

SEX PHEROMONE OF THE PAPAYA MEALYBUG

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Supplementary Information

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1. Preparation of ($\pm$)-*trans*-(2-isopropenyl-1-methylcyclobutyl)methanol

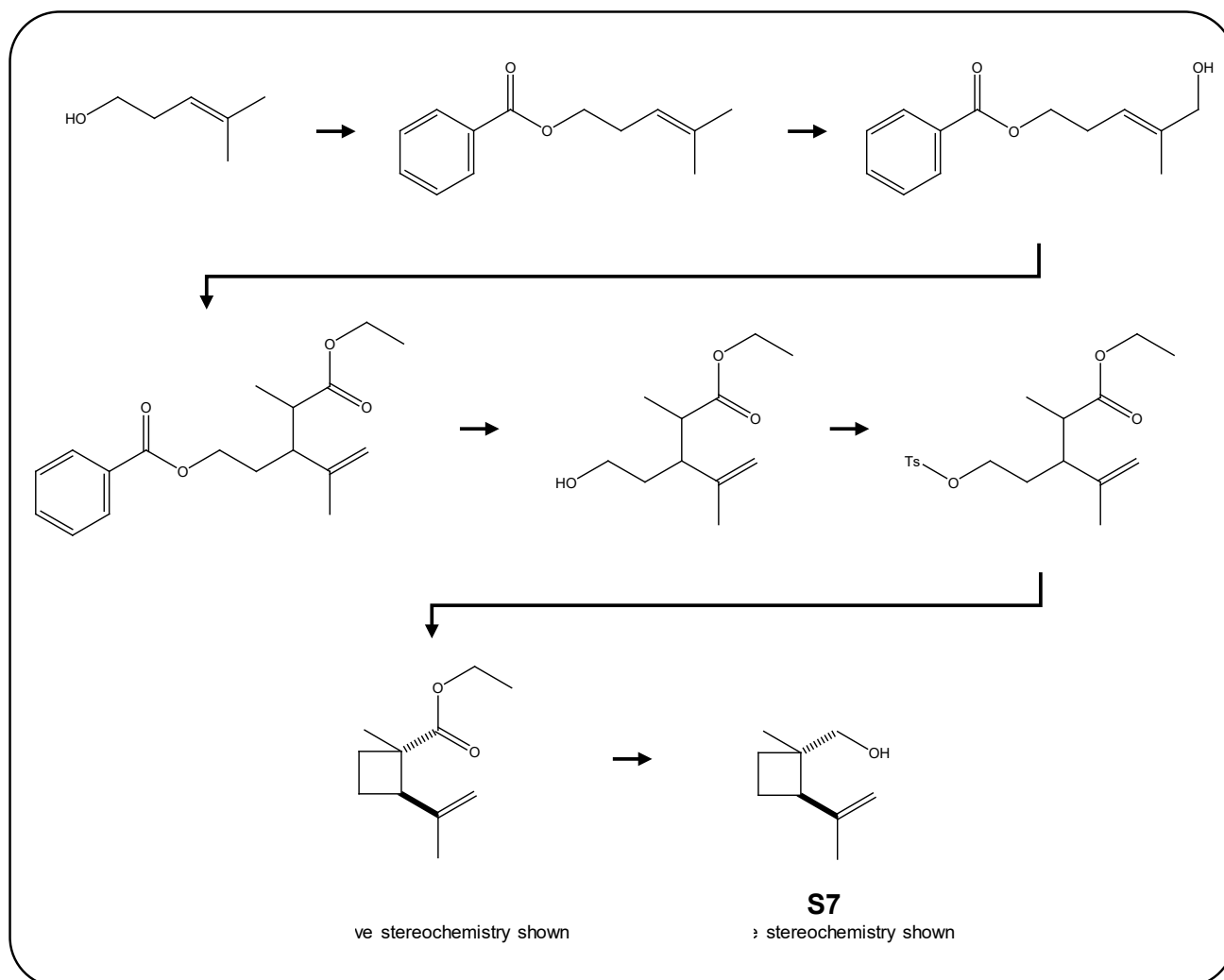


Figure S1. Scheme of synthesis

4-Methylpent-3-en-1-yl benzoate (S1)

4-Methylpent-3-en-1-ol (0.26 g; 2.4 mmol; Sigma-Aldrich, St. Louis, MO, USA), 4-dimethylaminopyridine (29 mg; Tokyo Chemical Industry, Tokyo, Japan), and pyridine (0.39 mL; Wako Pure Chemicals, Osaka, Japan) were dissolved in 3 mL of dry CH₂Cl₂ under argon. The mixture was stirred on ice, and 0.34 mL of benzoyl chloride (Wako) was added dropwise. The mixture was stirred overnight at room temperature and was then poured into 2 mL of H₂O. The organic phase was collected, and the aqueous phase was extracted three times with 2 mL of ethyl acetate. The combined

organic phase was washed successively with 1 M aqueous HCl, saturated aqueous NaHCO₃, and brine. It was then dried over Na₂SO₄ and concentrated in a rotary evaporator. The residue was purified by silica gel column chromatography with elution with 30:1 mixture of hexane and ethyl acetate to give **S1** (0.48 g; 99% yield). The MS and NMR spectra were in agreement with those in the literature (Kim et al. 1990).

(E)-5-Benzoyloxy-2-methylpent-2-en-1-ol (S2)

Selenium dioxide (0.23 g; Wako) and 70% *tert*-butyl hydroperoxide in water (1 mL; Wako) were dissolved in 4 mL of CH₂Cl₂ and stirred for 15 min at room temperature under argon. Then a solution of **S1** (0.48 g; 2.4 mmol) in 2 mL of CH₂Cl₂ was added to the mixture. After 24 h of stirring at room temperature, 0.4 mL of methyl sulfide was added to the mixture, which was further stirred for 30 min. The mixture was concentrated by a rotary evaporator, and the residue was dissolved in 2 mL of methanol. To this solution was added sodium borohydride (84 mg; Tokyo Chemical Industry) portionwise at 0 °C. After being stirred for 45 min, the mixture was poured into 10 mL of H₂O and was extracted three times with 10 mL of ethyl acetate. The extract was washed with brine, dried over Na₂SO₄, concentrated, and purified by silica gel column chromatography with elution with a 3:1 mixture of hexane and ethyl acetate to give **S2** (0.29 g; 55% yield). The MS and NMR spectra were in agreement with those in the literature (Kim et al. 1990).

Ethyl 5-benzoyloxy-3-isopropenyl-2-methylpentanoate (S3)

S2 (0.28 g; 1.3 mmol) and phenol (18 mg; Wako) were added to 6 mL of triethyl orthopropionate (Tokyo Chemical Industry), and the mixture was incubated at 150 °C for 24 h under argon. The mixture was then poured into 8 mL of 1 M aqueous HCl and extracted three times with 10 mL of ethyl acetate. The extract was successively washed with saturated aqueous NaHCO₃ and brine, dried over Na₂SO₄, concentrated, and purified by silica gel column chromatography with elution with a 15:1 mixture of hexane and ethyl acetate to give a 1:1 mixture of stereoisomers of **S3** (0.34 g; 88% yield). The MS and

NMR spectra were in agreement with those in the literature (Kim et al. 1990).

Ethyl 5-hydroxy-3-isopropenyl-2-methylpentanoate (S4)

S3 (0.31 g; 1.0 mmol) was dissolved into a combined solution of 3.5 mL of tetrahydrofuran, 0.87 mL of ethanol, and 0.84 mL of benzyltrimethylammonium hydroxide (40% in water; Tokyo Chemical Industry). The mixture was stirred for 18 h at 50 °C, and then 1 mL of ethyl acetate was added. After being stirred further at 50 °C for 4 h, the reaction mixture was concentrated in a rotary evaporator to remove the organic solvent and water. The residue was redissolved in 3 mL of dry tetrahydrofuran. To this solution was added 0.45 mL of ethyl iodide, and the mixture was stirred at 50 °C overnight under argon. Then the mixture was filtered, concentrated, and purified by silica gel column chromatography with elution with a 2.5:1 mixture of hexane and ethyl acetate to give **S4** (0.18 g; 90% yield). The MS and NMR spectra were in agreement with those in the literature (Kim et al. 1990).

Ethyl 3-isopropenyl-2-methyl-5-(toluene-*p*-sulfonyloxy)pentanoate (S5)

S4 (0.18 g; 0.90 mmol) was dissolved in 1.6 mL of dry CHCl₃. The mixture was cooled to 0 °C, and 0.18 mL of pyridine and 0.36 g of *p*-toluenesulfonyl chloride (1.89 mmol; Wako) were successively added. After 4 h of stirring at 0 °C, the mixture was poured into 1 mL of H₂O and extracted three times with 1 mL of ethyl acetate. The combined organic phases were washed successively with 1 M aqueous HCl, saturated aqueous NaHCO₃, and brine, dried over Na₂SO₄, concentrated, and purified by silica gel column chromatography with elution with a 10:1 mixture of hexane and ethyl acetate to give **S5** (0.25 g; 78% yield). The MS and NMR spectra were in agreement with those in the literature (Kim et al. 1990).

Ethyl *trans*-2-isopropenyl-1-methylcyclobutanecarboxylate (S6)

To a mixture of 0.7 mL of 14% potassium bis(trimethylsilyl)amide in toluene (Tokyo Chemical Industry) and 0.3 mL of dry tetrahydrofuran, a solution of **S5** (80 mg; 0.23 mmol) in 1.2 mL of dry

tetrahydrofuran was added dropwise at $-78\text{ }^{\circ}\text{C}$ under argon with vigorous stirring. The mixture was stirred for 1 h at $-78\text{ }^{\circ}\text{C}$ and then warmed to $0\text{ }^{\circ}\text{C}$ over 1 h. It was poured into 1 mL of saturated aqueous NH_4Cl and extracted three times with 1 mL of ethyl acetate. The combined extract was washed with brine, dried over Na_2SO_4 , concentrated, and purified by silica gel column chromatography with elution with a 15:1 mixture of hexane and ethyl acetate to give **S6** (33 mg; 80% yield). The MS and NMR spectra were in agreement with those in the literature (Kim et al. 1990).

***trans*-(2-Isopropenyl-1-methylcyclobutyl)methanol (S7)**

To a stirred suspension of 7.1 mg of lithium aluminum hydride (Tokyo Chemical Industry) in 1 mL of dry tetrahydrofuran, a solution of **S6** (30 mg; 0.16 mmol) in 1 mL of dry tetrahydrofuran was added at room temperature under N_2 . After 3 h of stirring at room temperature, 2 mL of saturated potassium sodium tartrate aq. was added dropwise on ice, and the mixture was stirred for 30 min. The organic phase was collected, and the aqueous phase was extracted three times with 2 mL of diethyl ether. The combined organic phases were washed with brine, dried over Na_2SO_4 , concentrated, and purified by silica gel column chromatography with elution with 15% diethyl ether in pentane to give **S7** (21 mg; 94% yield). The MS and NMR spectra were in agreement with those in the literature (Kim et al. 1990).

Reference

Kim D, Lee YK, Jang YM, Kim IO, Park SW (1990) Stereoselective synthesis of racemic fragranol by an intramolecular ester enolate alkylation. J Chem Soc Perkin Trans 1: 3221–3224

2. Responses of males in Petri dish bioassay

Male responses to each fraction from successive chromatography were assayed in a glass Petri dish (9 cm in diameter, 1.5cm deep). Ten newly emerged males were introduced to the dish along with one piece of filter paper impregnated with 1 female-day-equivalent of sample dissolved in 1 μ L of hexane. Before each assay, male responses to the paper with solvent only were examined as a blank test. The response behavior to each piece of filter paper was recorded for 20 min. The *P*-values of chi-squared tests of generalized linear models with binomial distributions to compare the effects of each fraction and the blank control are presented.

Table S1. Male responses to fractions from open column chromatography using different proportions of diethyl ether in hexane as an eluent.

Fraction	N	Response (mean % $\pm$ SEM)		<i>P</i> -value
		Treatment	Blank	
0% Ether	3	6.7 $\pm$ 3.3	3.3 $\pm$ 3.3	0.2904
5% Ether	3	23.3 $\pm$ 8.8	10.0 $\pm$ 0.0	0.1609
15 % Ether	5	66.0 $\pm$ 2.4	2.00 $\pm$ 2.00	0.0004033
50 % Ether	3	40.0 $\pm$ 17.3	13.3 $\pm$ 8.8	0.01747

Table S2. Male responses to fractions from high performance liquid chromatography

Fraction	N	Response (mean % $\pm$ SEM)		<i>P</i> -value
		Treatment	Blank	
Lc 1	3	3.3 $\pm$ 3.3	3.3 $\pm$ 3.3	1.000
Lc 2	3	3.3 $\pm$ 3.3	6.7 $\pm$ 3.3	0.5500
Lc 3	3	0	3.3 $\pm$ 3.3	0.3219
Lc 4	3	6.7 $\pm$ 3.3	6.7 $\pm$ 3.3	1.000
Lc 5	3	6.7 $\pm$ 3.3	3.3 $\pm$ 3.3	0.5500

Lc 6	3	0	3.3 ± 3.3	0.2362
Lc 7	3	3.3 ± 3.3	6.7 ± 3.3	0.5500
Lc 8	3	3.3 ± 3.3	3.3 ± 3.3	1.000
Lc 9	4	77.5 ± 4.8	2.5 ± 2.5	0.003895
Lc 10	3	16.7 ± 3.3	3.3 ± 3.3	0.0733

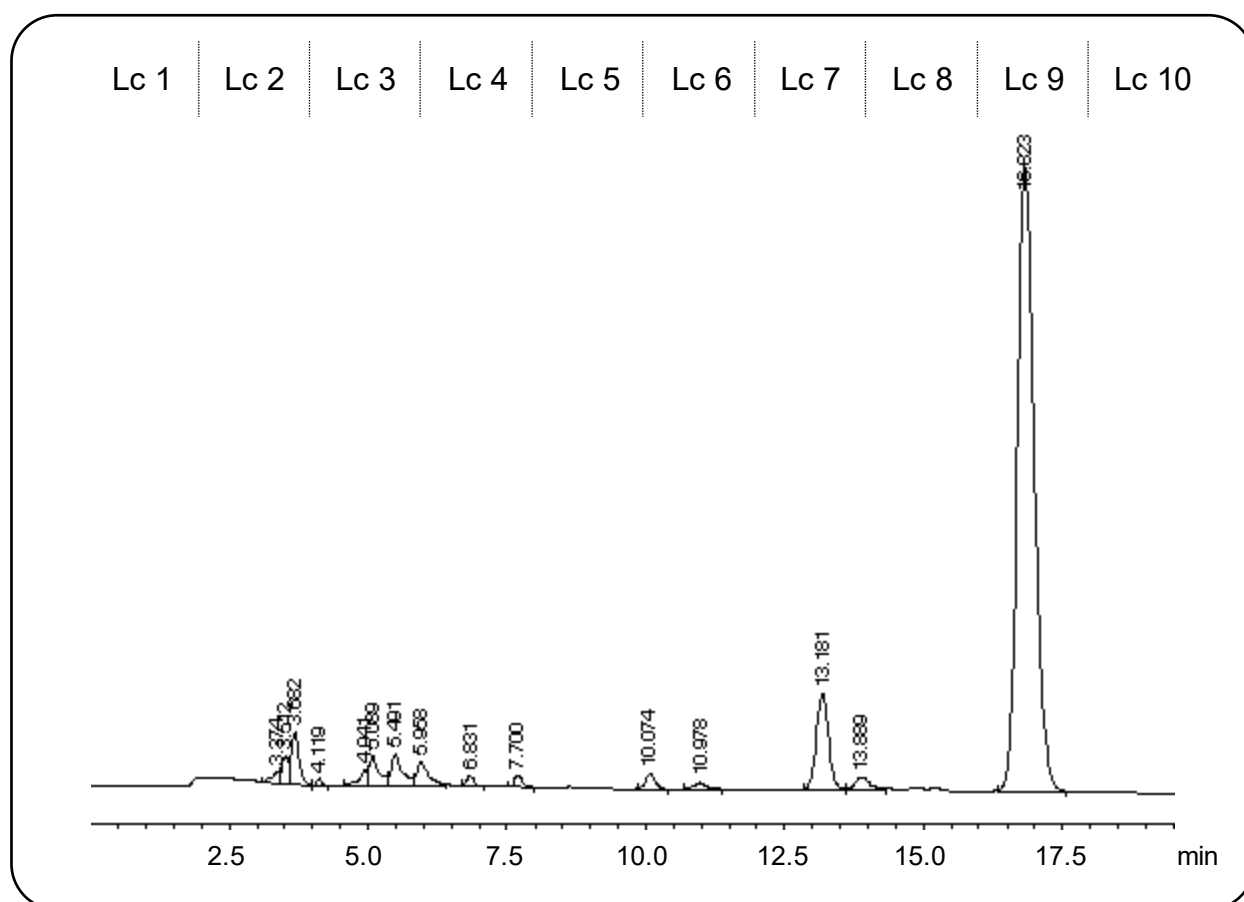


Figure S2. High performance liquid chromatogram of the 15% diethyl ether in hexane fraction from open column chromatography

Table S3. Male responses to fractions from gas chromatography

Fraction	N	Response (mean % $\pm$ SEM)		<i>P</i> -value
		Treatment	Blank	
Gc 1	3	3.3 ± 3.3	6.7 ± 3.3	0.5500

Gc 2	6	73.3 ± 3.3	3.3 ± 2.1	<0.0001
Gc 3	3	3.3 ± 3.3	10.0 ± 5.8	0.2903

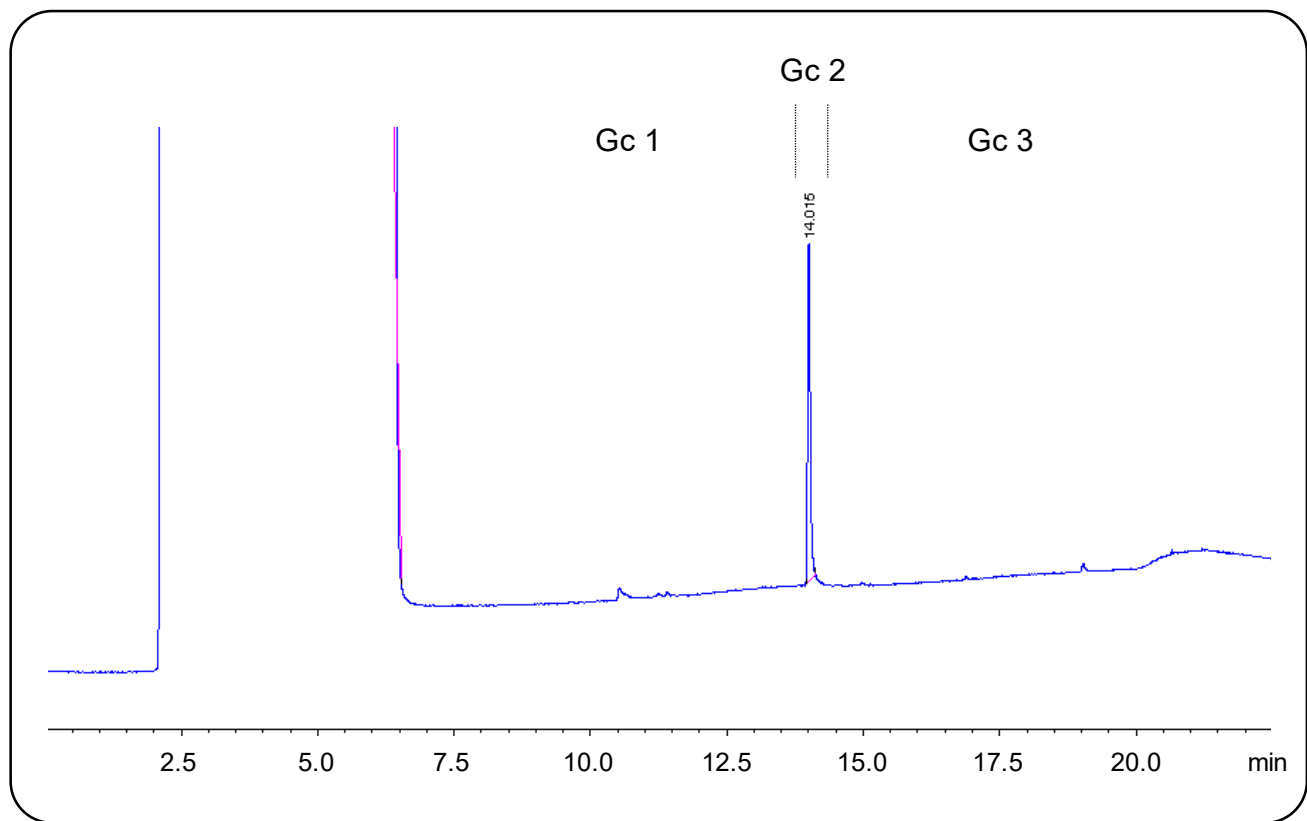


Figure S3. Gas chromatogram of the fraction Lc9 from HPLC

3. ^1H -NMR spectra of the synthetic and natural pheromone

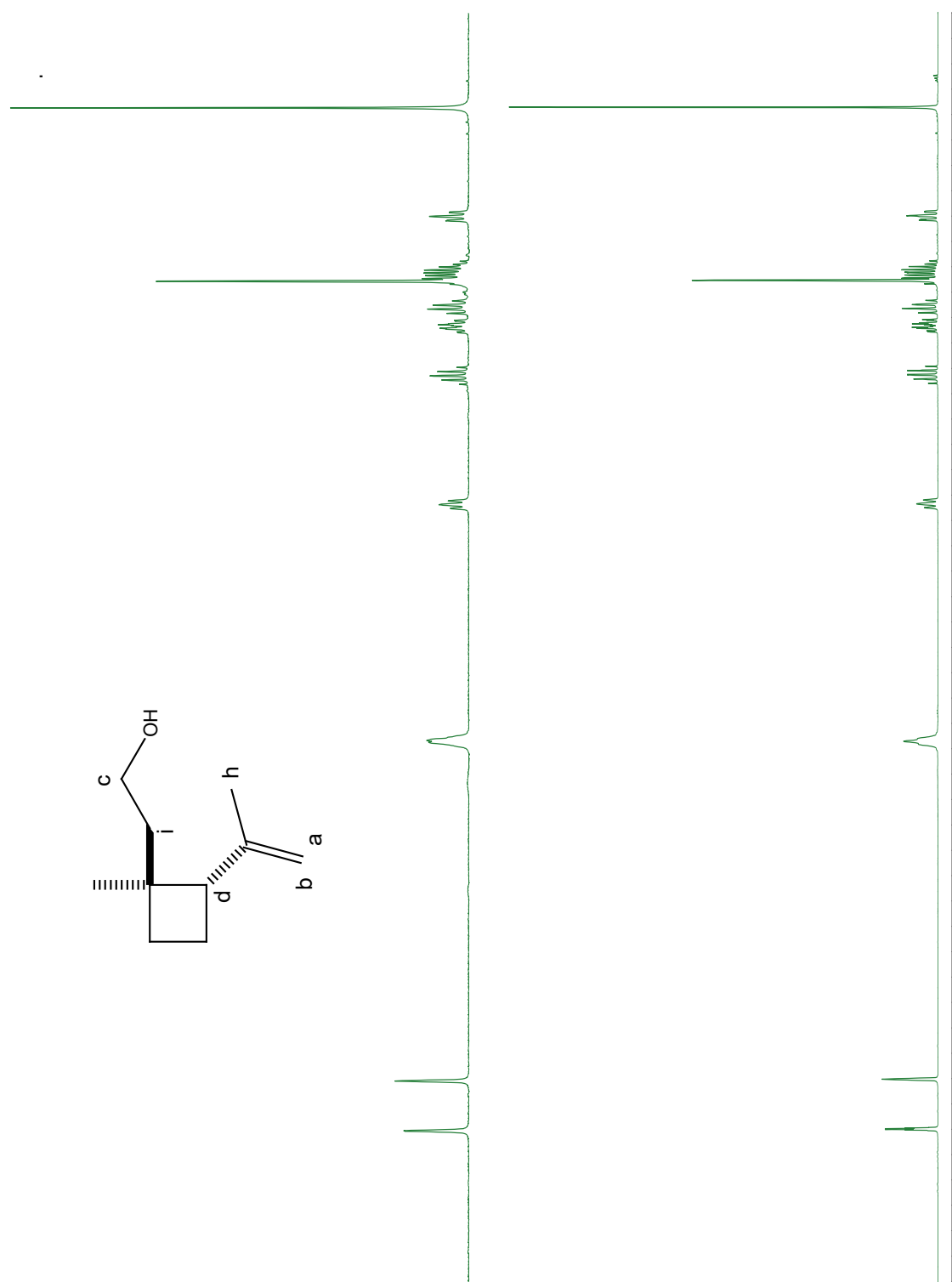


Figure S4. ^1H -NMR spectra of the natural and synthetic fragranol

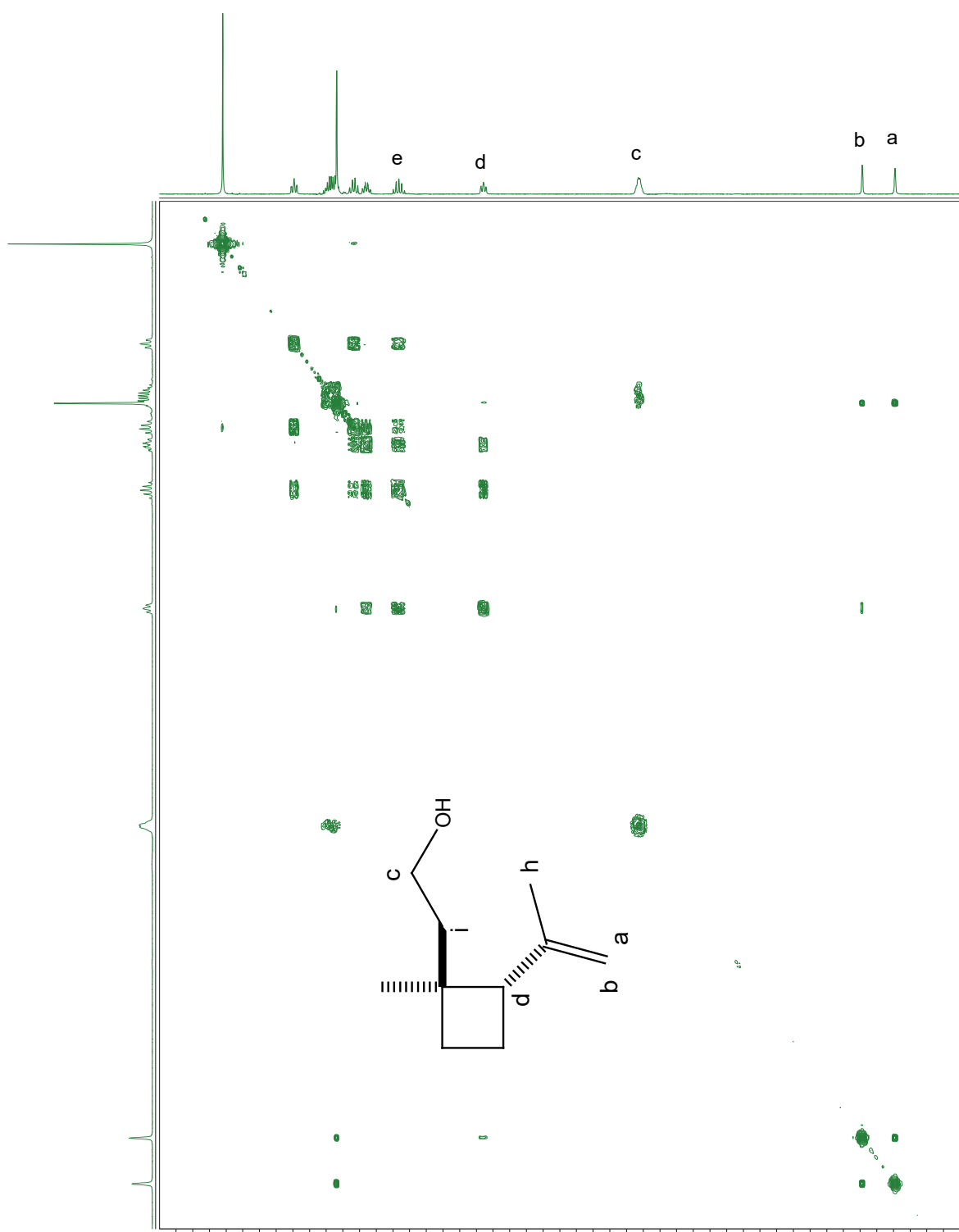


Figure S5. ^1H - ^1H COSY spectrum of the natural pheromone

4. Chiral HPLC of the natural pheromone, (±)-fragranol, and (±)-grandisol

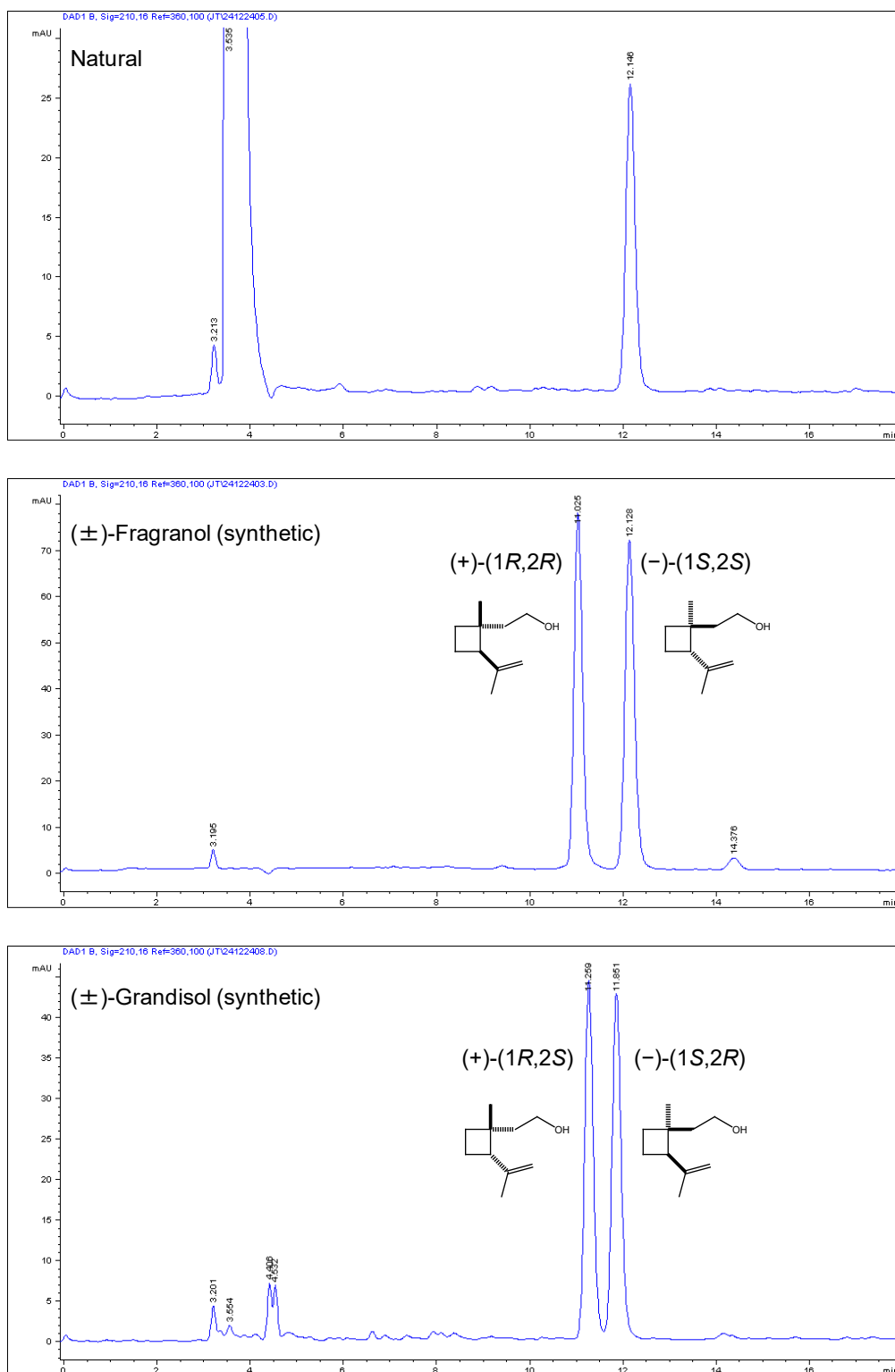


Figure S6. HPLC chromatograms using a CHIRALPAK IG column (4.6 mm I.D. × 250 mm, 5 µm particle size) eluted by a 100:1.5 mixture of hexane and ethanol at a flow rate of 1 mL/min.