Synthesis of *trans*-4,5-Epoxy-(*E*)-2-decenal and Its Deuterated Analog Used for the Development of a Sensitive and Selective Quantification Method Based on Isotope Dilution Assay with Negative Chemical Ionization

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ABSTRACT: The volatile compound *trans*-4,5-epoxy-(*E*)-2-decenal (**1**) was synthesized in two steps with good overall yields. The newly developed method is based on *trans*-epoxidation of (*E*)-2-octenal with alkaline hydrogen peroxide followed by a Wittig-type chain elongation with the ylide formylmethylene triphenylphosphorane. For the synthesis of [4,5-$^2H_2$]-*trans*-4,5-epoxy-(*E*)-2-decenal (***d*-1**), [2,3-$^2H_2$]-(*E*)-2-octenal was prepared by reduction of 2-octyn-1-ol with lithium aluminum deuteride and subsequent oxidation of [2,3-$^2H_2$]-(*E*)-2-octen-1-ol with manganese oxide. Compound ***d*-1** was used as internal standard for the quantification of **1** by isotope dilution assay. Among various mass spectrometry (MS) ionization techniques tested, negative chemical ionization with ammonia as reagent gas gave best results with respect to both sensitivity and selectivity. The detection limit was found to be at about 1 pg of the analyte introduced into the gas chromatography–MS system.

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trans-4,5-Epoxy-(*E*)-2-decenal (**1**) was first reported by Selke and coworkers (1) as a reaction product of autoxidized trilinolein. Model studies revealed 12,13-epoxy-9-hydroperoxy-10-octadecenoate, a degradation product of 13-hydroperoxy-9,11-octadecadienoic acid (13-HPOD), as a precursor of **1** (2). In addition, formation of **1** from 9-hydroperoxy-10,12-octadecadienoic acid (9-HPOD) and 2,4-decadienal as key intermediates has recently been reported in a systematic study (3). A reaction route was proposed for the formation of **1** from linoleic acid (4).

Compound **1** has been described in various food systems as a potent odorant eliciting metallic and green notes (5,6). It contributes to the development of off-flavors such as the green, hay-like flavor of soybean oil when stored in the dark (7) and "warmed-over" flavor of refrigerated cooked meat (8,9). Its sensorial relevance is due to low odor thresholds, i.e., 1.5 pg/L air (6), 15 ng/L water (10), and 1.3 [(μg/L oil (7)].

The role of **1** in biological systems has also been studied. Several α,β-unsaturated aldehydes formed upon lipid peroxidation have been shown to modify proteins, i.e., 4-hydroxy-2-alkenals (11) and 4,5-epoxy-2-alkenals (12). Compound **1** has been reported to be an intermediate in the formation of 4,5-dihydroxy-2-decenal (13), a cytotoxic aldehyde originating from the peroxidation of liver microsomal lipids (14). On the other hand, epoxyaldehydes such as *trans*-4,5-epoxy-(*E*)-2-heptenal and **1** may react with amino groups located in side chains of amino acids (15). Polymeric pyrrole derivatives identified in model systems containing such epoxyaldehydes and lysine are responsible for color formation and fluorescence (12,16). These reactions also generate 1-substituted pyrroles that have been proposed as indicators of oxidative stress in biological systems (12).

Compound **1** has recently been obtained by (salen)Mn(III)-catalyzed asymmetric epoxidation of (*E,Z*)-2,4-decenol with concomitant oxidation of the allylic alcohol to the aldehyde in 16% yield (17). However, most synthesis procedures reported so far are based on epoxidation of (*E,E*)-2,4-decadienal with 3-chloroperbenzoic acid (6,12). The deuterated analog (***d*-1**) was similarly prepared using labeled (*E,E*)-2,4-decadienal as starting material (4). Main limitations of the synthesis procedures are low yields, typically 5–10%, and time-consuming purification steps such as column chromatography (CC) and high-performance liquid chromatography.

In general, the amounts of **1** found in foods are low, i.e., up to ~11 μg/kg in refrigerated cooked meat (9,10), ~12 μg/kg in soybean oil stored in the dark (4), and 13–20 μg/kg in virgin olive oils (18). These values were obtained by the isotope dilution assay (IDA) quantification method (4,10), which is based on spiking the sample with known amounts of a labeled substance prior to sample preparation and analysis by gas chromatography–mass spectrometry (GC–MS) (19). In this way, losses can be accounted for because of the almost identical chemical and physical properties of the labeled internal standard and the analyte to be quantified. Unfortunately, quantification of **1** implies laborious cleanup steps such as distillation in high vacuum, CC and high-performance liquid chromatography

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Abbreviations: CC, column chromatography; COSY, homonuclear correlation spectroscopy; EI, electron ionization; GC, gas chromatography; HETCOR, heteronuclear correlation spectra; IDA, isotope dilution assay; MS, mass spectrometry; NCI, negative chemical ionization; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; PC, phosphatidylcholine; PCI, positive chemical ionization; PE, phosphatidylethanolamine; RI, retention index; SIM, selected ion monitoring; THF, tetrahydrofuran.

leading to extraction yields of about 1% (4). Thus, very sensitive detection methods are required for the quantification of **1**. Furthermore, neither **1** nor ***d*-1** is commercially available, but both are required for applying IDA. In this paper, we report a newly developed synthesis procedure of ***d*-1** and its unlabeled analog and an improved quantification method of **1** based on IDA using negative chemical ionization (NCI) as ionization technique.

EXPERIMENTAL PROCEDURES

Materials. Lithium aluminum deuteride ($LiAl^2H_4$, >99% deuterium), deuterium oxide (^2H_2O, heavy water, >99.8% ^2H), manganese oxide (MnO_2), formylmethylene triphenylphosphorane, anhydrous tetrahydrofuran (THF) (H_2O <0.005%, stored over molecular sieves), diethyl ether (Et_2O), methylenechloride (CH_2Cl_2), and Celite 545 were purchased from Fluka (Buchs, Switzerland). (*E*)-2-Octenal was obtained from Aldrich (Buchs, Switzerland) and 2-octyn-1-ol from Lancaster (Morecambe, England). Hydrogen peroxide (H_2O_2), salt (NaCl), sodium hydroxide (NaOH), sodium hydrogen carbonate ($NaHCO_3$), sodium sulfate (Na_2SO_4), hexane, heptane, methanol (MeOH), and silica gel 60 (70–230 mesh) were from Merck (Darmstadt, Germany). The egg phospholipids phosphatidylcholine (PC) and phosphatidyl-ethanolamine (PE) were obtained from Avanti Polar Lipids (Copenhagen, Denmark).

*Formation of **1** in heated phospholipids.* One gram of egg PC or egg PE was dispersed in a phosphate buffer (50 mL, 0.5 M, pH 5.6) by stirring magnetically. The dispersion was then heated for 30 min from room temperature to 145°C in a laboratory autoclave (Berghof, Eningen, Germany). After cooling, ***d*-1** (2.44 μg) was added to the reaction mixture that was then saturated with NaCl. The neutral compounds were continuously extracted from the aqueous phase for 15 h with Et_2O using a liquid-liquid extractor. The solvent extract was applied to high-vacuum transfer (20) in order to remove nonvolatile compounds. The condensate containing volatile compounds was concentrated to 1 mL before GC–MS analysis using a Vigreux column (50 × 1 cm).

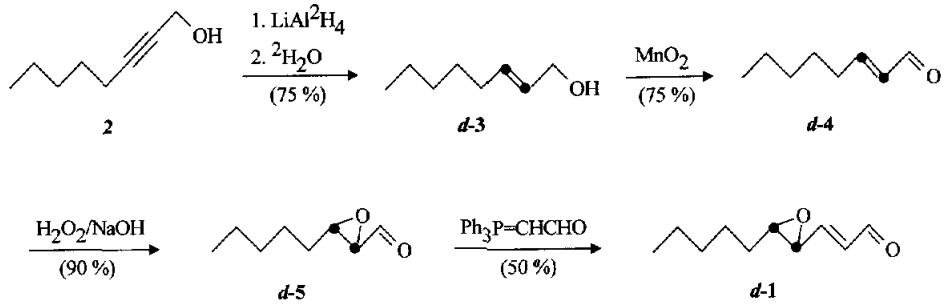
Capillary GC. This was performed on a Hewlett-Packard HP-5890 gas chromatograph (Geneva, Switzerland) equipped with a splitless injector and a flame-ionization detector. Fused-silica capillary columns were used, i.e., DB-5, DB-OV 1701, DB-FFAP, and DB-WAX (J&W Scientific, Folsom, CA; 30 m × 0.32 mm, film thickness 0.25 μm). Helium was used as carrier gas (100 kPa). The gas chromatograph was operated at an injector temperature of 250°C and a detector temperature of 250°C. The ovens were programmed as follows: 20°C, 70°C/min to 50°C, 4°C/min to 180°C, 10°C/min to 240°C (10 min) for DB-5; 20°C (2 min), 40°C/min to 50°C (2 min), 4°C/min to 150°C, 10°C/min to 240°C (15 min) for FFAP; and 20°C (1 min), 70°C/min to 60°C, 6°C/min to 180°C, 10°C/min to 240°C (15 min) for OV-1701 and DB-Wax. Linear retention indices (RI) were calculated (21).

GC–MS. Qualitative and quantitative GC–MS analysis was performed on a Finnigan SSQ 7000 mass spectrometer (Bremen, Germany) using the following conditions: electron ionization (EI) mass spectra were generated at 70 eV; positive chemical ionization (PCI) and negative chemical ionization (NCI) were carried out at 200 eV with ammonia or isobutane as reagent gas. Samples (2 μL) were introduced *via* a Hewlett-Packard HP-5890 gas chromatograph equipped with an HP-7673 autosampler using the following conditions: splitless injection at 250°C on fused-silica capillary column DB-OV 1701 described above. The carrier gas was helium (90 kPa). The temperature program was as follows: 60°C (2 min), 6°C/min to 180°C, 10°C/min to 240°C (10 min). Quantitative measurements were carried out in the selective ion monitoring (SIM) mode measuring characteristic ions of **1** and ***d*-1**. Calibration curves were obtained using mixtures of defined amounts of analyte (**1**) and labeled internal standard (***d*-1**) (4). Nine mixtures ***1/d*-1** were used, i.e., from 0.5 + 9.5, 1 + 9, 2 + 8, 3 + 7, and 5 + 5 to 7 + 3, 8 + 2, 9 + 1, and 9.5 + 0.5. The concentrations (μg/mL) of **1** and ***d*-1** used for preparing the mixtures were adapted to the varying sensitivity of the ionization modes used, i.e., 101.6/105.8 (PCI/isobutane), 10.2/10.6 (NCI/isobutane; PCI/ammonia), and 1.0/1.1 (NCI/ammonia). Samples for establishing the calibration curves and for quantification were injected twice.

Nuclear magnetic resonance (NMR) spectroscopy. The samples for NMR spectroscopy were prepared in WILMAD 528-PP 5-mm Pyrex NMR tubes, using as solvent about 0.7 mL of 99.8% C^2HCl_3 (Dr. Glaser AG, Basel, Switzerland) from a sealed vial. The NMR spectra were acquired on a Bruker AM-360 spectrometer (Karlsruhe, Germany), equipped with a quadrinuclear 5-mm probe head, at 360.13 MHz for ^1H and at 90.56 MHz for ^{13}C under standard conditions. The probe temperature was 21°C for the proton spectra and slightly higher for the carbon spectra, owing to heteronuclear composite pulse decoupling. All shifts are cited in ppm from the internal tetramethylsilane (TMS) standard. Where appropriate, proton nuclear Overhauser effect (NOE) difference spectra, distortionless enhancement by polarization transfer (DEPT) and/or fully proton-coupled spectra, and two-dimensional homonuclear and heteronuclear correlation spectra (COSY and HETCOR, respectively) were acquired as described earlier (22).

*Synthesis of [4,5-$^2H_2$]-trans-4,5-epoxy-(E)-2-decenal (**d-1**).* The synthesis procedure for ***d*-1** is shown in Scheme 1. Commercially available 2-octyn-1-ol (**2**) was used as starting material to prepare [2,3-$^2H_2$]-(*E*)-2-octen-1-ol (***d*-3**) by reduction with $LiAl^2H_4$ followed by deuterolysis (22). Oxidation of ***d*-3** with MnO_2 according to (23) resulted in [2,3-$^2H_2$]-(*E*)-2-octenal (***d*-4**). [2,3-$^2H_2$]-*trans*-2,3-Epoxyoctanal (***d*-5**) was obtained by reaction of ***d*-4** with alkaline H_2O_2 (24). Chain elongation was achieved by a Wittig reaction of ***d*-5** with the ylide formylmethylene triphenylphosphorane (25) resulting in ***d*-1** as the major product.

[2,3-$^2H_2$]-(E)-2-Octen-1-ol (**d-3**) (Scheme 1, step ***a***). In a three-necked reactor (200 mL) fitted with a reflux condenser and a thermometer, $LiAl^2H_4$ (4.2 g, 100 mmol) was suspended in anhydrous THF (50 mL). 2-Octyn-1-ol (10.0 g, 79 mmol) dissolved in anhydrous THF (50 mL) was slowly added to the



SCHEME 1

magnetically stirred solution. The mixture was refluxed for 1 h and then stored overnight at room temperature. GC analysis indicated complete reduction of **2** to ***d*-3**. On cooling in an ice bath, heavy water (25 mL) was added drop by drop, followed by aqueous H_2SO_4 (4 N, 80 mL) to dissolve any insoluble residual materials. The organic phase was separated and the aqueous solution was then extracted with Et_2O (3 × 50 mL). The combined organic phases were washed successively with saturated solutions of $NaHCO_3$ (2 × 10 mL) and NaCl (2 × 10 mL), then dried over anhydrous Na_2SO_4. After removing the solvent by evaporation, 7.7 g (59 mmol, 75% yield) of a colorless oil of ***d*-3** with a purity of >97% (GC) was obtained. GC: RI (DB-5) = 1169, RI (OV-1701) = 1188, RI (FFAP) = 1617, RI (DB-WAX) = 1615. MS (EI) *m/z* (rel%): 130 (1, $[M]^+$), 112 (6, $[M\text{-}H_2O]^+$), 97 (7), 96 (5), 85 (8), 84 (10), 83 (37), 82 (18), 71 (11), 70 (25), 69 (32), 68 (23), 59 (100), 58 (40), 57 (32), 56 (48), 55 (33), 45 (32), 44 (16). The molecular ion was confirmed by PCI, isobutane, *m/z* (rel%): 113 (100, $[M - H_2O + H]^+$).

[2,3-$^2H_2$]-(E)-2-Octenal (**d-4**) *(Scheme 1, step b).* MnO_2 (20 g) was suspended in a solution of ***d*-3** (2.0 g, 15.4 mmol) in hexane (50 mL). Oxidation of ***d*-3** to ***d*-4** was complete after stirring the mixture at room temperature for 18 h. The mixture was filtered through a short pad of Celite to remove the remaining solid, and the Celite was washed with Et_2O. The solvent was removed by evaporation, obtaining 1.5 g (11.7 mmol, 75% yield) of a pale yellow oil of ***d*-4** without further purification. GC: RI (DB-5) = 1059, RI (OV-1701) = 1171, RI (FFAP) = 1428, RI (DB-WAX) = 1424. MS (EI) *m/z* (rel%): 128 (1, $[M]^+$), 127 (2), 113 (5), 110 (5, $[M - H_2O]^+$), 100 (12), 99 (32, $[M - CHO]^+$], 97 (5), 96 (5), 95 (7), 94 (10), 86 (18), 85 (84), 84 (34), 83 (40), 82 (15), 81 (8), 73 (8), 72 (55), 71 (58), 70 (100), 69 (48), 68 (24), 67 (22), 59 (56), 58 (35), 57 (60), 56 (48), 55 (68), 53 (7), 44 (15), 43 (36), 42 (52), 41 (93), 40 (12), 39 (32). The molecular ion was confirmed by PCI, isobutane *m/z* (rel%): 129 (100, $[M + 1]^+$), 128 (10, $[M]^+$), 111 (10, $[M + H - H_2O]^+$). ^{1}H NMR δ (ppm, C^2HCl_3): 9.51 (1:1:1, pattern typical for coupling to ^{2}H, $^3J_{1\text{-}H,2\text{-}^2H}$ = 1.0 Hz, 0.9 H, intensity slightly reduced because of the long spin-lattice relaxation time T_1, 1-H); 2.33 (*t*, slightly broad, $^3J_{4\text{-}H,5\text{-}H}$ = 7.5 Hz, 2 H, 4-CH_2), 1.52 ("quintet," $^3J_{avg}$ = 7.3 Hz, ≥2 H, 5-CH_2), 1.40–1.25 (*m*, complex, ≥4 H, 6-CH_2 and 7-CH_2), 0.91 ("*t*," *J* ~ 6.9 Hz, ≥3 H, 8-CH_3). Residual signals 6.85 (*t* 1:1:1, $^3J_{3\text{-}H,4\text{-}H}$ = 6.7 Hz, $^3J_{3\text{-}H,2\text{-}^2H}$ = 2.3 Hz, 0.07 H, 3-H) and 6.12 (*m*, $^3J_{1\text{-}H,2\text{-}H}$ = 8.0 Hz and further couplings, 0.02 H, 2-H) indicated mono- or undeuterated (*E*)-2-octenal as by-products that accounted for about 9%. No evidence for the occurrence of (*Z*) isomer was seen in the NMR spectrum. NOE-difference experiments with unlabeled (*E*)-2-octenal proved the spatial vicinity of the 1-CHO and 3-CH protons, indicating the all-*trans* arrangement of the C=C and C=O double bonds. ^{13}C NMR (proton decoupled) δ (ppm, C^2HCl_3): 194.21 (*d*, 1-CHO), 158.67 (*s* 1:1:1, $^1J_{C^2H}$ = 23.2 Hz, 3-C^2H), 132.59 (*s* 1:1:1, $^1J_{C^2H}$ = 24.5 Hz, 2-C^2H), 32.55 (*t*, 4-CH_2), 31.30 (*t*, 6-CH_2), 27.49 (*t*, 5-CH_2), 22.41 (*t*, 7-CH_2), 13.95 (*q*, 8-CH_3). These chemical shifts corresponded well to the spectrum of the unlabeled compound (26). Small signals for the residual nondeuterated 2-C and 3-C in about 1:3 ratio were observed at 132.89 (*d*) and 159.04 ppm (*d*), respectively. The NMR assignments above were based on our data of undeuterated (*E*)-2-octenal (not cited here), the assignments of which were in turn derived from those of natural abundance and 2,3-deuterated (*E*)-2-nonenal (22).

[2,3-$^2H_2$]-trans-2,3-Epoxyoctanal (**d-5**). *(Scheme 1, step c).* A two-necked flask (100 mL) equipped with a thermometer was charged with a solution of ***d*-3** (2.6 g, 20 mmol) in MeOH (25 mL). After cooling the solution to 1–3°C in an ice bath, 6.6 g of a 30% aqueous H_2O_2 solution (6 mL, 60 mmol) was added. The mixture was stirred vigorously and kept well cooled in the ice bath. Then, 40 µL of a 15% aqueous NaOH solution (0.15 mmol) was added in one portion. After 1 h reaction at 3–5°C, brine (30 mL) was added and the resulting suspension was extracted with CH_2Cl_2 (3 × 50 mL). The extracts were dried over Na_2SO_4, and the solvent was removed by distillation through a Vigreux column, obtaining 2.9 g (20.1 mmol, 99% yield) of *trans*-2,3-epoxyoctanal (***d*-5**) with a purity of 90% (GC). The sample, containing the corresponding *cis* isomer as main by-product, was used as such for the Wittig reaction. GC: RI (DB-5) = 1093, RI (OV-1701) = 1218, RI (FFAP) = 1542, RI (DB-WAX) = 1533. MS (EI) *m/z* (rel%): 144 (1, $[M]^+$), 97 (3), 85 (3), 73 (100, $[M - C_5H_{11}]^+$), 72 (8), 69 (9), 59 (10), 57 (10), 56 (15), 55 (15), 45 (5), 43 (32), 42 (10), 41 (40), 40 (15), 39 (10). The molecular ions were confirmed by PCI, ammonia, *m/z* (rel%): 162 (100, $[M + NH_4]^+$), 146 (8, $[M + NH_4 - O]^+$); PCI, isobutane *m/z* (rel%): 145 (10, $[M + H]^+$), 129 (100, $[M + H - O]^+$), 128 (15), 127 (5, $[M + H - H_2O]^+$).

[4,5-$^2H_2$]-trans-4,5-Epoxy-(E)-2-decenal (**d-1**) *(Scheme 1, step d).* A solution of ***d*-5** (1.9 g, 13.2 mmol) in heptane (50 mL) was added to a suspension of formylmethylene triphenylphos-

phorane (4.1 g, 13.3 mmol) in heptane (50 mL). The reaction mixture was refluxed for 30 min. After cooling, the precipitated phosphorine oxide was filtered off. The filtrate was evaporated to remove the solvent. Four isomers of epoxydecenal were formed, with one predominant product: the *trans*-(*E*) isomer accounted for 87% of the total peak area. Major by-products were the *cis*-(*E*) and *trans*-(*Z*) isomers with 7 and 5%, respectively. Isomers were separated by CC using a glass column (20 × 2 cm) packed with a slurry of silica gel in hexane. Elution was carried out with hexane/Et_2O (95 + 5, vol/vol), collecting fractions of 10 mL. The target compound ***d*-1** was found by GC analysis in the fractions eluted from 200 to 400 mL. About 0.9 g (5.3 mmol, 40% yield) of the target compound was obtained as a colorless oil smelling intensely metallic with a purity of 95% [GC, 5% *cis*-(*E*) isomer]. GC: RI (DB-5) = 1380, RI (OV-1701) = 1558, RI (FFAP) = 2011, RI (DB-WAX) = 1995. MS, ^1H NMR, and ^{13}C NMR data are shown in Figure 1 and Tables 1–3.

Synthesis of trans-*4,5-Epoxy-*(E)*-2-decenal (1).* The unlabeled compound was prepared by adapting the procedure illustrated in Scheme 1 using commercially available (*E*)-2-octenal (**4**) as starting material.

trans-*2,3-Epoxyoctanal (5).* This was obtained in analogy to ***d*-5** by epoxidation of **4** (5.0 g, 40 mmol) with alkaline H_2O_2. About 5.1 g of **5** was obtained (36 mmol, 91% yield) by distillation under vacuum (130°C, 2 mbar) with a purity of 94% (GC). GC: RI (DB-5) = 1092, RI (OV-1701) = 1218, RI (FFAP) = 1540, RI (DB-WAX) = 1531. MS (EI) *m/z* (rel%): 142 (1, $[M]^+$), 95 (5), 83 (5), 72 (5), 71 (100, $[M - C_5H_{11}]^+$), 69 (15), 57 (25), 55 (50), 43 (35), 41 (65), 39 (20). The molecular ions were confirmed by PCI, ammonia, *m/z* (rel%): 160 (100, $(M + NH_4]^+$), 144 (5, $(M + NH_4–O]^+$); PCI, isobutane *m/z* (rel%): 143 (10, $(M + H]^+$), 127 (100, $(M + H - O]^+$), 125 (5, $[M + H - H_2O]^+$).

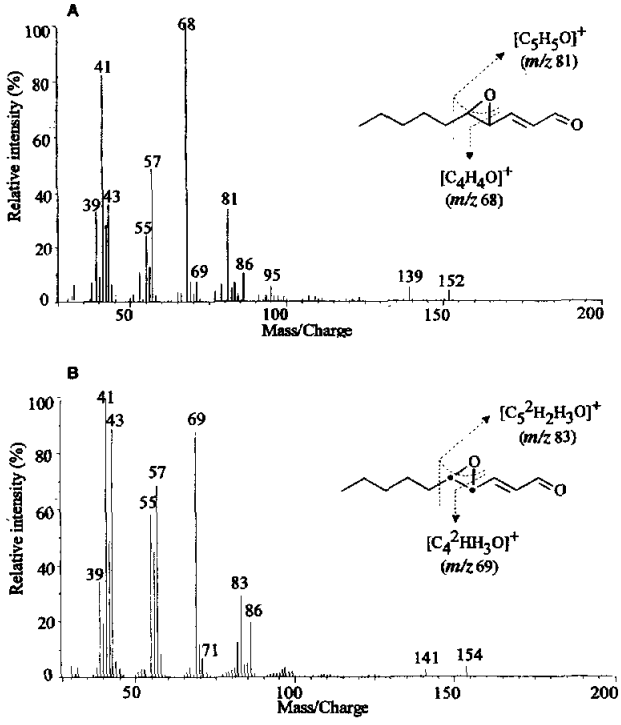


FIG. 1. Mass spectra of (A) *trans*-4,5-epoxy-(*E*)-2-decenal and (B) [4,5-$^2H_2$]-*trans*-4,5-epoxy-(*E*)-2-decenal obtained by electron impact ionization.

trans-*4,5-Epoxy-*(E)*-2-decenal* (**1**). This was obtained in analogy to ***d*-1** with 7.1 g (23 mmol) formylmethylene triphenylphosphorane and 3.3 g (23 mmol) of compound **5**. The *trans*-(*E*) isomer accounted for 92%, the *cis*-(*E*) isomer for 7% of the total peak area. After purification by CC using the same conditions as described above, about 1.5 g (8.9 mmol, 39% yield) of **1** was obtained as a colorless oil with a purity of 95% [GC, 5% *cis*-(*E*) isomer]. GC: RI (DB-5) = 1378, RI (OV-1701) = 1557, RI (FFAP) = 2010, RI (DB-WAX) = 1993. MS, ^1H NMR and ^{13}C NMR data are shown in Figure 1 and Tables 1–3.

RESULTS AND DISCUSSION

*Preparation and characterization of [4,5-$^2H_2$]-*trans*-4,5-epoxy-*(E)*-2-decenal (**d-1**) and* trans*-4,5-epoxy-*(E)*-2-decenal (1). (i) Synthesis procedure.* The newly developed synthesis procedure for ***d*-1** is based on the preparation of deuterated (*E*)-2-octenal (***d*-4**) followed by *trans*-epoxidation to 2,3-epoxyoctanal (***d*-5**) and subsequent chain elongation by a Wittig-type reaction (Scheme 1). In general, the preparative procedures and purification techniques used were simple and the yields satisfactory. Deuteration of commercially available 2-octyn-1-ol (**2**) resulted in [2,3-$^2H_2$]-(*E*)-2-octen-1-ol (***d*-3**) in 75% yields. As recently shown, reduction of α-acetylenic alcohols with $LiAl^2H_4$ leads preferably to *trans*-configured olefinic alcohols (22). The high stereospecificity was explained by geometrical constraints of cyclic intermediate structures. Oxidation of the primary allylic alcohol ***d*-3** with MnO_2 led to the corresponding aldehyde (***d*-4**) in 75% yield. The reaction of ***d*-4** with alkaline H_2O_2 was nearly quantitative. The resulting labeled 2,3-epoxyoctanal (***d*-5**) was obtained as a mixture of *trans* and *cis* isomers in the ratio 9:1. Wittig reaction of ***d*-5** gave rise to a mixture of epoxydecenal isomers in about 50% yield. As major isomer, the target compound (***d*-1**) was separated from isomeric by-products by CC. Similarly, the unlabeled compound (**1**) was synthesized in a two-step procedure in good overall yields using (*E*)-2-octenal (**4**) as starting material.

(ii) Structure characterization by MS. MS–PCI data indicated the incorporation of two deuterium atoms in ***d*-1**, i.e., at *m/z* 171 ($[M + H]^+$), compared to *m/z* 169 for **1**, when using isobutane as reagent gas (Table 1). Similarly, MS–PCI (ammonia) resulted in *m/z* 188 for ***d*-1** compared to *m/z* 186 for **1**. These ions are represented by the species $[M + NH_4]^+$ with abundances of about 50–90%. Fragmentation of these ions resulted in two major products that correspond to $[M + NH_4 - O]^+$ (100%) and $[M + H - O]^+$ (~60%), suggesting that the molecular ions are unstable and readily lose oxygen. This phenomenon was also observed when using isobutane as reagent gas. Apart from the molecular ions at *m/z* 171 and 169, the major signals were at *m/z* 155 and 153, representing $[M + H - O]^+$ of the labeled and unlabeled compound, respectively.

Loss of oxygen also generated characteristic fragments in the

TABLE 1
Ions Detected for *trans*-4,5-Epoxy-(*E*)-2-decenal (1) and [4,5-$^2H_2$]*trans*-4,5-Epoxy-(*E*)-2-decenal (*d*-1) Using Various Chemical Ionization Techniques[a]

Ionization mode	1	*d*-1
PCI (isobutane)	169 (25, $[M + H^+]$, 153 (100, $[M + H - O]^+$), 151 (10, $[M + H - H_2O]^+$)	171 (15, $[M + H^+]$, 155 (100, $[M + H - O]^+$), 153 (5, $[M + H - H_2O]^+$)
PCI (ammonia)	186 (90, $[M + NH_4]^+$), 170 (100, $[M + NH_4 - O]^+$), 169 (15, $[M + H]^+$), 153 (65, $[M + H - O]^+$)	188 (55, $[M + NH_4]^+$), 172 (100, $[M + NH_4 - O]^+$), 171 (20, $[M + H]^+$), 155 (55, $[M + H - O]^+$)
NCI (isobutane)	167 (40, $[M - H]^-$), 152 (30, $[M - O]^-$), 97 (100, $[M - C_5H_{11}]^-$)	168 (20, $[M - {}^2H]^-$), 154 (35, $[M - O]^-$), 99 (100, $[M - C_5H_{11}]^-$)
NCI (ammonia)	167 (20, $[M - H]^-$), 97 (100, $[M - C_5H_{11}]^-$)	168 (5, $[M - {}^2H]^-$), 99 (100, $[M - C_5H_{11}]^-$)

[a]Conditions of measurements are described in the Experimental Procedures section. Mass spectral data are given as ions (*m/z*) with intensities relative to the base peak (% relative abundance). PCI, positive chemical ionization; NCI, negative chemical ionization.

TABLE 2
^1H NMR Data of *trans*-4,5-Epoxy-(*E*)-2-decenal (1) and [4,5-$^2H_2$]-*trans*-4,5-Epoxy-(*E*)-2-decenal (*d*-1)[a]

Proton	1	*d*-1
1-CHO	9.57, *d*, $^3J_{1\text{-H},2\text{-H}}$ = 7.6 Hz, 1 H	9.56, *d*, $^3J_{1\text{-H},2\text{-H}}$ = 7.7 Hz, 1H
2-CH	6.39, *d d*, $^3J_{2\text{-H},3\text{-H}}$ = 15.7, $^3J_{1\text{-H},2\text{-H}}$ = 7.6 Hz, 1H	6.39, *d d*, *J* = 15.8, 7.7 Hz, 1H
3-CH	6.56, *d d*, $^3J_{2\text{-H},3\text{-H}}$ = 15.7, $^3J_{3\text{-H},4\text{-H}}$ = 6.9 Hz, 1 H	6.56, *d*, $^3J_{2\text{-H},3\text{-H}}$ = 15.7 Hz, 1H
4-CH	3.33, *d d*, $^3J_{3\text{-H},4\text{-H}}$ = 6.9, $^3J_{4\text{-H},5\text{-H}}$ = 2.1 Hz, 1 H	(residual signal 3.33, *d*, $^3J_{3\text{-H},4\text{-H}}$ = 6.7 Hz, ~0.02 H)
5-CH	2.96, *t d*, $^3J_{5\text{-H},6\text{-H}}$ = 5.5, $^3J_{4\text{-H},5\text{-H}}$ = 2.1 Hz, 1 H	(residual signal 2.96, *t*, $^3J_{5\text{-H},6\text{-H}}$ = 5.7 Hz, ~0.07 H)
6-CH_2	1.65, *m*, $^3J_{5\text{-H},6\text{-H}}$ = 5.5 Hz, 2 H	1.65, *m*, 2 H
7-CH_2	1.48, *m*, 2 H	1.48, *m*, 2 H
8,9-CH_2	~1.33, *m*, 4 H	~1.33, *m*, 4 H
10-CH_3	0.91, "*t*," 3 H	0.91, "*t*," 3 H

[a]Shifts in ppm from internal tetramethylsilane. Multiplicity abbreviations used to describe ^1H nuclear magnetic resonance (NMR) signals: *s* = singlet, *d* = doublet, *t* = triplet, *m* = complex multiplet. Quotation marks "..." mean approximate description of the multiplet.

MS–EI spectra, i.e., *m/z* 154 for ***d*-1** and *m/z* 152 for **1** (Fig. 1). In agreement with that, loss of oxygen has been reported for several 4,5-epoxy-2-alkenals of the chain length C_7–C_{11} (6). The fragment *m/z* 81 detected in the MS–EI spectra of this class of compounds, including **1**, is most likely a fragmentation product of $[M - O]^+$ with the composition C_5H_5O (12,27) that is formed by cleavage of the bonds at 5-C and 6-C. As ***d*-1** was labeled in the position 4-C and 5-C, the corresponding ion detected was *m/z* 83 with the composition $[C_5H_3{}^2H_2O]$. Therefore, the oxygen atom eliminated from the molecule is most likely originating from the oxirane ring.

However, the major ions did not reflect the shift of two units, i.e., *m/z* 69 and 68, which probably correspond to $[C_4{}^2HH_3O]^+$ and $[C_4H_4O]^+$, respectively. The data suggest that the said ions were generated by cleavage between 4-C and 5-C, which is in contrast to the fragmentation pattern proposed in the literature (6). Finally, the ions *m/z* 141 and 139 correspond to $[M - CHO]^+$. Loss of the aldehyde group has also been observed for 4,5-epoxy-2-heptenal (27) and 4,5-epoxy-2-nonenal (7).

(iii) Structure elucidation by NMR spectroscopy. NMR analysis was based on the non-deuterated compound **1**, for which a set of one- and two-dimensional spectra were acquired,

TABLE 3
^{13}C NMR Data of *trans*-4,5-Epoxy-(*E*)-2-decenal (1) and [4,5-$^2H_2$]-*trans*-4,5-Epoxy-(*E*)-2-decenal (*d*-1)[a]

Carbon	1	*d*-1
1-CHO	192.58, *d*	192.60, *d*
2-CH	133.51, *d*	133.51, *d*
3-CH	153.16, *d*	153.10, *d*
4-CH	56.19, *d*	(small residual signal 56.12, *d*)
4-C^2H	—	55.74, *s* (1:1:1), $^1J_{C^2H}$ = 27.2 Hz
5-CH	61.95, *d*	(small residual signal 61.88, *d*)
5-C^2H	—	61.48, *s* (1:1:1), $^1J_{C^2H}$ = 26.5 Hz
6-CH_2	31.87, *t*	31.69, *t*
7-CH_2	25.47, *t*	25.44, *t*
8-CH_2	31.50, *t*	31.50, *t*
9-CH_2	22.52, *t*	22.52, *t*
10-CH_3	13.96, *q*	13.96, *q*

[a]Shifts in ppm from internal tetramethylsilane. Multiplicity: abbreviations *s*, *d*, *t*, and *q* represent quaternary carbons, CH, CH_2, and CH_3 carbons, respectively. For abbreviation see Table 2.

including proton NOE difference spectra, COSY, and HETCOR for direct carbon-proton couplings. The structure of **1** could be confirmed, as shown in Tables 2 and 3. The coupling constant $J_{2\text{-H},3\text{-H}}$ 15.7 Hz revealed the *trans* configuration of the carbon-carbon double bond. In analogy to literature values on α-epoxy alcohols (28), the coupling constant $J_{4\text{-H},5\text{-H}} = 2.1$ Hz suggested a *trans* form of the oxirane ring. NOE-difference experiments with 10-s pre-irradiation and 4.23-s acquisition periods yielded signal enhancements, i.e., irradiation of 4-H: 7% on 2-H, 3% on 3-H, and 1.8% on 6-CH_2; irradiation of 5-H: 4.7% on 3-H, 2.2% on 2-H, and 1.4% on both 5-CH_2 and 6-CH_2. The absence of an NOE between 4-H and 5-H confirmed the *trans* arrangement of the oxirane ring. These NOE-difference results also suggested a non-coplanar arrangement of the enal group with respect to the oxirane ring. For the enal group, an all-*trans* conformation could be assumed, based on the close similarity of the $^3J_{1\text{-CHO},2\text{-H}}$ coupling constant in **1** and in (*E*)-2-octenal.

The NMR spectra also indicated the presence of about 7% of an isomer. Among the clearly distinguished, isolated ^{1}H-NMR signals of this isomer (between 10 and 2 ppm), the shifts of the putative 4- and 5-protons deviated more from the shifts of the main compound than those of any other proton. The coupling patterns of those same protons were the only ones to change, whereas the couplings of the 1-CHO and 3-H signals were practically the same for both isomers. The isomer is therefore presumably *cis*-4,5-epoxy-(*E*)-2-decenal. The low-field deviations (*ca.* 0.3 ppm) of the 4-H and 5-H signals could also indicate *cis* substitution of the oxirane ring, according to the shift rules established by Pierre *et al.* (28) for α-epoxy alcohols. Complete data for this second isomer were not available because of signal overlap with the dominating compound (especially for the 2-H and the aliphatic signals in the proton spectrum).

Both the ^{1}H NMR data of **1** in Table 2 and the ^{13}C-NMR data in Table 3 were in good agreement with those recently reported by Zamora and Hidalgo (12). The data of **1** are only given in this paper for closer comparison with the deuterated analog ***d*-1** that is reported for the first time. Other ^{1}H-NMR results on **1** (6) are not directly comparable with ours because of a different solvent used ($C^2H_2Cl_2$). Earlier ^{1}H NMR data of *trans*-4,5-epoxy-(*E*)-2-heptenal in C^2HCl_3 (27) are well compatible with ours on **1** for the unsaturated and oxirane part of the molecule, considering the different experimental temperature (35°C) and eventual concentration effects.

The NMR spectra of ***d*-1** clearly demonstrated a nearly quantitative deuteration at 4-C and 5-C (e.g., absence of couplings $^3J_{3\text{-H},4\text{-H}}$ and $^3J_{5\text{-H},6\text{-H}}$ on the 3-H and 6-H proton signals, characteristic deuterium patterns in the ^{13}C NMR spectrum). The proton spectrum indicated about 98 and 93% deuterium substitution, respectively. No undeuterated **1** could be detected in the proton spectrum. As in **1**, about 7% of the putative, equally 4,5-deuterated *cis* isomer were found in ***d*-1**, i.e., [4,5-$^2H_2$]-*cis*-4,5-epoxy-(*E*)-2-decenal.

Quantification of trans-*4,5-epoxy-*(E)*-2-decenal by IDA. (i) Mass spectra obtained by various ionization techniques.* The characteristic ions $[M - O]^+$ at *m/z* 152 (**1**) and 154 (***d*-1**) were not sufficiently abundant for quantification by IDA (Fig. 1). The $[M - CHO]^+$ fragments at *m/z* 139 and 141, showing the mass difference of two units, were also too weak. On the other hand, the more abundant ions were less characteristic (e.g., *m/z* 81/83, 55/57, 41/43) or they did not reflect the shift of two mass units (*m/z* 68/69). As no characteristic ions with sufficient abundance could be found in the MS–EI spectra of **1** and ***d*-1**, further ionization modes were tested to evaluate their suitability for IDA.

GC–MS using chemical ionization is known as a soft ionization method generating abundant molecular ions that are most characteristic of the compound to be analyzed. PCI using ammonia as a reagent gas resulted in characteristic and abundant ions for **1** and ***d*-1** (Table 1), i.e., at *m/z* 186/188 (~60–90%), 170/172 (100%), and 153/155 (~60%), respectively. The PCI spectra with isobutane generated mainly the ions at *m/z* 153/155 (100%). The NCI spectra of **1** and ***d*-1** obtained with ammonia and isobutane revealed abundant ions at *m/z* 97/99 (100%) which represent the characteristic part of the molecules. They correspond to the species $[C_5H_5O_2]^-$ and $[C_5H_3H_2O_2]^-$, respectively. Fragments formed by loss of oxygen ($[M - O]^-$) were also found, particularly when ionized with isobutane, i.e., at *m/z* 152 and 154. All these ions were estimated as suitable for IDA, and those finally selected were *m/z* 170/172 (PCI/ammonia), 153/155 (PCI/isobutane), and 97/99 (NCI with either ammonia or isobutane).

(ii) Sensitivity. Decreasing amounts of mixtures containing the analyte (**1**) and internal standard (***d*-1**) were analyzed by PCI and NCI in the SIM detection mode using isobutane or ammonia as reagent gas (Fig. 2). In general, NCI was found to result in more abundant signals than PCI. The sensitivity could further be improved when replacing isobutane by ammonia as a reagent gas. Signal-to-noise ratios (S/N) revealed NCI/ammonia as the most suitable method for the quantitative analysis of **1** (Fig. 2). The detection limit was determined to be at about 1 pg injected into the ion source with an acceptable S/N ratio of 3 (Table 4). PCI/isobutane was about 200 times less sensitive (detection limit: ~200 pg) whereas PCI/ammonia and NCI/isobutane gave comparable results, i.e., a detection limit of about 10 and 20 pg, respectively.

(iii) Selectivity. In addition to the high sensitivity, the NCI technique was also found to significantly improve selectivity in the detection of **1** and ***d*-1** (Fig. 3). When analyzing an aroma extract obtained from heated egg PE, no interferences were detected with NCI using either isobutane or ammonia. On the contrary, PCI resulted in further peaks, thus complicating data interpretation and calculation of reliable values. Therefore, NCI can be suggested as an ideal ionization technique for the quantitative analysis of **1**, particularly when using ammonia as a reagent gas.

(iv) Calibration curves. The calibration curve was established using mixtures containing defined amounts of analyte (**1**) and labeled internal standard (***d*-1**) in different ratios by measuring the characteristic ions of **1** and ***d*-1** (4). The ions indicated in Table 4 were monitored in the SIM mode. Linear curves were obtained for all of the ionization modes tested. As an example, the calibration curve using NCI/ammonia is presented in Figure 4A. Similar curves were obtained for the other ionization modes

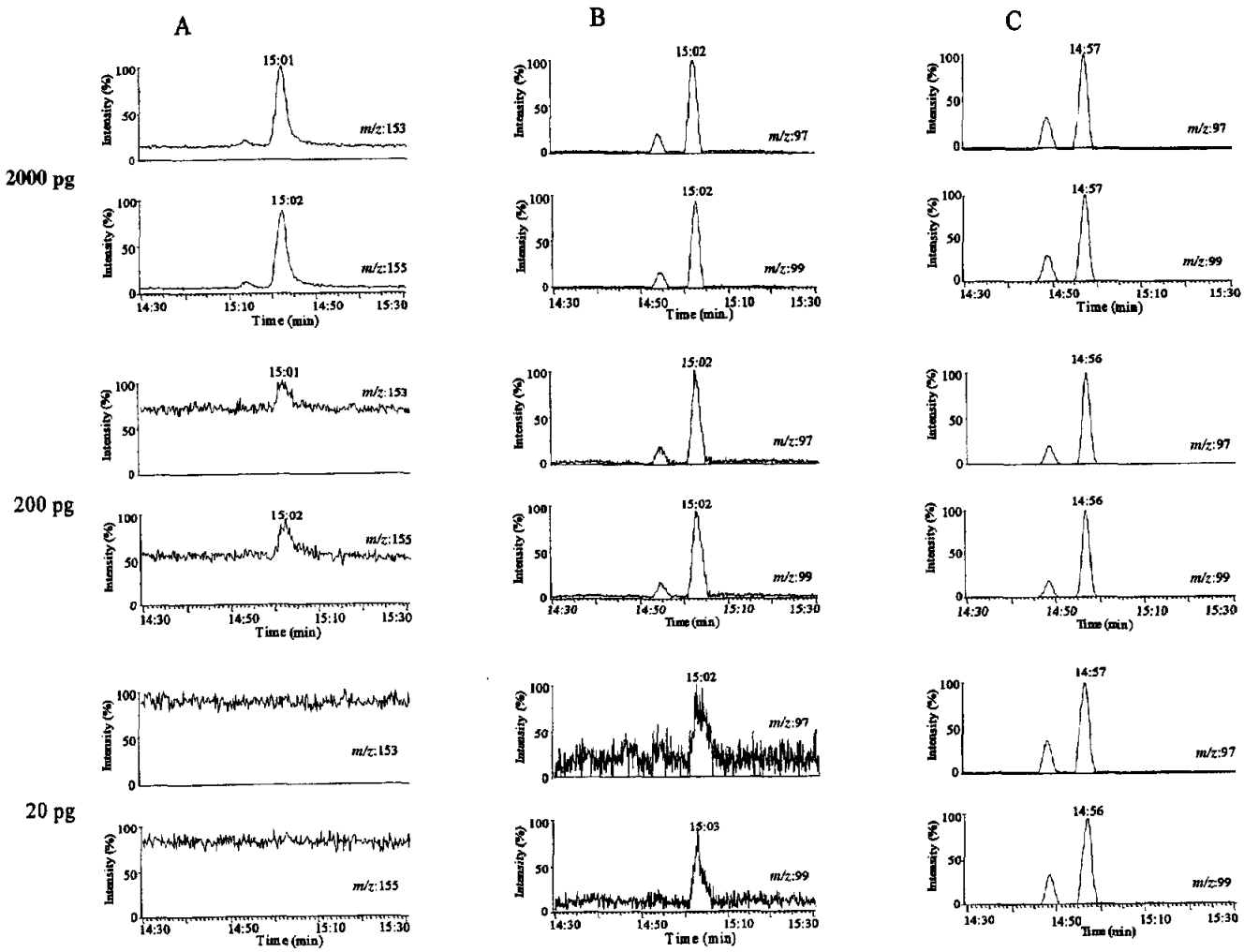


FIG. 2. Traces of decreasing amounts (2000, 200, and 20 pg) of *trans*-4,5-epoxy-(*E*)-2-decenal (**1**) and [4,5-$^2H_2$]-*trans*-4,5-epoxy-(*E*)-2-decenal (***d*-1**) using various ionization techniques in the selected ion monitoring (SIM) mode. (A) Positive chemical ionization (PCI), isobutane; (B) negative chemical ionization (NCI), isobutane; (C) NCI, ammonia. The major peak represents the *trans* isomer, the minor peak the *cis* isomer of **1** and ***d*-1**.

tested, i.e., $y = 0.8404x + 0.155$, $r^2 = 0.9952$ (PCI/isobutane), $y = 0.7181x + 0.1121$, $r^2 = 0.9966$ (PCI/ammonia), and $y = 0.6365x + 0.0732$, $r^2 = 0.9985$ (NCI/isobutane). It should be pointed out, however, that if the amount ratios were extended to values higher than 10, second-order curves were obtained because of the natural deuterium isotope peaks of **1**, which coincide with the acquired ions of ***d*-1** (29).

The accuracy of the measured values with the theoretical values was checked according to the procedure described by Staempfli and coworkers (30). On the basis of knowing the amount of **1** and ***d*-1** in the mixture, the theoretical enrichment of ***d*-1** was calculated for each calibration point and expressed in mol percent excess (MPE). The measured deuterium enrichment was plotted vs. theoretical deuterium enrichment, resulting in linear curves for the four ionization modes (see example with NCI/ammonia in Fig. 4B). The slopes of the calibration curves were close to 1.000 proving the accuracy of the measurement, i.e., 0.9558 (PCI/isobutane), 0.9723 (PCI/ammonia), and 0.9029 NCI/isobutane). The observed differences might be due to chemical impurity of **1**, chemical and isotopic impurities of ***d*-1**, and slight variation in the fragmentation pattern of **1** and ***d*-1**.

Quantification of trans*-4,5-epoxy-*(E)*-2-decenal (1) in heated phospholipids.* The linear ranges shown in Table 4 of the calibration curves were used to quantify **1** in heated aqueous dispersions of egg PC. Comparison of the data obtained from the same sample by the four ionization modes revealed good agreement in the results (Table 4), i.e., 1.61–1.67 μg **1** per g PC with an average of 1.64 ± 0.02, which correspond to a variation coefficient of less than 2%. As IDA has already been shown to be an accurate method for the quantification of **1** (4,9,10), the use of NCI/ammonia represents a significant improvement in terms of higher sensitivity and selectivity, thus allowing reduction of sample preparation time by applying simple cleanup procedures.

Conclusions. The newly developed synthesis procedure allows the preparation of both [4,5-$^2H_2$]-*trans*-4,5-epoxy-(*E*)-2-

TABLE 4
Comparison of Various Chemical Ionization Modes for the Quantification of *trans*-4,5-Epoxy-(*E*)-2-decenal (1) in Heated Egg Phosphatidylcholine (PC) by Isotope Dilution Assay[a]

Ionization mode (reagent gas)	Selected ions (*m/z*) 1	*d*-1	Detection limit (pg)[b]	Linearity (r^2)	Linear range (ratio **1**/***d*-1**)[c]	Concentration of 1 in egg PC (μg/g)[d]
PCI (isobutane)	153	155	200	0.999	0.05–9.0	1.67
PCI (ammonia)	170	172	10	0.999	0.05–9.0	1.61
NCI (isobutane)	97	99	20	0.999	0.05–4.0	1.64
NCI (ammonia)	97	99	1	0.998	0.05–4.0	1.62

[a][4,5-$^2H_2$]-*trans*-4,5-Epoxy-(*E*)-2-2decenal (***d*-1**) used as internal standard. Linear ranges and linearity were obtained from the calibration graphs using selected ions (see Experimental Procedures section).
[b]Detection limits correspond to a signal-to-noise ratio of 3.
[c]Linear range with $r^2 > 0.99$.
[d]Values represent the average of two injections using various chemical ionization techniques in the selected ion monitoring mode.

decenal (***d*-1**) and its unlabeled analog **1** in high purity and good yields. Optimization of mass spectrometric conditions, such as ionization mode and reagent gas, resulted in a powerful quantification method for **1** based on IDA. Comparison of various ionization modes revealed NCI as the most suitable technique with respect to both sensitivity and selectivity, particularly when

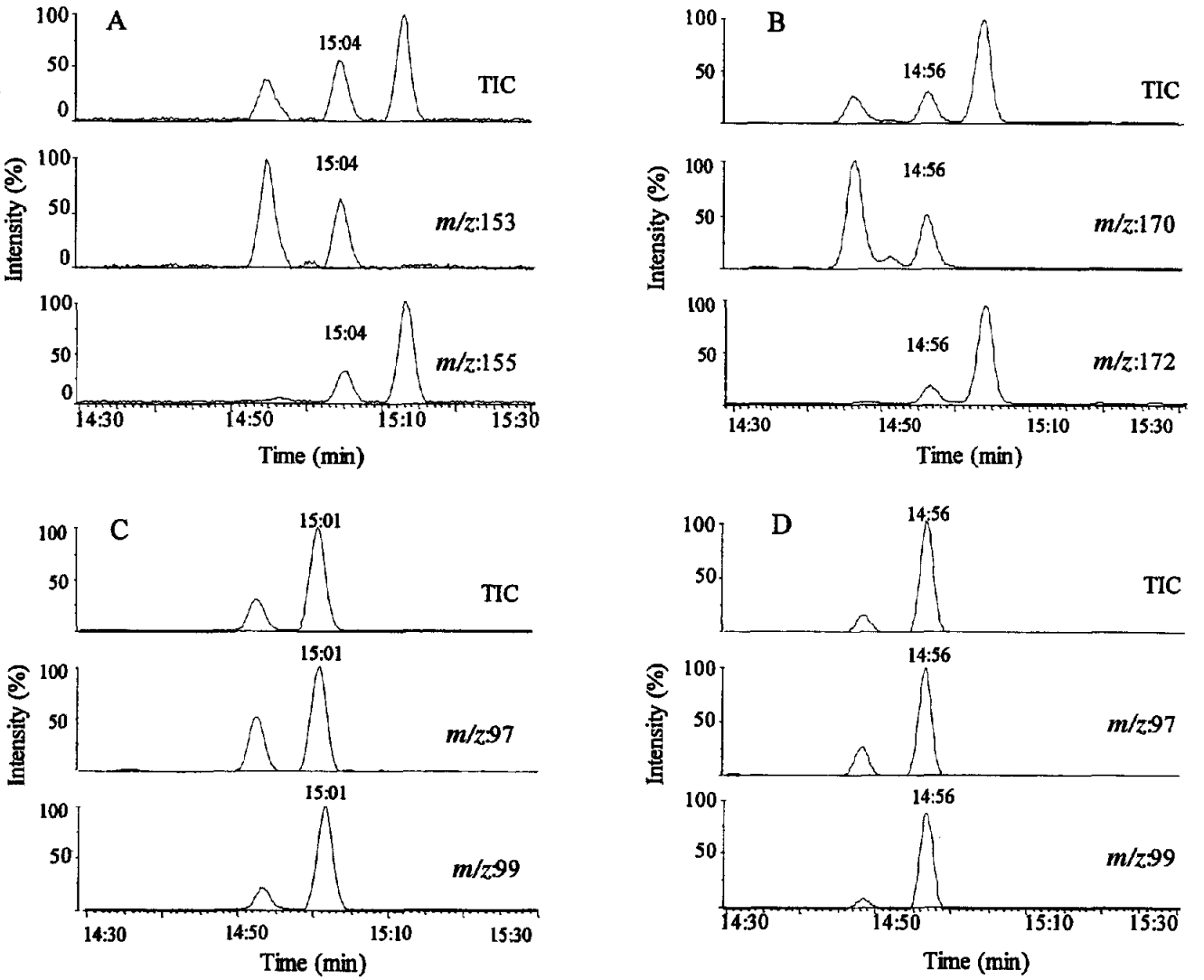


FIG. 3. Improvement of selectivity for the quantification of *trans*-4,5-epoxy-(*E*)-2-decenal (**1**) in heated egg phosphatidylethanolamine by using various chemical ionization methods in the SIM mode. (A) PCI, isobutane; (B) PCI, ammonia; (C) NCI, isobutane; (D) NCI, ammonia. TIC, total ion current; for other abbreviations see Figure 2.

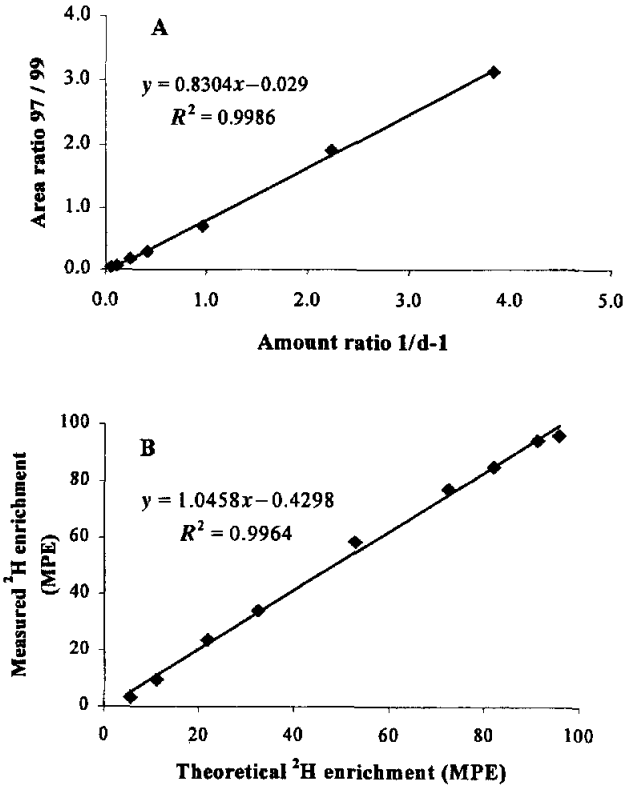


FIG. 4. (A) Calibration curve obtained for the quantification of *trans*-4,5-epoxy-(*E*)-2-decenal (**1**) using the ionization technique NCI/ammonia. The **1/d-1** ratios were 0.05, 0.11, 0.25, 0.43, 1.0, 2.33, 4.0, and 9.0. (B) Determination of the accuracy of the calibration curve obtained by using the ionization technique NCI/ammonia. The theoretical enrichments were calculated according to Wolfe (29) using known amounts of labeled and unlabeled *trans*-4,5-epoxy-(*E*)-2-decenal (**1**). MPE, mol percent excess; for other abbreviation see Figure 2.

using ammonia as a reagent gas. The low detection limit of 1–10 pg combined with increased selectivity by NCI are interesting features for obtaining reliable quantitative data of **1** and similar labile epoxyaldehydes at low concentration levels in complex mixtures allowing simplified clean-up procedures.

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