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Supporting Information

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Biosynthesis of the Off-flavor 2-Methylisoborneol by the Myxobacterium *Nannocystis exedens*

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Table S1. Compounds identified in the headspace extracts of *Nannocystis exedens* (Na e485, Na eB37) and *Nannocystis exedens* subsp. *cinnabarina* (Na c29).

GC[a]	Compound[b]	*I*[c]	Ident[d]	Na e485[e] VY/2	Na eB37 VY/2	Na eB37 Pol 0.3	Na c29 VY/2	Na c29 Pol
	Ethyl 2-methylpropionate		ms		xx	x		
	sec-Butyl acetate		ms				x	
	Ethyl 2-methylbutyrate	856	ms		x			
	Ethyl 3-methylbutyrate	862	ms		x	x		
	2-Furanemethanol	869	syn		x	x		x
	Pentan-4-olide	975	syn					x
a	2-Methylenebornane	989	ms	x				
b	2-Methyl-2-bornene	1022	ms	xx		x	x	x
	Limonene	1031	ms			x		x
	Benzyl alcohol	1051	syn		x	x	x	x
	Hexan-4-olide	1073	syn			x		x
	Methyl 2-furancarboxylate	1101	ms		x		x	
	2-Phenylethanol	1131	syn		xx		x	x

	Pinanol	1139	ms				x	x
	2,5-Dimethyl-3-(1-methylethyl)pyrazine	1148	ms	x			x	x
	Benzyl nitrile	1160	syn		x	x	x	x
	Heptan-4-olide	1173	syn		x			x
	Borneol	1186	ms					x
	Diethyl succinate	1193	ms		x			
c	2,5-Di-(1-methylethyl)pyrazine	1199	syn		x	x	x	xx
d	(–)-2-Methylisoborneol (**1**)	1200	syn, chgc	xxx	xx	xxx	xxx	xxx
	Benzothiazole	1249	ms		x		x	x
	Ethyl phenylacetate	1258	ms		x			
	2-(1-Methylethenyl)-5-(1-methylethyl)pyrazine	1267	syn				x	x
e	5-(1-Methylethyl)-2-(1-methylpropyl)pyrazine	1278	syn	x	x	x	x	x
	5-(1-Methylethyl)-2-(2-methylpropyl)pyrazine	1284	syn	x			x	x
f	Isobornyl acetate	1294	ms	x	x	x	x	x
	2-Aminoacetophenone	1324	syn			x		
g	β-Methylgeraniol	1330	ms			x	x	x
h	2,5-Di-(1-methylpropyl)pyrazine	1360	syn		x	x		x
	β-Ylangene	1423	ms, ri					x
i	(–)-Geosmin (**2**)	1429	syn, chgc	xx	xx	xxx	xx	xxx
j	unknown (B: 105, M: 204)	1437		xx	x		x	x
	Dodecan-1-ol	1483	syn		x		x	
k	Germacrene D	1488	nat			x	x	x
	unknown (M: 202, B: 105)	1501			x		x	
	unknown (M: 204, B: 108)	1504					x	
	unknown (M: 222, B: 161)	1523			x	x		

	(6*S**,10*S**)-6,10-Dimethylbicyclo[4.4.0]dec-1-en-3-one	1571	syn			x	x	x
l	(1(10)*E*,5*E*)-Germacradien-11-ol (**4**)	1654	nat	x	x	x	x	x
m	unknown (B: 191, M: 234)	1669		x	x		x	x
	Tetradecan-1-ol	1686	syn		x		x	
n	unknown (B: 189, M: 204)	1690		x	x			
	unknown (B: 137, M: 234)	1715		x				
	unknown (B: 119, M: 218)	1719		x			x	
	unknown (M: 220, B: 91)	1739					x	
o	unknown (B: 119, M: 218)	1807		xx	x	x	xx	x
	Hexadecan-1-ol	1889	syn		x		x	
	1-Phenyldecan-1-one	1899	syn			x		

[a] Marker in Figure 1. [b] For unidentified compounds the molecular ion (M) and base peak (B) in the EI mass spectrum are given. Compounds originating from the medium are not mentioned. [c] Retention index determined from a homologous series of alkanes. [d] Identification based upon mass spectrum (ms), retention index (ri), synthetic sample (syn), sample isolated from natural sources (nat), GC on chiral stationary phase (chgc). [e] Different experiments with strains Na e485, Na eB37, and Na c29 grown on VY/2 or Pol 0.3 medium. The relative amounts of the compounds are noted. x: trace compound (0-2%), xx: minor compound (2-8%), xxx: main compound (>8% of total area in GC).

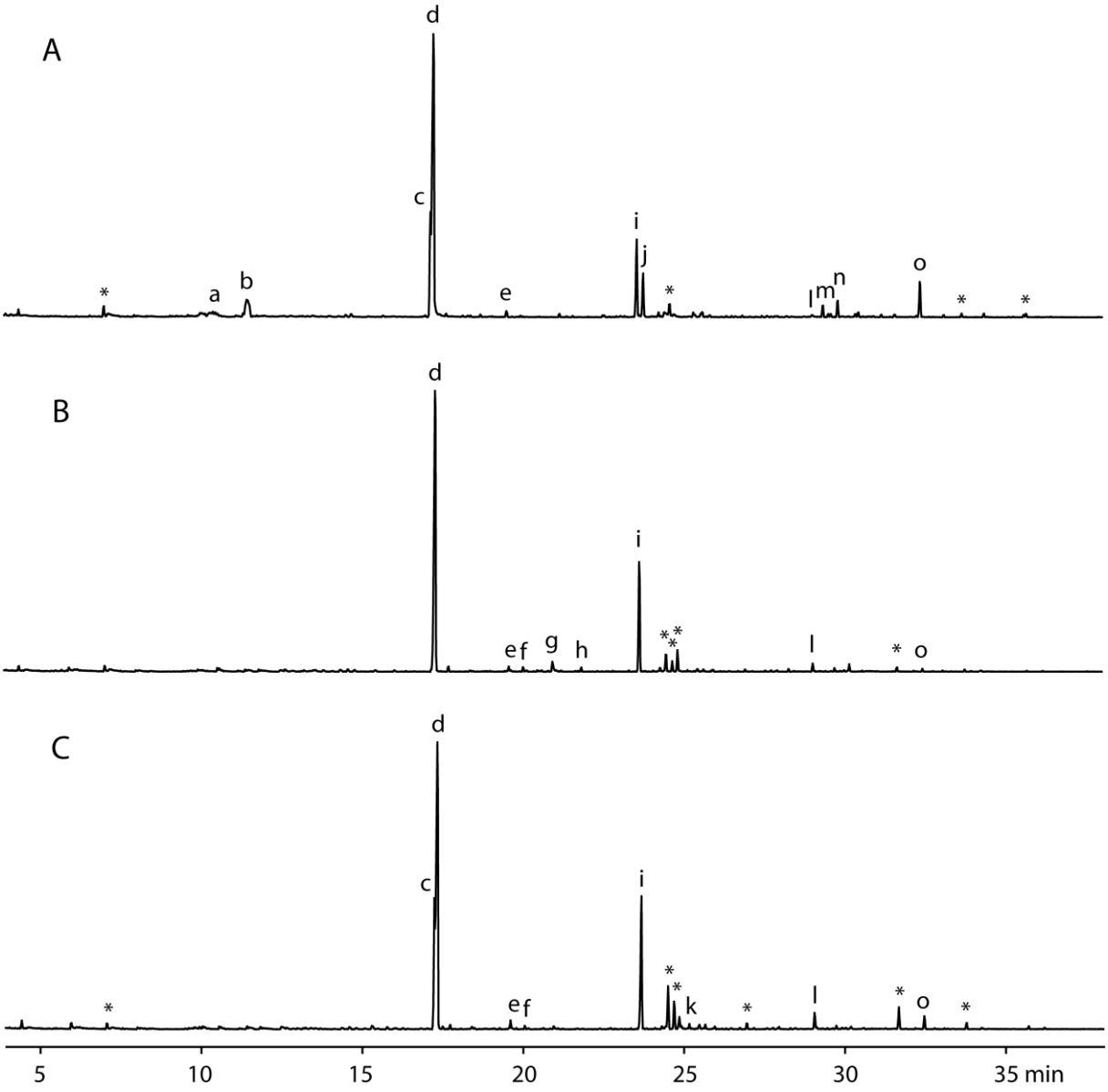


Figure S1. Total ion chromatograms of three headspace extracts of *Nannocystis exedens* Na e485 (A) and Na eB37 (B), as well as *Nannocystis exedens* subsp. *cinnabarina* Na c29 (C). Letters refer to compounds in Table 1. Artifacts are indicated by asterisks.

Table S2. Incorporation rates of isotopically labeled precursors into **1** in feeding experiments with *Nannocystis exedens* Na e485.

Compound	Incorporation rate[a]
[*methyl*-^{13}C]methionine	84%
[4,4,4,6,6-$^{2}H_5$]mevalolactone	16%
[5,5,6,6,6-$^{2}H_5$]mevalolactone	10%

[a] Incorporation rates determined by integration of ion chromatograms of the molecular ions of labeled and unlabeled isotopomers of **1**. In case of mevalolactones it was considered, that **1** is generated from two isoprene building blocks and that labeled isotopomers may arise by incorporation of either one or two units of labeled mevalolactone.

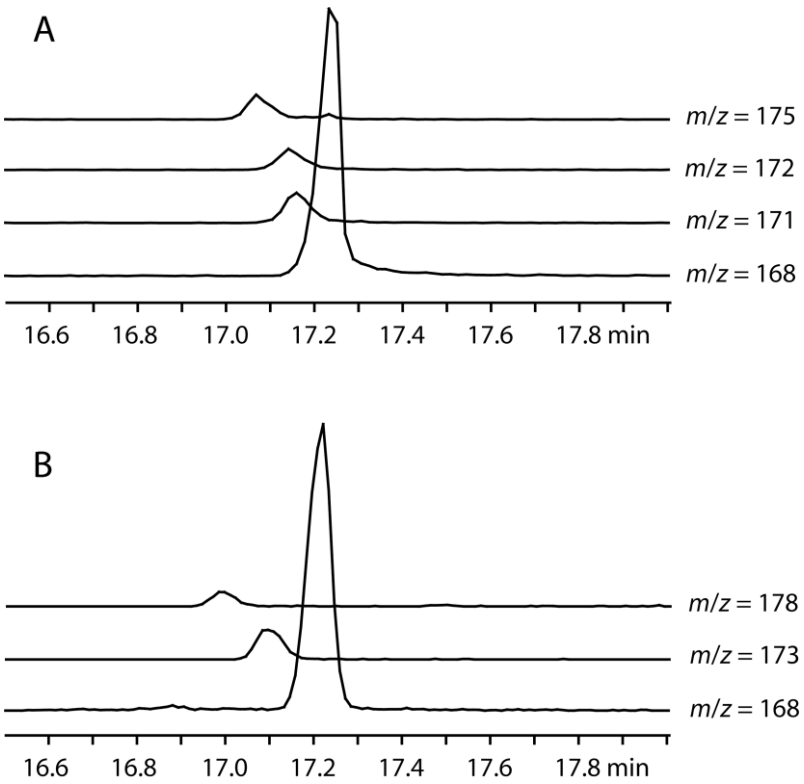


Figure S2. Ion chromatograms showing the molecular ions of different labeled and unlabeled isotopomers of **1** after incorporation of [4,4,6,6,6-$^{2}H_5$]mevalolactone (A) and after incorporation of [5,5,6,6,6-$^{2}H_5$]mevalolactone (B).

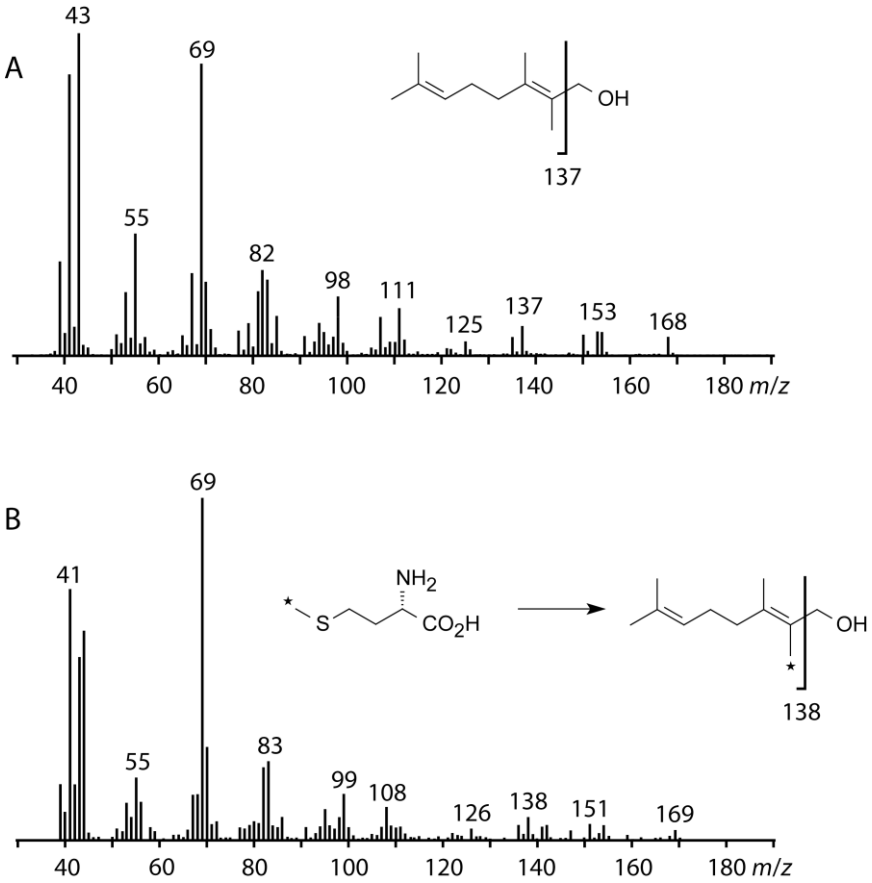


Figure S3. Mass spectra of (*E*)-**5** (A) and [^{13}C]-(*E*)-**5** after feeding of [*methyl*-^{13}C]-methionine (B).

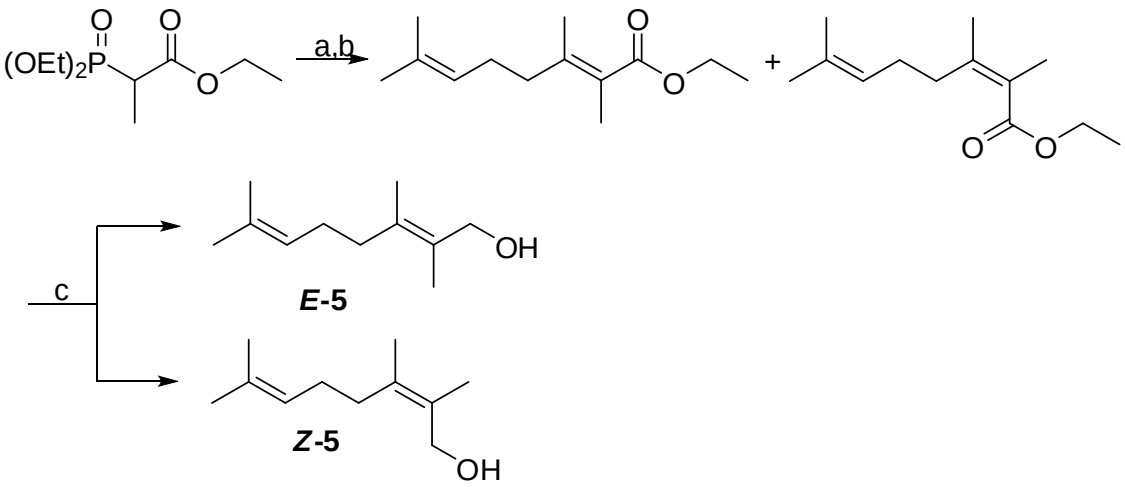
Strains, Culture Conditions, Feeding experiments. The strains Na e485, Na eB37, and Na c29, respectively, were grown as liquid cultures in MD1 medium composed of 3 gL^{-1} peptone from casein, tryptically digested (Merck), 2 gL^{-1} $MgSO_4 \cdot 7H_2O$, 0.5 gL^{-1} $CaCl_2 \cdot 2H_2O$, pH 7.0. Batch cultures of 100 ml in 250-ml Erlenmeyer flasks were incubated at 30 ^{0}C on a gyratory shaker at 160 rpm for about 4 days. The strains were also grown analysis of volatiles on both VY/2 agar plates (5 gL^{-1} baker yeast, 0.1 gL^{-1} $CaCl_2 \cdot 2H_2O$, 0.5 mgL^{-1}, vitamin B_{12}, 15 gL^{-1} agar, pH 7.2). and Pol 0.3 agar plates (3 gL^{-1} Probion (single cell protein prepared from *Methylomonas clarae*; Hoechst A.G.), 3 gL^{-1} soluble starch, 2 gL^{-1} $MgSO_4 \cdot 7H_2O$, 0.5 gL^{-1} $CaCl_2 \cdot 2H_2O$, 50 mM HEPES, 15 gL^{-1} agar, pH 7.2). The feeding experiments were performed in plastic petri dishes containing 25 ml of VY/2 agar. The corresponding labeled precursors (1 mM final concentration) were striked out on the surface area of the agar plates. Afterwards aliquots of about 250 µl of a well grown liquid culture were placed central on the prepared agar plates and dried. The plates were incubated at 30 ^{0}C for 5 to 10 days and then analyzed.

Sampling. The volatiles emitted by agar plate cultures of *Nanocystis exedens* were collected using the CLSA technique.[1] The volatiles were adsorbed on charcoal (Chromtech, Precision Charcoal Filter, 5 mg) for 24 hours, and eluted with 30 μL of dichloromethane. The extracts were immediately analyzed by GC-MS and stored at -80°C.

GC-MS. GC-MS analyses were carried out on a HP 6890 Series GC System connected to a HP 5973 Mass Selective Detector (Hewlett-Packard) fitted with a BPX5 fused-silica capillary column (25 m x 0.22 mm i. d., 0.25 μm film, SGE). Conditions were as follows: inlet pressure: 77.1 kPa, He 23.3 mL min^{-1}; injection volume: 1 μL; transfer line: 300°C; electron energy: 70 eV. The GC was programmed as follows: 5 min at 50°C, then increasing at 5°C min^{-1} to 300°C, and operated in splitless mode (60 s valve time). The carrier gas was He at 1 mL min^{-1}. Retention indices *I* were determined from a homologous series of alkanes (C_8 - C_{25}). Identification of compounds was performed by comparison of mass spectra to the Wiley 6 Library and the Essential Oils Library (Massfinder) and by comparison with synthetic standards (see Table 1 for details).

Synthesis of (+)- and (–)-1. As described by Dimitrov et al.,[2] anhydrous $CeCl_3$ (492 mg, 2 mmol) was dried at 140°C in vacuo (< 0.1 mbar) for 6 h, cooled to room temperature, and then suspended in dry THF (10 mL). A solution of D-(+)-camphor (760 g, 5 mmol) in dry THF (10 mL) was added dropwise. The reaction mixture turns to yellow while stirring at room temperature for 1 h. A solution of methylmagnesium chloride in THF (2 mL, 3 mol L^{-1}, 6 mmol) was added dropwise, causing an immediate decolouration. Stirring was continued for 48 h, and then the reaction mixture was quenched by the addition of sat. NH_4Cl (100 mL). The mixture was extracted with diethyl ether (3 × 100 mL). The combined organic layers were dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography on silica gel with pentane/diethyl ether (5:1) to give (–)-**1** (570 mg, 3.39 mmol, 68%) as a colorless solid. The (+)-enantiomer was obtained in an identical procedure from L-(–)-camphor. TLC: R_F = 0.32 (pentane/diethyl ether 5:1); GC: *I* = 1203; $[\alpha]_D^{20}$= -6.4 (c 0.085, $CHCl_3$) Lit: $[\alpha]_D^{21}$= -5 (c 2.2,, $CHCl_3$)[3], δ = 0.84 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 1.10 (s, 3H, CH_3), 1.24 (s, 3H, CH_3), 1.37-1.41 (m, 4H, 2xCH_2), 1.69-1.72 (m, 2H, CH_2), 2.06 (dt, 1H, $^3J_{H,H}$ = 3.8 Hz, 13.1 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 9.9 (CH_3), 21.1 (CH_3), 21.4 (CH_3), 26.8 (CH_2), 27.0 (CH_3), 31.3 (CH_2), 45.4 (CH), 47.3 (CH_2), 48.9 (C), 51.9 (C), 79.6 (C); EI-MS: *m/z* (%) = 168

(2) $[M]^+$, 150 (4) $[M–H_2O]^+$, 135 (9), 125 (3), 121 (3), 108 (21), 95 (100), 79 (8), 67 (16), 55 (16), 43 (42).



Scheme S1. a) NaH, THF, 40°C, 1 h, 24%/71%; b) 6-methylhept-en-2-one, 65°C, 24 h; c) DIBAH, toluene, -78°C, 15 h, 64%/44%.

Synthesis of (E)- and (Z)-2,3,7-trimethylocta-2,6-dienoic acid ethyl ester. According to the procedure of Ortiz de Montellano et al.,[4] NaH (200 mg, 4.99 mmol, 60% in mineral oil) was suspended in THF (5 ml) and cooled to 0°C. Triethylphosphonopropionate (1.20 g, 4.99 mmol) dissolved in THF (3ml) was added dropwise to this suspension. The temperature was raised to 40°C and the mixture was stirred for 1 h. After cooling to 0°C, 6-methylhept-5-en-2-one (629 mg, 4.99 mmol) was added. Finally, the reaction mixture was stirred for 24 h under reflux and then quenched with an excess of aq. HCl (2 M). Then the mixture was extracted three times with diethyl ether, the combined organic phases dried with $MgSO_4$, and concentrated. Column chromatography on silica gel using pentane/diethyl ether (35:1) was performed to give the (*Z*)- and the (*E*)-ester as a light yellow oil.

(Z)-2,3,7-trimethylocta-2,6-dienoic acid ethyl ester

Yield: 24%; TLC: R_F = 0.20 (pentane/diethyl ether 35 :1); GC: *I* = 1418 ; ^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.29 (t, 3H, $^3J_{H,H}$ = 7.1 Hz, CH_3), 1.61 (s, 3H, CH_3), 1.68 (d, 3H, $^3J_{H,H}$ = 1.0 Hz, CH_3), 1.79 (m, 3H, CH_3), 1.84 (d, 3H, $^3J_{H,H}$ = 0.9 Hz, CH_3), 2.10-2.17 (m, 2H, CH_2), 2.35 (m, 2H, CH_2), 4.18 (q, 2H, $^3J_{H,H}$ = 7.1 Hz, CH_2), 5.08-5.14 (m, 1H, CH) ; ^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 14.3 (CH_3), 15.9 (CH_3), 17.6 (CH_3), 20.2 (CH_3), 25.7 (CH_3), 27.1 (CH_2), 36.4 (CH_2), 60.0 (CH_2), 123.1 (C),

124.0 (CH), 131.8 (C), 145.5 (C), 169.8 (C); EI-MS: *m*/*z* (%) = 210 (5) $[M]^{+,}$ 195 (1), 182 (1), 165 (20), 142 (28), 137 (31), 96 (52), 69 (100), 41 (48).

(*E*)-2,3,7-trimethylocta-2,6-dienoic acid ethyl ester

Yield: 71%; TLC: R_F = 0.18 (pentane/diethyl ether 25 :1) ; GC: *I* = 1448 ; ^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.30 (t, 3H, $^3J_{H,H}$ = 7.2 Hz, CH_3), 1.61 (d, 3H, $^3J_{H,H}$ = 0.5 Hz, CH_3), 1.69 (d, 3H, $^3J_{H,H}$ = 0.8 Hz, CH_3), 1.86 (q, 3H, $^3J_{H,H}$ = 1.5 Hz, CH_3), 1.99 (q, 3H, $^3J_{H,H}$ = 1.4 Hz, CH_3), 2.07-2.17 (m, 4H, 2xCH_2), 4.19 (q, 2H, $^3J_{H,H}$ = 7.1 Hz, CH_2), 5.07-5.15 (m, 1H, CH) ; ^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 14.3 (CH_3), 15.3 (CH_3), 17.6 (CH_3), 20.9 (CH_3), 25.7 (CH_3), 25.9 (CH_2), 36.2 (CH_2), 60.0 (CH_2), 122.9 (C), 123.5 (CH), 132.3 (C), 146.0 (C), 170.0 (C); EI-MS: *m*/*z* (%) = 210 (4) $[M]^+$, 195 (1), 182 (1), 165 (23), 142 (24), 137 (24), 96 (36), 69 (100), 41 (39).

Synthesis of (E)- and (Z)-2,3,7-trimethylocta-2,6-dien-1-ol (5). Following the procedure of Ley et al.,[5] a solution of 2,3,7-trimethylocta-2,6-dienoic acid ethyl ester (164 mg, 0.78 mmol) in dry toluen (4 ml) was cooled to –78°C and a solution of diisobutylaluminium hydride in toluene (1 mol/l, 2.3 mmol) was added dropwise. The reaction mixture was then stirred for 15 h and the temperature was allowed to warm to room temperature. The mixture was quenched with sat. potassium-sodium tartrate and extracted three times with diethyl ether. The combined organic layers were dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography on silica gel with pentane/diethyl ether (3:1) to give the (*E*)- and the (*Z*)-alcohol as a colourless oil.

(*Z*)-2,3,7-Trimethylocta-2,6-dien-1-ol

Yield**:** 64%; TLC: R_F = 0.28 (pentane/diethyl ether 3 :1) ; GC: *I* = 1319; ^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.59 (d, 3H, $^3J_{H,H}$ = 0.6 Hz, CH_3), 1.69 (d, 6H, $^3J_{H,H}$ = 0.8 Hz, 2xCH_3), 1.74 (q, 3H, $^3J_{H,H}$ = 0.8 Hz, CH_3), 2.03-2.16 (m, 4H, 2xCH_2), 4.07 (s, 2H, CH_2), 5.08-5.14 (m, 1H, CH). ^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 16.7 (CH_3), 17.5 (CH_3), 18.7 (CH_3), 25.6 (CH_3), 27.1 (CH_2), 34.0 (CH_2), 63.4 (CH_2), 124.1 (CH), 128.4 (C), 132.3 (C), 132.8 (C). EI-MS: *m*/*z* (%) = 168 (11) $[M]^+$, 153 (18), 150 (11), 137 (9), 135 (16), 107 (29), 98 (34), 82 (37), 69 (100), 43 (93), 41 (80).

(*E*)-2,3,7-Trimethylocta-2,6-dien-1-ol

Yield: 44%; TLC : R_F = 0.19 (pentane/diethyl ether 3:1) ; GC: *I* = 1333 ; ^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.61 (d, 3H, $^3J_{H,H}$ = 0.8 Hz, CH_3), 1.69 (s, 3H, CH_3), 1.74 (t, 3H, $^3J_{H,H}$ = 1.3 Hz, CH_3), 1.76 (t, 3H, $^3J_{H,H}$ = 1.4 Hz, CH_3), 2.05 (s, 2H, CH_2), 2.06 (s,

2H, CH_2), 4.12 (s, 2H, CH_2), 5.10-5.15 (m, 1H, CH) ; ^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 16.2 (CH_3), 17.6 (CH_3), 17.9 (CH_3), 25.7 (CH_3), 26.4 (CH_2), 34.9 (CH_2), 64.1 (CH_2), 124.1 (CH), 127.9 (C), 131.7 (C), 133.0 (C); EI-MS: *m*/*z* (%) = 168 (6) $[M]^+$, 153 (8), 150 (7), 137 (11), 135 (7), 107 (12), 98 (19), 82 (30), 69 (100), 43 (88), 41 (68).

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