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Elucidating the Formation Pathway of the Off-Flavor Compound 6-Propylbenzofuran-7-ol

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Supporting Information

ABSTRACT: In a previous work, we identified 6-propylbenzofuran-7-ol as an off-flavor compound formed from ascorbic acid and (*E*)-hex-2-enal in a test apple beverage. In this study, we elucidate the pathway by which 6-propylbenzofuran-7-ol formed. Isotope labeling studies revealed that the propyl group of 6-propylbenzofuran-7-ol derives from (*E*)-hex-2-enal and that 6-propylbenzofuran-7-ol contains carbons 2−6 of ascorbic acid. Two compounds, namely, 2,3-dihydro-6-propylbenzofuran-3,7-diol and 3-(2-furoyl)hexanal, were identified as byproducts of a model reaction of ascorbic acid and (*E*)-hex-2-enal. Each of these compounds was dissolved in an aqueous solution of citric acid and stored at 60 °C for 1 week. After storage, 6-propylbenzofuran-7-ol was detected from a solution of 2,3-dihydro-6-propylbenzofuran-3,7-diol, but not from 3-(2-furoyl)hexanal. 6-Propylbenzofuran-7-ol was formed by isolating tricyclic hemiacetal lactone derived from the Michael addition of ascorbic acid to (*E*)-hex-2-enal, mixing the tricyclic hemiacetal lactone with the aqueous solution of citric acid, and applying heat. This confirmed that 6-propylbenzofuran-7-ol was formed via the Michael adduct.

KEYWORDS: *apple beverage, off-flavor, ascorbic acid, (E)-hex-2-enal, isotope labeling study, 6-propylbenzofuran-7-ol, Michael addition*

INTRODUCTION

Ascorbic acid is a water-soluble vitamin that plays many biological roles and is contained in many foods. Ascorbic acid is often added to processed foods as an antioxidant or nutritional supplement. But on the other hand, it is known that degradation of ascorbic acid causes problems such as nonenzymatic browning and off-flavor .[1−11]

Kurata et al.[1] reported that L-ascorbic acid was degraded to furfural with the formation of 3-deoxy-L-pentose as an intermediate in an acidic condition. This acid-catalyzed degradation reaction took place without oxygen and under the storage or cooking condition of foodstuffs. Tatum et al.[2] identified fifteen compounds as degradation products from ascorbic acid.

Ascorbic acid reacts with other constituents of foods or beverages and causes off-flavors. In experiments by König et al.[12] with citrus soft drinks, the reaction of ascorbic acid with ethyl alcohol during beverage storage produced 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon) and caused on off-flavor. In follow-up studies using isotopically labeled ethyl alcohol and ascorbic acid, König et al.[12] hypothesized that soloton could be formed by either of two pathways, that is, formation from one molecule of ascorbic acid with two molecules of ethyl alcohol or with one molecule of ethyl alcohol.

(*E*)-Hex-2-enal has a fresh, green odor, and occurs naturally in many foods. In processed foods, (*E*)-hex-2-enal is used as a flavor ingredient to impart freshness. The compound is regarded as an important flavor component of apple juice.[13,14]

In a previous work, we identified 6-propylbenzofuran-7-ol as a medicinal off-flavor compound present after the storage of a test apple beverage at 40 °C for 8 weeks.[15] Among the constituents of the test apple beverage, ascorbic acid and (*E*)-hex-2-enal were identified as important constituents of the off-flavor compound formed. This off-flavor may develop in any of the various foods found to contain the compounds ascorbic acid and (*E*)-hex-2-enal. By clarifying the mechanisms by which 6-propylbenzofuran-7-ol forms, we may have better guidance for preventing the occurrence of this off-flavor in foods with these compounds.

This study seeks to clarify the pathway by which 6-propylbenzofuran-7-ol forms. We describe our investigations using isotope-labeled precursors and the isolation of reaction intermediates for the formation of 6-propylbenzofuran-7-ol.

MATERIALS AND METHODS

Chemicals. The following chemicals were obtained commercially: (*E*)-hex-2-enal (PFW Aroma Chemicals B.V., Barneveld, Netherlands), ascorbic acid (DSM Nutrition Japan K.K., Tokyo, Japan), citric acid (IWATA CHEMICAL CO., Ltd., Shizuoka, Japan), [$^{13}C_6$]-ascorbic acid and [1-^{13}C]-ascorbic acid (Omicron Biochemicals, Inc., South Bend, IN), and [D_{10}]-*n*-butanol (C/D/N ISOTOPES, Inc., Quebec, Canada). [D_{6-8}]-(*E*)-hex-2-enal was synthesized in our laboratory. All other reagents and solvents were of analytical grade.

Synthesis. *[D_8]-n-Butanal (**2**).* Dess-Martin periodinane (75.6 g, 178 mmol) was added to a solution of [D_{10}]-*n*-butanol (**1**, 10.0 g, 118 mmol) and pyridine (23.5 g, 297 mmol) in

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CH_2Cl_2 (100 mL) at 0 °C. After 30 min of stirring at 0 °C, the reaction mixture was warmed to room temperature and stirred for 30 min, cooled back down to 0 °C (the temperature of the mixture rose spontaneously to over 50 °C during the stirring), and stirred for another 30 min at 0 °C. Then, the reaction mixture was warmed to room temperature and stirred for 30 min. Next, more Dess-Martin periodinane (5.0 g, 11.9 mmol) was added, the mixture was stirred for another 2.5 h, and saturated aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$ were added gradually, and stirred for a while. The mixture was then extracted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, and dried over $MgSO_4$. This CH_2Cl_2 solution was directly used in the next step.

MS-EI (*m*/*z*: intensity in %) 78 (54), 60 (20), 48 (55), 47 (11), 46 (100), 45 (36), 44 (35), 42 (13).

*[D_{6-8}]-(E)-Hex-2-enoic Acid (**3**).* In a flask equipped with distillation apparatus, the CH_2Cl_2 solution of [D_8]-*n*-butanal (**2**) obtained in the previous step was added to a solution of malonic acid (18.6 g, 178.2 mmol) in pyridine (22.0 g) at 50 °C. The mixture was then warmed to 80 °C as removing CH_2Cl_2 by distillation. After stirring for 1.5 h at 80 °C, the mixture was cooled to room temperature, malonic acid (9.3 g, 89 mmol), pyridine (11.0 g), and the previously removed CH_2Cl_2 solution (containing unreacted [D_8]-*n*-butanal) were added, and the mixture was warmed to 80 °C again as removing CH_2Cl_2. After stirring for 4 h at 80 °C, the mixture was cooled to room temperature, the removed CH_2Cl_2 solution (containing unreacted [D_8]-*n*-butanal) was added, and the mixture was stirred for 3 h at 80 °C. The mixture was poured into water, extracted with diethyl ether, washed with HCl (2 mol/L), and concentrated under reduced pressure. The residue was dissolved in *n*-hexane and extracted with 10% aqueous NaOH (47.5 g, 119 mmol), and the aqueous NaOH layer was washed with *n*-hexane, acidified with 50% aqueous H_2SO_4 (12.8 g, 131 mmol), and extracted with diethyl ether. The diethyl ether layer was washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure into a crude oil of [D_{6-8}]-(*E*)-hex-2-enoic acid (**3**, 15.7 g). This crude product was directly used in the next step.

MS-EI (*m*/*z*: intensity in %) 122 (19), 121 (9), 120 (7), 104 (27), 103 (21), 102 (17), 77 (33), 76 (100), 75 (53), 74 (17), 62 (18), 61 (21), 60 (17), 57 (33), 56 (19), 48 (48), 47 (35), 46 (41), 45 (58), 44 (29).

*[D_{6-8}]-Ethyl (E)-Hex-2-enoate (**4**).* Concd H_2SO_4 (1.6 g) was added to a solution of crude [D_8]-(*E*)-hex-2-enoic acid (**3**, 15.7 g) in EtOH (157 g) at room temperature and refluxed for 3 h. Then, EtOH was removed by distillation under atmospheric pressure. After cooling, the mixture was poured into water, extracted with diethyl ether, washed with saturated aqueous $NaHCO_3$, water and brine, dried over $MgSO_4$, and concentrated in a high vacuum. The residue (15.5 g) was distilled (58−61 °C/1.0 kPa) into a colorless oil of [D_{6-8}]-ethyl (*E*)-hex-2-enoate (**4**, 6.2 g; yield, 35% for 3 steps; purity, 90.5%).

MS-EI (*m*/*z*: intensity in %) 150 (2), 149 (2), 148 (2), 122 (8), 121 (7), 120 (6), 105 (78), 104 (74), 103 (57), 100 (100), 77 (18), 76 (40), 75 (26), 57 (100), 56 (54), 45 (39).

*[D_{6-8}]-(E)-Hex-2-enol (**5**).* Under N_2 atmosphere, a solution of $AlCl_3$ (8.0 g, 60 mmol) in diethyl ether (100 mL) was added dropwise to a stirred suspension of $LiAlH_4$ (1.5 g, 40 mmol) in diethyl ether (100 mL) for 30 min at 0 °C. After stirring for 30 min at 0 °C, a solution of (*E*)-hex-2-enoate (**4**, 6.0 g, 40 mmol) in diethyl ether (50 mL) was added dropwise to the mixture for 1 h at 0 °C, and then stirred for 1 h at 0 °C. After the addition of water, HCl (2 mol/L) was added, the organic layer was separated, and the aqueous layer was extracted with diethyl ether. The combined organic layer was washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated by distillation under atmospheric pressure. CH_2Cl_2 (100 mL) was added to the residue and then directly used in that form in the next step.

MS-EI (*m*/*z*: intensity in %) 108 (0.7), 107 (0.5), 89 (9), 88 (8), 87 (4), 59 (28), 58 (100), 45 (25), 44 (28).

*[D_{6-8}]-(E)-Hex-2-enal (**6**).* Under N_2 atmosphere, MnO_2 (21.6 g) was added to CH_2Cl_2 solution of [D_{6-8}]-(*E*)-hex-2-enol (**5**) from the previous step and stirred at room temperature for 14.5 h. MnO_2 was added to the mixture twice in equal portions of 21.6 g, with stirring for 4.5 and 2.5 h after the first and second additions, respectively. After filtration, a grain of 2,6-di-*tert*-butyl-4-methylphenol (BHT) was added, and concentrated under reduced pressure (30 °C, 70 kPa). The residue was purified by silica gel column chromatography eluted with *n*-pentane/diethyl ether (50:1, v/v) into a crude product (**6**, 3.2 g). The crude product was distilled under reduced pressure in a Kugelrohr apparatus (110 °C, 2.0 kPa) to give a colorless oil of [D_{6-8}]-(*E*)-hex-2-enal (**6**, 2.4 g; yield, 57% for 2 steps; purity, 93.3%). Arbitrary numbering of carbon atoms in NMR data refers to Figure 1.

[D_{6-8}]-(*E*)-hex-2-enal **6**; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.25 and 2.27 (s, s, total 1H, H−C4 of [D_6]-**6** and [D_7]-**6**); 6.09 (d, J = 8.0, 1H, H−C2); 9.48 (d, 1H, J = 8.0, H−C1). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.4 (septet, C6), 20.0 (m, C5), 33.3 (m, C4 of [D_8]-**6**), 33.9 (t, C4 of [D_7]-**6**), 34.3 (C4 of [D_6]-**6**), 133.0 (C2), 158.3 (t, C3), 194.1 (C1). MS-EI (*m*/*z*: intensity in %) 106 (18), 105 (19), 104 (17), 88 (54), 87 (40), 86 (34), 76 (20), 75 (21), 73 (29), 72 (35), 71 (24), 60 (100), 59 (59), 58 (40), 57 (74), 56 (45), 48 (50), 47 (43), 46 (92), 45 (95), 44 (72), 42 (52), 41 (55), 40 (37).

Labeling Experiments. Three labeling experiments were performed. Solutions with the following composition were prepared: ascorbic acid (20 mg), citric acid (2 mg), (*E*)-hex-2-enal (2 mg) and deionized water (20 mL). (*E*)-Hex-2-enal was substituted with [D_{6-8}]-(*E*)-hex-2-enal in one solution, ascorbic acid was substituted with [$^{13}C_6$]-ascorbic acid in another solution, and ascorbic acid was substituted with [1-^{13}C]-ascorbic acid in a third solution. The three solutions were poured into glass bottles, and stored at 60 °C in darkness for 1 week, and extracted with diethyl ether (3 × 10 mL). The organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, concentrated with a Vigreux column under atmospheric pressure, and analyzed by GC-MS.

Model Reaction. Ascorbic acid (200 mg), citric acid (20 mg) and (*E*)-hex-2-enal (20 mg) were dissolved in deionized water (200 mL) to give a model solution with a pH of about 3.2. The solution was poured into glass bottle and stored at 60 °C in darkness for 1 week. After storage, the solution was extracted with diethyl ether (3 × 20 mL). Diethyl ether solution of tridecane (0.2 g/L, 1.0 mL) was added to the organic layer as an internal standard (IS), and the organic layer was dried over anhydrous sodium sulfate, filtered, concentrated with a Vigreux column under atmospheric pressure, and analyzed by GC-MS.

Isolation of Diol (8) and Ketoaldehyde (9). Ascorbic acid (1.0 g, 5.7 mmol), citric acid (0.1 g, 0.52 mmol) and (*E*)-hex-2-enal (1.0 g, 10.2 mmol) were dissolved in deionized water (1000 mL). The solution was poured into a glass bottle,

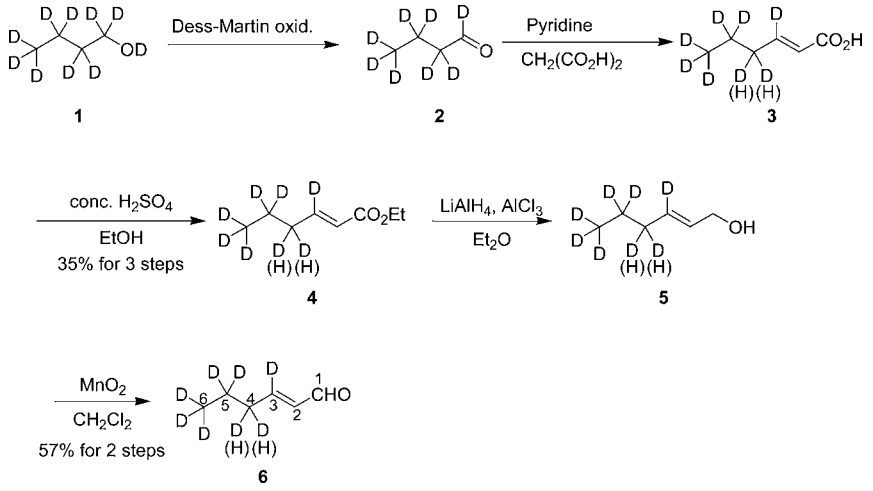

Figure 1. Synthesis of labeled (*E*)-hex-2-enal.

stored at 70 °C in darkness for 1 week, and extracted with diethyl ether (3 × 200 mL). The organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue (0.52 g) was purified by silica gel chromatography using *n*-hexane/ethyl acetate (5:1, v/v) and *n*-hexane/ethyl acetate (2:1, v/v) in sequence into a yellow oil of 3-(2-furoyl)hexanal (**9**, 152 mg, yield: 13.8%, purity: 93.7%) and a colorless solid of 2,3-dihydro-6-propylbenzofuran-3,7-diol (**8**). This solid was recrystallized in CH_2Cl_2 to give colorless needles of 2,3-dihydro-6-propylbenzofuran-3,7-diol (**8**, 38 mg, yield: 3.4%, purity: 98.7%). Arbitrary numbering of carbon atoms in NMR data refers to Figure 6.

3-(2-Furoyl)hexanal **9**; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (t, J = 7.6, 3H, H−C12); 1.30 (m, 2H, H−C11); 1.47 (m, 1H, H−C10); 1.69 (m, 1H, H−C10); 2.61 (dd, J = 4.4, 18.8, 1H, H−C8); 3.07 (dd, J = 8.8, 18.8, 1H, H−C8); 3.71 (dddd, J = 4.4, 6.8, 6.8, 8.8, 1H, H−C9); 6.53 (dd, J = 1.6, 3.4, 1H, H−C5); 7.23 (d, J = 3.6, 1H, H−C4); 7.59 (d, J = 1.6, 1H, H−C6); 9.73 (s, 1H, H−C7). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0 (C12), 20.3 (C11), 34.5 (C10), 40.6 (C9), 45.2 (C8), 112.4 (C5), 117.8 (C4), 146.6 (C6), 152.4 (C3), 191.2 (C2), 200.5 (C7). MS-EI (*m*/*z*: intensity in %) 194 (M+, 0.2), 152 (44), 123 (76), 95 (100), 55 (10), 39(12).

2,3-Dihydro-6-propylbenzofuran-3,7-diol **8**; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.94 (t, J = 7.3, 3H, H−C12); 1.60 (tq, J = 7.3, 7,3, 2H, H−C11); 2.56 (t, J = 7.3, 2H, H−C10); 3.8 (m, 2H, H−C6); 4.86 (dd, J = 5.0, 5.92, 1H, H−C5); 5.75 (br, 1H, HO-C2); 6.43 (d, J = 7.8, 1H, H−C7); 6.60 (d, J = 7.8, 1H, H−C8); 8.39 (br, 1H, HO-C5). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (C12), 22.8 (C11), 31.7 (C10), 66.3 (C6), 75.8 (C5), 117.7 (C7), 120.7 (C4), 121.1 (C8), 128.8 (C9), 142.3 (C3), 143.3 (C2). MS-EI (*m*/*z*: intensity in %) 194 (M+, 36), 176 (3), 165 (100), 147 (13), 137 (13), 123(12), 91 (12), 77 (7).

Gas Chromatography−Mass Spectrometry (GC-MS). The GC-MS analyses were performed with an Agilent 7890 gas chromatograph (GC) combined with an Agilent MSD5975 quadrupole mass spectrometer equipped with a TC-1 capillary column (0.25 mm i.d. × 60 m, 0.25 μm film thickness; GL Sciences Co., Tokyo, Japan). Each sample was injected in 0.2 μL volumes in a split mode (30: 1) at a constant temperature of 250 °C. The oven temperature was held at 40 °C for the initial 3 min and then increased to 250 °C at a rate of 3 °C/min, with a constant carrier helium gas flow of 1.8 mL/min. Mass spectra in the electron impact (EI) mode were recorded at 70 eV ionization energy.

Gas Chromatography (GC). The GC analyses were performed with an Agilent 6890 GC equipped with a flame ionization detector (FID; 250 °C). The column, sample volume, split ratio, injection temperature, oven temperature program, carrier gas, and flow rate were all the same as those set for the GC-MS analysis described above. The purity of the compounds was calculated by integration of the chromatogram obtained by the FID.

Nuclear Magnetic Resonance (NMR) Spectra. ^{1}H, ^{13}C, HMQC, and HMBC experiments were performed on a JEOL JNM-ECX400 spectrometer, using $CDCl_3$ or DMSO-d_6 as solvent. The chemical shifts were measured from the signal of tetramethylsilane used as an internal standard. The chemical shifts (δ) and coupling constants (J) are expressed in parts per million (ppm) and hertz (Hz), respectively.

Fourier Transformation Infrared Spectroscopy (FTIR). The IR spectrum was recorded on a JUSCO FT/IR-4100 FTIR spectrometer from 4000 to 400 cm^{-1}.

High Resolution fast atom bombardment Mass Spectra (HR-FAB-MS). The HR-FAB-MS were recorded on a JEOL JMS-700 with a matrix of glycerol and PEG200.

Reaction of Diol (8). Diol (**8**, 20 mg) and citric acid (1.0 g) were dissolved in deionized water (100 mL) to give a solution with a pH of about 3. The solution was poured into a glass bottle, stored at 60 °C in darkness for 1 week and extracted with diethyl ether (3 × 20 mL). The organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, concentrated with a Vigreux column under atmospheric pressure, and analyzed by GC-MS.

Reaction of Ketoaldehyde (9). Ketoaldehyde (**9**, 20 mg) and citric acid (1.0 g) were dissolved in deionized water (100 mL) to give a solution with a pH of about 3. The solution was stored and the volatile fractions were isolated by the method described above.

Synthesis of Tricyclic Hemiacetal Lactone (11). Under nitrogen atmosphere, ascorbic acid (17.6 g, 0.10 mol) was dissolved in deionized water (100 mL). Hydrochloric acid (0.5 mL) and (*E*)-hex-2-enal (10.8 g, 0.11 mol) were added and then stirred for 1 week at room temperature. The reaction mixture was extracted by *n*-hexane (30 mL), and then extracted by ethyl acetate (6 × 30 mL). The ethyl acetate layers were combined, dried over $MgSO_4$, and concentrated under high

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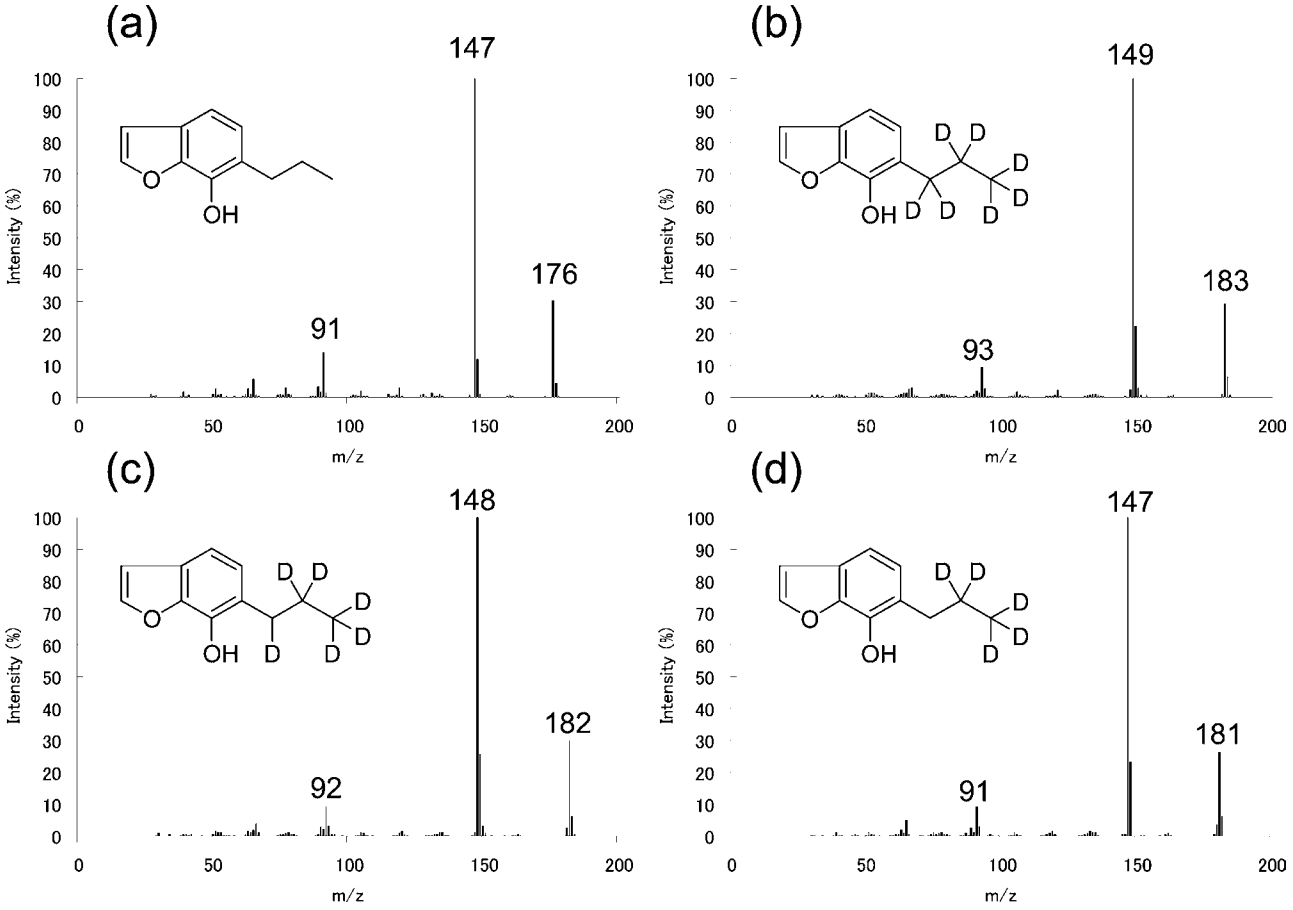

Figure 2. Mass spectra of (a) unlabeled 6-propylbenzofuran-7-ol, (b) [D_7]-6-propylbenzofuran-7-ol, (c) [D_6]-6-propylbenzofuran-7-ol, and (d) [D_5]-6-propylbenzofuran-7-ol formed from [D_{6-8}]-(*E*)-hex-2-enal and unlabeled ascorbic acid.

vacuum to yield a yellow oil (16.8 g). The crude product was recrystallized from methyl *tert*-butyl ether (MTBE)/*n*-hexane to give colorless needles of 1,3,7-trioxa-8-oxo-5,9,12-trihydroxy-10-propyltricyclo-[4.3.2.$0^{2,6}$.$0^{2,9}$]-dodecane (**11**, 10.1 g, yield: 36.9%). Arbitrary numbering of carbon atoms in NMR data refers to Figure 6.

1,3,7-Trioxa-8-oxo-5,9,12-trihydroxy-10-propyltricyclo-[4.3.2.$0^{2,6}$.$0^{2,9}$]-dodecane **11**; mp, 77 °C. $[\alpha]_D^{20}$ = +18.2° (*c* 0.01, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.88 (t, *J* = 6.8, 3H, H−C12); 1.18−1.26 (m, 2H, H−C11); 1.30−1.42 (m, 2H, H−C10); 1.72 (ddd, *J* = 5.5, 12.4, 12.4, 1H, H−C8); 1.92 (dd, *J* = 7.3, 12.4, 1H, H−C8); 2.73−2.82 (m, 1H, H−C9); 3.81 (dd, *J* = 5.0, 9.6, 1H, H−C6); 4.15 (dd, *J* = 6.8, 9.6, 1H, H−C6); 4.25 (ddd, *J* = 4.1, 5.0, 6.8, 1H, H−C5); 4.41 (s, 1H, H−C4); 5.49 (dd, *J* = 5.5, 5.5, 1H, H−C7); 5.58 (d, *J* = 4.1, 1H, HO-C5); 6.44 (d, *J* = 5.5, 1H, HO-C7); 6.73 (s, 1H, HO-C2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.1 (C12), 21.4 (C11), 30.9 (C10), 38.5 (C9), 40.3 (C8), 74.1 (C6), 74.4 (C4), 88.4 (C5), 89.0 (C2), 98.1 (C7), 106.0 (C3), 174.3 (C1). IR (KBr): cm^{-1} 3419, 2961, 2935, 2874, 1793, 1634, 1467, 1341, 1023. HRMS (FAB) found 275.1133, calculated for $C_{12}H_{19}O_7$ $[M + H]^+$ 275.1131.

Reaction of Tricyclic Hemiacetal Lactone (11). Tricyclic hemiacetal lactone (**11**, 200 mg) and citric acid (2.0 g) were dissolved in deionized water (200 mL) to give a solution with a pH of about 3. The solution was poured into a glass bottle, stored at 60 °C in darkness for 1 week, and extracted with diethyl ether (3 × 40 mL). The organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated with a Vigreux column under atmospheric pressure, and analyzed by GC-MS.

RESULTS AND DISCUSSION

Synthesis of Labeled (*E*)-Hex-2-enal. The synthesis of labeled (*E*)-hex-2-enal is outlined in Figure 1. [D_{10}]-*n*-Butanol (**1**) was oxidized with Dess-Martin periodinane to give [D_8]-*n*-butanal (**2**), whereupon the Doebner modification of [D_8]-*n*-butanal (**2**) gave [D_{6-8}]-(*E*)-hex-2-enoic acid (**3**). This step was thought that to form not only [D_8]-(*E*)-hex-2-enoic acid, but also [D_{6-7}]-(*E*)-hex-2-enoic acid, because the α-deuterium of [D_8]-*n*-butanal (**2**) was abstracted by pyridine and protonated by malonic acid. Next, **3** was esterified with ethanol to produce [D_{6-8}]-ethyl (*E*)-hex-2-enoate (**4**), whereupon **4** was reduced with AlH_3 prepared from $LiAlH_4$ and $AlCl_3$ to give [D_{6-8}]-(*E*)-hex-2-enol (**5**), then **5** was oxidized with MnO_2 to give [D_{6-8}]-(*E*)-hex-2-enal (**6**). The carbon atom on which deuterium was attached was confirmed by NMR. This [D_{6-8}]-(*E*)-hex-2-enal (**6**) was used for the labeling experiment.

Labeling Experiments. [D_{6-8}]-(*E*)-Hex-2-enal (**6**) was added to an aqueous solution of unlabeled ascorbic acid, and the mixture was stored at 60 °C for 1 week. GC-MS analysis detected [D_{5-7}]-6-propylbenzofuran-7-ol (Figure 2). A fragment ion of unlabeled 6-propylbenzofuran-7-ol at *m*/*z* 147 [M − 29] indicated cleavage of an ethyl group. Meanwhile, [D_7]-6-propylbenzofuran-7-ol gave a corresponding fragment ion at *m*/*z* 149 [M − 34], which indicated cleavage of an [D_5]-ethyl

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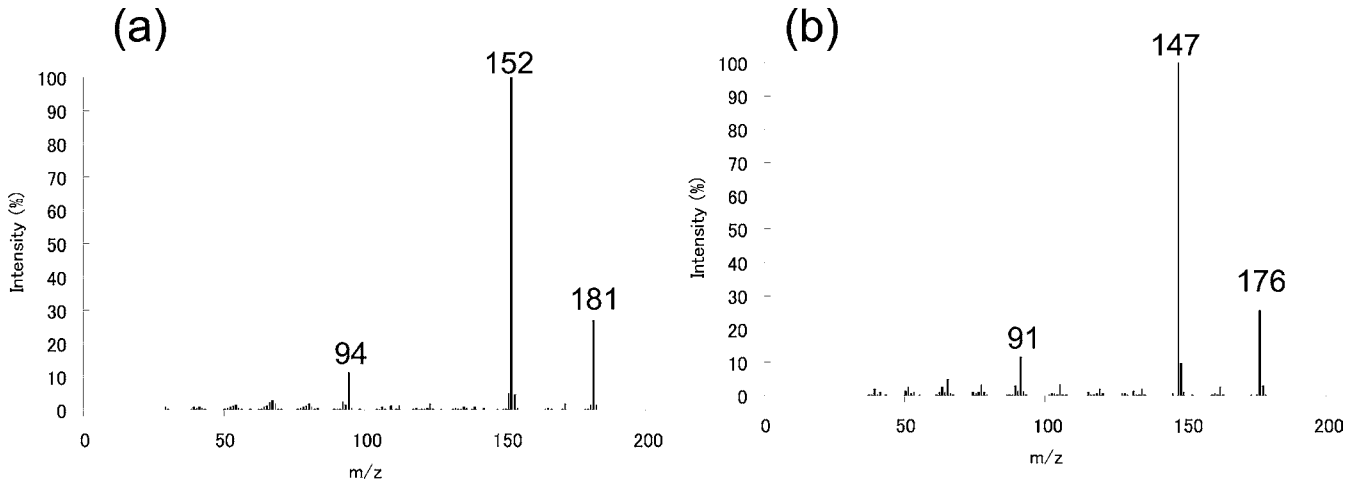

Figure 3. Mass spectra of 6-propylbenzofuran-7-ol formed from: (a) [$^{13}C_6$]-ascorbic acid and unlabeled (*E*)-hex-2-enal; (b) [1-^{13}C]-ascorbic acid and unlabeled (*E*)-hex-2-enal.

group. This indicated deuterium atoms must be located at the propyl group.

Next, when we carried out the same reaction using solution of [$^{13}C_6$]-ascorbic acid with unlabeled (*E*)-hex-2-enal, GC-MS analysis revealed the incorporation of five labeled carbons in 6-propylbenzofuran-7-ol (Figure 3a). Thus, one carbon was lost from the ascorbic acid. In earlier investigations on the degradation pathway of ascorbic acid in an acidic condition,[1,5] the pathway involved a decarboxylation step resulting in the loss of carbon 1 of the ascorbic acid. Similarly, we postulated that carbon 1 of the acid was lost as CO_2 via decarboxylation in our reaction. To confirm this hypothesis, we carried out the reaction using a solution of [1-^{13}C]-ascorbic acid with unlabeled (*E*)-hex-2-enal. GC-MS analysis detected unlabeled 6-propylbenzofuran-7-ol from this reaction (Figure 3b), which confirmed that 6-propylbenzofuran-7-ol contained carbons 2−6 of ascorbic acid. Compared with the spectra of unlabeled and [$^{13}C_5$]-6-propylbenzofuran-7-ol, three labeled carbons were incorporated in the benzene ring of [$^{13}C_5$]-6-propylbenzofuran-7-ol (*m*/*z* 91 versus 94). Therefore, two remaining labeled carbons were corresponded to the two tertiary carbons in the furan ring.

Model Reaction. To gain insight on the formation of 6-propylbenzofuran-7-ol, we identified other reaction products of ascorbic acid with (*E*)-hex-2-enal. After storage of the model solution at 60 °C for 1 week, the solution was extracted with diethyl ether and analyzed. As shown in Table 1, eight compounds were identified as major components. After storage, (*E*)-hex-2-enal decreased to about one-seventh of its initial amount. (*E*)-2-Hex-2-enoic acid was increased by oxidation of (*E*)-hex-2-enal. Furfural, 3-hydroxy-2-pyrone, and 2-furoic acid had been previously identified as degradation products of ascorbic acid in aqueous solution.[5] 2,3-Dihydro-6-propylbenzofuran-3,7-diol (**8**) and 3-(2-furoyl)hexanal (**9**) had never been reported and we identified as reaction products of ascorbic acid with (*E*)-hex-2-enal. From the structure of these compounds, it seems possible to form 6-propylbenzofuran-7-ol (**10**) by dehydration of 2,3-dihydro-6-propylbenzofuran-3,7-diol (**8**) or the intramolecular cyclization of 3-(2-furoyl)hexanal (**9**). Hence, we dissolved each of these compounds in an aqueous solution of citric acid and stored the solutions at 60 °C for 1 week. After storage, 6-propylbenzofuran-7-ol was detected from the 2,3-dihydro-6-propylbenzofuran-3,7-diol (**8**) solution, but not from the 3-(2-furoyl)hexanal (**9**). Our experiment thus demonstrated that 2,3-dihydro-6-propylbenzofuran-3,7-diol (**8**) was a precursor of 6-propylbenzofuran-7-ol (Figure 4).

Table 1. Compounds Identified from a Model Solution of Ascorbic Acid and (*E*)-Hex-2-enal after Storage at 60 °C for 1 Week

compound	RI on TC-1	ratio to IS[a] initial	ratio to IS[a] after storage
furfural	804	nd	0.61
(*E*)-hex-2-enal	834	142.88	20.50
3-hydroxypyran-2-one	965	nd	9.46
(*E*)-hex-2-enoic acid	1032	3.80	12.51
2-furoic acid	1074	nd	2.57
3-(2-furoyl)hexanal (**9**)[b]	1440	nd	1.02
6-propylbenzofuran-7-ol (**10**)[b]	1463	nd	0.26
2,3-dihydro-6-propylbenzofuran-3,7-diol (**8**)[b]	1627	nd	0.08

[a]nd, not detected. The IS was added to the extract at a 1.0 ppm concentration. [b]The compound numbering corresponds to that in Figure 6.

Possible Pathways for the Formation of 6-Propylbenzofuran-7-ol. It is known that ascorbic acid undergoes Michael-type addition to α,β-unsaturated aldehydes such as acrolein and gives the tricyclic hemiacetal lactone (Figure 5).[16] This addition takes place below pH 4 of ascorbic acid, and no basic catalyst is necessary. For this study, we assumed that the Michael addition of ascorbic acid to (*E*)-hex-2-enal took place in the same way and led, in turn, to the formation of 6-propylbenzofuran-7-ol via tricyclic hemiacetal lactone. We thus attempted to isolate the tricyclic hemiacetal lactone. (*E*)-Hex-2-enal was added to an aqueous solution of ascorbic acid containing a small amount of hydrochloric acid as catalyst, and stirred at room temperature under nitrogen atmosphere for 1 week. According to an earlier report on the similar reaction with acrolein, the tricyclic hemiacetal lactone formed via the Michael addition was precipitated as a crystalline product.[16] In our experiment, no crystalline product was precipitated by the reaction of ascorbic acid and (*E*)-hex-2-enal. Next, the reaction mixture was extracted with *n*-hexane to remove unreacted (*E*)-hex-2-enal, and then extracted with ethyl acetate. After the ethyl acetate was removed, the crude product was recrystallized from

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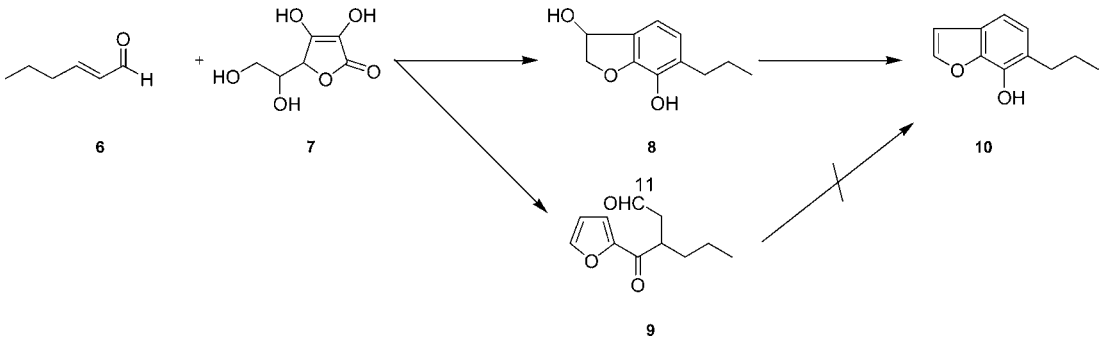

Figure 4. Formation pathway of 6-propylbenzofuran-7-ol from ascorbic acid and (*E*)-hex-2-enal.

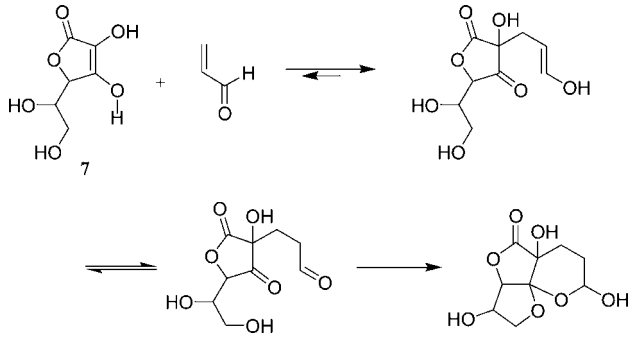

Figure 5. Michael addition of ascorbic acid to acrolein.[16]

MTBE/*n*-hexane and the crystalline product could be isolated. NMR analyses identified the crystalline product as tricyclic hemiacetal lactone **11**.

Next, **11** was dissolved in an aqueous solution of citric acid and stored at 60 °C for 1 week. A GC-MS study after the 1 week of storage detected 6-propylbenzofuran-7-ol (**10**), 2,3-dihydro-6-propylbenzofuran-3,7-diol (**8**), and 3-(2-furoyl)-hexanal (**9**). All of these compounds were confirmed to have been formed via tricyclic hemiacetal lactone **11**.

On the basis of the foregoing, we proposed the following as a possible reaction pathway (Figure 6). The first step is an acid-catalyzed Michael addition of the ascorbic acid carbanion to (*E*)-hex-2-enal to form lactone **11**. After rearrangement into **12**, decarboxylation of **12** leads to diketone **13**. The reaction pathway is divided into two routes from this intermediate **13**.

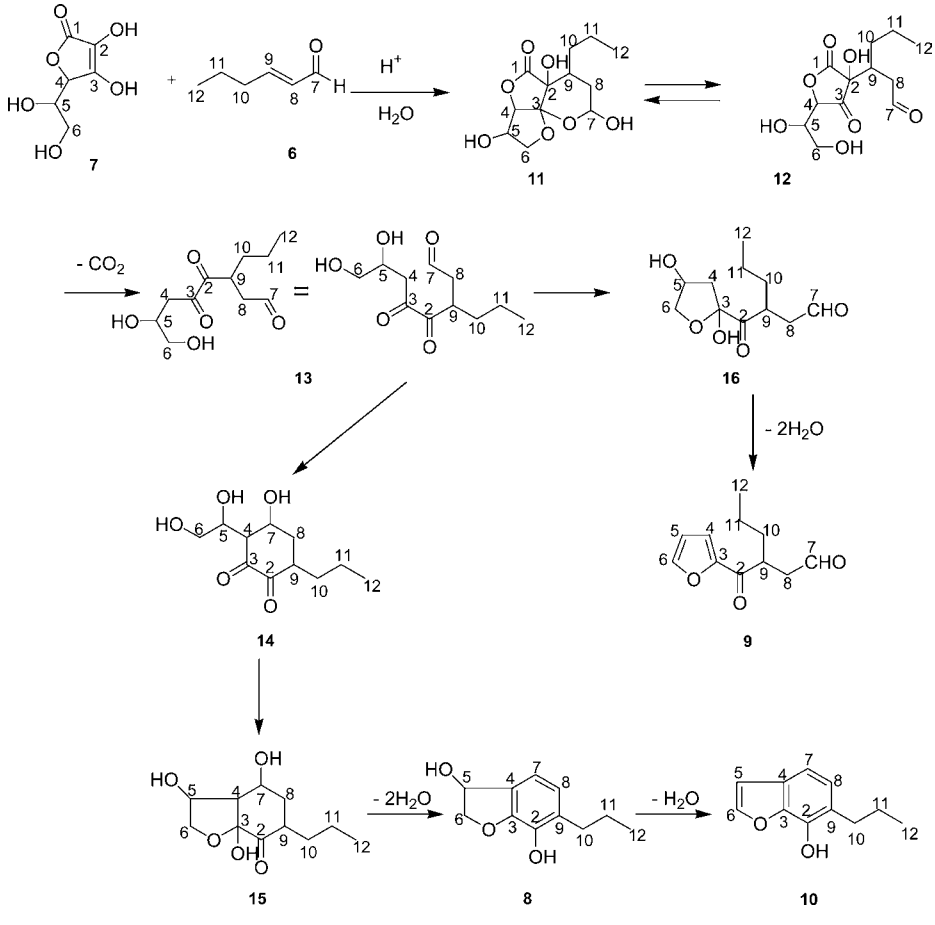

Figure 6. Possible reaction pathway of the formation of 6-propylbenzofuran-7-ol from ascorbic acid and (*E*)-hex-2-enal.

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Subsequent cyclization by an intramolecular aldol reaction yields compound **14**. Next, compound **15** is formed by the further cyclization of **14** via the intramolecular addition of the terminal hydroxyl group to the carbonyl group, and the subsequent dehydration of **15** yields 6-propylbenzofuran-7-ol via diol **8**. If diketone **13** undergoes cyclization by an intramolecular addition of the terminal hydroxyl group to the carbonyl group, then compound **16** is formed. Subsequent dehydration yields ketoaldehyde **9**.

In conclusion, we elucidated the possible reaction pathway of 6-propylbenzofuran-7-ol which is an off-flavor compound formed from (*E*)-hex-2-enal and ascorbic acid by the investigations using isotope labeled precursors and by isolation of the reaction intermediates. By learning details on the generation of 6-propylbenzofuran-7-ol, its formation pathway, and its reaction intermediates, we may be able to find ways to control or prevent this off-flavor. (*E*)-Hex-2-enal and ascorbic acid are widely found in natural foods, as well as in various beverages. By confirming how reaction parameters influence the formation of 6-propylbenzofuran-7-ol, we can better prevent the characteristic off-flavor that appears in foods containing (*E*)-hex-2-enal and ascorbic acid. Studies to clarify the generation conditions of 6-propylbenzofuran-7-ol are in progress.

■ ASSOCIATED CONTENT

Supporting Information

Total ion chromatogram and mass chromatogram of $[D_{6-8}]$-(*E*)-hex-2-enal synthesized according to the route outlined in Figure 1. Total ion chromatogram and mass chromatogram of $[D_{5-7}]$-6-propylbenzofuran-7-ol formed from $[D_{6-8}]$-(*E*)-hex-2-enal and unlabeled ascorbic acid. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

GC-MS, gas chromatography−mass spectrometry; NMR, nuclear magnetic resonance; MTBE, methyl *t*-butyl ether; RI, retention index; HMQC, heteronuclear multiple quantum correlation; HMBC, heteronuclear multiple bond correlation; FTIR, Fourier Transformation Infrared Spectroscopy; HRMS, High Resolution Mass Spectra; FAB, fast atom bombardment.

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