Volatile Constituents of Kiwi Fruit Flowers: Simultaneous Distillation and Extraction versus Headspace Sampling

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The volatile components of the kiwi fruit flower (*Actinidia chinensis* Planch.) were analyzed by capillary gas chromatograpy and gas chromatography-mass spectrometry. Samples were prepared by simultaneous steam distillation and solvent extraction (dichloromethane). A total of 87 components were identified in the extract including 32 carbonyls, 24 alcohols, 23 hydrocarbons, 6 esters, 1 acid, and 2 miscellaneous components. A dynamic headspace technique was employed for isolating the flower volatiles by using a Tenax GC trap. Headspace analysis of the flower volatiles yielded the identification of 50 components including 22 hydrocarbons, 10 carbonyls, 10 esters, and 9 alcohols.

Key words: volatile compounds, kiwi fruit flower, simultaneous steam distillation and solvent extraction (SDE), dynamic headspace sampling, capillary GC-MS.

Kiwi fruit (*Actinidia chinensis* Planch.) is a native of China. Although it was first developed as a commercial crop in New Zealand, it is now grown in several other countries. Recent papers dealt with the flavor volatiles of kiwi fruit (Takeoka et al., 1986; Bartley and Schwede, 1989), but none have investigated the floral fragrance of kiwi fruit. Palmer-Jones and Clinch (1974, 1976) investigated the role played by honey bees (*Apis mellifera* L.) in the pollination of kiwi fruit. Nectar secretion was not observed in male or female kiwi fruit flowers, but honey bees usually visit male and female flowers to collect pollen. Both citrus trees and white clovers, which produce nectar, are competing plants of kiwi fruit for the attraction of honey bees (Palmer-Jones and Clinch, 1974). It was suggested that the satisfactory pollination by the honey bees' visitation produces a good harvest of fruit (Clinch, 1984). The relationship between orchid floral fragrance and euglossine bee attraction has been summarized by Williams and Whitten (1983). Honey bee-plant relationships are based on a conditioning process in which olfactory and gustatory cues are closely linked, leading to a selective foraging behavior (Pham-Delegue et al., 1987). We were interested in the relationship between the volatile components of the flowers and honey bee attraction in connection with the pollination of kiwi fruit. The aim of this work was to obtain knowledge on the volatile comoponents emitted by kiwi fruit flowers. The volatile components of kiwi fruit flowers concentrated by two different sampling techniques, simultaneous steam distillation/solvent extraction (SDE) and dynamic headspace sampling, were identified by gas chromatography-mass

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spectrometry.

EXPERIMENTAL PROCEDURES

Materials. Kiwi fruit flowers (*A. chinensis* Planch.) were collected from vines on the farm of our college in Kawanishi-shi, Hyogo, Japan, during May 1988 and May 1989. Ultrahigh-quality water generated with a water purification system (Elgastat UHQ; Elga Ltd., Lane End, U.K.) was used in the concentration of volatiles by simultaneous steam distillation/solvent extraction. Dichloromethane of special grade was obtained from Wako Pure Chemical Industries Ltd. (Osaka 540, Japan). High-purity synthetic air (99.99% pure) in a bomb obtained from Sumitomo Seika Chemicals Co., Ltd. (Osaka 541, Japan) was used in the dynamic headspace collection. Tenax GC (60-80 mesh) was obtained from Enka NV, Arnhem, The Netherlands. Kieselgel 60 (70-230 mesh) was obtained from E. Merk AG. β-Phorone was synthesized by the deconjugation of isophorone (Meinwald and Hendry, 1971).

Sampling Techniques. *1. Simultaneous Steam Distillation and Solvent Extraction (SDE).* Thefresh flowers (600 g) and water (100 mL) in a 3-L flask were subjected to simultaneous steam distillation/solvent extraction for 1 h using a modified Likens-Nickerson apparatus (Likens and Nickerson, 1964; Schultz et al., 1977). The extracting solvent was dichloromethane (50 mL). The extract was dried by shaking with powder anhydrous sodium sulfate for 1 h and then carefully concentrated with a Kuderna-Danish evaporating concentrator to a final volume of approximately 0.3 mL (pot temperature 40℃).

2. Dynamic Headspace Sampling Procedure. The fresh flowers (350 g) were placed in a 500-mL sample glass bottle at room temperature (27℃). High-purity air was led into the sample bottle via a Teflon tube and passed over the flowers and out of the sample bottle through a Tenax GC column (9-cm length×0.5-cm i.d., 0.25 g) for 1 h. After collection of volatiles, the Tenax column was purged with a stream of high-purity nitrogen (50 mL/min for 30 min) to remove water.

3. Column Chromatography. The concentrated extract from the SDE was fractionated on a silica gel column (20-cm×1-cm i.d., 5 g, Kieselgel 60) with a pentane/ether gradient. Silica gel was activated for 4 h at 120-130℃ prior to use. Fraction I (pentane, 200 mL), fraction II (p/e, 1:99 v/v, 200 mL), fraction III (p/e, 1:9 v/v, 200m L), fraction IV (p/e, 3:7 v/v, 200m L), and fraction V (ether, 200m L) were obtained. Each fraction was concentrated with a Kuderna-Danish evaporating concentrator to a final volume of approximately 0.3 mL (pot temperature 40℃).

Gas Chromatography. A Shimadzu GC-9A gas chromatograph (Shimadzu, Kyoto, Japan) with a FID, equipped with a 60-m×0.25-mm i.d. DB-Wax column (df=0.25 μm, bonded poly(ethylene glycol) phase) was employed. The column temperature was programmed from 40℃ (5 min isothermal) to 200℃ at 3℃/min and then held at the upper limit. Helium carrier gas was used at a flow velocity of 25 cm/s (40℃). The injector and detector were maintained at 260℃. An injection splitter SPL-G9 (Shimadzu) was used at a split ratio of 1:28. A data processor C-R4A (Shimadzu) was used at a split ratio of 1:28. A data processor C-R4A (Shimadzu) was used for the calculation of retention indices of authentic

reference standards and flower volatile components. The fused silica columns were obtained from J&W Scientific, Folsom, CA.

Gas Chromatography-Mass Spectrometry. A GCMS-QP1000 system (Shimadzu) equipped with a 60-m×0.25-mm i.d. DB-Wax column ($df = 0.25\ \mu m$) was employed. The column temperature was programmed in the same manner as for the GC analysis. Helium carrier gas was used at a flow velocity of 27 cm/s (40℃). The injector and ion source were maintained at 260 and 250℃, respectively. The injection splitter SPL-G9 was used at a split ratio of 1:17. In all cases, the outlet of the column was directly coupled to the ion source of the quadrupole mass spectrometer. In the electron impact mode (EI), the mass spectrometer was scanned from m/z 20 to 300 in a 1-s interval. The instrument was operated at an ionization voltage of 70 eV. In the chemical ionization mode (CI), the mass spectrometer was scanned from m/z 60 to 300 in a 2-s interval. Isobutane was used as a regent gas.

For the headspace experiments, a flush sampler FLS-3 (Shimadzu) was used for thermal desorption/backflushing of a loaded Tenax GC trap. The loaded Tenax column was placed in a furnace of the flush sampler attached to the GC-MS inlet. Helium carrier gas was passed through both the loaded Tenax GC column and the DB-Wax capillary column for 30 min to remove nitrogen, and then the Tenax column was heated to 250℃ within 1 min. The volatiles were thermally desorbed from the Tenax GC and directly introduced into the capillary column via a splitter (Tatsuka et al., 1988). The column temperature control program of the GS-MS was started at the same time as the start of heating of the flush sampler. Peak area percent values were calculated from EI ion intensity of individual comoponents without response factor correction (Tatsuka et al., 1987).

Component Identification. Sample components were tentatively identified by mass spectra matching with a mass spectra library collection using a library search system LSS-20 (Shimadzu). The reference library is basically the NIH/EPA collection, supplemented by our previous work. The *Wiley/NBS Registry of Mass Spectral Data* (1989) was also used for tentative identification of sample components by the manual search with the help of M^+ data obtained from the CI mass spectra. Tentative MS identifications were verified by comparison of components' experimental retention index with that of an authentic reference standard. Experimental retention index values were calculated from retention time data obtained by the coinjection of the normal hydrocarbon reference series with an experimental sample. The retention index system proposed by Kovs (1958, 1965) was utilized. A useful discussion on the difference of the experimental retention index values from the corresponding reference retention index values determined with an authentic standard was given by Takeoka et al. (1988).

RESULTS AND DISCUSSION

The volatile constituents of kiwi fruit flowers were examined by two different sampling methods. The first method was SDE, while the second method was dynamic headspace sampling. Both qualitative and quantitative differences were found between the constituents analyzed by

Table I. Volatile Constituents of Kiwi Fruit Flowers: Simultaneous Steam Distillation and Solvent Extraction

peak no.[a]	constituent	Kovàts index DB-Wax exptl	Kovàts index DB-Wax ref	% area[b]	peak no.[a]	constituent	Kovàts index DB-Wax exptl	Kovàts index DB-Wax ref	% area[b]
1	*n*-pentane	500	500	0.14	54	furfural	1466	1467	0.12[d]
2	*n*-hexane	600	600	0.34		(*Z*)-3-hexenyl butyrate	1466		–
3	*n*-heptane	700	700	0.03	55	*cis*-furan linalool oxide	1478	1480	0.04
4	(dichloroethene)[e]	730			56	(*E*, *E*)-2, 4-heptadienal	1497	1497	0.04
5	*n*-octane	800	800	0.06	57	*n*-pentadecane	1500	1500	4.49[d]
6	2-propanone	814	814	0.02		decanal	1500	1502	–
7	2-propanone	863[f]	814	0.41	58	benzaldehyde	1528	1529	0.07
8	2-methyl propanal	884[f]	812	0.04	59	unknown	1535		0.02
9	*n*-nonane	900	900	2.08	60	(*E*)-2-nonenal	1542	1542	0.27
10	dichloromethane[e]	925	931		61	linalool	1552	1552	2.57
11	2, 3-butanedione	977	978	0.31[d]	62	1-octanol	1564	1564	1.08
	pentanal	979	979	–	63	((*E*, *Z*)-2, 6-nonadienal)[c]	1591		0.02
12	*n*-decane	1000	1000	0.04	64	isophorone	1600	1595	0.72[d]
13	tetrachloroethene[e]	1022	1022			*n*-hexadecane	1600	1600	–
14	2-butanol	1030	1030	0.02	65	undecanal	1609	1609	0.12
15	toluene	1040	1041	0.03	66	unknown	1617		0.04
16	2, 3-pentanedione	1060	1061	0.02	67	(C_{18} H_{32} hydrocarbon)[c]	1622		0.16
17	(2-methyl-1-penten-3-one)[c]	1068		0.02	68	phenylacetaldehyde	1650	1646	0.21
18	hexanal	1083	1083	1.11	69	(7-octen-4-ol)[c]	1656		0.06
19	2-methyl-1-propanol	1092	1094	0.08[d]	70	1-nonanol	1666	1666	1.19
	unknown	1092		–	71	(3-nonen-1-ol)[c]	1685		0.16
20	*n*-undecane	1100	1100	0.02	72	unknown	1690		0.12
21	isoamyl acetate	1126	1126	0.04[d]	73	*α*-terpineol	1695	1698	0.28
	3-penten-2-one	1126	1127	–	74	*n*-heptadecane	1700	1700	2.13
22	(*E*)-2-pentenal	1130	1130	0.03	75	dodecanol	1709	1710	0.03
23	*m*-xylene	1142	1143	0.01	76	(C_{17} H_{34} hydrocarbon)[c]	1725		8.95
24	1-butanol	1150	1150	0.01	77	*α*-farnesene	1755	1754	7.42
25	1-penten-3-ol	1164	1165	0.09	78	(hydrocarbon)[c]	1764		4.82
26	(cyclopentanecarboxaldehyde)[c]	1170		0.03	79	(hydrocarbon)[c]	1767		0.07
27	(hydrocarbon)[c]	1175		0.08	80	(hydrocarbon)[c]	1773		0.04
28	2-heptanone	1185	1184	0.04[d]	81	(2, 2, 6-trimethyl-1, 4-cyclohexanedione)[c]	1787		0.10
	o-xylene	1185	1185	–					
	pyridine	1185	1187	–	82	*n*-octadecane	1800	1800	0.06
29	heptanal	1188	1186	9.07	83	nerol	1806	1807	0.11
30	3-methyl-1-butanol	1211	1211	0.19[d]	84	(*E*, *E*)-2, 4-decadienal	1819	1821	0.02
	1, 8-cineole	1211	1212	–	85	*β*-phenylethyl acetate	1825	1826	1.09[d]
31	(*E*)-2-hexenal	1219	1219	0.30		(hydrocarbon)[c]	1825		–
32	(2-hexanol)[c]	1226		0.03	86	nerylacetone	1835	1838	0.04
33	(2-pentylfuran)[c]	1235		0.02	87	geraniol	1853	1854	1.82
34	(*E*)-ocimene	1255	1255	0.12[d]	88	geranylacetone	1862	1865	1.10
	1-pentanol	1255	1256	–	89	benzyl alcohol	1885	1886	0.15
35	hexyl acetate	1276	1276	0.03	90	unknown	1893		0.13
36	acetoin	1287	1287	0.04	91	*n*-nonadecane	1900	1900	3.38
37	octanal	1291	1291	2.15	92	*β*-phenylethyl alcohol	1922	1923	4.45[d]
38	*n*-tridecane	1300	1300	0.03		(C_{11} H_{16} hydrocarbon)[c]	1922		–
39	(2-vinylcrotonaldehyde)[c]	1313		0.04	93	phenylacetonitrile	1938	1941	0.04
40	(*Z*)-3-hexenyl acetate	1320	1321	0.12	94	*β*-ionone	1953	1954	0.04
41	(*Z*)-2-pentenol	1325	1325	0.12	95	*cis*-jasmone	1960	1961	1.01
42	unknown	1332		0.02	96	*β*-phenylethyl *n*-butyrate	1975	1978	0.08
43	6-methyl-5-hepten-2-one	1342	1341	0.54	97	*n*-eicosane	2000	2000	0.34
44	1-hexanol	1359	1359	0.88	98	(octanoic acid)[c]	2070		0.21
45	(*E*)-3-hexenol	1369	1370	0.01	99	(sesquiterpene)[c]	2091		0.41
46	(*Z*)-3-hexenol	1389	1389	0.49	100	*n*-heneicosane	2100	2100	7.84
47	nonanal	1400	1396	12.57[d]	101	(hydrocarbon)[c]	2117		0.82
	n-tetradecane	1400	1400	–	102	nonanoic acid	2169	2171	1.52
48	(*E*)-2-hexenol	1411	1410	0.06	103	*n*-docosane	2200	2200	0.42
49	*β*-phorone	1416	1416	1.51	104	unknown	2257		1.00
50	(*E*)-2-octenal	1434	1434	0.03	105	unknown	2287		0.62
51	*trans*-furan linalool oxide	1450	1452	0.04	106	*n*-tricosane	2300	2300	2.98
52	1-octen-3-ol	1456	1456	0.06	107	unknown	2305		0.29
53	1-heptanol	1462	1461	1.12					

[a] The peak numbers correspond to the numbers in Figure 1. [b] Peak area percentage calculated from EI ion intensity excluding the solvent peaks (assuming all response factors of 1). [c] Tentative identifications enclosed in parentheses. [d] Total area percentage of overlapped peaks. [e] Solvent and solvent contaminant. [f] Retarding of the elution by a large quantity of dichloromethane.

the two different sampling techniques. Table I lists the volatile constituents identified in the extracts prepared by SDE. A reconstructed ion chromatogram from GC-MS analysis of the flower volatiles isolated by SDE is shown in Figure 1. A total of 33 hydrocarbons (47.31%) composing 23 identified hydrocarbons (31.96%) and 10 tentatively identified hydrocarbons (15.35%) were found in the extract. Tentatively identified hydrocarbons are eluants of the pentane fraction obtained by the silica gel column chromatography. The qualitative differences of hydrocarbons between the SDE volatiles and the headspace volatiles were not so large except for hydrocarbons with large retention index. Major volatile constituents were *n*-nonane (2.08%), *n*-pentadecane (4.49%), *n*-heptadecane (2.13%), *n*-nonadecane (3.38%), *n*-heneicosane (7.84%), *n*-tricosane (2.98%), and α-farnesene (7.42%). Carbonyls occupied 31.98% of the total area. The differences may be due to both enzymic and thermal formation of secondary volatiles caused by disruption and heating of the flower tissues during the SDE. Both the aliphatic aldehydes and the aliphatic

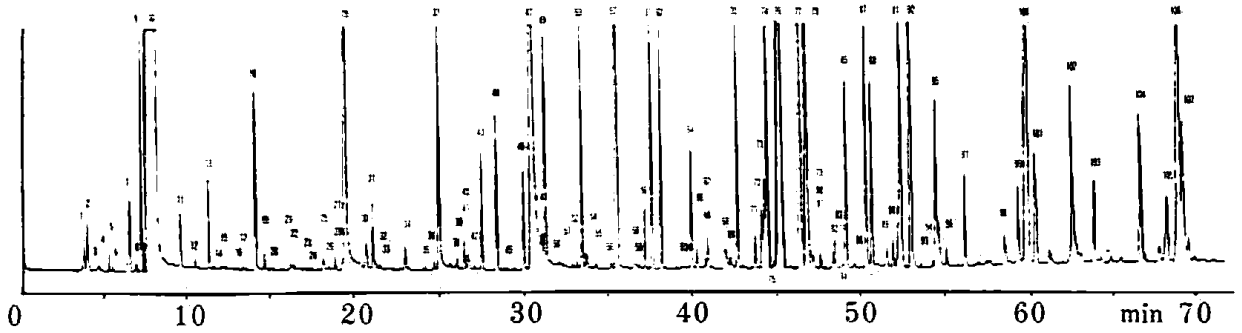

Fig.1. Reconstructed ion chromatogram from GC-MS analysis of kiwi fruit flower volatiles obtained by simultaneous steam distillation and solvent extraction (dichloromethane). Temperature was programmed from 40°C (5 min isothermal) to 200°C at 3°C/min on a 60-m × 0.25-mm (i.d.) DB-Wax column. The peak numbers correspond to the numbers in Table I.

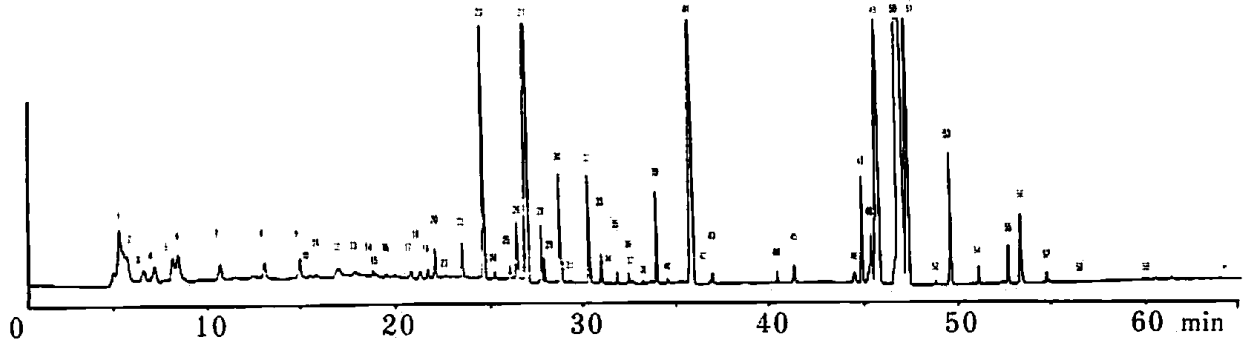

Fig.2. Reconstructed ion chromatogram from GC-MS analysis of kiwi fruit flower headspace volatiles. Temperature was programmed from 40°C(5 min isothermal) to 200°C at 3°C/min on a 60-m × 0.25-mm (i.d.) DB-Wax column. The peak numbers correspond to the numbers in Table II.

alcohols were derived from enzyme-induced and thermal oxidative breakdown of unsaturated fatty acids, that is, oleic acid, linoleic acid, and linolenic acid. These unsaturated fatty acids are an essential part of every plant cell (Buttery, 1981; Frankel, 1982). From these considerations, it would be concluded that the SDE volatiles consist of both the essential volatiles of the flowers and the secondary volatiles formed during the extraction process. Major volatile constituents in the carbonyls include hexanal (1.11%), heptanal (9.07%), octanal (2.15%), nonanal (12.57%), β-phorone (1.51%), isophorone (0.72%), geranylacetone (1.10%), and *cis*-jasmone (1.01%). Relative large amounts of isophorone and β-phorone were detected in the SDE volatiles in our 1988 and 1989 experiments. Isophorone containing a small amount of β-phorone as impurity (Kawahashi et al., 1986) is used in industrial solvent mixtures. Only a few papers have reported the existence of isophorone and β-phorone in nature. Isophorone was described as a volatile compound of oat groats (Heydanek and McGorrin, 1981), while β-phorone was described as a volatile constituent of females of the spruce bark beetle (Birgersson et al., 1984). Geranylacetone and 6-methyl-5-hepten-2-one could result from the oxidative cleavage of carotenoids (Stevens, 1970; Buttery, 1981). Alcohols (14.86%) include 1-hexanol (0.88%), 1-heptanol (1.12%), 1-octanol (1.08%), 1-nonanol (1.19%), linalool (2.57%), and geraniol (1.82%) as major comoponents. A major volatile constituent of esters is β-phenylethyl acetate (1.09%).

Components identified in the headspace volatile are listed in Table II. A reconstructed ion chromatogram from GC-MS analysis of the headspace volatiles is shown in Figure 2. A total area percentage of hydrocarbons including tentatively identified hydrocarbons was 63.38%. Major volatile constituents include *n*-pentadecane (9.06%) and α-farnesene (29.70%). Alcohols (4.95%) include 1-hexanol (1.60%), (*Z*)-3-hexenol (1.54%), and β-phenylethyl alcohol (1.35%) as major constituents. A total carbonyl area percentage (2.88%) of headspace volatiles was very low in comparison with that (31.98%) of the SDE volatiles. In contrast with this, a total ester area percentage (26.98%) of headspace volatiles was very high in comparison with that (1.28%) of the SDE volatiles. Major volatile constituents of esters include (*Z*)-3-hexenyl acetate (16.52%), hexyl acetate (5.47%), and β-phenylethyl acetate (1.90%).

Koltermann (1969) concluded that scent was more important in conditioning honey bees than color, form, or time of day. Waller et al. (1973) developed a bioassay test for determining the olfactory responses of honey bees to the scent of alfalfa flowers. They found that honey bees conditioned to collect sucrose solution near the scent of ocimene would respond to the scent of alfalfa flowers but not to the scent of flowers of red clover, which do not contain ocimene. The olfactory discrimination by honey bee of terpenes of alfalfa flower was reported by Waller et al. (1974). (*E*)-β-Ocimene is a major component found in alfalfa flowers by Tenax trapping (Buttery et al., 1982). The most dominant headspace constituent found in kiwi fruit flowers is α-farnesene (29.70%), which was known as a strong attractant for larvae of codling moth (Sutherland and Hutchins, 1972). Ocimene is a monoterpene hydro-

Table II. Headspace Constituents of Kiwi Fruit Flowers

peak no.[a]	constituent	Kovâts index DB-Wax exptl	Kovâts index DB-Wax ref	% area[b]
1	*n*-hexane	600	600	2.05
2	*n*-heptane	700	700	0.71
3	*n*-octane	800	800	0.38
4	methyl acetate	828	827	0.57
5	ethyl acetate	884	887	0.74
6	2-butanone	900	900	0.96
7	3-pentanone	974	976	0.52[d]
	2-pentanone	974	978	–
8	toluence	1037	1041	0.38
9	hexanal	1080	1083	0.45
10	2-methyl-1-propanol	1094	1094	0.07
11	*n*-undecane	1100	1100	0.08
12	(monoterpene)[c]	1122		0.43[d]
	isoamyl acetate	1124	1126	–
	ethylbenzene	1126	1128	–
13	*m*-xylene	1141	1143	0.17[d]
	(2-methyl-4-pentenal)[c]	1141		–
14	1-penten-3-ol	1161	1165	0.11
15	myrcene	1165	1167	0.04
16	*n*-amyl acetate	1175	1177	0.08
17	D-limonene	1200	1198	0.19[d]
	n-dodecane	1200	1200	–
18	3-methyl-1-butanol	1209	1211	0.20[d]
	1, 8-cineole	1209	1212	–
19	(*E*)-2-hexenal	1218	1219	0.19
20	(1-methoxy-3-methylene-2-pentanone)[c]	1225		0.45
21	(*Z*)-ocimene	1236	1238	0.04
22	(*E*)-ocimene	1252	1255	0.53
23	hexyl acetate	1274	1276	5.47
24	acetoin	1286	1287	0.09
25	*n*-tridecane	1300	1300	0.24
26	(2-methyl-6-methylene-1, 7-octadien-2-one)[c]	1308		1.00
27	(*Z*)-3-hexenyl acetate	1319	1321	16.52
28	(*E*)-2-hexenyl acetate	1336	1338	0.52
29	6-methyl-5-hepten-2-one	1339	1341	0.26
30	1-hexanol	1356	1359	1.60
31	(*E*)-3-hexenol	1366	1370	0.04
32	(*Z*)-3-hexenol	1387	1389	1.54
33	*n*-tetradecane	1400	1400	0.34
34	(*E*)-2-hexenol	1409	1410	0.04
35	*n*-hexyl *n*-butyrate	1419	1420	0.13
36	(hydrocarbon)[c]	1431		0.13
37	unknown	1435		0.04
38	unknown	1447		0.07
39	(*Z*)-3-hexenyl butyrate	1464		1.05
40	(sesquiterpene)[c]	1476		0.09
41	*n*-pentadecane	1500	1500	9.06
42	unknown	1524		0.04
43	benzaldehyde	1527	1529	0.13[d]
	(C_{15} H_{30} hydrocarbon)[c]	1527		–
44	*n*-hexadecane	1600	1600	0.11
45	(C_{16} H_{32} hydrocarbon)[c]	1620		0.28
46	unknown	1691		0.18
47	*n*-heptadecane	1700	1700	1.54
48	(sesquiterpene)[c]	1712		0.91
49	(C_{17} H_{34} hydrocarbon)[c]	1722		8.57
50	*α*-farnesene	1752	1754	29.70
51	(hydrocarbon)[c]	1763		6.92
52	*n*-octadecane	1800	1800	0.07
53	*β*-phenylethyl acetate	1822	1826	1.90
54	geranylacetone	1859	1865	0.17
55	*n*-nonadecane	1900	1900	0.35
56	*β*-phenylethyl alcohol	1919	1923	1.35
57	*cis*-jasmone	1955	1961	0.11
58	*n*-eicosane	2000	2000	0.03
59	*n*-heneicosane	2100	2100	0.04

[a] The peak numbers correspond to the numbers in Figure 2. [b] Peak area percentage calculated from EI ion intensity (assuming all response factors of 1). [c] Tentative identifications enclosed in parentheses. [d] Total area percentage of overlapped peaks.

carbon, while *α*-farnesene is a sesquiterpene hydrocarbon, and the chemical structures of the two compounds are similar. The major oxygenated components of headspace volatiles included (*Z*)-3-hexenyl acetate, hexyl acetate, and *β*-phenylethyl acetate. *β*-Phenylethyl acetate is a good attractant for male euglossine bees (Williams and Whitten, 1983). Pham-Delegue et al. (1987) reported that only one polar fraction composed of 28 compounds can be considered an active fraction of sunflower aroma for honey bee attraction. *α*-Farnesene and the oxygenated components may be active compounds for honey bee attraction. Since the kiwi fruit flowers do not produce nectar, honey bee attraction may be mainly attributed to aroma (olfaction) and pollen (gustation).

In the GC-MS analysis, a large quality of dichloromethane is introduced in the DB-Wax capillary column; this has an effect upon the retention behavior of 2-propanone and 2-methylpropanal, retarding the elution of the polar compounds eluted in the front of dichloromethane. The reference and experimental Kovâts index values of the two compounds differ by 49 and 72 units, respectively, but a small peak of 2-propanone also appears at the normal elution time. The retardation of elution of the two compounds was reproduced by the model experiments.

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LITERATURE CITED

Bartley, J. P.; Schwede, A. M. Production of Volatile Compounds in Ripening Kiwi Fruit (*Actinidia Chinensis*). *J. Agric. Food Chem.*, 37, 1023−1025 (1989).

Birgersson, G.; Schlyter, F.; Löfqvist, J.; Bergstrom, G. Quantitative Variation of Pheromone Components in The Spruce Bark Beetle *Ips typographus* from Different Attack Phases. *J. Chem. Ecol*, 10, 1029−1055 (1984).

Buttery, R. G. Vegetable and Fruit Flavors. In *Flavor Research Recent Advances*; Teranishi, R., Flath, R. A., Sugisawa, H., Eds.; Dekker: New York, pp. 175−216 (1981).

Buttery, R. G.; Kamm, J. A.; Ling, L. C. Volatile Components of Alfalfa Flowers and Pods. *J. Agric. Food Chem.*, 30, 739−742 (1982).

Clinch, P. G. Kiwifruit Pollination by Honey Bees 1. Tauranga Observations, 1978−81. *N. Z. J. Exp. Agric.*, 12, 29−38 (1984).

Frankel, E. N. Volatile Lipid Oxidation Products. *Prog. Lipid Res.*, 22, 1−33 (1982)

Heydanek, M. G.; McGorrin, R. J. Gas Chromatography-Mass Spectroscopy Investigations on the Flavor Chemistry of Oat Groats. *J. Agric. Food Chem.*, 29, 950−954 (1981).

Kawahashi, K.; Shimada, G.; Uragami, M. Purification of Isophorone. Japanese Patent. 61289055 (1986).

Koltermann, R. *Z. Vgl. Rhysiol.*, 63, 310−334 (1969).

Kováts, E. *Helv. Chim. Acta*, 41, 1915−1932 (1958).

Kováts, E. Gas Chromatographic Characterization of Organic Substances in the Retention Index System. *Adv. Chromatogr.*, 1, 229−247 (1965).

Likens, S. T.; Nickerson, G. B. Detection of Certain Hop Oil Constituents in Brewing Products. *Proc. Am. Soc. Brew. Chem.*, 5, 5 (1964).

Meinwald, J.; Hendry, L. The Deconjugation of Isophorone. *J. Org. Chem.*, 36, 1446−1447 (1971).

Palmer-Jones, T.; Clinch, P. C. Observations on the Pollination of Chinese Gooseberries Variety Hayward. *N. Z. J. Exp. Agric.*, 2, 455−458 (1974).

Palmer-Jones, T.; Clinch, P. G. Effect of Honey Bee Saturation on The Pollination of Chinese Gooseberries Variety Hayward. *N. Z. J. Exp. Agric.*, 4, 255−256 (1976).

Pham-Delegue, M. H.; Etievant, P.; Masson, C. Molecular Parameters Involved in Bee-Plant Relationships: A Biological and Chemical Approach. *Biochimie*, 69, 661−670 (1987).

Schultz, T. H.; Flath, R. A.; Mon, T. R.; Eggling, S. B.; Teranishi, R. Isolation of Volatile Components from a Model System. *J. Agric. Food Chem.*, 25, 446−449 (1977).

Stevens, M. A. Relationship Between Polyene-Carotene Content and Volatile Compound Composition of Tomatoes. *J. Am. Soc. Hortic Sci.*, 95, 461−464 (1970).

Sutherland, O. R. W.; Hutchins, R. F. N. α-Farnesene, An Natural Attractant for Codling Moth Larvae. *Nature*, 239, 170 (1972).

Takeoka, G. R.; Güntert, M.; Flath, R. A.; Wurz, R. E.; Jennings, W. Volatile Constituents of Kiwi Fruit (*Actinidia chinensis* Planch.). *J. Agric. Food Chem.*, 34, 576−578 (1986).

Takeoka, G. R.; Flath, R. A.; Güntert, M.;

Jennings, W. Nectarine Volatiles: Vacuum Steam Distillation versus Headspace Sampling. *J. Agric. Food Chem.*, 36, 553−560 (1988).

Tatsuka, K.; Kohama, M.; Suekane, S.; Mori, D. Volatile Components in Kamaboko (Seasoned Steamed Minced Fish Paste) and Its Supporting Wooden Plate by Gas Chromatography Mass Spectrometry. *Nippon Nōgeikagaku Kaishi*, 61, 587−598 (1987).

Tatsuka, K.; Kohama, M.; Suekane, S. Floral Fragrance Components of *Zygopetalum mackayi (Orchidaceae)*. *Agric. Biol. Chem.*, 52, 1599−1600 (1988).

Waller, G. D.; Loper, G. M.; Berdel, R. L. A Bioassay for Determining Honey Bee Responses to Flower Volatiles. *Environ. Entomol.*, 2, 255−259 (1973).

Waller, G. D.; Loper, G. M.; Berdel, R. L. Olfactory Discrimination by Honeybees of Terpenes Identified from Volatiles of Alfalfa Flowers. *J. Apic. Res.*, 13, 191−197 (1974).

Wiley/NBS Registry of Mass Spectral Data; Wiley: New York, 1989.

Williams, N. H.; Whitten, W. M. Orchid Floral Fragrances and Male Euglossine Bees: Methods and Advances in The Last Sesquidecade. *Biol. Bull.*, 164, 355−395 (1983).

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Registry No. *n*-Pentane, 109-66-0; *n*-hexane, 110-54-3; *n*-heptane, 142-82-5; *n*-octane, 111-65-9; 2-propanone, 67-64-1; 2-methylpropanal, 78-84-2; *n*-nonane, 111-84-2; 2, 3-butanedione, 431-03-8; pentanal, 110-62-3; *n*-decane, 124-18-5; 2-butanol, 78-92-2; toluene, 108-88-3; 2, 3-pentanedione, 600-14-6; 2-methyl-1-penten-3-one, 25044-01-3; hexanal, 66-25-1; 2-methyl-1-propanol, 78-83-1; *n*-undecane, 1120-21-4; isoamyl acetate, 123-92-2; 3-penten-2-one, 625-33-2; (*E*)-2-pentenal, 1576-87-0; *m*-xylene, 108-38-3; 1-butanol, 71-36-3; 1-penten-3-ol, 616-25-1; cyclopentanecarboxaldehyde, 872-53-7; 2-heptanone, 110-43-0; *o*-xylene, 95-47-6; pyridine, 110-86-1; heptanal, 111-71-7; 3-methyl-1-butanol, 123-51-3; 1, 8-cineole, 470-82-6; (*E*)-2-hexenal, 6728-26-3; 2-hexanol, 626-93-7; 2-pentylfuran, 3777-69-3; (*E*)-ocimene, 3779-61-1; 1-pentanol, 71-41-0; hexyl acetate, 142-92-7; acetoin, 513-86-0; octanal, 124-13-0; *n*-tridecane, 629-50-5; 2-vinylcrotonaldehyde, 20521-42-0; (*Z*)-3-hexenyl acetate, 3681-71-8; (*Z*)-2-pentenol, 1576-95-0; 6-methyl-5-hepten-2-one, 110-93-0; 1-hexanol, 111-27-3; (*E*)-3-hexenol, 928-97-2; (*Z*)-3-hexenol, 928-96-1; nonanal, 124-19-6; *n*-tetradecane, 629-59-4; (*E*)-2-hexenol, 928-95-0; *β*-phorone, 471-01-2; (*E*)-2-octenal, 2548-87-0; *trans*-furan linalool oxide, 34995-77-2; 1-octen-3-ol, 3391-86-4; 1-heptanol, 111-70-6; furfural, 98-01-1; (*Z*)-3-hexenyl butyrate, 16491-36-4; *cis*-furan linalool oxide, 5989-33-3; (*E*, *E*)-2, 4-heptadienal, 4313-03-5; *n*-pentadecane, 629-62-9; decanal, 112-31-2; benzaldehyde, 100-52-7; (*E*)-2-nonenal, 18829-56-6; linalool, 78-70-6; 1-octanol, 111-87-5; (*E*, *Z*)-2, 6-nonadienal, 557-48-2; isophorone, 78-59-1; *n*-hexadecane, 544-76-3; undecanal, 112-44-7; phenylacetaldehyde, 122-78-1; 7-octen-4-ol, 53907-72-5; 1-nonanol, 143-08-8; 3-nonen-1-ol, 51494-28-1; *α*-terpineol, 98-55-5; *n*-heptadecane, 629-78-7; dodecanal, 112-54-9; *α*-farnesene, 502-61-4; 2, 2, 6-trimethyl-1, 4-cyclohexanedione, 20547-99-3; *n*-octadecane, 593-45-3; nerol, 106-25-2; (*E*,

E)-2, 4-decadienal, 25152-84-5; β-phenylethyl acetate, 103-45-7; nerylacetone, 3879-26-3; geraniol, 106-24-1; geranylacetone, 3796-70-1; benzyl alcohol, 100-51-6; *n*-nonadecane, 629-92-5; β-phenylethyl alcohol, 60-12-8; phenylacetonitrile, 140-29-4; β-ionone, 14901-07-6; *cis*-jasmone, 488-10-8; β-phenylethyl *n*-butyrate, 103-52-6; *n*-eicosane, 112-95-8; octanoic acid, 124-07-2; *n*-heneicosane, 629-94-7; nonanoic acid, 112-05-0; *n*-docosane, 629-97-0; *n*-tricosane, 638-67-5; methyl acetate, 79-20-9; ethyl acetate, 141-78-6; 2-butanone, 78-93-3; 3-pentanone, 96-22-0; 2-pentanone, 107-87-9; ethylbenzene, 100-41-4; 2-methyl-4-pentenal, 5187-71-3; myrcene, 123-35-3; *n*-amyl acetate, 628-63-7; *D*-limonene, 5989-27-5; *n*-dodecane, 112-40-3; 1-methoxy-3-methylene-2-pentanone, 55956-45-1; (*Z*)-ocimene, 27400-71-1; 2-methyl-6-methylene-1, 7-octadien-3-one, 41702-60-7; (*E*)-2-hexenyl acetate, 2497-18-9; 6-methyl-5-hepten-2-one, 110-93-0; *n*-hexyl *n*-butyrate, 2639-63-6.