Isolation and Identification of a Volatile Compound in Habanero Pepper (Capsicum chinense)

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Abstract: A volatile compound was isolated from the fruit of Habanero pepper (Capsicum chinense) and related varieties and identified as 6-methyl-(E)-4-heptenyl 3-methylbutyrate by ¹H and ¹³C NMR spectroscopy and two-dimensional NMR experiments, including HMOC, HMBC, and ¹H-¹H COSY. The compound has a retention index of 1387 and is one of the major volatile compounds in Habanero pepper. This compound would be useful as a new flavor.

Key words: Capsicum chinense, Habanero, volatile compound, 6-methyl-(E)-4-heptenyl 3-methylbutyrate

1 INTRODUCTION

Habanero chili peppers, such as Habanero Red Sabina (Capsicum chinense cv. Red Savina) and Orange (C. chinense cv. Habanero Orange) (Fig. 1), are extremely hot because they have high contents of capsaicinoids, such as capsinin. Bhut Jolokia (C. chinense × C. frutescens cv. Bhut Jolokia) is hotter than Habanero pepper. These chili peppers also have a fruity aroma. They are cultivated in Kameoka City and Kyotanba-cho, Kyoto, Japan.

Volatiles compounds in C. chinense were identified and quantified by Pino et al.²⁻⁶ Cachucha pepper (C. chinense Jacq.) grown in Cuba contains 136 volatile compounds.² Habanero chili pepper (C. chinense Jacq. cv. Habanero) contains 102 volatile compounds.³ By headspace solid-phase microextraction, Habanero chili pepper (C. chinense Jacq.) was analyzed, and 53 volatile compounds were detected.³ A recent report described biological activities of volatile extracts from two varieties of Habanero pepper and their quantification of volatile components.⁶

Volatiles compounds in five varieties of C. annum, one variety of C. chinense, and three varieties of C. frutescens cultivated in Valencia, Spain were precisely examined and a total of 107 esters and 74 terpenoids were found.⁷

These compounds were identified by comparing their mass spectra and retention indices (RIs) with those of standards, with database or literature, or with mass spectral database. We also identified major compounds in Habanero pepper cultivated in Kyoto Prefecture, Japan. One of the most abundant compounds having RIMS = 1387 in our study was identified as 2,3-dimethylcyclohexanol (RI = 1389) or 3,3-dimethylcyclohexanol (RI = 1392) by Pino et al.²⁻⁶ However, their standards had different RI values (2,3-dimethylcyclohexanol RI = 1011 ~ 1054; three peaks in the GC/MS chromatogram, 3,3-dimethylcyclohexanol RI = 1002), although their mass spectra were very similar to that of the compound having RI = 1387. The compound was also one of the major compounds in their works²⁻⁶. While, the compound was identified as 6-methyl-(E)-4-heptenyl 3-methylbutyrate by the mass spectrum and the comparison of RI with homologues⁷⁻⁹. However, MS spectrum of the substance having RI = 1387 did not clearly show the characteristic m/z ions (103: C₉H₁₅O₂H and 85: C₈H₁₄O₂H).
C₅H₆COOH) derived from 3-methylbutyryl group. So, it was not
evident to be 6-methyl-\((E)\)-4-heptenyl 3-methylbutyrate.
Thus, we tried to isolate this compound and analyze its
structure.

2 EXPERIMENTAL PROCEDURES

2.1 Chemicals

2.3-Dimethylcyclohexanol and 3,3-dimethylcyclohexa-
one were purchased from Tokyo Chemical Industry
(Tokyo, Japan). Other chemicals were reagent grade.

2.2 Materials

Habanero Red Sabina and Habanero Orange were pro-
vided by Shino Farm Limited Co from 2011 to 2015.

2.3 Standard sample preparation

2.3.1 4-Methylpentyl 3-methylbutyrate

To a solution of 4-methylpentan-1-ol (12 \( \mu \)L; 96.5 \( \mu \)mol)
in methylene chloride (1 mL) was added triethylamine (13.5
\( \mu \)L; 96.8 \( \mu \)mol) at 4 °C, then 3-methylbutyl chloride (11
\( \mu \)L; 90 \( \mu \)mol) was added. The reaction mixture was stirred
overnight then \( n \)-hexane (9 mL) was added. The resulting
solution was washed with 1M NaOH and then washed with
water three times, and dried over anhydrous Na₂SO₄. After
the solution was diluted 100 times with \( n \)-hexane, the
sample was analyzed by GC/MS.

\( n \)-Hexyl 3-methylbutyrate, 4-methylpentyl valerate,
\( n \)-hexyl valerate, 5-methylhexyl 3-methylbutyrate, \( n \)-
heptyl 3-methylbutyrate, \( n \)-octyl 3-methylbutyrate, and (Z)-3-
hexenyl 3-methylbutyrate were prepared by the same
method, and analyzed by GC/MS.

2.3.2 4-Methylpentyl 4-methylvalerate

To a solution of 4-methylvaleric acid (10 \( \mu \)L; 79.5 \( \mu \)mol)
in methylene chloride (1 mL) was added 1-[3-(dimethyl-
amino)propyl]-3-ethylcarbodiimide (15.5 \( \mu \)L; 87.7 \( \mu \)mol)
under cooling with ice, and then 4-methylpentan-1-ol (11
\( \mu \)L; 88.4 \( \mu \)mol). The reaction mixture was stirred overnight
and then \( n \)-hexane (9 mL) and 1 M HCl (5 mL) were added.
The resulting solution was washed with water three times,
then dried over anhydrous Na₂SO₄. The solution was
 diluted 100 times with \( n \)-hexane and analyzed by GC/MS.

2.3.3 3-Methylbutyl 8-methyl-(Z)-6-nonoate

Potassium bis(trimethylsilyl) anide (4.0 g, 20 mmol) was
added dropwise to a suspension of 5-(carboxypentyl)tri-
phenylphosphonium bromide (4.57 g, 10 mmol) in anhy-
drous THF (40 mL) at 0°C under a nitrogen atmosphere
and the reaction mixture was stirred for 30 min at 0°C. 2-Meth-
ylpropionaldehyde (1.8 mL, 20 mmol) in anhydrous THF (10
mL) was added dropwise to the reaction mixture and then
the reaction mixture was warmed to room temperature
and stirred for 2 h at the same temperature. The reaction
mixture was quenched by adding saturated NH₄Cl solution.

After phase separation, the aqueous layer was extracted
with Et₂O and the combined organic layer was washed with
brine, dried over anhydrous Na₂SO₄, and concentrated under
reduced pressure. The obtained crude product was
purified by flash chromatography on silica gel using \( n \)-hex-
ane–ethyl acetate (96:4 v/v) as the eluent to afford 5.0 g of
crude 8-methyl-\((Z)\)-6-nonoic acid.

Crude 8-methyl-\((Z)\)-6-nonoic acid (3.0 g, 17.6 mmol),
3-methylbutan-1-ol (9.06 mL, 88 mmol) and \( p \)-toluenesul-
fonic acid monohydrate (500 mg, 2.9 mmol) were dissolved
in toluene (100 mL) and the reaction mixture was warmed
up to 120°C. After stirring at 120°C for 5 h, the reaction
mixture was allowed to cool to room temperature. The re-
action mixture was quenched by adding saturated NaHCO₃
solution. After phase separation, the aqueous layer was
extracted with toluene and the combined organic layer was
washed with brine, dried over anhydrous Na₂SO₄ and then
concentrated under reduced pressure. The crude product
was purified by flash chromatography on silica gel using \( n \)-hex-
ane–ethyl acetate (20:1 v/v) as the eluent to afford 520
mg of crude 3-methylbutyl 8-methyl-\((Z)\)-6-nonoate.

2.0 M NaNO₂ solution (1.9 mL) and HNO₃ solution (6.0 M,
0.87 mL) were added to crude 3-methylbutyl 8-methyl-\((Z)\)
-6-nonoate⁰⁰. The reaction mixture was warmed to 75°C
and stirred at the same temperature for 2 h under a nitro-
gen atmosphere. The reaction mixture was allowed to cool
to room temperature and quenched by adding saturated
NaHCO₃ solution. Extraction was carried out with Et₂O and
the organic layer was washed with brine, dried over anhy-
drous Na₂SO₄ and concentrated under reduced pressure.
The crude product was purified by flash chromatography
on silica gel using \( n \)-hexane–ethyl acetate (50:1 v/v) as the
eluent to afford 108 mg of 3-methylbutyl 8-methyl-\((E)\)
-6-nonoate in 7.5% yield for three steps. MS: \( m/z \) (M + H) +
= 241.2150, calcd.; \( m/z \) (M + H) + = 241.2168 for
C₉H₁₆O₂. \( ^{1}H\) NMR (CDCl₃): δ 0.92 (d, \( J = 6.9 \) Hz, 6H), 0.96
(\( d, J = 6.9 \) Hz, 6H), 1.37 (tt, \( J = 7.6, 7.6 \) Hz, 2H), 1.52 (td, \( J
= 6.9, 6.9 \) Hz, 2H), 1.62 (tt, \( J = 7.6, 6.2 \) Hz, 2H), 1.68 (qt, \( J
= 6.9, 6.2 \) Hz, 1H), 1.99 (td, \( J = 7.6, 6.9 \) Hz, 2H), 2.22 (dq, \( J
= 6.9, 6.2 \) Hz, 1H), 2.29 (t, \( J = 7.6 \) Hz, 2H), 4.10 (t, \( J = 6.9 \)
Hz, 2H), 5.32 (td, \( J = 15.8, 6.2 \) Hz, 1H), 5.38 (dd, \( J = 15.8,
6.2 \) Hz, 1H). \(^{13}C\) NMR (CDCl₃): δ 22.5 (2C), 22.7 (2C), 24.6,
25.1, 29.2, 31.1, 32.2, 34.4, 37.4, 63.0, 126.6, 138.1, 174.0.
2.3.4 3,3-Dimethylcyclohexanol

3,3-Dimethylcyclohexanol (100 \( \mu \)L, 0.72 mmol) was dis-
solved in ethanol (10 mL), and NaBH₄ (28 mg, 0.74 mmol)
was added in two portions. The reaction mixture was
stirred for 2 h. Water (2 mL) was added to the reaction
mixture, and this was followed by extraction with methy-
lene chloride (5 mL). The extract was washed with satu-
rated NaCl solution twice and dried over anhydrous
Na₂SO₄. The methylene chloride solution (10 \( \mu \)L) was
diluted with \( n \)-hexane (20 \( \mu \)L), which was analyzed by GC/MS.

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2.4 Habanero (Red Sabina and Orange) sample preparation

Thawed Habanero Red Sabina (2.5 g) diced in 0.5 mm size was subjected to absorption with Monolithic Silica Adsorbent (MonoTrap DCC, GL Sciences Inc., Tokyo) in a sealed tube without contact with the sample for 1 d. The absorbent was extracted with methylene chloride (500 μL) three times. The extract was evaporated by atmospheric distillation to yield a residue that was dissolved in n-hexane (500 μL). Then, the hexane solution was analyzed by GC/MS.

In the same manner, the Habanero Orange sample was prepared from thawed Habanero Orange (3.0 g), and analyzed by GC/MS.

2.5 Purification of volatile component having RI = 1387 (UK-A)

Habanero fruits (1.0 kg) were chopped in distilled water (1 L) with a food processor. The slurry was extracted with 1 L of n-hexane with overnight stirring at 500 rpm. After phase separation, the organic layer was dried over anhydrous Na2SO4 and then concentrated under reduced pressure to yield Habanero extract (5.4 g). The extract was purified by flash chromatography on silica gel using 650 mL of 10% ethyl acetate/n-hexane. The fraction containing the compound having RI = 1387 was evaporated to a residue (4.1 g). The residue was dissolved in n-hexane (50 mL) and washed with saturated NaHCO3 (25 mL) five times to remove fatty acid. Then, it was dried and evaporated to dryness and chromatographed on silica gel using 25 mL of ethyl acetate/n-hexane as eluent. Each fraction was monitored by GC/MS. Fractions containing the compound were concentrated (0.4 g) and purified by preparative HPLC in two steps (1st step: elution with 3% ethyl acetate/n-hexane, 2nd step: elution with 1.5% ethyl acetate/n-hexane). Then, 3 mg of the pure sample was obtained. This compound exhibited a fruity aroma. MS: m/z (M + H)+ = 213.1872, calcd.; m/z (M + H)+ = 213.1855 for C13H25O2. 1H NMR (CDCl3) and 13C NMR: shown in Table 1. IR (nujol): ν 2963, 1736, 1469, 1193, 1158, 971 cm⁻1.

Table 1  Chemical shifts of the compound having RI = 1387 (UK-A) in 13C and 1H NMR spectrum in CDCl3.

| position | 13C δ/ppm | 1H δ/ppm | multi. | J/Hz | HMBC (H to C) |
|----------|------------|----------|--------|------|---------------|
| K-methyl | 4, 5       | 22.57    | 0.96   | d    | 7 C2, C3      |
| J-methyl | 7, 8       | 22.75    | 0.96   | d    | 7 C2, C6      |
| I-methylene | 3     | 25.90    | 2.10   | m    | C1, C2, C4, C5|
| H-methylene | 2'    | 28.71    | 1.68   | quint. | 7 C1, C3, C6 |
| G-methylene | 3'    | 28.98    | 2.05   | q    | 7 C1, C2, C4, C5 |
| F-methylene | 6'    | 31.13    | 2.23   | m    | C4, C5, C7, C8 |
| E-methylene | 2     | 43.69    | 2.18   | d    | 7 C1, C4, C6 |
| D-methylene | 1'    | 63.82    | 4.06   | t    | 7 C2, C5, C6 |
| C-methylene | 4'    | 125.74   | 5.33   | dt   | 15, 7 C2, C3, C5, C6, C7, C8 |
| B-methylene | 5'    | 138.83   | 5.41   | dd   | 15, 7 C2, C3, C5, C6, C7, C8 |
| A-carbon | 1        | 173.42   | –      | –    | –             |

2.6 Analysis methods

2.6.1 GC/MS and GC/TOF MS methods

GC was conducted by a 6890N or a 7890 (Agilent Technologies Inc., Santa Clara, CA, USA) in the split-less mode using an HP-5MS capillary column (0.25 mm i.d. x 30 m, 0.25 μm film thickness, Agilent Technologies Inc.) and a temperature gradient from 60°C (2 min hold) to 290°C (5 min hold) at 10°C/min. Mass spectra were measured using an Agilent Technologies 5975 Inert XL Mass Selective Detector operated at 70 eV or using GCT Premier (Waters Corporation, Milford, MA, USA) by chemical ionization (reactant gas: methane) at 20 eV.

2.6.2 Preparative HPLC methods

The HPLC system consisted of a column (Chemcosorb 58I φ 10 mm x 300 mm, Chemco Pak®, Chemco Plus Scientific Co., Ltd., Osaka), a pump (880-PU Intelligent HPLC Pump, JASCO Corporation, Tokyo) and a refractive index (RI) Detector (RI-930 Intelligent RI Detector, JASCO Corporation, Tokyo). n-Hexane/ethyl acetate (97:3 or 99:1) was used as the eluent and flow rate was 2.5 mL/min.

2.6.3 NMR analysis

1H and 13C NMR spectra were recorded in CDCl3 on a JEOL JNM-E600 spectrometer (JEOL Ltd., Tokyo) at 600 and 150 MHz, respectively, with tetramethylsilane as the internal standard.
The structure of the isolated product was elucidated by assigning all the protons and carbons on the basis of the COSY (Correlation Spectroscopy), \(^1\)H-\(^1\)H correlation spectra, HMQC (Heteronuclear Multiple Quantum Coherence), and HMBC (Heteronuclear Multiple Bond Correlation) \(^1\)H-\(^13\)C correlation spectra.

3 RESULTS AND DISCUSSION

3.1 Total ion chromatograms (TICs) of Habanero samples

The TICs of Habanero Red Sabina and Orange \((C. chinense)\) are shown in Figs. 2 and 3, respectively. The mass spectra and RIs of 4-methylpentyl \((RI = 1208)\), (Z)-3-hexenyl \((RI = 1238)\), \(n\)-hexyl \((1243)\), 5-methylhexyl \((1304)\), \(n\)-heptyl \((1341)\), and \(n\)-octyl \((1349)\) 3-methylbutyrate agreed with those of synthetic standards. The mass spectra and RIs of 4-methylpentyl 4-methylhexanoate \((1315)\) and 3-methylbutyl 8-methyl-\((E)-6\)-nonenoate \((1592)\) were also agreed with those of synthetic standards. The mass spectra and RIs of 2-methyltridecane \((1364)\), \(n\)-tetradecane \((1400)\), 2-methyl-1-tetradecene \((1446)\), 2-methyltetradecane \((1463)\), \(n\)-pentadecane \((1499)\), 2-methylpentadecane \((1563)\), and \(n\)-hexadecane \((1599)\) agreed with those in NIST 05 database and RI database \((\text{Aroma Office v. 2})\).

As shown in Figs. 2 and 3, almost all of compounds were detected in both TICs, although there was the difference of each amount of content between them. For example, 4-methylpentyl and \(n\)-hexyl 3-methylbutyrate in Red Sabina were relatively lower content than in Orange. 4-Methylpentyl 3-methylbutyrate, \(n\)-hexyl 3-methylbutyrate, and a compound having RI = 1387 (UK-A) were the major components in Bhut Jolokia, the hottest pepper (data not shown). Caribbean Red \((C. chinense \text{ cv. Caribbean Red})\) gave 4-methylpentyl 3-methylbutyrate as the most abundant component (data not shown). In Habanero Red Sabina, 2-methyl-1-tetradecene was approximately the same content as UK-A (Fig. 2).

UK-A was one of the most abundant in our Habanero varieties and the other Habanero varieties in previous reports. The compound was assigned to 3,3-dimethylcyclohexanol by Pino et al. Actually, its mass spectrum was very similar to that of 2,3-dimethylcyclohexanol \((RI = 1011 \sim 1054)\) and/or 3,3-dimethylcyclohexanol (Fig. 4), but its RI was quite different retention time \((RI = 1002)\). The Spanish and German group identified the compound as 6-Methyl-\((E)-4\)-heptenyl 3-methylbutyrate by comparing its mass spectrum and RI with those of its homologues. However, the mass spectrum of the compound having RI = 1387 did not clearly show the characteristic \(m/z\) ions \((103: C_7H_4CO_2H_2\) and \(85: C_6H_5CO)\) derived from 3-methylbutyryl group. Then, we tried to isolate UK-A. It could be isolated and purified by silica gel chromatography and subsequent HPLC. The isolated compound was subjected to NMR measurement.

3.2 Structure elucidation of the compound having RI = 1387 (UK-A)

The compound exhibited an experimental positive \(m/z\) of 213.1872 in GC/TOF MS, thus indicating a molecular weight of 212 associated with the chemical formula \(C_{13}H_{25}O_2\). This experimentally determined value is in excellent agreement with the theoretical positive \(m/z\) \((M + H)^+)\) of 213.1855 for \(C_{13}H_{25}O_2\).

In the \(^1\)H spectrum of the compound indicated 24 hydrogen atoms, including four doublet methyl groups near 1 ppm, which were assigned to two methyls \((K\text{-methyl})\) and two methyls \((J\text{-methyl})\), four methylene groups at 1.68 \((J\text{-methylene})\), 2.05 \((E\text{-methylene})\), and 4.06 ppm \((D\text{-methylene})\); and four methine groups at 2.10 \((I\text{-methine})\), 2.23 \((F\text{-methine})\), 5.33 \((C\text{-olefinic methine})\), and 5.41 ppm \((B\text{-olefinic methine})\) (see Fig. 4 and Table 1). Both \(B\) and \(C\)-olefinic proton gave \(J = 15 \text{ Hz}\), which indicated \(E\text{-(trans)}\) configuration. Signals for 11 carbon atoms were observed in the \(^13\)C NMR spectrum (Fig. 4). However, those signals could be assigned to 13 carbons, i.e., the two methyl carbon signals are assigned to four methyl groups. In the HMQC spectrum, 12 carbons had corresponding cross signals except the carbon at 173 ppm \((C = \text{O}, \text{A-carbon})\). These \(^13\)C and \(^1\)H signals were assigned (see Table 1).

In the \(^1\)H-\(^1\)H COSY spectrum, two J-methyl groups \((d)\) coupled with F-methine \((m)\); F-methine \((m)\) coupled with J-methyl \((d)\) and B-olefinic methine \((dd)\); B-olefinic methine \((dd)\) coupled with F-methine \((m)\) and C-olefinic methine \((dt)\); C-olefinic methine \((dt)\) coupled with G-methylenne \((q)\) and B-olefinic methine \((dd)\); G-methylenne \((q)\) coupled with H-methylene (quint.) and C-olefinic methine \((dt)\); H-methylene (quint.) coupled with G-methylenne \((q)\) and D-methylenne \((t)\); and D-methylenne \((t)\) coupled with H-methylene \((q)\). Further, two K-methyl groups \((d)\) coupled with I-methine \((m)\); I-methine \((m)\) coupled with K-methyl \((d)\) and E-methylene \((d)\); and E-methylene \((d)\) coupled with I-methine \((m)\). Thus, \(\text{C}_6\text{H}_5\text{CO}_2\text{H}_2\) was identified.
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Fig. 2  GC/MS chromatogram (TIC) of Habanero Red Sabina.

Fig. 3  GC/MS chromatogram (TIC) of Habanero Orange.
CHH2-CDH2-and CKH3̅ moieties were determined.

In the HMBC spectrum, J-methyl protons correlated with F- and B-carbon; F-methine proton correlated with J-, B-, and C-carbon; B-olefinic proton correlated with F-, J-, C-, and G-carbon; C-olefinic proton correlated with B-, F-, G-, and H-carbon; G-methylene protons correlated with C-, B-, H-, and D-carbon; H-methylene protons correlated with G-, C-, and D-carbon; and D-methylene protons correlated with H-, G-, and A-carbon. Further, K-methyl protons correlated with I and E-carbon; I-methine proton correlated with K, E, and A-carbon; E-methylene protons correlated with I-, K-, and A-carbon. From the correlations of A-carbon (carbonyl carbon), E-carbon formed a bond with A-carbon directly, whereas D-carbon formed a bond with A-carbon through an oxygen atom. The downfield chemical shift of D-methylene protons is evidence of this result. The HMBC correlation was shown in Table 1.

From these data, the compound having RI = 1387 (UK-A) was determined to be 6-methyl-ʫ(E)ולם-4-heptenyl 3-methylbutyrate (Fig. 5). Although this compound has already been assigned on the basis of comparison of its mass spectrum and RI with those of homologs, it was confirmed by purification and NMR measurement in this study. 6-Methyl-ʫ(E)ולם-4-hepten-1-ol which is produced by hydrolysis of the compound may be a precursor of acid moiety of capsaicin (8-methyl-ʫ(E)ולם-6-nonenoic acid) as they have the same moiety. In the mass spectrum of the compound, if the O-C bond of alcoholic moiety of the ester is cleaved, 6-methyl-1,4-heptadiene (C8H14, m/z = 110) would be generated. Thus, the mass spectrum might be similar to those of 3,3- or 2,3-dimethylcyclohexanol (Fig. 6). In the mass spectrum of UK-A (6-methyl-ʫ(E)ולם-4-heptenyl 3-methylbutyrate), both m/z = 103 ion (C4H9CO2H2) and m/z = 85 ion (C4H9CO) were slightly observed (Fig. 6). These ions may be derived from 3-methylbutyrate moiety, although the other 3-methylbutyrate derivatives such as 4-methylpentyl 3-methylbutyrate gave the clear ion peaks (data not shown). Both 3,3- and 2,3-dimethylcyclohexanol had also m/z = 85 ion, but it was supposed to be C6H13. The m/z = 85 ion from C6H13 might not be generated by 6-methyl-ʫ(E)ולם-4-hepten-1-ol moiety,
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which possibly generated C6H10\(\frac{m}{z} = 82\) and C6H11\(\frac{m}{z} = 83\) as shown in Fig. 6.

4 CONCLUSION

A volatile component present as one of major components in Habanero peppers (C. chinense) and related varieties was determined to be 6-methyl-(E)-4-heptenyl 3-methylbutyrate by isolation and purification from Habanero fruit, followed by identification by NMR measurement. It was also present in both Bhut Jolokia and Caribbean Red (C. chinense cv. Caribbean Red) as a major component. This compound giving the fruity aroma could be useful as a new flavor by chemical synthesis.

REFERENCES

1) http://shinofarm.jp/ Home Page of Shino Farm Limited Co.
2) Pino, J.; Sauri-Duch, E.; Marbot, R. Changes in volatile compounds of Habanero chile pepper (Capsicum chinense Jack. cv. Habanero) at two ripening stages. Food Chem. 94, 394-398 (2006).
3) Pino, J.; González, M.; Ceballos, L.; Centurión-Yah, A.R.; Trujillo-Aguirre, J.; Latournerie-Moreno, L.; Sauri-Duch, E. Characterization of total capsaicinoids, colour and volatile compounds of Habanero chilli pepper (Capsicum chinense Jack.) cultivars grown in Yucatan. Food Chem. 104, 1682-1686 (2007).
4) Pino, J.; Fuentes, V.; Barrios, O. Volatile constituents of Cachucha peppers (Capsicum chinense Jacq.) grown in Cuba. Food Chem. 125, 860-864 (2011).
5) Cuevas-Glory, L.F.; Sosa-Moguel, O.; Pino, J.; Sauri-Duch, E. GC–MS Characterization of volatile compounds in Habanero pepper (Capsicum chinense Jacq.) by optimization of headspace solid-phase microextraction conditions. Food Anal. Methods 8, 1005-1013 (2015).
6) Sosa-Moguel, O.; Pino, J.A.; Ayora-Talavera, G.; Sauri-Duch, E.; Cuevas-Glory, L. Biological activities of volatile extracts from two varieties of Habanero pepper (Capsicum chinense Jacq.). Int. J. Food Prop. 20, S3042-S3051 (2017).
7) Rodrigez-Burruezo, A.; Kollmannsberger, H.; González-Mas, M.C.; Nitz, S.; Nuez, F. HS-SPME Comparative analysis of genotypic diversity in the volatile fraction and aroma-contributing compounds of capscium fruits from the annuum-chinense-frutescens complex. J. Agric. Food Chem. 58, 4388-4400 (2010).
8) Zellner, B. d’A.; Bicchi, C.; Dugo, P.; Rubiolo, P.; Dugo, G.; Mondello, L. Linear retention indices in gas chromatographic analysis: A review. Flavour FrAGR. J. 23, 297-314 (2008).
9) Kollmannsberger, H.; Rodrigez-Burruezo, A.; Nitz, S.; Nuez, F. Volatile and capsaicinoid composition of aji (Capsicum baccatum) and rocoto (Capsicum pubescens), two Andean species of chile peppers. J. Sci. Food Agric. 91, 1598-1611 (2011).
10) Kaga, H; Goto, K.; Fukuda, T.; Orito, K. An alternate synthesis of the capsaicinoide. Biosci. Biotech. Biochem. 56, 946-948 (1992).
11) Ochiai, M.; Iwaki, S.; Ukita, T.; Nagao, Y. Hypervalent organoiodine-promoted oxidative ring expansions of tributylstannyl lactols. New and general synthesis of unsaturated medium-ring lactones. Chem. Lett. 133-136 (1987).

ACKNOWLEDGMENT

We thank Mr. Yoshihira Owari, Mr. Daiki Amaoka, Mr. Takashi Iwahashi, Mr. Naoki Mimura, and Mr. Yuta Harada for assistance in research.

Fig. 6 MS spectra of the compound having RI = 1387 (UK-A, 3,3-, and 2,3-dimethylcyclohexanol.)