Synthesis of Deuterated Volatile Lipid Degradation Products To Be Used as Internal Standards in Isotope Dilution Assays. 2. Vinyl Ketones

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The isotopically labeled compounds [5,6-$^2H_2$]-(*Z*)-1,5-octadien-3-one (**d-I**) and [1-$^2H_{1;2}$,2-$^2H_{1;1}$]-1-octen-3-one (**d-II**), as well as the unlabeled reference compound (*Z*)-1,5-octadien-3-one (**I**) were prepared by improved synthesis procedures. Labeling position, chemical purity, and isotopic distribution of the compounds were characterized by various MS and NMR techniques. These molecules are used as internal standards in quantification experiments based on isotope dilution assay. The newly prepared compound **d-II** was synthesized in a simple two-step procedure, and formation of the main isotopomers was studied in model systems.

Keywords: *Synthesis; isotope labeling; deuteration; flavor compounds; lipid degradation products; [1-$^2H_{1;2}$,2-$^2H_{1;1}$]-1-octen-3-one; [5,6-$^2H_2$]-(Z)-1,5-octadien-3-one; NMR; GC/MS; isotope dilution assay (IDA)*

INTRODUCTION

Lipid-derived volatile compounds such as (*Z*)-1,5-octadien-3-one (**I**) and 1-octen-3-one (**II**) (Figure 1) contribute to the characteristic and desired flavor of food products but can also cause off-flavors depending on their concentration. The metallic, geranium-like smelling odorant **I** was shown to participate in the reversion flavor of soybean oil (Ullrich and Grosch, 1988a). Compound **II**, smelling mushroom-like and metallic, was identified as a key odorant of mushrooms (Fischer and Grosch, 1987). These flavor compounds are formed from linolenate (Ullrich and Grosch, 1988b) and linoleate (Grosch, 1987), respectively.

Because odorants such as **I** and **II** often occur in low concentrations in foods, special efforts must be made to obtain reliable quantitative results. Isotope dilution assay (IDA) has been shown to be a powerful quantification method in flavor research [Schieberle and Grosch, 1987; review by Grosch (1994) and Schieberle (1995)]. IDA is based on the use of labeled internal standards added to the product prior to sample preparation to compensate for losses during the cleanup procedure. The labeled internal standard and the analyte are monitored by mass spectrometry (MS). Both labeled and unlabeled flavor compounds of known purity must be available for establishing the calibration curves.

As recently discussed by Milo and Blank (1998), the availability of labeled compounds is still the limiting factor in the development of IDA. The aim of this work was to prepare deuterated analogues of **I** and **II** and characterize their chemical and isotopic purity. The following compounds were synthesized (Figure 1): [5,6-$^2H_2$]-(*Z*)-1,5-octadien-3-one (**d-I**) and [1-$^2H_{1;2}$,2-$^2H_{1;1}$]-1-octen-3-one (**d-II**), as well as the commercially unavailable (*Z*)-1,5-octadien-3-one (**I**).

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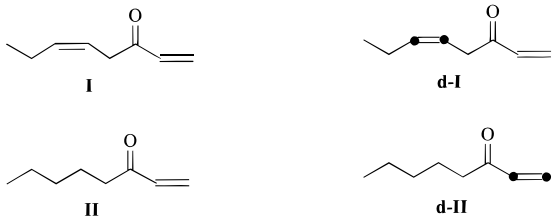

Figure 1. Chemical structures of (*Z*)-1,5-octadien-3-one (**I**), 1-octen-3-one (**II**), and their corresponding deuterated analogues (● indicates the labeling position; the abbreviation "d" means only that the compound is deuterated, without specifying number or position of the deuterium atoms).

EXPERIMENTAL PROCEDURES

Materials and Reagents. The unlabeled flavor compound 1-octen-3-one (**II**) was commercially available from Oxford Chemicals (purity = 98%, Brackley, England). The starting materials for the syntheses were of highest purity: (*Z*)-3-hexen-1-ol (**IV**, 95%) was from Fluka (Buchs, Switzerland), 1-octyn-3-ol (**VII**, 98%) was from Lancaster (Morecambe, England), and 3-hexyn-1-ol (**III**, 98%) was from Aldrich (Buchs, Switzerland). The $^2H_2$-labeled compounds deuterium oxide (^2H_2O, >99.8% ^2H), lithium aluminum deuteride ($LiAl^2H_4$, >99% ^2H), and deuteromethanol (MeO^2H, >99.5% ^2H) were obtained from Fluka. Deuterium gas ($^2H_2$, >99.8% ^2H) was from Carbagas (Lausanne, Switzerland) and deuterochloroform (C^2HCl_3, 99.8%) from Dr. Glaser AG (Basel, Switzerland). The following reagents were used: chromium(VI) oxide (CrO_3, >99%) and pyridinium chlorochromate (PCC, ≥98%) from Fluka; vinylmagnesium bromide ($BrMgC_2H_3$, 1 M in THF) and palladium on $CaCO_3$ (deactivated with lead, Lindlar's catalyst) from Aldrich.

Anhydrous dichloromethane (CH_2Cl_2), diethyl ether (Et_2O), pyridine, and tetrahydrofuran (THF) stored over molecular sieves (H_2O <0.005%) as well as ethanol (EtOH, >99.8%), methanol (MeOH, >99.5%), sulfuric acid (H_2SO_4, 98%), and Florisil adsorbent for chromatography (100−200 mesh) were purchased from Fluka. Sodium hydrogencarbonate ($NaHCO_3$), sodium sulfate (Na_2SO_4), sodium acetate (NaOAc), and silica gel 60 were from Merck (Darmstadt, Germany).

Analytical Methods. *Gas Chromatography (GC).* This was performed on a Hewlett-Packard HP-5890 gas chromatograph

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(Geneva, Switzerland) equipped with a splitless injector and a flame ionization detector (FID). J&W fused silica capillary columns were employed: DB-5, DB-1701, DB-FFAP, and DB-Wax (30 m × 0.32 mm, film thickness = 0.25 μm) using different oven temperature programs (Lin et al., 1999). The carrier gas was helium (100 kPa). Linear retention indices (RI) were calculated according to the method of van den Dool and Kratz (1963).

GC/MS. This was performed on a Finnigan MAT 8430 mass spectrometer (Bremen, Germany). Electron impact (EI) mass spectra were generated at 70 eV and positive chemical ionization (PCI) at 150 eV with ammonia as the reagent gas. Volatile components were sampled via a Hewlett-Packard HP-5890 gas chromatograph (Geneva, Switzerland) equipped with a cold on-column injector. DB-1701 or DB-Wax fused silica capillary columns were used (Lin et al., 1999). The carrier gas was helium (90 kPa). Relative abundances of the ions are given in percent. Selected ion monitoring (SIM) of $[M + NH_3]^+$ ions was used for determining the distribution of doubly and triply deuterated 1-octen-3-ol.

For the determination of isotopic purity, GC/MS was performed on a Finnigan SSQ 7000 using PCI at 200 eV and isobutane as reagent gas (Lin et al., 1999). The samples were introduced by splitless injection (1 μL) using the DB-1701 capillary column described above. Helium was used as carrier gas (70 kPa).

Nuclear Magnetic Resonance (NMR) Spectroscopy. Samples were analyzed in WILMAD 528-PP 5 mm Pyrex NMR tubes using deuterated chloroform as solvent. If not otherwise specified, the NMR spectra were acquired on a Bruker AM-360 spectrometer at 360.13 MHz for ^{1}H and at 90.56 MHz for ^{13}C under standard conditions (Lin et al., 1999). All shifts are cited in parts per million from the internal tetramethylsilane (TMS) standard. Proton NOE difference spectra (**d-I**), selective homonuclear decoupled spectra (**IV** and **VI**), DEPT spectra (**d-I** and **d-II**), and two-dimensional COSY (**VI**) and HETCOR (**d-I** and **VI**) were acquired as described earlier (Lin et al., 1999) to make unequivocal assignments (detailed experimental conditions can be obtained upon request). The SPECINFO online program SPECAL (Lin et al., 1999) was applied to some of the ^{13}C NMR spectra for the assignment of equivocal signals.

Chemical and Isotopic Purity. This was determined on the basis of GC-FID, GC/MS (PCI), and NMR data (Rohwedder, 1985; Lin et al., 1999). The nondeuterated substances were analyzed by GC/MS (PCI) for isotope correction of the labeled compounds.

Synthesis of [5,6-^{2}H$_2$]-(*Z*)-1,5-Octadien-3-one (d-I). *[3,4-^{2}H$_2$]-(Z)-3-Hexen-1-ol (**d-IV**).* A solution of 3-hexyn-1-ol (**III**, 4.0 g, 41 mmol) in MeO^{2}H (100 mL) was deuterated with Lindlar's catalyst (200 mg) under normal pressure at room temperature. Samples were taken and analyzed by GC periodically to monitor the process of deuteration that was complete after 3.5 h. The reaction was stopped, and the catalyst was removed by filtration. After removal of MeOH, the residue was distilled under vacuum (51−52 °C, 12 mbar), giving rise to 3.7 g (36 mmol, 88% yield) of a colorless oil of **d-IV** with a purity of 98% (GC): ^{1}H NMR (400 MHz, C^{2}HCl$_3$) δ 3.62 (t, J = 6.8 Hz, 2H, 1-CH$_2$), 2.30 (t, J = 6.8 Hz, 2H, 2-CH$_2$), 2.07 (q, J = 7.7 Hz, 2H, 5-CH$_2$), 0.98 (t, J = 7.7 Hz, 3H, 6-CH$_3$); ^{13}C NMR (100 MHz, C^{2}HCl$_3$) δ 134.8 (s 1:1:1, J_{C^2H} = 23 Hz, 3-C^{2}H), 124.4 (s 1:1:1, J_{C^2H} = 24 Hz, 4-C^{2}H), 62.0 (t, 1-CH$_2$), 30.4 (t, 2-CH$_2$), 20.4 (t, 5-CH$_2$), 14.0 (q, 6-CH$_3$). These spectra, compared to those acquired with a 360 MHz instrument of commercial nondeuterated **IV** (data not shown), unambiguously proved the above molecular structure and deuteration pattern.

*[3,4-^{2}H$_2$]-(Z)-3-Hexenal (**d-V**).* Collin's reagent was prepared by adding CrO_3 (25.2 g, 0.25 mol) to a stirred solution composed of anhydrous pyridine (40 g, 0.5 mol) and CH_2Cl_2 (600 mL). The mixture was stirred for 15 min at a temperature not exceeding 25 °C. A solution of **d-IV** (4.2 g, 41 mmol) in anhydrous CH_2Cl_2 (10 mL) was added in one portion. After an additional 15 min of stirring at room temperature, the solution was decanted from the residue and the latter was washed with dry Et_2O (2 × 100 mL). The combined organic layers were filtered through a short pad of Florisil, and the filtrate was washed with cold HCl (1 N) until pH 2−3 and then with saturated NaCl until neutral. The solvent was removed by distillation on a Vigreux column (50 × 1 cm). The residue was further distilled under vacuum (30−31 °C, 21 mbar), obtaining 1.84 g (18.4 mmol, 45% yield) of **d-V** with a purity of 97% (GC): GC RI(DB-5) = 798, RI(DB-1701) = 885, RI(FFAP) = 1129, RI(DB-Wax) = 1138; MS-EI 100 (34, M^+), 99 (7), 86 (2), 85 (30), 84 (5), 83 (4), 82 (19), 81 (13), 80 (4), 72 (11), 71 (75), 70 (21), 69 (8), 68 (5), 58 (8), 57 (45), 56 (18), 55 (11), 54 (8), 53 (5), 52 (4), 44 (9), 43 (78), 42 (100), 41 (31), 40 (25), 39 (12); MS-CI (NH_3) 118 (100, $[M + NH_4]^+$), 100 (19, M^+).

*[5,6-^{2}H$_2$]-(Z)-1,5-Octadien-3-ol (**d-VI**).* **d-V** (1.8 g, 18 mmol) in dry THF (30 mL) was added dropwise to a stirred solution of $BrMgC_2H_3$ (1 M, 2 0 mL) cooled at 10 °C in an ice bath. The mixture was stirred overnight and then hydrolyzed using a saturated solution of NH_4Cl. The sample was extracted with Et_2O (2 × 50 mL). The solvent was removed, and the residue was distilled under vacuum (105 °C, 13−14 mbar, Büchi GKR-50), resulting in 1.2 g (9.4 mmol, 51% yield) of **d-VI** with a purity of 93% (GC): GC RI(DB-5) = 972, RI(DB-1701) = 1075, RI(FFAP) = 1483, RI(DB-Wax) = 1486; MS-EI 110 (2), 72 (65), 71 (23), 70 (8), 58 (8), 57 (100), 56 (7), 55 (6), 43 (12), 42 (11), 41 (5); MS-CI (NH_3) 164 (1, $[M + NH_3 + NH_4]^+$), 146 (7, $[M + NH_4]^+$), 128 (100, M^+ or $[M + NH_4 - H_2O]^+$).

*[5,6-^{2}H$_2$]-(Z)-1,5-Octadien-3-one (**d-I**).* Octadienol **d-VI** (0.9 g, 7.0 mmol) was oxidized with PCC to the corresponding ketone using the procedure described for the oxidation of [5,6-^{2}H$_2$]hexan-1-ol (Lin et al., 1999). About 270 mg (2.1 mmol, 30% yield) of **d-I** was obtained with a purity of 99% (GC). The isotopic purity of **d-I** was 98%, with 0.4% of [^{2}H]-**I** and 1.6% of [^{2}H$_3$]-**I**: GC RI(DB-5) = 977, RI(DB-1701) = 1080, RI(FFAP) = 1364, RI(DB-Wax) = 1371; MS-CI (NH_3) 144 (100, $[M + NH_4]^+$), 126 (20, M^+).

Synthesis of (*Z*)-1,5-Octadien-3-one (I). *(Z)-3-Hexenal (**V**).* In analogy to the synthesis of **d-V**, 3.9 g (38 mmol, 32% yield) of **V** was prepared with a purity of 85% (GC) using commercially available **IV** (12.0 g, 120 mmol) as starting material: GC RI(DB-5) = 798, RI(DB-1701) = 891, RI(FFAP) = 1141, RI(DB-Wax) = 1138; MS-EI 98 (20, M^+), 97 (7), 84 (18), 80 (20), 70 (11), 69 (48), 55 (28), 53 (18), 43 (39), 42 (100), 40 (40); MS-CI (NH_3) 116 (100, $[M + NH_4]^+$), 98 (18, M^+).

*(Z)-1,5-Octadien-3-ol (**VI**).* In analogy to the synthesis of **d-VI**, 2.1 g (16.7 mmol, 60% yield) of **VI** was prepared with a purity of 97% (GC) using **V** as starting material (2.9 g, 28 mmol): GC RI(DB-5) = 977, RI(DB-1701) = 1077, RI(FFAP) = 1486, RI(DB-Wax) = 1488; MS-EI 108 (2), 70 (70), 69 (27), 57 (100), 55 (45), 42 (17), 41 (43), 39 (15); MS-CI (NH_3) 162 (1, $[M + NH_3 + NH_4]^+$), 144 (7, $[M + NH_4]^+$), 126 (100, M^+ or $[M + NH_4 - H_2O]^+$).

*(Z)-1,5-Octadien-3-one (**I**).* The octadienol **VI** (2.0 g, 16 mmol) was oxidized to **I** in the same way as described for the oxidation of [5,6-^{2}H$_2$]hexan-1-ol (Lin et al., 1999). About 370 mg (3.0 mmol, 19% yield) **I** was obtained with a purity of 95% (GC): GC RI(DB-5) = 979, RI(DB-1701) = 1085, RI(FFAP) = 1365, RI(DB-Wax) = 1371; MS-CI (NH_3) 142 (100, $[M + NH_4]^+$), 124 (28, M^+).

Synthesis of [1-^{2}H$_{1;2}$,2-^{2}H$_{1;1}$]-1-Octen-3-one (d-II). *[1-^{2}H$_{1;2}$,2-^{2}H$_{1;1}$]-1-Octen-3-ol (**d-VIII**).* In a 200 mL three-neck reactor fitted with a reflux condenser and a thermometer was suspended 2.0 g (48 mmol) of Li^{2}H$_4$ in anhydrous THF. Compound **VII** (5.0 g, 40 mmol) dissolved in anhydrous THF (10 mL) was slowly added to the magnetically stirred solution. The mixture was refluxed under nitrogen. GC analysis indicated complete reduction of **VII** to **d-VIII** after 18 h. On cooling in an ice bath, 20 mL of heavy water was added drop by drop, followed by 80 mL of aqueous H_2SO_4 (4 N) to dissolve any insoluble residues. The organic phase was separated from the water phase, and the aqueous solution was then extracted with Et_2O (3 × 50 mL). The combined organic solutions were washed successively with saturated solutions of $NaHCO_3$ (2 × 10 mL) and NaCl (2 × 10 mL) and then dried over anhydrous Na_2SO_4. After removal of the solvent by evaporation and distillation under vacuum (59−61 °C, 10 mbar), 4.8 g (37 mmol, 92% yield) of a colorless oil of **d-VIII** with a purity of

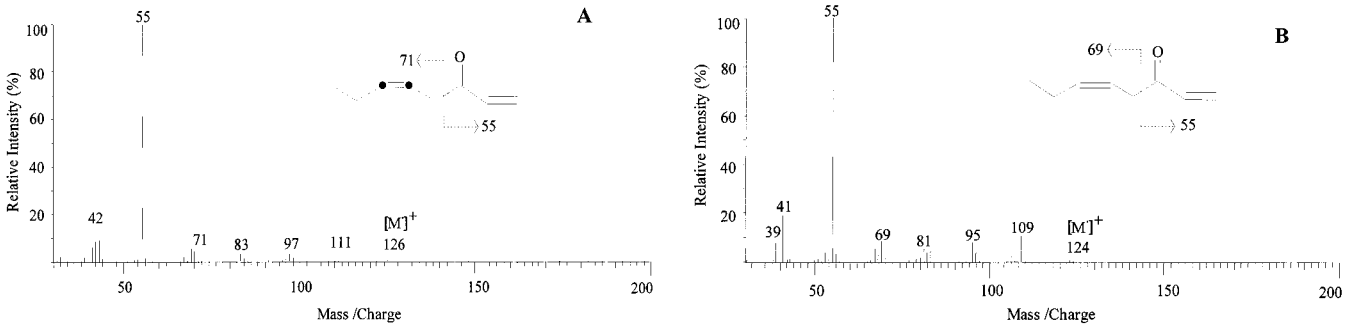
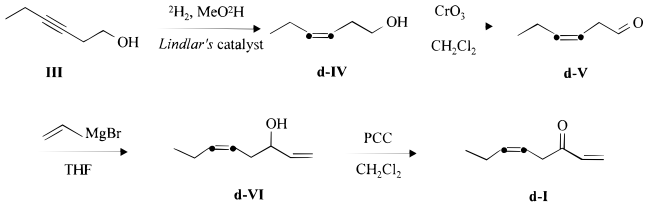


Figure 2. Mass spectrum (EI) of [5,6-$^2H_2$]-(*Z*)-1,5-octadien-3-one (**d-I**, A) and (*Z*)-1,5-octadien-3-one (**I**, B) (● indicates the labeling position).

Scheme 1. Synthesis of [5,6-$^2H_2$]-(*Z*)-1,5-Octadien-3-one (d-I) and (*Z*)-1,5-Octadien-3-one (I)[a]



[a] ● indicates the labeling position; the abbreviation "d" means that the compound is deuterated, without specifying number or position of the deuterium atoms.

97% (GC) was obtained: GC RI(DB-5) = 984, RI(DB-1701) = 1076, RI(FFAP) = 1545, RI(DB-Wax) = 1456; MS-EI 113 (1), 112 (1), 100 (2), 99 (10), 88 (10), 87 (9), 85 (4), 84 (7), 83 (10), 82 (5), 76 (2), 75 (19), 74 (18), 71 (7), 70 (8), 69 (8), 68 (5), 61 (7), 60 (100), 59 (84), 58 (8), 57 (12), 56 (8), 55 (12), 44 (6), 43 (19), 42 (5), 41 (11); MS-CI (NH_3) 149/148 (40/48, $[M + NH_4]^+$), 131/130 (100/85, M^+ or $[M + NH_4 - H_2O]^+$).

*[1-$^2H_{1;2}$,2-$^2H_{1;1}$]-1-Octen-3-one (**d-II**).* Oxidation of **d-VIII** (4.75 g, 36.5 mmol) was performed as described for the oxidation of [5,6-$^2H_2$]-hexan-1-ol (Lin et al., 1999) using 11.8 g (55 mmol) of PCC and 0.9 g (11 mmol) of anhydrous NaOAc suspended in anhydrous CH_2Cl_2. Distillation under vacuum (56−60 °C, 16 mbar) gave ∼1.9 g of a yellow oil with an intense metallic smell. The oil was further purified by column chromatography on silica gel by eluting first with pentane (100 mL) and then with pentane/Et_2O (95 + 5, v/v), resulting in 1.6 g (12.2 mmol, 34% yield) of compound **d-II** with a purity of 99% (GC). **d-II** was composed of [$^2H_2$]-**II** (55%), [$^2H_3$]-**II** (44 %), and [^2H]-**II** (1%): GC RI(DB-5) = 973, RI(DB-1701) = 1064, RI(FFAP) = 1291, RI(DB-Wax) = 1301; MS-CI (NH_3) 147/146 (100/90, $[M + NH_4]^+$), 130/129 (4/4, $[M + H]^+$).

Reduction of 1-Octyn-3-ol (VII) with Different Amounts of $LiAl^2H_4$. 1-Octyn-3-ol (**VII**, 0.6 g, 5 mmol) and various amounts of $LiAl^2H_4$ were refluxed in 10 mL of anhydrous THF as described aboved in the preparation of **d-VIII**. The molar ratios of $LiAl^2H_4$ to **VII** were 1:4, 1:2, 1:1, and 2:1. Aliquots of the reaction mixtures were taken after refluxing for 0.5, 1, 2, 4, and 18 h. The reaction products were first hydrolyzed with ^2H_2O (1 mL) and then treated with aqueous H_2SO_4 (4 N, 2 mL). The aqueous layers were extracted with Et_2O. The solvent extracts were dried over Na_2SO_4 and then diluted for GC-FID and GC/MS analysis. The solvent was completely evaporated for NMR measurements.

RESULTS AND DISCUSSION

Synthesis and Characterization of [5,6-$^2H_2$]-(*Z*)-1,5-Octadien-3-one (d-I) and (*Z*)-1,5-Octadien-3-one (I). Compounds **d-I** and **I** were prepared as shown in Scheme 1 by adapting the method reported by Ullrich and Grosch (1988b) and Guth and Grosch (1990). Lindlar's catalyst was used for partial deuteration of 3-hexyn-1-ol (**III**) to [3,4-$^2H_2$]-(*Z*)-3-hexen-1-ol (**d-IV**) via stereospecific syn-addition. According to Rakoff and Rohwedder (1992), derivatization of the OH group in **III** to eliminate replaceable hydrogen is not required prior to the deuteration step. Oxidation of **d-IV/IV** was carried out with Collin's reagent to avoid isomerization of **d-V/V** due to the reagent's lower acidity compared to PCC (Kajiwara et al., 1975). Chain elongation was achieved by a Grignard reaction of the aldehydes (**d-V/V**) with vinylmagnesium bromide followed by oxidation of the resulting alcohols (**d-VI/VI**) with PCC.

The MS-EI data of the intermediate reaction product **VI** were identical with those of (*Z*)-1,5-octadien-3-ol reported by Tressl et al. (1982). MS-CI of **d-VI** indicated incorporation of two deuterium atoms. The mass spectra of **d-I** and its unlabeled analogue (**I**) are shown in parts A and B of Figure 2, respectively. These data were in good agreement with those reported by Guth (1991) and Swoboda and Peers (1977), respectively. The shift of two units in **d-I** was indicated by the fragments at *m/z* 111, 97, 83, and 71. MS-CI of **d-I** confirmed incorporation of two deuterium atoms.

The ^1H NMR data of **VI**, **I**, and **d-I** are summarized in Table 1 and the ^{13}C NMR data of **VI** and **d-I** in Table 2. Spectral interpretation of **VI** helped to assign the NMR signals of **I** and **d-I**. Besides the exchangeable hydroxyl proton at 1.7 ppm, the proton spectrum of **VI** (Figure 3) represented nine different single spins or groups of magnetically equivalent spins forming a coupling network not easily separated into subgroups. Gaussian resolution enhancement was applied to the free induction decay to better resolve the complex line patterns (resolution enhanced spectrum not shown). A COSY spectrum permitted unambiguous assignment of both ends of the molecule. However, selective homonuclear decoupling experiments were required for unequivocal attribution of the 5-CH and 6-CH signals. Irradiation of the 4-CH_2 signal produced a loss of doublet coupling of ∼7.5 Hz on the 5-CH signal and a loss of triplet coupling of ∼1.5 Hz on the 6-CH signal, whereas irradiation of the 7-CH_2 signal produced a loss of doublet coupling of ∼7.5 Hz on the 6-CH signal and a loss of triplet coupling of ∼1.5 Hz on the 5-CH signal. The coupling of 10.9 Hz between 5-CH and 6-CH demonstrates cis configuration of the double bond. Carbon assignments were derived from those of the protons via a one-bond HETCOR experiment.

Proton NMR data of **I** (Table 1) obtained at 400 MHz are in reasonable agreement with those reported by Swoboda and Peers (1977), who were, however, not able to distinguish the 5-H and 6-H signals at 90 MHz spectral frequency. No NMR data for **d-I** were found in

Table 1. ^{1}H NMR Data of (*Z*)-1,5-Octadien-3-ol (VI), (*Z*)-1,5-Octadien-3-one (I), and [5,6-$^2H_2$]-(*Z*)-1,5-Octadien-3-one (d-I)[a]

proton	(*Z*)-1,5-octadien-3-ol (**VI**)	(*Z*)-1,5-octadien-3-one (**I**)[b]	[5,6-$^2H_2$]-(Z)-1,5-octadien-3-one (**d-I**)
1-CH_{cis}	5.26, d t, J = 17.2, 1.5 Hz, 1 H	6.26, d d, J = 18.0, 1.5 Hz, 1 H	6.26, d d, J = 17.6, 1.4 Hz 1 H
1-CH_{trans}	5.13, d d d[c], J = 10.5, 1.5, 1.3 Hz, 1 H	5.84, d d, J = 10.5, 1.5 Hz, 1 H	5.84, d d, J = 10.4, 1.4 Hz, 1 H
2-CH	5.90, d d d, J = 17.2, 10.5, 5.8 Hz, 1 H	6.38, d d, J = 18.0, 10.5 Hz, 1 H	6.39, d d, J = 17.6, 10.3 Hz, 1 H
3-H	4.15, d t t[c], J = 6.8, 5.8, 1,4 Hz, 1 H		
3-OH	1.7, broad, >1 H (includes fast exchanging H^2HO protons from solvent)		
4-CH_2	2.32, complex multiplet, ≥25 lines[c], 2 H	3.35, d, J = 7.0 Hz, 2 H	3.35, s (slightly broad), 2 H
5-CH	5.36, d t t, J = 10.9, 7.5, 1.6 Hz, 1 H	5.54[d], m, 1 H	—[e]
6-CH	5.58, d t t, J = 10.9, 7.3, 1.5 Hz, 1 H	5.62[d], m, 1 H	—[e]
7-CH_2	2.08, quintet of d t[c], J = 7.5, 1.6, 0.7 Hz, 1 H	2.08, quintet, J = 7.5 Hz, 2 H	2.07, q (slightly broad), J = 7.5 Hz, 2 H
8-CH_3	0.97, t, J = 7.5 Hz, 3 H	0.98, t, J = 7.6 Hz, 3 H	0.99, t, J = 7.5 Hz, 3 H

[a] Chemical shift δ in ppm from internal TMS. The multiplicity abbreviations used to describe NMR signals are s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). For the proton spectra, d t means doublet of triplets, with decreasing values of the absolute coupling constants (here: $|J|_{doublet} > |J|_{triplet}$). [b] Spectrum measured on a Bruker 400 MHz instrument in C^2HCl_3 with TMS as internal standard (room temperature, not precisely specified). Shifts and coupling constants for the 400 MHz spectra were determined graphically and may be less precise than the 360 MHz data. [c] Gaussian resolution enhancement required to resolve these lines. [d] Assignments not verified, might have to be interchanged. [e] The total integral value in the range 5.7−5.4 ppm was ~0.04 H. These trace signals are not necessarily due to residual 5-CH and/or 6-CH of the title compound, but may also be caused by other impurities.

Table 2. ^{13}C NMR Data of (*Z*)-1,5-Octadien-3-ol (VI) and [5,6-$^2H_2$]-(*Z*)-1,5-Octadien-3-one (d-I)[a]

carbon	(*Z*)-1,5-octadien-3-ol (**VI**)	[5,6-$^2H_2$]-(*Z*)-1,5-octadien-3-one (**d-I**)
1-CH_2	114.7, t	128.5, t
2-CH	140.5, d	136.0, d
3-C=O		198.6, s
3-C−OH	72.4, d	
4-CH_2	35.0, t	38.5, t
5-CH	123.7, d	
5-C^{2}H		119.6, s, 1:1:1, $^1J_{C^2H}$ = 24.5 Hz
6-CH	135.3, d	
6-C^{2}H		135.0, s, 1:1:1, $^1J_{C^2H}$ = 23.6 Hz
7-CH_2	20.7, t	20.8, t
8-CH_3	14.2, q	13.9, q

[a] For ^{13}C NMR spectra, s, d, t, and q denominate quaternary, CH, CH_2, and CH_3 carbons, respectively.

the literature. All of the signals of **d-I** could be individually assigned. On the basis of the spectrum of **I**, the resonances of the residual 5-H and 6-H protons would be expected in the region of 5.7−5.4 ppm. Among the spurious small signals in this region, no resonances clearly displaying the expected characteristics were found. Because of the deuteration, the (*Z*)-character of the 5/6-C double bond could not be demonstrated from the coupling constants. NOE difference spectra (irradiation of 4-CH_2) yielded a small NOE on the 7-CH_2 signals, supporting the cis arrangement. Most carbon signals of **d-I** were assigned via correlation to the proton signals by a two-dimensional HETCOR experiment. The two deuterated carbons were assigned by shift prediction with the SPECINFO SPECAL program and by comparison with **VI**.

Synthesis and Characterization of [1-$^2H_{1;2}$,2-$^2H_{1;1}$]-1-Octen-3-one (d-II). Compound **d-II** was prepared in a newly developed simple two step procedure (Scheme 2) by $LiAl^2H_4$ reduction of 1-octyn-3-ol (**VII**) to deuterated 1-octen-3-ol (**d-VIII**) followed by subsequent oxidation with PCC following the general working procedures reported by Bates et al. (1954), Snyder (1967), and Corey and Suggs (1975). This synthesis route was chosen to obtain stable molecules that are not susceptible to ^{2}H/H exchange provoked by enoliza-

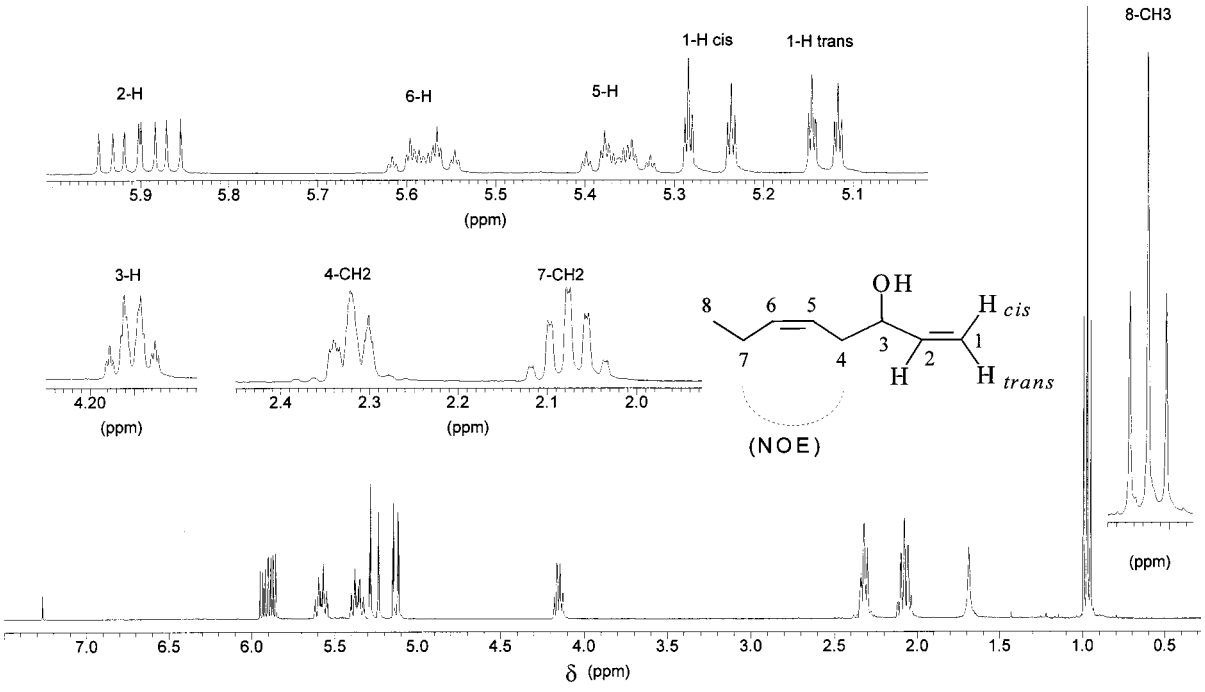
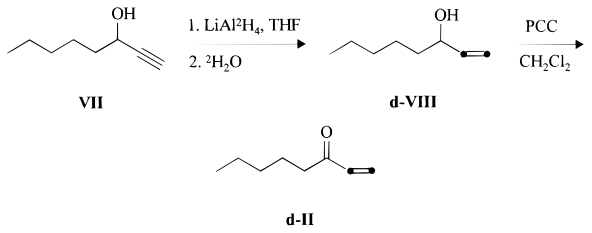


Figure 3. ^{1}H NMR spectrum of (*Z*)-1,5-octadien-3-ol (**VI**) with multiplet expansions (for explanation, see text).

Scheme 2. Synthesis of a Mixture of [1,2-$^2H_2$]-1-Octen-3-one and [1,1,2-$^2H_3$]-1-Octen-3-one (d-II)[a]



[a] ● indicates the labeling position; the abbreviation "d" means that the compound is deuterated, without specifying number or position of the deuterium atoms.

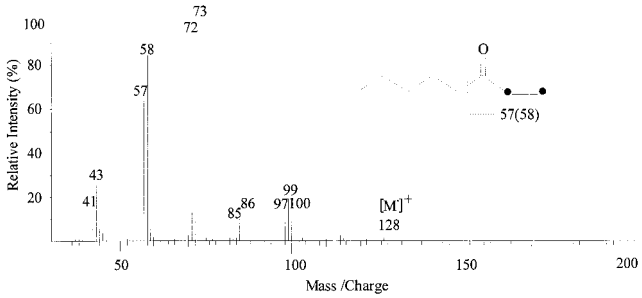


Figure 4. Mass spectrum (EI) of **d-II** composed of a mixture of [1,2-$^2H_2$]-1-octen-3-one and [1,1,2-$^2H_3$]-1-octen-3-one (● indicates the labeling position without specifying the number of deuterium atoms).

tion. Such phenomena have been observed for the compound [4,5-$^2H_2$]-1-octen-3-one (Guth and Grosch, 1990).

The presence of 1-octen-3-one was indicated by the RI values on different stationary phases. The MS-CI data suggested **d-II** to be a mixture of doubly and triply deuterated 1-octen-3-ones with nearly equivalent distribution. The shift of two or three units was indicated in the MS-EI spectrum of **d-II** (Figure 4) by the fragments at *m/z* 99/100, 85/86, 72/73 (McLafferty rearrangement), and 57/58.

^{1}H NMR and ^{13}C NMR data of **d-II** are shown in Table 3. The assignment of the aliphatic part was straightforward. The olefinic protons were identified by comparison with the proton spectrum of **I**. Our results for **d-II** agree well with earlier ^{1}H NMR data on **II**

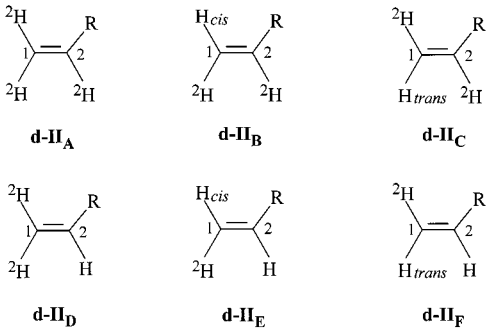


Figure 5. Structures of various isotopomers of **d-II** identified by NMR spectroscopy [the rest R represents $-CO-(CH_2)_4-CH_3$].

reported by Swoboda and Peers (1977) and with ^{13}C NMR shifts of the nonlabeled compound given by Pritzkow et al. (1979). The integral values of the olefinic protons demonstrated that **d-II** contained about half [1,1,2-$^2H_3$]-1-octen-3-one (**d-II$_A$** in Figure 5) and half [1,2-$^2H_2$]-1-octen-3-one; the deuterium atom at 1-C was nearly equally distributed between the cis (**d-II$_B$**) and trans (**d-II$_C$**) isomers. Small percentages of cis and trans [1-^{2}H]-1-octen-3-one were also present, that is, **d-II$_E$** and **d-II$_F$** (Figure 5). The NMR spectra in the olefinic region are rationalized as discussed in the following.

^{1}H NMR (Figure 6A) revealed signals at ∼6.2 ppm generated by 1-CH protons in the cis position on the double bond with respect to the rest of the molecule. The three bigger lines are equidistant with a splitting of 2.6 Hz (**d-II$_B$**). They represent a 1:1:1 signal with additional line broadening probably due to an unresolved small deuterium splitting (the fact that the middle line is higher than the outer ones is caused by their incomplete separation). Therefore, it can be postulated that this signal is due to a double bond substituted with two deuterium atoms, where $^3J_{H^2H}$ is 2.6 Hz and $^2J_{H^2H}$ much smaller. The two small "satellite" lines have a distance of 17.6 Hz, typical for a trans vinyl $^3J_{HH}$ and thus are due to a single cis proton coupling to a 2-CH (**d-II$_E$**).

The same reasoning applies for the signal at 5.8 ppm, which represents 1-CH protons trans to the rest of the molecule, with $^3J_{H^2H}$ = 1.6 Hz (**d-II$_C$**) and $^3J_{HH}$ = 10.7 Hz (**d-II$_F$**). The integrals of the signal groups near 6.2

Table 3. ^{1}H NMR and ^{13}C NMR Data of [1-$^2H_{1;2}$,2-$^2H_{1;1}$]-1-Octen-3-one (d-II)[a] (for Assignment of Isotopomers, See Text)

proton	^{1}H NMR[b]	carbon	^{13}C NMR
1-CH_{cis}[c]	6.20, ∼1:1:1, J = 2.6 Hz (**d-II$_B$**) and d (small), J = 17.6 Hz (**d-II$_E$**), total 0.2−0.3 H[d]	1-C^{2}H	127.49[e], d (∼1:1:1), $^1J_{C^2H}$ = 24.4 Hz (**d-II$_B$** or **d-II$_C$**), 127.46[e], d (∼1:1:1), $^1J_{C^2H}$ = 24.5 Hz (**d-II$_B$** or **d-II$_C$**)
1-CH_{trans}[c]	5.80, ∼1:1:1, J = 1.6 Hz (**d-II$_C$**) and d (small), J = 10.7 Hz (**d-II$_F$**), total 0.2−0.3 H[d]	1-C$^2H_2$	127.2, s (probably 1:2:3:2:1), $^1J_{C^2H}$ 24.4 Hz (**d-II$_A$**, overlapping with the 1-C^{2}H signals)
2-CH	6.3−6.4 (several signals superimposed[f], **d-II$_D$**, **d-II$_E$**, **d-II$_F$**)	2-CH	136.56, 136.54, 136.47, all d, all low intensity (**d-II$_{D-F}$**)
		2-C^{2}H	136.23[g], s, 1:1:1, $^1J_{C^2H}$ = 24.6 Hz (**d-II$_B$** or **d-II$_C$**) 136.22[g], s, 1:1:1, $^1J_{C^2H}$ = 24.5 Hz (**d-II$_B$** or **d-II$_C$**) 136.14[g], s, 1:1:1, $^1J_{C^2H}$ = 24.5 Hz (**d-II$_A$**)
		3-C=O	∼201.14, s, ≥3 unresolved lines
4-CH_2	2.58, t, J = 7.5 Hz, 2 H	4-CH_2	39.60, t
5-CH_2	1.63, m (quintet), J_{av} = 7.5 Hz, 2 H	5-CH_2	23.72[h], t
6,7-CH_2	1.2−1.4, complex m, 4 H	6-CH_2	31.46, t
		7-CH_2	22.50[h], t
8-CH_3	0.90, t, J = 6.9 Hz, 3 H	8-CH_3	13.94, q

[a] Chemical shift δ in ppm from internal TMS. The multiplicity abbreviations used to describe ^{1}H NMR signals are s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). For ^{13}C NMR spectra, s, d, t, and q denominate quaternary, CH, CH_2, and CH_3 carbons, respectively. [b] Interpulse spacing ≈ 220 s. [c] Cis and trans refer to the proton position at 1-C with respect to the aliphatic rest of the molecule at 2-C. [d] Superimposed signals of at least two isotopomers. [e] Assignment to either cis or trans proton isotopomer not attempted. [f] Signals correspond to 1-CH_2, 1-C^{2}H, and 1-C$^2H_2$ isotopomers with total <0.05 H. [g] For assignment of these signals, see text. [h] Most likely assignment for 5-CH_2 and 7-CH_2 based on SPECAL spectra prediction.

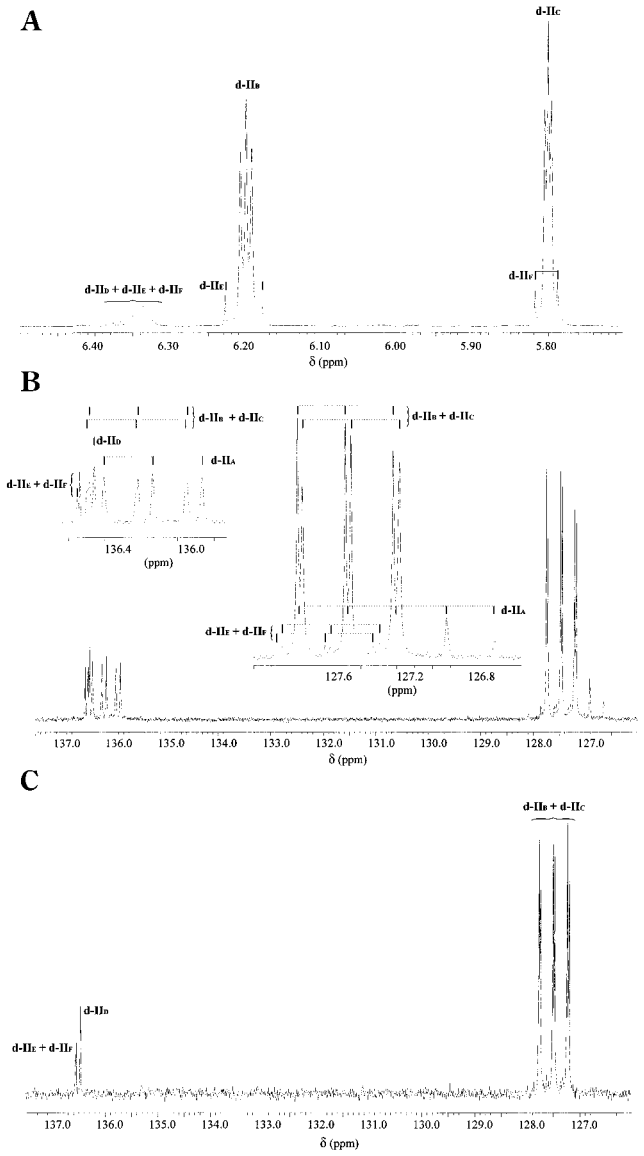


Figure 6. NMR spectra of the olefinic region of deuterated 1-octen-3-one (**d-II**) with expansions (for explanation, see text): (A) ^{1}H NMR; (B) ^{13}C NMR; (C) ^{13}C-DEPT.

and 5.8 ppm amount to ~0.25 proton values each. The complex residual signal between 6.4 and 6.3 ppm amounts to ~0.05 proton equivalents and stands for byproduct isotopomers (**d-II$_D$**, **d-II$_E$**, and **d-II$_F$**) bearing a proton instead of a deuterium atom in the 2-position.

The olefinic part of the ^{13}C NMR spectrum is shown in Figure 6B. The dominant 1-C signals are two sets of doublet 1:1:1 line patterns near 127.5 ppm, which correspond to [1,2-$^2H_2$]-**II** with deuterium at either the cis (**d-II$_B$**) or the trans (**d-II$_C$**) position of 1-C. For [1,1,2-$^2H_3$]-**II** (**d-II$_A$**), a singlet 1:2:3:2:1 line pattern near 127.2 ppm with lower intensity would be expected (upfield one-bond isotope effect of ~0.3 ppm). Because of overlap with the 1:1:1 patterns, only its two rightmost lines can be observed. Note that the ^{13}C relaxation behavior of different isotopomers can be very different, so that these signals should not be used for quantification. With sufficient digital resolution (i.e., by zero-filling of the free induction decay), three overlapping dominating 1:1:1 patterns can be found in the 2-C region near 136.2 ppm. They represent **d-II$_A$**, **d-II$_B$**, and **d-II$_C$**, which do not show up in the DEPT spectrum (Figure 6C). As for 1-C, we expect an upfield two-bond deuterium isotope effect of ~0.1 ppm. Therefore, the 2-C 1:1:1 signal at highest field is tentatively assigned to **d-II$_A$**, whereas the hardly resolved two signal groups at lower field can be attributed to **d-II$_B$** and **d-II$_C$**. As for 1-C, it is not easy to say which signal belongs to cis and which to trans.

Table 4. Formation of Deuterated 1-Octen-3-ol (d-VIII) by Reduction of 1-Octyn-3-ol (VII) with Different Amounts of LiAl$^2H_4$ at Various Reflux Times[a]

	ratio LiAl$^2H_4$/**VII**		reflux time				
sample	molar basis	$^2H/H_{acidic}$	0.5 h	1 h	2 h	4 h	18 h
1	1:4	1:2	0	0	0	0	0
2	1:2	1:1	50	50	50	50	50
3	1:1	2:1	60	70	80	90	100
4	2:1	4:1	30	35	40	50	100

[a] Approximate yields (in percent) were obtained by GC analysis. Abbreviation "d" only means that the compound is deuterated, without specifying number or position of the deuterium atoms.

The three downfield doublet signals appearing in the DEPT spectrum (Figure 6C) are due to three more isotopomers containing a 2-CH proton instead of a 2-C^{2}H deuterium (**d-II$_D$**, **d-II$_E$**, and **d-II$_F$**). Because they occur in very small quantities only, they are not further discussed. The absence of negative (triplet) signals near 127.5 ppm in the DEPT spectrum indicates that isotopomers bearing two terminal vinyl protons do not occur in appreciable amounts.

Formation Mechanism of [1-$^2H_{1;2}$,2-$^2H_{1;1}$]-1-Octen-3-one (d-II). In the synthesis of **d-II**, deuterium was introduced by reduction of 1-octyn-3-ol (**VII**) with LiAl$^2H_4$ (Scheme 2). A set of experiments was carried out to study the formation of doubly and triply deuterated 1-octen-3-ol, that is, [1,2-$^2H_2$]-**VIII** and [1,1,2-$^2H_3$]-**VIII**. Two aspects were of special interest: the overall yields of labeled 1-octen-3-ol obtained and the distribution of isotopomers formed.

Reduction of **VII** to deuterated 1-octen-3-ol (**d-VIII**) was monitored by GC analysis (Table 4). In sample 1, the reaction did not result in any **d-VIII** when using LiAl$^2H_4$/**VII** in a ratio of 1:4. Partial reduction of **VII** was observed by increasing the ratio LiAl$^2H_4$/**VII** to 1:2, and about half of the starting material was reacted to 1-octen-3-ol after 0.5 h (sample 2). However, further reflux time did not increase yields of the alcohol, indicating that the amount of LiAl$^2H_4$ was not sufficient. For a complete reduction, the molar ratio of starting material to reagent should be at least 1:1 (sample 3). 1-Octen-3-ol was then continuously generated over time and the reaction was completed after 18 h. The reaction was found to be slower when using an excess of LiAl$^2H_4$; that is, yields of **d-VIII** were only 40% after 2 h, whereas 80% was produced in the presence of equimolar amounts of LiAl$^2H_4$ and alkynol (sample 4).

GC/MS analysis of sample 1 revealed that the starting material 1-octyn-3-ol (**VII**) was transferred into the monodeuterated analogue (**d-VII**). The exact labeling position was determined by ^{1}H NMR (δ/ppm): **VII** showed characteristic signals at 2.47 (d, J = 2.2 Hz, 1 H, 1-CH) and 4.37 (broad t d, J = 6.6, 1.8 Hz, 1 H, 3-CH), and **d-VII** was characterized by signals at 2.47 (d, J = 2.2 Hz, 0.04 H, 1-CH) and 4.37 (broad t, J = 6.4 Hz, 1 H, 3-CH). In both **VII** and **d-VII**, the 3-CH signals were broad, so that coupling constant could not exactly be determined. To obtain quantitative proton signals, relaxation delays of at least 80 s had been used. The

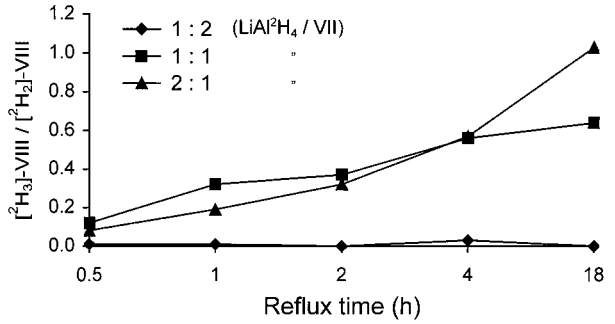


Figure 7. Formation of [$^2H_3$]-1-octen-3-ol ([$^2H_3$]-**VIII**) and [$^2H_2$]-1-octen-3-ol ([$^2H_2$]-**VIII**) by reacting $LiAl^2H_4$ with 1-octyn-3-ol (**VII**) at the $LiAl^2H_4$/**VII** molar ratios of 1:2, 1:1, and 2:1 for various reflux times monitored by GC/MS.

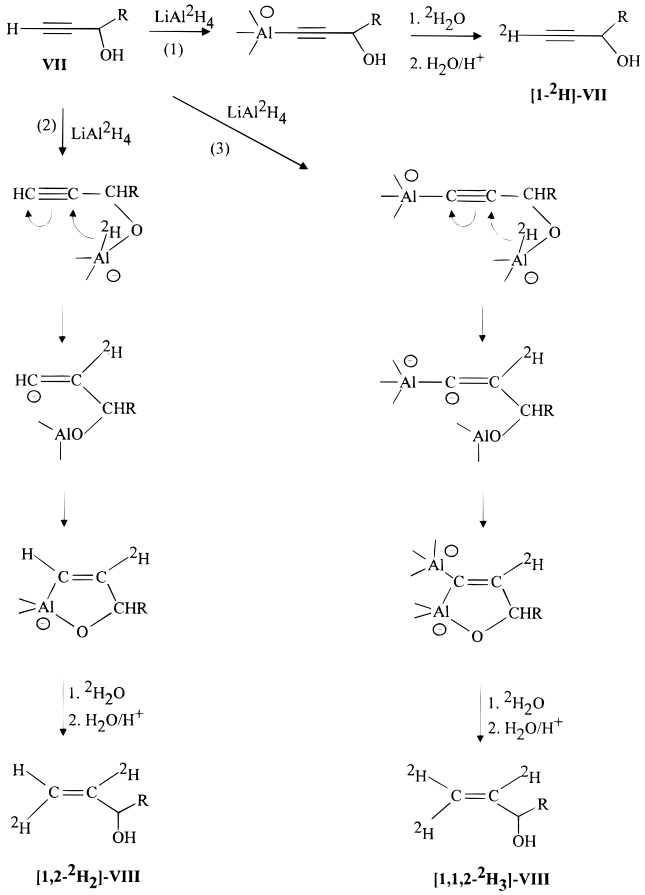
signal intensity of the acetylenic proton in **d-VII** was reduced to 0.04 H and the signal multiplicity of the proton attached to C-3 changed from a triplet of doublets to a triplet pattern. On the basis of these data, it was concluded that the deuterium atom was located at the terminal alkyne carbon (1-C), resulting in ~96% of [1-^{2}H]-**VII**. Alkynic monodeuteration was also confirmed by ^{13}C NMR spectra (data not shown).

The relative distribution of doubly or triply deuterated 1-octen-3-ol was analyzed by GC/MS in the CI mode using NH_3 as reagent gas by monitoring the $[M + NH_4]^+$ ions. Ratios of [$^2H_3$]-**VIII** to [$^2H_2$]-**VIII** were obtained after isotope correction (Rakoff and Rohwedder, 1992). As shown in Figure 7, doubly deuterated 1-octen-3-ol was exclusively obtained when using a $LiAl^2H_4$/**VII** molar ratio of 1:2. When the $LiAl^2H_4$/**VII** molar ratio was raised to 1:1 or 2:1, the amounts of triply deuterated 1-octen-3-ol increased with reaction time up to 40 and 50% of the total composition, respectively. A slight excess of $LiAl^2H_4$ over **VII** (molar ratio of 1.2:1, conditions described under Experimental Procedures) also resulted in **d-II** containing about equal proportions of doubly and triply deuterated isotopomers.

The formation of different isotopomers of **II** may be explained by the structural particularity of the α-alkynol used as the starting material. In general, metal hydrides preferably react with "active" hydrogen atoms, liberating hydrogen gas. In **VII** there are two distinct regions with active hydrogen atoms, that is, the hydroxyl group and the acetylenic hydrogen. Experimental findings suggest that the favored reaction at a $LiAl^2H_4$/**VII** molar ratio of 1:4 was the replacement of the acetylenic hydrogen (sample 1 in Table 4), thus forming [1-^{2}H]-**VII** upon deuterolysis (pathway 1 in Scheme 3). As shown by Mole and Surtees (1963), the acetylenic deuterium is stable toward ^{2}H/H exchange under the conditions of hydrolysis. In agreement with that, additional washing of a sample containing [1-^{2}H]-**VII** and unlabeled 1-octyn-3-ol (**VII**) with aqueous acid did not change the ^{2}H/H ratio (data not shown).

At a $LiAl^2H_4$/**VII** molar ratio of 1:2 (sample 2), the formation of [$^2H_2$]-1-octen-3-ol can be explained by attack of $LiAl^2H_4$ at the hydroxyl group (pathway 2 in Scheme 3). This reaction corresponds to reduction of α-alkynols to olefinic alcohols (Bates et al., 1954). The preferred formation of trans-configured olefinic alcohols may be due to geometrical constraints of the cyclic intermediate postulated by Snyder (1967), which rapidly undergoes deuteride transfer to the C-2 acetylenic carbon. Subsequent deuterolysis results in [1,2-$^2H_2$]-**VIII** in 50% yields (Table 4), whereas the remaining

Scheme 3. Schematic Formation Mechanism of [1-^{2}H]-1-Octyn-3-ol, [1,2-$^2H_2$]-1-Octen-3-ol, and [1,1,2-$^2H_3$]-1-Octen-3-ol by Reacting $LiAl^2H_4$ with 1-Octyn-3-ol (R = C_5H_{11}) at Different Molar Ratios[a]



[a] In the reaction intermediates, aluminum may bind to deuterium atoms (^{2}H) and/or to the starting molecule 1-octyne-3-ol via Al−O or Al−C bonds, depending on the molar ratio of $LiAl^2H_4$ to 1-octyn-3-ol.

50% of the reaction mixture was [1-^{2}H]-**VII** formed via reaction pathway 1.

[$^2H_3$]-**VIII** was generated in the presence of an excess of active ^{2}H compared to active H (samples 3 and 4 in Table 4) favoring the attack of $LiAl^2H_4$ at both active hydrogens in **VII**. Deuteride transfer and deuterolysis led to [1,1,2-$^2H_3$]-1-octen-3-ol as shown in pathway 3 (Scheme 3).

These results suggest that [1,1,2-$^2H_3$]-1-octen-3-ol can be obtained with high isotopomeric purity by first treating **VII** with $LiAl^2H_4$ (ratio 4:1) followed by complete reduction of [^{2}H]-**VII** to [$^2H_3$]-**VIII** with an excess of $LiAl^2H_4$. Alternatively, [1,2-$^2H_2$]-1-octen-3-ol can be obtained by reacting $LiAl^2H_4$ and **VII** in the molar ratio of 1:2. However, the maximal yield is 50% and removal of residual **VII** is required.

Conclusions. Two labeled vinyl ketones and one nonlabeled vinyl ketone were prepared by applying new or modified synthesis procedures. Their yields as well as chemical and isotopic purity were characterized using MS and NMR techniques. Mixtures of several isotopomers were obtained in the synthesis of deuterated 1-octen-3-one. However, this does not preclude its use as an internal standard in IDA. In view of the likely future demand for such labeled reference compounds, more work on optimization of synthesis procedures from a yield, time, and cost perspective may be justified.

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LITERATURE CITED

Bates, E. B.; Jones, E. R. H.; Whiting, M. C. Research on acetylenic compounds. Part XLII. Reduction with lithium aluminium hydride. *J. Chem. Soc.* **1954**, 1854−1860.

Corey, E. J.; Suggs, J. W. Pyridinium chlorochromate. An efficient reagent for oxidation of primary and secondary alcohols to carbonyl compounds. *Tetrahedron Lett.* **1975**, *31*, 2647−2650.

Fischer, K.-H.; Grosch, W. Volatile compounds of importance in the aroma of mushrooms (*Psalliota bispora*). *Lebensm. Wiss. Technol.* **1987**, *20*, 233−236.

Grosch, W. Reactions of hydroperoxides−Products of low molecular weight. In *Autoxidation of Unsaturated Lipids*; Chan, H. W.-S., Ed.; Academic Press: London, U.K., 1987; pp 95−139.

Grosch, W. Determination of potent odourants in foods by aroma extract dilution analysis (AEDA) and calculation of odour activity values (OAVs). *Flavour Fragrance J.* **1994**, *9*, 147−158.

Guth, H. Verderb von Sojaöl unter Einwirkung von Licht und Sauerstoff − Identifizierung der Aromastoffe und Vorläufer. Ph.D. Dissertation, Technical University of Munich, Germany, 1991 (in German).

Guth, H.; Grosch, W. Deterioration of soya-bean oil: Quantification of primary flavour compounds using a stable isotope dilution assay. *Lebensm. Wiss. Technol.* **1990**, *23*, 513−522.

Kajiwara, T.; Harada, T.; Hatanaka, A. Isolation of Z-3-hexenal in tea leaves, *Thea sinensis*, and synthesis thereof. *Agric. Biol. Chem.* **1975**, *39*, 243−247.

Lin, J.; Arce Vera, F.; Welti, D. H.; Fay, L. B.; Blank, I. Synthesis of deuterated volatile lipid degradation products to be used as internal standards in isotope dilution assays. 1. Aldehydes. *J. Agric. Food Chem.* **1999**, *47*, 2813−2821.

Milo, C.; Blank, I. Quantification of impact odorants in food by isotope dilution assay. Strength and limitations. In *Flavor Analysis—Developments in Isolation and Characterization*; Mussinan, C. J., Morello, M. J., Eds.; ACS Symposium Series 705; American Chemical Society: Washington, DC, 1998; pp 250−259.

Mole, T.; Surtees, J. R. Reaction of monosubstituted acetylenes with trialkylaluminums. *Chem. Ind.* **1963**, 1727−1728.

Pritzkow, W.; Radeglia, R.; Schmidt-Renner, W. Studies on the autoxidation of the isomeric *n*-octenes. *J. Prakt. Chem.* **1979**, *321* (5), 813−826 (in German); *Chem. Abstr.* **1980**, *92*, 75520c.

Rakoff, H.; Rohwedder, W. K. Catalytic deuteration of alkynols and their tetrahydropyranyl ethers. *Lipids* **1992**, *27*, 567−569.

Rohwedder, W. K. Mass spectrometry of lipids labeled with stable isotopes. *Prog. Lipid Res.* **1985**, *24*, 1−18.

Schieberle, P. New developments in methods for analysis of volatile flavor compounds and their precursors. In *Characterization of Food: Emerging Methods*; Gaonkar, A. G., Ed; Elsevier Science: Amsterdam, The Netherlands, 1995; pp 403−431.

Schieberle, P.; Grosch, W. Quantitative analysis of aroma compounds in wheat and rye bread crusts using stable isotope dilution assay. *J. Agric. Food Chem.* **1987**, *35*, 252−257.

Snyder, E. I. Stereochemistry and mechanism of carbon−carbon double-bond reduction in methyl cinnamate by lithium aluminium hydride. *J. Org. Chem.* **1967**, *32*, 3531−3534.

Swoboda, P. A. T.; Peers, K. E. Metallic odour caused by vinyl ketones formed in the oxidation of butterfat. The identification of octa-1,*cis*-5-dien-3-one. *J. Sci. Food Agric.* **1977**, *28*, 1019−1024.

Tressl, R.; Bahri, D.; Engel, K. H. Formation of eight-carbon and ten-carbon components in mushrooms (*Agaricus campestris*). *J. Agric. Food Chem.* **1982**, *30*, 89−93.

Ullrich, F.; Grosch, W. Flavour deterioration of soya-bean oil: Identification of intense odour compounds formed during flavour reversion. *Fat Sci. Technol.* **1988a**, *90*, 332−336.

Ullrich, F.; Grosch, W. Identification of the most intense odor compounds formed during autoxidation of methyl linolenate at room temperature. *J. Am. Oil Chem. Soc.* **1988b**, *65*, 1313−1317.

van den Dool, H.; Kratz, P. A generalization of the retention index system including linear temperature programmed gas−liquid partition chromatography. *J. Chromatogr.* **1963**, *24*, 463−471.

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