detected *cis*-vaccenic acid in their pulp lipids (Shibahara, A., et al., unpublished data). According to these findings, it can be concluded that *cis*-vaccenic acid is a usual fatty acid in fruit pulp lipids. Among the fruit pulps we have examined, the pulp lipids of Japanese persimmon is the richest source of *cis*-vaccenic acid. The details of Japanese persimmon pulp lipids will be discussed in our next paper.

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