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Isolation and Identification of Banana-like Aroma from Banana Shrub (*Michellia figo* Spreng)

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Banana shrub (*Michelia figo* Spreng) flowers were collected in late April 1980. The aroma constituents of the petals of these flowers were extracted with organic solvents and analyzed by using GC/MS techniques. The banana-like aroma of this flower was due to the high content of ester derivatives. The composition of the first fraction obtained from ethanol distillation was 65% esters, 1.8% monoterpenes, and 19.1% sesquiterpenes. The main component, which gives a fruity banana-like aroma, was isobutyl acetate (47.1%). One novel ketone, (8*Z*,11*Z*,14*Z*)-8,11,14-heptadecatrien-2-one, was isolated and identified by high- and low-resolution MS, ^{13}C NMR, ^{1}H NMR, and IR. This ketone could be derived from linolenic acid, which came from the wax of the flowers.

Banana shrub (*Michelia figo* Spreng) is an evergreen low-growing shrub (approximately 5 m) native to Southern China. The aroma constituents of its flowers have not been analyzed prior to this study. The flower is approximately 3 cm in diameter and consists of six light yellow petals. It blooms in March and April and gives a pleasant banana-like fragrance on warm, clear days. In its native country, China, it is called the "flower containing a laugh" and has been used as an accessory by young girls for their hair. The aroma from banana shrub flowers has long attracted people, and its fruity, banana-like fragrance is quite unusual among flower aromas. In this study, the volatile chemicals related to the banana-like fragrances were isolated and identified by GC/MS techniques. A novel ketone isolated from this organic solvent extract was identified by using high- and low-resolution MS, ^{1}H NMR, ^{13}C NMR, and IR. The volatile aroma constituents of natural banana and those of banana shrub were compared.

EXPERIMENTAL SECTION

Sample Preparation. Fresh flowers of the banana shrub were collected in Nishinoomote City, Kagoshima, Japan, at the end of April. The flower petals (1.5 kg) were placed in a 5-L round-bottomed flask with 1 L of ethanol. The flask was connected to a condenser, and the volatile constituents were distilled from the ethanol solution.

The distillates were collected into five fractions (100 mL each). Each fraction was marked as 1-5 according to its elution order. Distilled water (100 mL) was added to each fraction. Each fraction was extracted with 100 mL of isopentane and subsequently reextracted with 100 mL of an isopentane-ether (65:35) solution by using a liquid-liquid continuous extractor. Extraction were continued for 48 h each. The isopentane and isopentane-ether extracts were combined, and the solvents were removed by distillation. The yields of oils (obtained from each fraction) relative to the weight of petals used and the odor descriptions of each fraction are shown in Table I.

Table I. Yields and Odor Description of Fractions 1-5

fraction	yield, %[a]	odor description[b]
1	0.007	banana-like, floral
2	0.017	spicy
3	0.011	sweet
4	0.005	woody
5	0.004	sweet-woody

[a] (Quantity of oil recovered)/(quantity of petals used) × 100. [b] Examined by five trained perfumers.

Identification of the Aroma Constituents. The oil obtained from fraction 1, which gave the most banana-like odor, was analyzed by GC/MS. Identification of gas chromatographic peaks of the oil was made by comparison of their mass spectra and gas chromatographic retention indexes to those of authentic compounds. For some compounds, standard samples were not available to confirm positive identification. If the mass spectrum matched precisely that of published data and the retention could be estimated from the published data, the compound was listed as tentatively identified.

A Hewlett-Packard Model 5710A gas chromatograph equipped with a flame ionization detector was used for routine work. The gas chromatograph was fitted with an all-glass injector splitter of our own design to avoid any contact with metal surfaces in order to avoid artifacts and was operated with an injector split ratio of 100:1. Peak

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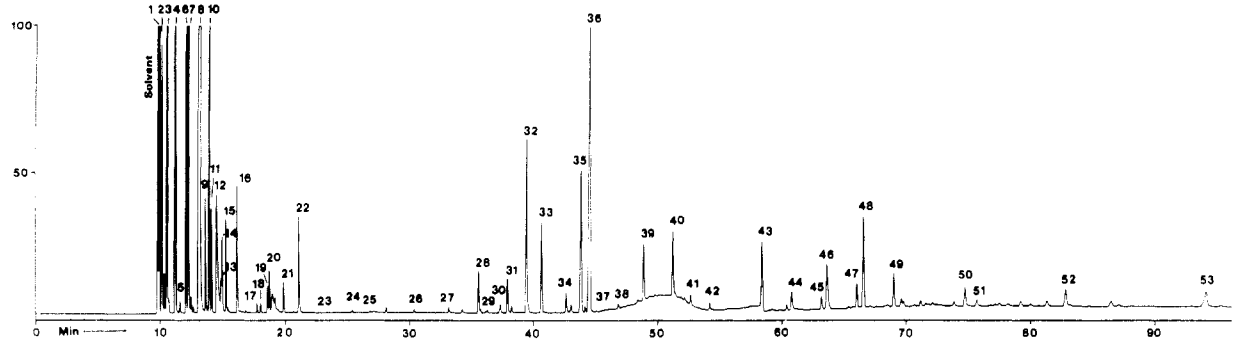


Figure 1. A typical gas chromatogram of the oil obtained from fraction 1. A glass capillary column (50 m × 0.28 i.d.) coated with Carbowax 20M was used. The column temperature was programmed from 80 to 200 °C at 2 °C/min. The injector and detector temperatures were 250 °C. For peak identification, see Table III.

area were integrated by using a Hewlett-Packard Model 3385-A automation system combined with the above gas chromatograph. A Hitachi Model RMU-6M combination mass spectrometer–gas chromatograph (Hitachi Model M-6010) equipped with a Hitachi Model 10 II/A data system was used for gas chromatographic components under the following conditions: ionization voltage, 20 eV; emission current, 80 μA; acceleration voltage, 3100 V; ion source temperature, 200 °C. The gas chromatographic column and oven conditions were as described for the Hewlett-Packard instrument (see Figure 1).

Isolation of Novel Ketone. The flower petals (4.4 kg) were placed in a 18-L glass container and soaked in 8 L of acetone for 48 h. After the petals were filtered off, acetone was distilled off to give 12.7 g of oily extract. The acetone extract was dissolved into 50 mL of diethyl ether, and undissolved materials were filtered off. The ether was removed by distillation to give a light green oil (4.7 g). This oil was placed onto the top of a glass column (40 × 2 cm i.d.) packed with 72 g of silica gel (Wako gel C-200; 100–200 mesh) and developed with the solvents shown in Table II. The sample recovered from fraction 5 (Table II) was further purified by using preparative thin-layer chromatography (Merck silica gel; size, 20 cm × 20 cm; solvent, *n*-hexane–ether = 4:1). A colorless and odorless oil [yield = 0.01% (390 mg) relative to the amount of petals used] was obtained from the TLC at the R_f = 0.5 and was shown by GLC to consist of a single component. The structure of this compound was elucidated by high-resolution MS (Hitachi Model M-80), ^{1}H NMR (JEOL FX-100), ^{13}C NMR (JEOL FX-100), and IR (Hitachi Model EPI-G3) in addition to GC/MS.

Materials. The standard sesquiterpenes which are not commercially available were obtained from the essential oils containing the objective sesquiterpenes: α-cubebene, cubeb oil (Ohta et al., 1966); δ-elemene, black pepper oil (Muller and Jennings, 1967); α-copaene, black pepper oil (Muller and Jennings, 1967; Debrauwere and Verzele, 1976); β-cubebene, cubeb oil (Ohta et al., 1966); β-elemene, basil oil (Lawrence et al., 1971) and cubeb oil (Ohta, 1966); β-caryophyllene, black pepper oil (Muller and Jennings, 1967; Debrauwere and Verzele, 1976); γ-elemene, juniperberry oil (de Pascual et al., 1976); α-humulene, black pepper oil (Muller and Jennings, 1967; Debrauwere and Verzele, 1976); γ-muurolene, black pepper (Debrauwere and Verzele, 1976). All other chemicals were obtained from reliable commercial sources.

RESULTS AND DISCUSSION

Constituents of Banana-like Aroma. The volatile aroma constituents identified in fraction 1 obtained from the oil of the banana shrub flower are shown in Table III. The aroma descriptions of the fractions obtained from column chromatography are shown in Table I. A typical gas chromatogram of fraction 1 is shown in Figure 1. It is obvious that most volatile chemicals are present in fraction 1, which gave a strong, floral, banana-like odor. The gas chromatogram of the fractions also indicated that the main volatiles are concentrated in fraction 1. Fraction 1 thus represent the banana-like aroma of this flower. The major components of fraction 1 are isobutyl acetate (47.1%), ethyl isobutyrate (8.1%), β-caryophyllene (5.7%), ethyl acetate (4.7%), and β-elemene (3.2%).

Table II. Column Chromatography of the Oil Obtained from an Acetone Extract

fraction	solvent	quantity of eluent, mL	quantity of oil recovered, g
1	*n*-hexane	100	0.10
2	5% ether–95% *n*-hexane	200	0.41
3	5% ether–95% *n*-hexane	200	0.18
4	10% ether–90% *n*-hexane	200	0.07
5	10% ether–90% *n*-hexane	200	0.38
6	20% ether–80% *n*-hexane	100	0.91
7	40% ether–60% *n*-hexane	100	0.20
8	60% ether–40% *n*-hexane	100	0.37
9	ether	100	0.18
10	ether	100	0.44
11	5% ethyl acetate–95% ether	100	0.50
12	5% ethyl acetate–95% ether	100	0.04
13	10% ethyl acetate–90% ether	200	0.03
14	10% ethyl acetate–90% ether	200	0.03
15	20% ethyl acetate–80% ether	200	0.01
16	methanol	100	0.40

The oil obtained from fraction 1 consisted mainly of ester (65.5%). On the other hand, the quantity of monoterpenes, which are the main constituents of most essential oils, is less than 2% in this flower. These facts explain the fruity rather than greenish aroma of this oil. Natural banana aroma consists of esters; no hydrocarabons have, however, been reported in natural banana volatiles (Wick et al., 1969. On the other hand, banana shrub oil contained some hydrocarbons (total peak area percent = 9.96). A relatively large quantity of sesquiterpene hydrocarbons (total peak area percent = 23.64) was also found in banana shrub oil. The main constituent of natural banana extract is isoamyl acetate (Chou, 1978). The banana shrub flower's main component is isobutyl acetate. Both isobutyl acetate and isoamyl acetate possess a banana-like aroma of isoamyl acetate. The image of tropical fruit given by this banana shrub flower may be due to the high content of odorous ester derivatives.

It is interesting that oils of *Magnolia kobus* DC. and *Magnolia sieboldii* K. Koch (*Magnolia parviflora* Sieb. et Zucc.; *Magnolia oyama* Kort), which belongs to the same botanical family (Magnolia) as the Banana shrub, contain

Table III. Compounds Identified in Fraction 1 Obtained from the Oil of *Michelia figo* Spreng

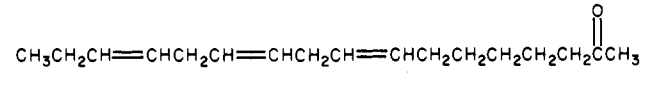
peak no.	compound	peak area, %	I_u[a]	I_k[b]
1	*n*-hexane	3.29	600	600
2	*n*-heptane	1.26	700	700
3	methylcyclohexane	5.41	780	778
4	ethyl acetate	4.75	858	856
5	acetaldehyde diethyl acetal	0.06	867	866
6	ethyl *n*-propionate	1.36	900	903
7	ethyl isobutyrate	8.06	931	934
8	isobytyl acetate	47.09	951	953
9	*n*-butyl acetate	0.45	1000	1002
10	ethyl 2-methylbutyrate	2.46	1034	1031
11	camphene	0.39	1040	1042
12	isobutyl alcohol	1.25	1053	1057
13	unknown	0.04	1068	
14	unknown	0.33	1071	
15	isobutyl isobutyrate	0.39	1081	1083
16	isoamyl acetate	0.57	1102	1105
17	unknown	0.04	1140	
18	myrcene	0.06	1151	1150
19	isobutyl isovalerate	0.30	1165	1167
20	*n*-heptanal	0.32	1167	1134
21	limonene	0.20	1190	1192
22	ethyl *n*-hexanoate	0.52	1216	1213
23	isoamyl isovalerate	trace	1277	1280
24	perillene (tentative)	0.02	1289	*c*
25	*n*-hexanol	0.02	1305	1308
26	*n*-nonanal	0.03	1367	1369
27	ethyl *n*-octanoate	0.03	1412	1415
28	α-cubebene	0.52	1443	1447
29	δ-elemene	0.15	1451	1455
30	α-ylangene (tentative)	0.10	1467	*c*
31	α-copaene	0.44	1475	1478
32	linalool	1.12	1506	1508
33	β-cubebene	1.12	1516	1519
34	bornyl acetate	0.13	1546	1549
35	β-elemene	3.18	1562	1565
36	β-caryophyllene	5.72	1568	1570
37	γ-elemene	trace	1608	1609
38	α-humulene	0.13	1632	1634
39	γ-muurolene	0.72	1669	1672
40	$C_{15}H_{24}$ (sesquiterpene; M = 204)	1.36	1717	
41	$C_{15}H_{24}$ (sesquiterpene; M = 204)	0.29	1738	
42	$C_{15}H_{24}$ (sesquiterpene; M = 204)	0.15	1749	
43	$C_{15}H_{24}$ (sesquiterpene; M = 204)	2.06	1777	
44	$C_{15}H_{24}$ (sesquiterpene; M = 204)	0.11	1783	
45	$C_{15}H_{24}O$ (M = 220)	0.09	1812	
46	γ-cadinene (tentative)	0.34	1830	*c*
47	$C_{15}H_{24}O$ (M = 220)	0.15	1858	
48	$C_{15}H_{24}O$ (M = 220)	0.54	1879	
49	β-bisabolene (tentative)	0.25	1911	*c*
50	$C_{15}H_{24}O$ (M = 220)	0.09	1967	
51	$C_{15}H_{24}O$ (M = 220)	0.08	1990	
52	$C_{15}H_{24}O$ (M = 220)	0.14	2320	
53	8,11,14-heptadecatrien-2-one	0.45	2611	

[a] Kovats index of unknown. [b] Kovats index of authentic compound. [c] Authentic sample is not available; tentatively identified.

mainly mono- and sesquiterpenes and that their ester content is very low (Fujita et al., 1975). The presence of sesquiterpenes was easily recognized by the MS fragmentation pattern. It was not, however, possible to confirm some of the structures because authentic samples were not available. The role of the sesquiterpenes in the aroma nature of this flower is, therefore, not yet completely understood.

In the sesquiterpene range of the gas chromatogram of fraction 1, peak no. 53 (Figure 1) showed different mass spectral fragmentation from that of a sesquiterpene. This compound was investigated further.

Identification of a Novel Ketone. The colorless and odorless sample obtained by the procedure described under Experimental Section was proved to be the same compound as peak no. 53 of the gas chromatogram of fraction 1 (Figure 1) by GC/MS analysis. The data obtained from the instrumental analysis of this compound are shown in Table IV. The formula of this compound was found by high-resolution mass spectra (M_r found: 248.2174. Calcd: 248.2140; Δ4.1 mass units) and proton decoupled and off-resonance ^{13}C NMR to be $C_{17}H_{28}O$. The presence of the $-C(=O)CH_3$ moiety was observed in IR [1720 cm^{-1} (carbonyl)], ^1H NMR [δ 2.15 (s, $-CH_3$)], and ^{13}C NMR [δ 209.46 (s, 29.12, q)]. This acetyl group was found to be attached to the [$-(CH_2)_4-$] group by ^1H NMR [δ 2.46 (2 H, t, J = 7 Hz), 1.2–1.8 (6 H, m)], and ^{13}C NMR [δ 23.77 (t), 29.39 (t, 43.77)]. Three —CH=CH— groups can be elucidated from ^1H NMR [δ 5.39 (64, m)] and ^{13}C NMR [δ 127.20 (d), 128.06 (d), 128.32 (d), 128.85 (d), 130.12 (d), 132.10 (d)]. The partial structure, $-CH=CHCH_2CH=CHCH_2CH=CH-$, was elucidated by ^1H NMR decoupling [a broad triplet (4 H, J = 6 Hz) at δ 2.84 was changed to a broad singlet by the decoupling on the protons δ 5.39] and by ^{13}C NMR [δ 25.66 [t (two peaks)]]. The presence of a terminal methyl group was proposed from ^1H NMR [δ 1.00 (3 H, t, J = 7 Hz)] and ^{13}C NMR [δ 14.20 (q)]. The presence of two kinds of allylic methylene groups was observed in ^1H NMR [δ 1.9–2.3 (4 H, m)] and ^{13}C NMR [δ 20.58 (t, $CH_3CH_2CH=CH-$), 27.23 (t, $-CH=CHCH_2CH_2-$)]. Combining the above results, the following structure was elucidated:



The configuration of the double bonds were determined to be all Z by comparing the ^{13}C NMR chemical shift values of their allylic carbon atoms to those available in the literature (Table V). The ^{13}C NMR spectrum of authentic linolenic acid showed identical signals on C_5–C_{17}

Table IV. Spectral Data of (8*Z*,11*Z*,14*Z*)-8,11,14-Heptadecatrien-2-one

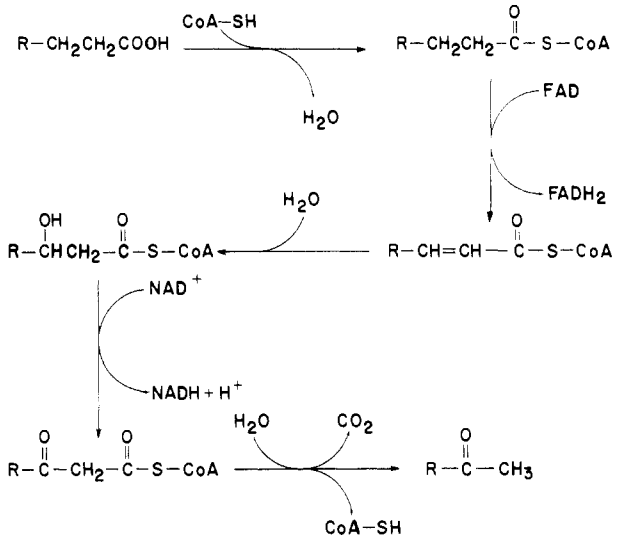
instrument	spectral data
low-resolution MS	M^+ = 248 (10), 192 (8), 135 (13), 121 (23), 108 (43), 95 (68), 79 (100), 67 (38), 43 (93)
high-resolution MS	M^+ = 248.2174 ($C_{17}H_{28}O$), 230 ($C_{17}H_{26}$), 79 (C_6H_7), 67 (C_5H_7)
IR (in CCl_4)	3010, 2950, 1720, 1470, 1415, 720 cm^{-1}
^1H NMR (in $CDCl_3$)	δ 1.00 (3 H, t, J = 7 Hz), 1.2–1.8 (6 H, m), 1.9–2.3 (4 H, m), 2.15 (3 H, s), 2.46 (2 H, t, J = 7 Hz), 2.84 (4 H, br t, J = 6 Hz), 5.39 (6 H, m)
^{13}C NMR (in $CDCl_3$)	δ 14.20 (q), 20.58 (t), 23.77 (t), 25.66 (t), 27.23 (t), 29.12 (q), 29.39 (t), 29.75 (t), 43.77 (t), 127.20 (d), 128.06 (d), 128.32 (d), 128.85 (d), 130.12 (d), 132.10 (d), 209.46 (s)

Table V. Chemical Shift (ppm) of Allylic Carbon Atoms in ^{13}C NMR

allylic carbon atom	cis or trans	literature[a,b]	observed
$-CH=CHCH_2-$	trans	32.67	
	cis	27.23	27.30
$-CH=CHCH_2CH=CH-$	cis, cis	25.72	25.66
	cis, trans	30.56	

[a] Gunstone et al. (1977). [b] Breitmaier et al. (1979).

Scheme I. Proposed Enzymatic Formation Pathways of a Methyl Ketone from a Fatty Acid



to that of this compound. This unknown ketone was, therefore, identified as (8*Z*,11*Z*,14*Z*)-8,11,14-heptadecatrien-2-one.

It is known that methyl ketones are readily produced from fat and oils by lipolytic bacteria, fungi, and yeasts (Tuynenburg Muys, 1965; Cantoni et al., 1967; Scott, 1968). The proposed formation pathway of a methyl ketone from the correspoinding fatty acid is shown in Scheme I.

Crossley et al. (1962) reported that heat treating triglycerides proposed methyl ketone, which possesses one less carbon atom than the corresponding fatty acid, by β-oxidation. This reaction occurs at 190 °C in the presence of oxygen.

The above two possible pathways can be proposed for the formation of the novel ketone; however, the temperature did not exceed 45 °C throughout the experiment. It is, therefore, more reasonable to conclude that this novel ketone is formed from linolenic acid ($C_{17}H_{29}COOH$) by a metabolic breakdown.

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Headspace Gas Chromatographic Analysis of Foods for Volatile Halocarbons

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A headspace technique for determining volatile (boiling point less than 150 °C) halocarbons (VHCs), such as chloroform, 1,1,1-trichloroethane, carbon tetrachloride, trichloroethylene, and tetrachloroethylene, in foods was developed. Food samples were placed in a septum-capped vial, 20 N H_2SO_4 was added to digest the sample, and water was added as a diluent. Some samples were analyzed without preliminary treatment, depending upon the matrix. The vial was equilibrated at 90 °C for 1 h. An aliquot of headspace was injected into a gas chromatograph equipped with an electron capture detector. VHCs were detected at sub-part-per-billion levels in aqueous foods, while for lipid-containing matrices, detection limits were in the 10–50-ppb range. By use of external standards or the method of standard additions, relative standard deviations of 20% or less were achieved. Fish and several processed foods, including jelly, chocolate sauce, ice cream, and mayonnaise, were analyzed by using the headspace technique.

Volatile halocarbons (VHCs), such as chloroform ($CHCl_3$), carbon tetrachloride (CCl_4), 1,1,1-trichloroethane (MC), trichloroethylene (TCE), and tetrachloroethylene (PCE), are used as solvents and chemical intermediates; hundreds of millions of pounds of each are produced annually (International Trade Commission, 1979). Some of these compounds are animal carcinogens (Environmental Protection Agency, 1978). They have been reported in ground and surface water (Deinzer et al., 1978; Zoeteman et al., 1980) and also in foods (McConnell et al., 1975; Page and Charbonneau, 1978). These compounds may enter the food supply through contamination of water used in food processing, as cleaning solvents for food processing equipment, through direct uptake from the environment (e.g., by fish), or through contact with packaging materials.

Because of the frequency of reports of VHCs in ground water and the potential for entering the food supply, a method was needed for the analysis of foods for the

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