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(5*E*/*Z*,7*E*,9)-Decatrien-2-ones, Pineapple-like Flavors from *Fomitopsis betulina* - Structure Elucidation and Sensorial Properties

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ABSTRACT

During the cultivation of the edible mushroom *Fomitopsis betulina* on agro-industrial side streams, a pleasant flavor strongly reminiscent of pineapple was perceived. Aroma extract dilution analyses identified two flavor components with a distinct pineapple odor. Based on mass spectrometric data, a Wittig reaction of (*E*)-penta-2,4-dien-1-yltriposponium bromide with ethyl levulinate was conducted. The resulting (*5E/Z,7E,9*)-decatrien-2-ones were identical to the compounds isolated from the fungal culture. Some structurally related methyl ketones were synthesized, confirmed by NMR and MS, and their odor characterized. The lowest odor threshold and most characteristic pineapple-like odor was found for (*5Z,7E,9*)-decatrien-2-one. Global minimum energy calculation of the methyl ketones and the comparison with (*1,3E,5Z*)-undecatriene, a character impact compound of fresh pineapple, showed that a chain length of at least ten carbon atoms and a terminal double bond embedded in an “L”-shaped conformation were common to compounds imparting an intense pineapple-like odor. Both (*5E/Z,7E,9*)-decatrien-2-ones have not been described as natural flavor compounds.

Keywords: Pineapple flavor, (*5E/Z,7E,9*)-decatrien-2-ones, structure-activity relationship

INTRODUCTION

Pineapple is one of the most popular tropical fruits because of its unique flavor. More than 380 volatiles including esters, heterocycles and polyunsaturated hydrocarbons were reported.¹⁻³ The key aroma compounds are 4-hydroxy-2,5-dimethyl-3(2H)-furanone, ethyl-2-methylbutanoate, ethyl-2-methylpropanoate, methyl-2-methylbutanoate and (1*E*/3,5)-undecatriene.² Takeoka et al. stated that besides the key aroma compounds ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl 2-methylpropanoate, methyl hexanoate and methyl butanoate are required to create the exotic sweet flavor of fresh pineapple, whereas Pino stated that solely the addition of ethyl 3-(methylthio)propanoate and (1,3*E*,5*Z*,8*Z*)-undecatetraene was required.⁴⁻⁶ However, a characteristic genuine impact compound, such as vanillin for vanilla, has not yet been identified.

During the cultivation of numerous basidiomycetous fungi on various agro-industrial side streams the formation of a pleasant flavor, strongly reminiscent of pineapple and honey, was perceived in cell cultures of the edible mushroom *Fomitopsis betulina* (formerly *Piptoporus* or *Polyporus betulinus*) when grown on cabbage cuttings. Aroma extract dilution analyses resulted in the identification of six important flavor components, among them one with distinct pineapple odor.⁷ Hence, the fungi generated an aroma compound that evoked a strong pineapple odor impression on its own.

The aims of this study were to elucidate the unknown chemical structure of the flavor compounds based on total synthesis, to determine their sensory properties and to model the structure-activity relationship based on analogues synthesized using the same route. This work not only identified a new impact aroma compound, but also contributes to the understanding of pineapple flavor perception.

MATERIALS AND METHODS

Chemicals and substances. Chemicals used were purchased in *p.a.* quality from Carl Roth (Karlsruhe, Germany) and Merck (Darmstadt, Germany). Dec-9-en-2-one was from Sigma-Aldrich (St. Louis, Missouri, USA) in a quality > 95 % and used without further purification. All solvents were purified in house by rectification.

Synthesis of (5*E*/*Z*,7*E*,9)-decatrien-2-ones and structural related compounds

(5*E*)-Bromopenta-1,3-diene (1). To a hydrogen bromide (HBr) solution (3.4 mL, 48 % (v/v), 30.3 mmol) at 5 °C penta-1,4-dienol (2.50 g, 98 %, 29.1 mmol) was added dropwise within 5 min and the reaction mixture was stirred for another 3 h at room temperature (RT). During this time diethyl ether (8 mL) and another portion of hydrogen bromide were added. Afterwards the organic layer was separated, washed with cold water (3 x 10 mL) and dried over magnesium sulphate (MgSO₄). After filtration the solvent was removed under reduced pressure using a rotary evaporator. The product (3.82 g, 26.0 mmol, 89 % yield) was obtained as a yellow oil.

RI: 1250 (DB-WAX UI), 885 (VF-5ms)

EI-MS: (C₅H₇Br, M = 147 g mol⁻¹): *m/z* (%) = 148 (M⁺, 11), 146 (M⁺, 11), 94 (1), 92 (1), 81 (2), 79 (2), 67 (100), 65 (15), 51 (4), 41 (39), 39 (25).

(*E*)-Penta-2,4-dien-1-yltriphenylphosphonium bromide (2). (5*E*)-Bromopenta-1,3-diene (5.18 g, 35.2 mmol) was added dropwise to a mixture of triphenylphosphine (9.85 g 98.5 % (v/v), 37.0 mmol) dissolved in toluene (23 mL) under nitrogen atmosphere. After stirring the mixture at room temperature for 22 h the crystalline (*E*)-penta-2,4-dien-1-yltriphenylphosphonium bromide was separated *via* filtration (10.75 g, 26.3 mmol, 75 % yield) as a colourless solid.

ESI(+)-MS/MS: (C₂₃H₂₂P, M⁺ = 329 g/mol): *m/z* (%) = 329 (M⁺, 10), 262 (100), 185 (13), 183 (50), 108 (87), 107 (6), 67 (13).

Ethyl-3-(2-methyl-1,3-dioxolan-2-yl)propanoate (3). A solution of ethyl levulinate (10.0 g, 69.4 mmol), ethylene glycol (7.0 ml, 125.5 mmol), and *p*-toluenesulfonic acid monohydrate (0.13 g, 0.7 mmol) in cyclohexene (40 mL) was heated in a Dean-Stark apparatus for 4 h under reflux. After cooling to RT saturated sodium hydrogen carbonate solution (NaHCO₃) (20 mL) was added. The organic phase was washed with water (3 x 20 ml) and dried over MgSO₄. The solvent was removed with a rotary evaporator and the product (5.41 g, 91 % purity, 28.7 mmol, 41 % yield) was obtained as a colorless oil.⁸

EI-MS: (C₉H₁₆O₄, M = 188 g/mol): *m/z* (%) = 173 (18), 143 (30), 129 (4), 99 (48), 87 (100), 45(3), 43 (35)

3-(2-Methyl-1,3-dioxolan-2-yl)propanal (4). Under N₂-atmosphere the acetal (3) (2.50 g, 13.3 mmol) was dissolved in dry dichloromethane (DCM, 74 mL) at -78 °C. To this solution 13.9 mL 1 M diisobutyl aluminiumhydride (DIBAL, 13.9 mmol) in *n*-hexane was added dropwise. After 45 min under continuous stirring at -78 °C, 2.5 ml methanol (MeOH) and 10.0 mL saturated sodium chloride solution (NaCl) were added and stirred for another 1 h at the same temperature. Then the reaction mixture was stirred over night at RT. After addition of 12.5 g Na₂SO₄ and stirring for 1 h the organic phase was filtered, the filter washed with DCM (3 x 10 ml) and finally the solvent was removed with the rotary evaporator. The residue was purified by flash chromatography (silica gel, elution with pentene/diethyl ether (PE; 1:1.12 *n/n*) and diethyl ether (DE), PE 3:1 → DE). The purified product (1.13 g, 7.8 mmol, 59 % yield) was obtained as a colorless oil.⁹

RI: 1623 (DB-WAX UI), 1079 (VF-5ms)

95 EI-MS: (C₇H₁₂O₃, M = 144 g/mol): *m/z* (%) = 129 (27), 87 (100), 85 (26), 71 (6), 57 (7),
96 55 (11), 43 (64).

97 **2-Methyl-2-((5*E*)-octa-3,5,7-trien-1yl)-1,3-dioxolane (5a,b).** Under N₂-
98 atmosphere the aldehyde (**4**) (0.50 g, 3.5 mmol) and the phosphonium salt (**2**) (1.56 g,
99 3.8 mmol) were dissolved in dry dimethyl formamide (DMF, 8 mL). After dropwise
100 addition of sodium methanolate (0.7 mL, 30 % in methanol, 3.6 mmol) at 5 °C the
101 reaction mixture was stirred for 2 h at constant temperature. Saturated ammonium
102 chloride solution (NH₄Cl, 30 mL) and *n*-hexane (30 mL) were added prior to filtration.
103 The organic phase of the filtrate was separated and the aqueous phase was extracted
104 with *n*-hexane (3 x 30 mL). The combined organic phase was washed with water and
105 saturated NaCl-solution (3 x 30 mL each), dried over MgSO₄ and then mixed with
106 butylated hydroxyl toluene (BHT, 10 mg) for stabilization. The solvent was removed
107 with a rotary evaporator under reduced pressure and the residue was purified by flash
108 chromatography (silica gel, elution PE, PE 20:1 → PE 8:1). The purified product
109 (0.51 g, 2.6 mmol, 76 % yield) was obtained as a light yellow oil.⁸

110 3(*Z*)-Isomer (**5b**)

111 RI: 1948 (DB-WAX UI), 1462 (VF-5ms)

112 EI-MS: (C₁₂H₁₈O₂, M = 194 g/mol): *m/z* (%) = 194 (M⁺, 19), 179 (7), 93 (19), 91 (35),
113 87 (100), 79 (16), 77 (27), 43 (75).

114 3(*E*)-Isomer (**5a**)

115 RI: 1991 (DB-WAX UI), 1487 (VF-5ms)

116 EI-MS: (C₁₂H₁₈O₂, M = 194 g/mol): *m/z* (%) = 194 (M⁺, 22), 179 (7), 93 (23), 91 (39),
117 87 (100), 79 (16), 77 (30), 43 (78).

(5 *E/Z*,7*E*,9)-Decatrien-2-one (6a,b). To a mixture of 3 ml perchloric acid (HClO₄, 35 %) and 1 ml DE at 5 °C, 2 mL of a solution of acetal (**5a,b**) in DE (0.20 g, 1 mmol) was added dropwise within 10 min. After 20 min at constant temperature (5 °C) the reaction mixture was neutralized with saturated NaHCO₃-solution. The organic phase was separated and the aqueous phase was extracted with DE (3 x 20 mL). The combined organic phase was washed with water and saturated NaCl-solution (3 x 30 mL), dried over MgSO₄ and stabilized with BHT (10 mg). The residue was purified by flash chromatography (silica gel, elution with PE 10:1). The purified product (57 mg, 0.38 mmol, 37 % yield) was obtained as a colorless oil.⁸

5(*Z*)-Isomer (6b)

RI: 1826 (DB-WAX UI), 1268 (VF-5ms)

EI-MS: (C₁₀H₁₄O, M = 150 g/mol): *m/z* (%) = 150 (M⁺, 35), 135 (1), 107 (16), 93 (14), 91 (81), 79 (57), 71 (3), 67 (11), 65 (18), 43 (100).

5(*E*)-Isomer (6a)

RI: 1846 (DB-WAX UI), 1278 (VF-5ms)

EI-MS: (C₁₀H₁₄O, M = 150 g/mol): *m/z* (%) = 150 (M⁺, 37), 135 (1), 107 (16), 93 (15), 91 (81), 79 (55), 71 (3), 67 (11), 65 (18), 43 (100).

¹H-NMR (6a,b): (400 MHz, CDCl₃): δ 2.14, 2.14 (s_{a,b}, 6H, 1_{a,b}), 2.35-2.49 (m, 4H, 4_{a,b}), 2.51-2.55 (m, 4H, 3_{a,b}), 5.05, 5.10 (d_{a,b}, *J*₁ = 10.09 Hz, *J*₂ = 10.25 Hz, 2H, 10_{a,b}), 5.18, 5.23 (d_{a,b}, *J*₁ = 16.70 Hz, *J*₂ = 16.82 Hz, 2H, 10_{a,b}), 5.42 (dt, *J*₁ = 7.47 Hz, *J*₂ = 10.68 Hz, 1H, 5_b), 5.69 (dt, *J*₁ = 7.01 Hz, *J*₂ = 14.76 Hz, 1H, 5_a), 6.01- 6.03 (m, 8H, 6_{a,b}, 7_{a,b}, 8_{a,b}, 9_{a,b}).

141 **¹³C-NMR (6a,b):** (100 MHz, CDCl₃): δ 22.3, 27.0, 30.1, 43.2, 43.5, 116.9, 117.6,
142 128.2, 129.5, 130.9, 131.2, 133.2, 133.6, 133.9, 137.1, 137.2,
143 208.06, 208.14.

144 **5,9-Decadien-2-one (7a,b).** This compound was synthesized along the same
145 route as the decatrienones (**6a,b**) with pent-4-en-1-yltriposponiumbromide used in
146 the Wittig reaction. The product (100 mg, 0.6 mmol, 53 % yield) was obtained as a
147 colorless oil.⁸

148 **5(Z)-Isomer (7b)**

149 RI: 1582 (DB-WAX UI), 1171 (VF-5ms)

150 EI-MS: (C₁₀H₁₆O, M = 152 g/mol): *m/z* (%) = 152 (M⁺, 1), 111 (5), 109 (2), 97 (1), 94
151 (14), 81 (2), 79 (13), 71 (4), 43 (100), 41 (12).

152 **5(E)-Isomer (7a)**

153 RI: 1173 (VF-5ms)

154 EI-MS: (C₁₀H₁₆O, M = 152 g/mol): *m/z* (%) = 152 (M⁺, 1), 111 (5), 109 (2), 97 (1), 94
155 (12), 81 (2), 79 (10), 71 (3), 43 (100), 41 (10).

156 **5-Decen-2-one (8a,b).** This compound was synthesized along the same route
157 as the decatrienones (**6**) with pentyltriposponiumbromide used in the Wittig reaction.
158 The product (70 mg, 0.45 mmol, 67 % yield) was obtained as a colorless oil.⁸

159 **5(Z)-Isomer (8b)**

160 RI: 1530 (DB-WAX UI), 1179 (VF-5ms)

161 EI-MS: (C₁₀H₁₆O, M = 152 g/mol): *m/z* (%) = 152 (M⁺, 1), 111 (5), 109 (2), 97 (1), 94
162 (14), 81 (2), 79 (13), 71 (4), 43 (100), 41 (12).

163 **5(E)-Isomer (8a)**

164 RI: 1183 (VF-5ms)

165 EI-MS: (C₁₀H₁₆O, M = 152 g/mol): *m/z* (%) = 152 (M⁺, 1), 111 (5), 109 (2), 97 (1), 94
166 (12), 81 (2), 79 (10), 71 (3), 43 (100), 41 (10).

167 **(5,7*E*)-Nonadien-2-one (9a,b)**. This compound was synthesized along the
168 same synthetic route as the decatrienones (**6**) with (*E*)-buten-2-en-1-
169 yltriphosponiumbromide used in the Wittig reaction. A product mixture of **9a,b** (80 mg,
170 81 % purity, 0.47 mmol, 59 % yield) and its constitutional isomer, 6-methylocta-5,7(*E*)-
171 dien-2-one, **10a,b** (80 mg, 16 % purity, 0.092 mmol, 59 % yield) was obtained as a
172 light yellow oil.⁸

173 5(*Z*)-Isomer (**9b**)

174 RI: 1596 (DB-WAX UI), 1139 (VF-5ms)

175 EI-MS(C₉H₁₄O, M = 138 g/mol): *m/z* (%) = 138 (M⁺, 29), 123 (5), 95 (41), 81 (31), 79
176 (38), 67 (29), 55 (21), 43 (100), 41 (22).

177 5(*E*)-Isomer (**9a**)

178 RI: 1605 (DB-WAX UI), 1145 (VF-5ms)

179 EI-MS: (C₉H₁₄O, M = 138 g/mol): *m/z* (%) = 138 (M⁺, 31), 123 (5), 95 (40), 81 (32), 79
180 (38), 67 (28), 55 (20), 43 (100), 41 (21).

181 5(*Z*)-Isomer (**9c**)

182 RI: 1613 (DB-WAX UI), 1150 (VF-5ms)

183 EI-MS(C₉H₁₄O, M = 138 g/mol): *m/z* (%) = 138 (M⁺, 27), 123 (5), 95 (40), 81 (29), 79
184 (38), 67 (27), 55 (20), 43 (100), 41 (21).

185 5(*E*)-Isomer (**9d**)

186 RI: 1617 (DB-WAX UI), 1154 (VF-5ms)

187 EI-MS: (C₉H₁₄O, M = 138 g/mol): *m/z* (%) = 138 (M⁺, 23), 123 (5), 95 (40), 81 (28), 79
188 (37), 67 (27), 55 (20), 43 (100), 41 (21).

189 **5,7-Octadien-2-one (10a,b).** This compound was synthesized along the same
190 route as the decatrienones (**6**) with allyltriphosphoniumbromide used in the Wittig
191 reaction. The product (80 mg, 0.47 mmol, 59 % yield) was obtained as a colorless oil.⁸

192 5(*Z*)-Isomer (**10b**)

193 RI: 1463 (DB-WAX UI)

194 5(*E*)-Isomer (**10a**)

195 RI: 1465 (DB-WAX UI)

196 EI-MS (*E*) und (*Z*) isomers: (C₈H₁₂O, M = 124 g/mol): *m/z* (%) = 124 (M⁺, 12), 109 (5),
197 81 (33), 79 (22), 71 (1), 67 (20), 53(12), 43 (100).

198 **Purification of the (5*E*,7*E*,9)-Decatrien-2-one (6a).** Product purification was
199 performed via preparative gas chromatography. A Hewlett-Packard 5890 (Hewlett-
200 Packard, Palo Alto) gas chromatograph equipped with a HP 7673 auto sampler
201 (Hewlett-Packard, Palo Alto), a cold injection system (CIS 3; Gerstel, Mülheim,
202 Germany) and a multi column switching system II (Gerstel, Mülheim, Germany).
203 Separation was performed on a polar Optima-Wax precolumn (5 m, 0.53 mm, 2 μm,
204 Macharey & Nagel Düren, Germany) and an Optima Wax preparative column (25 m,
205 0.53 mm, 2 μm, Macharey & Nagel Düren, Germany) with a hydrogen flow of
206 8.9 mL/min. For separation, the following temperature program was used: 40 °C hold
207 for 3 min; 1 °C min⁻¹ until 168 °C, 20 °C min⁻¹ until 240 °C held for 15 min. The product
208 was collected with the multi column switching system II. The purified product was used
209 for NMR characterization.

Gas chromatography mass spectrometry. All products synthesized with the exception of the respective Wittig salts were diluted to an appropriate concentration with diethyl ether and analyzed by means of high resolution capillary gas chromatography coupled to a mass selective detector (GC-MS). GC-analyses were performed on two stationary phases of different polarity. Retention indices were calculated according to Van den Dool and Kratz using homologue alkanes from C₈ to C₃₀. Samples (0.5 µL) were injected on-column.¹⁰ The GC-MS device consisted of a 7890B GC-system (Agilent Technologies, Santa Clara) combined with an Agilent 5977A mass selective detector (interface: 230 °C, ion source: 200 °C, quadrupole: 100 °C, electron impact ionization: 70 eV, scan range *m/z* 33–300 amu; Agilent Technologies, Santa Clara). Stationary phases DB-WAX UI (30 m, 0.25 mm, 0.25 µm, Agilent J&W GC Columns, Santa Clara) and VF-5ms (30 m, 0.25 mm, 0.25 µm, Agilent J&W GC Columns, Santa Clara) were installed. All analyses were performed with a volumetric flow rate of 1 mL/min Helium using the following temperature programs: 40 °C hold for 3 min; 8 °C/min until 230 °C, 230 °C hold for 10 min (DB-WAXms) and 40 °C hold for 3 min, 8 °C/min until 230 °C, 25 °C/min until 325 °C, 325 °C hold for 10 min (VF 5ms).

High resolution GC was done with an Agilent 6890 gas chromatograph equipped with an Quasar AMD (electron impact ionization: 70 eV scan range *m/z* 33–500 amu; Agilent Technologies, Santa Clara). Separation was performed on a DB WAX column (30 m, 0.32 mm, 0.25 µm, Agilent J&W GC Columns, Santa Clara) using the conditions as described above.

Gas chromatography Olfactometry. Sensory evaluation and determination of the perception threshold mass of the decatrien-2-ones was carried out using GC-Olfactometry (GC-O) combined with aroma extract dilution analysis. The same

chromatographic conditions were used as described, but with hydrogen as the carrier gas. The effluent was splitted at the end of the column (1:1). One part was directed towards a flame ionization detector (FID), the second towards the olfactory detection port (ODP) where the effluent was sniffed by panelists (five trained subjects). The stock solution contained all synthesized compounds and the commercial 9-decen-2-one (0.1 g/L in DE). Sensory evaluation started with a 1:10 dilution of the stock solution and was continued with further 1:10 dilution steps until no odor of any of the flavor compounds was perceived. The individual thresholds were expressed as the ultimate mass (m_T) injected effectively on-column which was still perceived by the majority of the panelists. The median of the individual thresholds was calculated.

High performance liquid chromatography tandem mass spectrometry (HPLC-MS2). The synthesis of Wittig salts was confirmed using high performance liquid chromatography coupled to a triple quadrupole mass analyzer (Varian 212 LC pump, Pro Star 325 UV/Vis detector, 320 TQ-MS mass spectrometer). The MS was conducted in the ESI positive (ESI(+)) mode with the selective mass of the respective Wittig cation for the first quadrupole and a scan range of m/z 50 to the respective mass of the cation in the third quadrupole. MS parameters were: capillary voltage +40 V, needle voltage 5000 V, nebulizer gas (N_2) 379 kPa, drying gas 207 kPa at 350 °C. The collision cell was operating with 267 mPa Argon as the collision gas and 40 V collision energy. For HPLC, water and acetonitrile (MS-grade), both containing 0.1 % formic acid, were used as the mobile phase. The following linear gradient was used: 10 % acetonitrile for three min, up to 90 % acetonitrile within 20 min, hold for five minutes and back to start conditions. The separation was performed on a Nucleodur C18 HD Pyramid-column (250×4 mm, 5 μ m, Macherey-Nagel) at a flow rate of 0.3 mL/min.

259 Samples were dissolved in acetonitrile/water (1:1), diluted to appropriate
260 concentrations, 20 μ L injected manually *via* a six port valve.

261 **Structure-activity relationship.** The compounds used in the olfactory analysis
262 were compared to the structure of the genuine pineapple impact aroma volatiles
263 (1,3*E*,5*Z*)-undecatriene and (1,3*E*,5*Z*,8*Z*)-undecatetraene. To identify the most
264 abundant molecular conformation of each compound, structures were energy-
265 minimized using the DREIDING force field. These data were used for Quenched
266 Dynamics Simulation (1 ns, 300 K) to obtain the most likely geometric spatial
267 conformation of the odor compounds.¹¹

268 **Nuclear magnetic resonance spectroscopy.** Structure elucidation was
269 performed by ^1H and ^{13}C 1D-spectra with broadband decoupling as well as 2D-Spectra
270 like DQF-COSY for proton correlations and ^{13}C -edited HSQC and HMBC spectra for
271 proton carbon correlations. All measurements were performed on a Bruker Ultrashield
272 500 MHz spectrometer with DRX-console and cryo TCI ^1H , $^{13}\text{C}/^{15}\text{N}$ Probe with z-
273 gradients. For measurements the products were dissolved in 0.7 mL deuterated
274 trichloromethane and measured at 298.0 K. The residual solvent signal of
275 trichloromethane was used as internal standard. All data were evaluated by
276 MestReNova and Topspin software.

277 RESULTS AND DISCUSSION

278 **Retrosynthesis of (5*E*/Z,7*E*,9)-decatrien-2-ones.** The retrosynthesis of the
279 target (5*E*/Z,7*E*,9)-decatrien-2-ones is shown in Figure 1. The decatrien-2-one
280 skeleton was built along a Wittig reaction of two C5 units, an aldehyde with a methyl
281 ketone group and a triphenylphosphonium salt bearing the conjugated pentadiene unit.
282 To avoid side reactions unprotected 4-ketopentanal was not reacted directly with the
283 respective Wittig salt. Thus, the keto group was converted to its acetal (**3**). Subsequent

a reductive acetal deprotection (DIBAL) was required after the Wittig reaction to yield the targeted decatrien-2-ones (**6a,b**).

The triene moiety of the decatrien-2-one was introduced *via* the phosphonium salt of (1,3*E*)-pentadiene which was generated by the nucleophilic substitution (S_N1') of 1,4-pentadienol with hydrogen bromide. This reaction proceeds stereo-selectively under formation of 1,3(*E*)-conjugated double bonds. The second C5-unit for the Wittig reaction, aldehyde (**4**), providing the methyl ketone unit, was synthesized starting with ethyl levulinate. The keto group was protected by acetal formation with ethylene glycol and the ester subsequently reduced with DIBAL to give aldehyde (**4**).

Both C5-units were coupled in a Wittig reaction resulting the targeted C10-skeleton and the third double bond in conjugation to the already present conjugated diene system. In general the (*E/Z*) selectivity of the Wittig reaction depends on the stabilization of the mesomeric ylide and ylene intermediates. Stabilization of the ylide favors the formation of the (*E*)-isomer, whereas non-stabilized give the (*Z*)-isomer. In case of preferred ylene formation in the transition state bulky substituents favor the formation of the (*Z*)-isomer, whereas repellant electronegative groups the (*E*)-isomer. Here, no preference for one of the 2-(5(*E*)-hepta-3,5-dien-1-yl)-2-methyl-1,3-dioxolane isomers (**5a,b**, (*E/Z*) = 44:56; assignment by $^1\text{H-NMR}$) was found. Hence, after removal of the protection group the targeted (5*E/Z*,7*E*,9)-decatrien-2-ones (**6a,b**) were obtained with an almost equimolar isomeric distribution.

Separation of the two isomers succeeded *via* GC-MS analysis (CW20M: 1842 and 1863, respectively). However, an assignment to the respective 5(*E/Z*) on the basis of the electron impact (EI) mass spectra was not possible. Re-evaluation of extracts from the fungal culture supernatant at the day of maximum product concentration (1.1 mg/L 5(*E*) isomer and 0.07 mg/L 5(*Z*) isomer; at day five) revealed the presence

of both compounds. The mass spectra of the synthesized isomers were nearly identical to each other and to the mass spectra of the pineapple flavor compounds found in culture medium of the basidiomycete *F. betulina*.

Structure elucidation of (5*E*/Z,7*E*,9)-decatrien-2-ones. Both isomers were clearly separated on GC-columns of different polarity, but the EI-mass spectra showed no significant differences. Ionization at the carbonyl oxygen resulted in neutral losses of m/z 15 (CH_3) and m/z 43 ($\text{CH}_3\text{C}=\text{O}$), indicative of the terminal methyl ketone group. As soon as the conjugated triene moiety of the molecule becomes ionized, fragmentation occurs preferentially after rearrangements. The intensity of the signals at m/z 91/92 and 77/79 indicated the formation of stable cyclic ions as they are known for cyclohexene derivatives, such as limonene. A similar behavior was observed while analyzing polyenes, *inter alia* (1,3,5)-undecatriene. However, due to the ionization of the conjugated triene system information about the stereochemistry of the double bonds ($\Delta 5$ and $\Delta 7$) was lost.

Accurate mass determination resulted 150.096 amu, corresponding to an empirical formula of $\text{C}_{10}\text{H}_{14}\text{O}$ (150.104 calc.). The pineapple-like smelling diastereomers were identified as (5*E*/Z,7*E*/Z,9)-decatrien-2-ones, but the geometry of the $\Delta 5$ and $\Delta 7$ double bonds remained unknown. Data on decatrien-2-ones are rare in literature. In 2010, Nakanishi and Watanabe published a patent and Tomita a research article dealing with an undecatrien-3-one (no exact structure given) identified in the *Yuzu* fruit (*Citrus junos* Sieb. Ex Tanaka). This compound was claimed as an ingredient of fresh fruit-like aroma compositions.^{12–14} Both authors presented (5,7,9)-decatrien-2-one as a fruity flavor.^{13,14} Neither further publications nor spectral data were published so far (SciFinder structural search, Sept. 08, 2019). Re-analysis of fresh pineapple, juices as well as canned products *via* solvent extraction techniques as well as SBSE did not

indicate the presence of decatrienones (data not shown; 200 g of pineapple sample; LOD: according to perceived threshold masses shown in table 1).

Preparative gas chromatography (GC) was used for the accumulation of one pure isomer from pooled extracts of the fungal culture medium (as described elsewhere).⁷ Purified product was subsequently subjected to NMR analysis. As both isomers were present after synthesis and further preparative separation was not undertaken, they were measured together. Thus the ¹H-NMR gave a double set of signals in the spectrum. For both isomers the proton at C5 showed ³J-couplings with protons at C4 and C6 revealing a multiplet (doublet, triplet), which was confirmed by a 2D proton correlation spectrum (DQF-COSY). In general, coupling constants (³J) of *E*-isomers are higher than those of *Z*-isomers due to the torsion angle of 180°. The multiplet at 5.42 ppm (*J* = 10.09 Hz) was assigned to the 5(*Z*) and that at 5.69 ppm (*J* = 14.76 Hz) to the 5(*E*)-isomer. Comparison of the intensity of both signals gave a ratio of 44(*E*):56(*Z*), the same as was found with GC-MS. The stereochemistry of the double bond at C7 could only be assigned for the 5(*Z*)-isomer via NMR. The coupling constant (*J* = 15.0 Hz) reveals a 7(*E*) configuration. Because of overlapping signals for the 5(*E*)-isomer, the stereochemistry of the double bond at C7 was not assigned by NMR. According to the synthetic route based on the Wittig reaction the stereochemistry at the C7 double bond was defined as trans and suggested that the 7(*E*)-isomer dominated in the cell cultures. This is explained by an isomerization of the 5(*Z*)-isomer to the thermodynamically more stable 5(*E*)-form during prolonged incubation at elevated temperature (25 °C).

Figure 2 compares the mixed ¹H-NMR of the synthesized decatrien-2-ones with the spectrum of the pineapple-like impact compound isolated earlier from the culture medium of *F. betulina*.⁷ The multiplet at 5.7 ppm, assigned to the proton at C5, in the

spectrum of the isolated decatrien-2-one showed that the isomer found in the culture medium of *F. betulina* was unequivocally (5*E*,7*E*,9)-decatrien-2-one.

Sensorial characterization of (5*E/Z*,7*E*,9)-decatrien-2-ones and structurally related compounds. Structure related methyl ketones were synthesized along the same route. One reactant of the Wittig reaction, the protected ketopentanal was kept constant, whereas the counterpart was varied in chain length as well as number and position of double bonds. The respective structures were confirmed with GC-MS and ¹H and ¹³C-NMR analyses. A sensorial evaluation of all compounds synthesized and the commercial 9-decen-2-one was undertaken using GC-O, and perception thresholds were determined by AEDA. If a sufficient chromatographic separation of certain (*E/Z*)-isomers was not achieved, the mixed impression of both isomers was recorded (Table 1). In general, all flavor compounds were described as fruity. More detailed descriptions resulted in a highly diverse set of attributes. 5,7-octadien-2-ones (**10a,b**) not only reminded of vanilla, but were also described as soil-like and fruity. Solely the isomers of (5*E/Z*,7*E*)-decatrien-2-one (**6a,b**) were clearly identified as pineapple aroma by the majority of panelists (4 out of 5). The 5(*Z*) isomer required a 800fold lower mass for perception (0.028 ng) compared to the 5(*E*)-isomer with 22 ng. The isomer dominating in the culture medium of *F. betulina* was the (5*E*,7*E*,9)-decatrien-2-one with the higher perception threshold mass. However, the relatively low threshold of the *E*-isomer together with its actual concentration were sufficient to provoke the distinct pineapple flavor of the fungal cell culture. Besides the (5,7,9)-decatrien-2-ones further chemosynthesized methyl ketones, such as dec-9-en-2-one and 5,9-decadien-2-ones (**7a,b**), imparted a slight pineapple-like odor impression.

In order to get insight into structural requirements for a pineapple odor impression a comparison of global energy minimized conformations (quenched

dynamics simulation, DREIDING force field,¹¹) of the respective methyl ketones was performed. The calculated conformations are shown in Figure 3 and compared to each other and to (1,3*E*,5*Z*)-undecatriene, a long-known genuine character impact compound of fresh pineapple with a low threshold of 7.7 ng/kg water.¹⁵ All compounds characterized as typically pineapple-like (**6a,b**; **7a,b** and **11**) provided a curved structure consisting of a skeleton of at least 10 carbon atoms with a terminal double bond opposite to the methyl ketone terminus. Since no variation of the keto function was done, its impact was not evaluated directly. However, (1,3*E*,5*Z*)-undecatriene and (1,3*E*,5*Z*,8*Z*)-undecatetraene were described as important constituents of fresh pineapple flavor.^{15,16} Their odor was described as fruity, pineapple-like for undecatriene and fresh, citrus-like for undecatetraene.¹⁷ Both olefins do not possess a ketone function, but an “L”-shaped conformation, which is more pronounced for the (1,3*E*,5*Z*)-undecatriene (Figure 4). The data suggest that the keto group was not a mandatory structural element to evoke the pineapple sensation. The pineapple-like odor impression was lost, if the length of the carbon chain was shortened (**9a-d**, **10a,b**). Although compounds **9b**, **9c** and **10b** show the same “L”-shaped conformation like (5*Z*,7*E*,9)-decatrien-2-one (**6b**), and additionally **10b** shows the same conjugated pentadienyl system with a terminal double bond, the pineapple character of the flavor was completely lost. Alteration of the double bond system (number, position and stereochemical orientation) while maintaining the carbon skeleton led to an increase of the thresholds (less intense) for all compounds compared to **6b**, but the pineapple impression remains with one exception. Unlike the others, 5-decen-2-one (**8a,b**) lacks a terminal double bond, which seems to be an essential molecular feature to evoke a pineapple-like flavor.

Since a long time, hexanoic acid prop-2-enyl ester ("allyl caproate"), absent from pineapple volatilome, has been used to imitate pineapple flavor.^{18,19} It shows a nine carbon skeleton, a carbonyl (ester) function and a terminal double bond. Still in use to round off citrus, peach, apricot and apple blossom perfume compositions, it lacks a convincing pineapple note.

Fungal production of (5*E*/Z,7*E*,9)-decatrien-2-ones. It remains a matter of speculation, why a higher fungus should synthesize these compounds. However, in their natural habitats, wood-destructing fungi are exposed to many microbial and animal enemies as well as to organisms spreading their spores. Thus, it is not surprising that they interact with their highly populated environment through volatile chemical signals.²⁰ Like the pineapple constituents (1,3*E*,5*Z*)-undecatriene and (1,3*E*,5*Z*,8*Z*)-undecatetraene, which are known to act as algal pheromones, the (5*E*/Z,7*E*,9)-decatrien-2-ones with their highly similar three dimensional structure may serve as signaling compounds in fungi.²¹

The biogenesis of the decatrienones is obscure. Although first detected in cell cultures supplemented with cabbage cuttings in concentrations of 1.1 mg/L, a particular precursor role of this nutrient is doubtful. Fatty acids may serve as precursor molecule for the formation of methyl ketones along the β -oxidation.²² Fruity constituents of high structural similarity, such as undecatrien-3-one in *Yuzu* (*Citrus junos*), may be formed along this pathway. An analogous origin of the decatrienones of *F. betulina* would require an odd-numbered unsaturated fatty acid, as they in fact occur in basidiomycetes. Supplementing labeled precursors will clarify this question. Another aim of future research could to investigate in detail the interaction of the decatrienones with human odorant receptor proteins.

433 **ABBREVIATIONS USED**

434	AEDA	Aroma extract dilution analysis
435	BHT	Butylated hydroxyl toluene
436	DCM	Dichloromethane
437	DE	Diethyl ether
438	DIBAL	Diisobutyl aluminiumhydride
439	DMF	Dimethyl formamide
440	FID	Flame ionization detector
441	GC-MS	Gas chromatography coupled with mass spectrometry
442	HBr	Hydrogen bromide
443	LOD	Limit of detection
444	MeOH	Methanol
445	NMR	Nuclear magnetic resonance spectroscopy
446	ODP	Olfactory detection port
447	<i>p.a.</i>	<i>pro analysi</i>
448	PE	Pentene/diethyl ether
449	RT	Room temperature
450	SBSE	Stir bar sorptive extraction

451

452 **Acknowledgement**

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FIGURE CAPTIONS

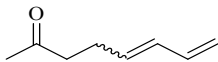
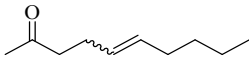
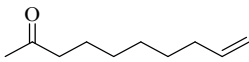
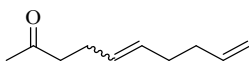
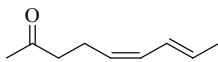
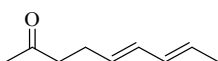
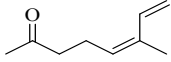
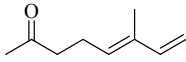
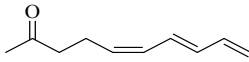
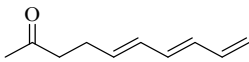
Figure 1: Retrosynthesis of (5*E*/*Z*,7*E*,9)-decatrien-2-one and structurally related methyl ketones (numbering corresponds to Table 1; * = commercial product; ° number of the respective final product)

Figure 2: Comparison of ¹H-NMR spectra of synthesized isomeric mixture of (5*E*/*Z*,7*E*,9)-decatrien-2-one **6a**, **6b** (top) and isolated (5*E*,7*E*,9)-decatrien-2-one **6a** from *F. betulina* (bottom). Signals in frames correspond to the respective proton(s) at C5.

Figure 3: Energy minimized structures of methyl ketones prepared (numbering corresponds to Table 1). Bold red numbers indicate compounds with a pineapple-like flavor sensation (quenched dynamics simulation. DREIDING force field.¹¹)

Figure 4: Comparison of (5*Z*,7*E*,9)-decatrien-2-one (**6b**) with 1.(3*E*,5*Z*)-undecatriene (**12**) and 1.(3*E*,5*Z*,8*Z*)-undecatetraene (**13**). Energy minimized structures (quenched dynamics simulation. DREIDING force field.¹¹)

Table 1: Odor impressions and perceived threshold masses of (5*E*/*Z*,7*E*,9)-decatrien-2-one and structurally related compounds

		m_T [ng]	Odor impression
	10a.b	5,0	Vanilla, soil like, fruity
	8a.b	0,5	Grapefruit, mandarin, orange, fruity, green
	12	5,0	Pineapple (fermented), rubber, plastic, bitter, fruity
	7a.b	5,0	Pineapple, bitter lemon, flowers, grassy, fruity
	9b	23	Citrus fruits, linalool, fruity, green,
	9a	18,5	Citrus fruits
	9c	0,195	Mandarin, fruity
	9d	5,0	Citrus fruits, flowers, fruity
	6b	0,028	Pineapple, fresh breath
	6a	22	Pineapple, fruity, pungent

m_T = threshold of perceived mass injected on-column and still perceived at the GC-sniff-port (median of five panellists)

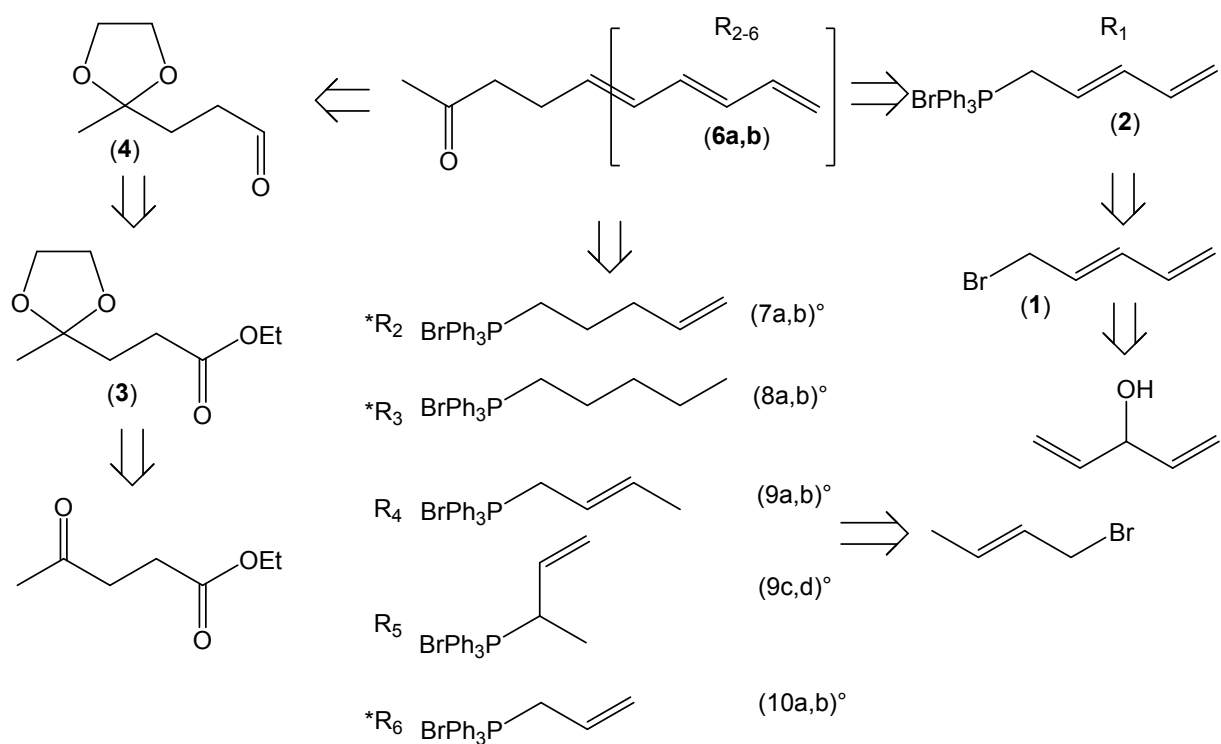


Figure 1

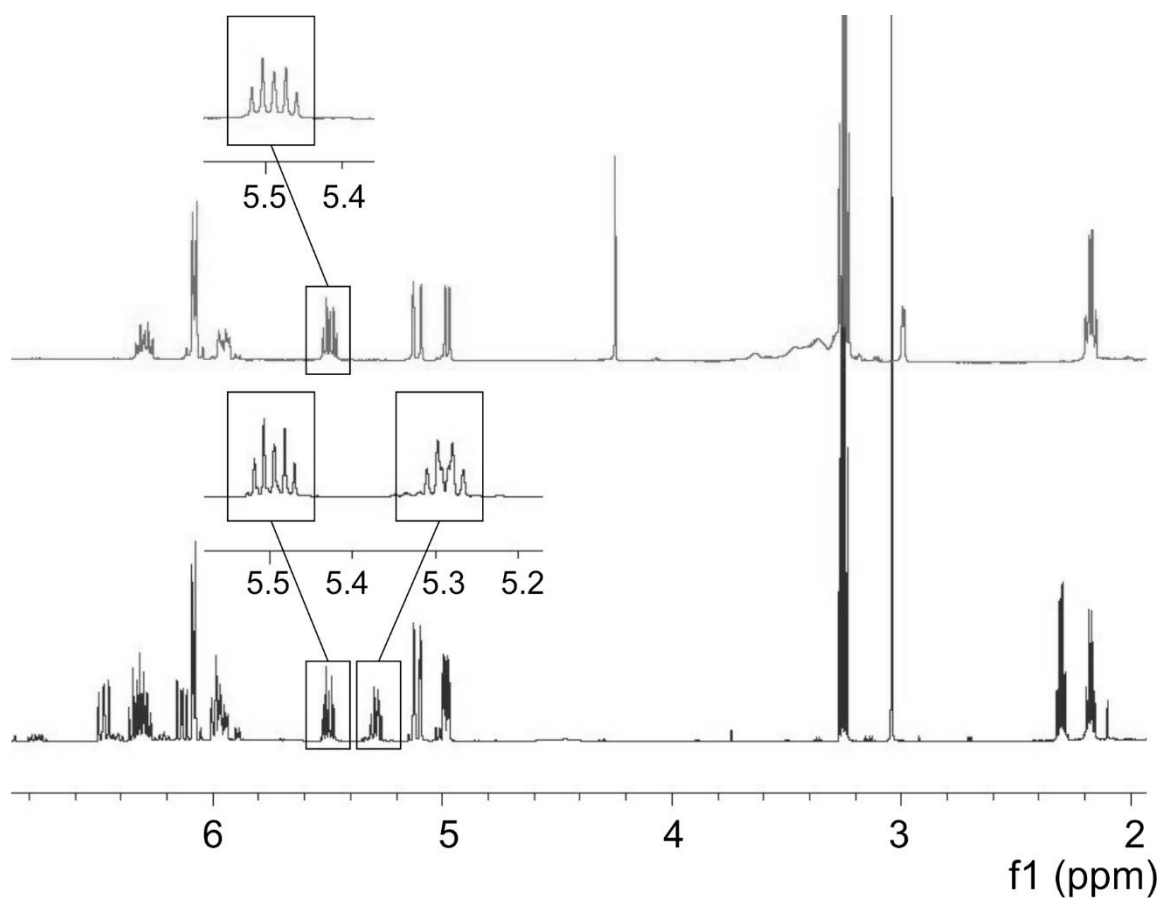


Figure 2

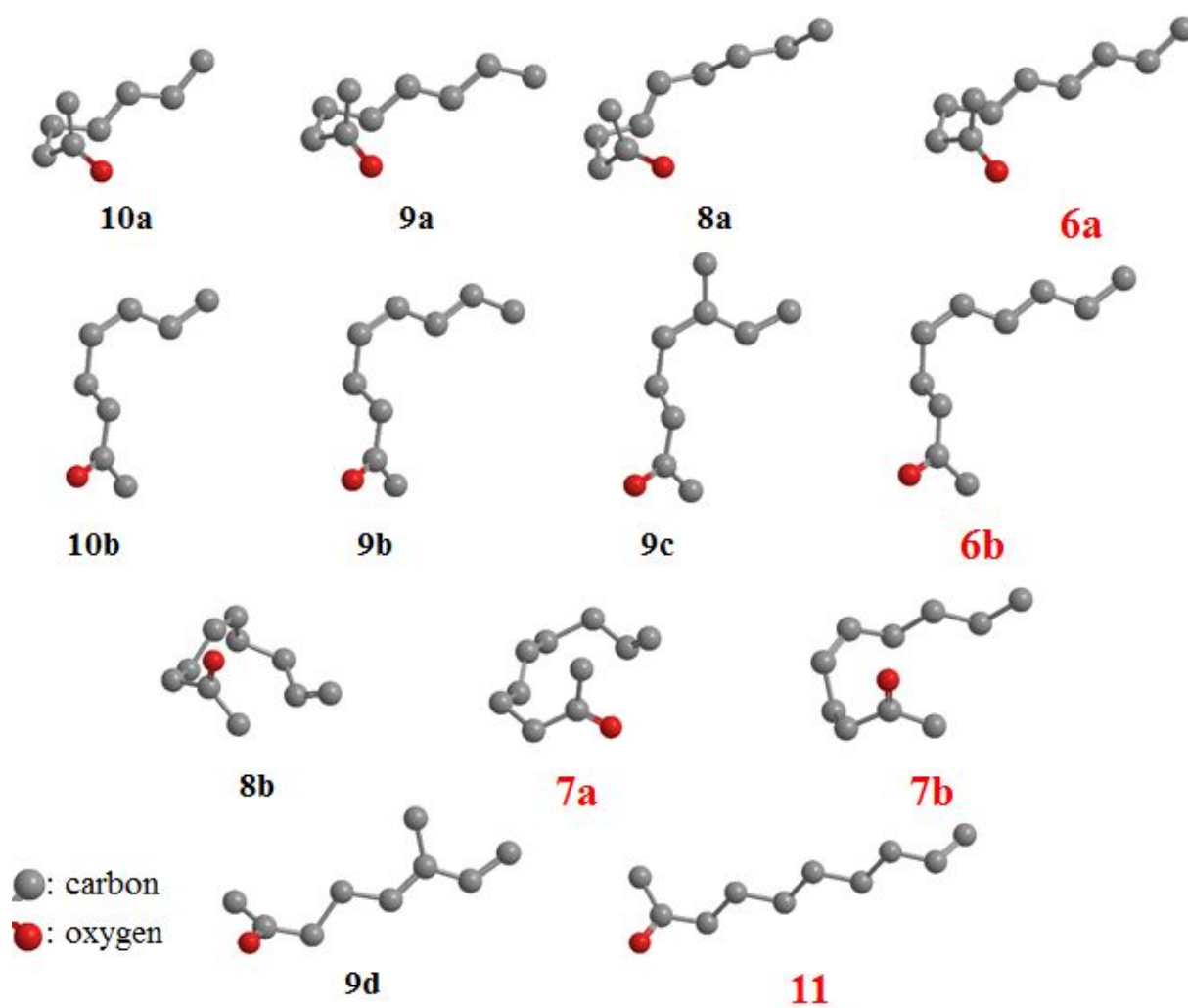


Figure 3

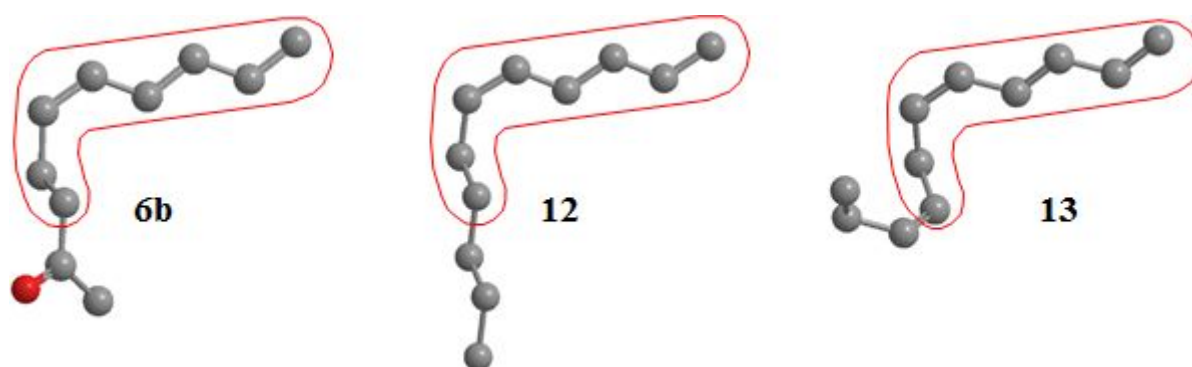
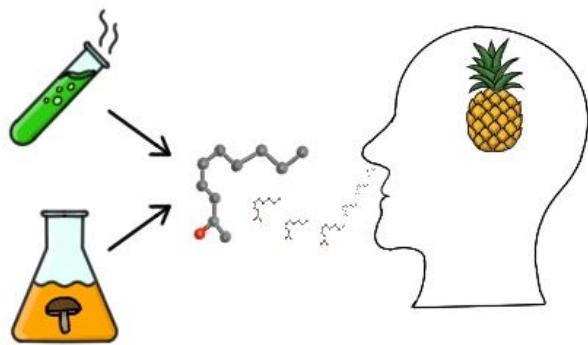


Figure 4



TOC graphic