

Identification of Potent Odorants Formed by Autoxidation of Arachidonic Acid: Structure Elucidation and Synthesis of (*E,Z,Z*)-2,4,7-Tridecatrienal

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The aroma composition of autoxidized arachidonic acid was characterized by aroma extract dilution analysis. The most potent odorant was *trans*-4,5-epoxy-(*E*)-2-decenal followed by 1-octen-3-one, (*E,Z*)-2,4-decadienal, (*E,Z,Z*)-2,4,7-tridecatrienal, (*E,E*)-2,4-decadienal, and hexanal. (*E,Z,Z*)-2,4,7-Tridecatrienal was unequivocally identified by mass spectrometry and nuclear magnetic resonance (NMR) data. The stereochemistry of its extended double-bond system was elucidated on the basis of NMR measurements. The target compound was synthesized in four steps starting with bromination of 2-octyn-1-ol, followed by copper-catalyzed coupling of the bromide with ethylmagnesium bromide and (*E*)-2-penten-4-yn-1-ol. Partial hydrogenation of the resulting C₁₃-compound with triple bonds in the positions C-4 and C-7 gave rise to (*E,Z,Z*)-2,4,7-tridecatrien-1-ol, which was finally oxidized to the target compound. It exhibits a typical egg-white-like, marine-like odor at low concentrations, and an intense orange-citrus, animal-like odor at higher concentrations. Its odor threshold was estimated by gas chromatography-olfactometry to be 0.07 ng/L air, which is of the same order of magnitude as that reported for 1-octen-3-one and (*E,E*)-2,4-decadienal.

Keywords: Arachidonic acid; autoxidation; aroma extract dilution analysis; key odorants; (*E,Z,Z*)-2,4,7-tridecatrienal; synthesis; flavor dilution factor

INTRODUCTION

Arachidonic acid is an ω -6 polyunsaturated fatty acid (20:4), which is very susceptible to oxidation by atmospheric oxygen, and thus brings about losses in nutritional quality and gives rise to off-flavors. Although Frankel (1) and Grosch (2) have published extensive reviews on oxidation products of unsaturated lipids, little is known about volatile compounds formed by autoxidation of arachidonic acid or methyl arachidonate. Various volatile compounds have been identified depending on the starting material, reaction conditions, and analytical techniques used. Ellis and co-workers (3) found hexanal as the dominating degradation product of methyl arachidonate, followed by heptenal and further unsaturated aldehydes. In a study on cold-storage flavor defects of butter, Badings (4) extracted a mixture of aldehydes and ketones from autoxidized arachidonic acid and identified them by mass spectrometry as their dinitrophenyl derivatives. Hexanal, (*E*)-2-heptenal, (*E,Z*)-2,4-decadienal, and 2,4,7-tridecatrienal were the major aldehydes reported. Various volatile degradation products were isolated from the headspace of autoxidized methyl arachidonate (5). 1-Octen-3-ol, hexanal, (*E*)-2-heptenal, and 1-octen-3-one were identified as the major compounds. In a study related to off-flavors in cooked meat, Artz and co-workers (6) found hexanal, methyl 5-oxopentanoate, pentane, and 2,4-decadienal as the

dominating volatile degradation products of autoxidized methyl arachidonate.

Application of aroma extract dilution analysis (7) to autoxidized linoleic acid led to the identification of hexanal, (*Z*)-2-octenal, and (*E*)-2-nonenal as the most important odorants. The same procedure applied to methyl linolenate revealed (*E,Z*)-2,6-nonadienal, (*Z*)-1,5-octadien-3-one, (*E,Z*)-3,5-octadien-2-one, and (*Z*)-3-hexenal as the most odor-active compounds (8). The aroma composition of autoxidized arachidonic acid has recently been characterized (9). Several aldehydes and ketones were found to contribute to the overall aroma. In this paper we report on the identification of the most potent odorants with special emphasis on (*E,Z,Z*)-2,4,7-tridecatrienal whose structure and double bond stereochemistry was elucidated for the first time on the basis of nuclear magnetic resonance (NMR) and mass spectrometry (MS) data. In addition, a new synthesis pathway was developed to confirm the odorant.

MATERIALS AND METHODS

Materials. The following chemicals were commercially available: (*E*)-2-nonenal (Agipal, Paris, France); arachidonic acid (99%), ethylmagnesium bromide, 2,4-nonadienal (*E,E*: 95%; *E,Z*: 5%), (*E,Z*)-2,6-nonadienal, (*E*)-2-octenal (containing traces of the *Z*-isomer), 1-octen-3-ol, palladium on CaCO₃ (Lindlar's catalyst) (Aldrich, Buchs, Switzerland); hydrogen gas (Carbagas, Lausanne, Switzerland); hexanal, cuprous chloride (CuCl), manganese(IV) oxide (MnO₂), phosphorus tribromide (PBr₃), pyridine (H₂O < 0.005%), tetrahydrofuran (THF, H₂O < 0.005%) (Fluka, Buchs, Switzerland); 2,4-decadienal (*E,E*: 95%; *E,Z*: 5%; Fontarom, Cergy Pontoise, France); deuterated chloroform (99.8%, Dr. Glaser AG, Basel, Switzerland); diethyl ether (Et₂O), hexane, hydrogen peroxide

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(H₂O₂), pentane, potassium hydroxide (KOH), silica gel 60, sodium carbonate (Na₂CO₃), sodium chloride (NaCl), sodium hydrogen carbonate (NaHCO₃), anhydrous sodium sulfate (Na₂SO₄), sulfuric acid (H₂SO₄) (Merck, Darmstadt, Germany); (*E,E*)-2,4-dodecadienal, 2-octyn-1-ol, (*E*)-2-penten-4-yn-1-ol, (*E,E*)-2,4-undecadienal (Lancaster, Morecambe, England); and 1-octen-3-one (Oxford, Brackley, UK). *trans*-4,5-Epoxy-(*E*)-2-decenal was synthesized as recently described (10). (*Z*)-2-Nonenal was a gift from Prof. W. Grosch (Techn. University, Munich, Germany).

Autoxidation of Arachidonic Acid. A solution of arachidonic acid (500 mg) in freshly distilled Et₂O (50 mL) was pipetted into a 250-mL Erlenmeyer flask. The solvent was removed with a stream of nitrogen to obtain a thin layer of the lipid material. The flask was filled with oxygen and sealed. After storing the sample in the dark for 48 h at room temperature, the flask was flushed with nitrogen.

Peroxide Value. Peroxides were measured with the Fe test (11), indicating the presence of about 60 mol % of total peroxides.

Isolation of Volatile Compounds. The aroma extract used in AEDA was obtained by high vacuum transfer at 10⁻³–10⁻⁵ mbar (12). After autoxidation, Et₂O (100 mL) was added to the reaction mixture. The solution was introduced dropwise into the vacuum system via a dropping funnel. Volatile compounds were transferred to two glass traps cooled with liquid nitrogen. The procedure was repeated by adding Et₂O (30 mL) to the residue. The condensates were combined and concentrated to 0.5 mL on a Vigreux column (50 × 1 cm).

Column Chromatography (CC). Purification of (*E,Z,Z*)-2,4,7-tridecatrienal (odorant 15) was achieved by CC using a water-cooled glass column (20 × 1 cm) packed with a slurry of silica gel 60, which was treated according to Esterbauer (13). Elution was performed with the following pentane/Et₂O mixtures (by volume): 98/2 (fraction F1), 95/5 (fraction F2), 90/10 (fraction F3), 80/20 (fraction F4), and 50/50 (fraction F5). Odorant 15 was enriched in fraction F3.

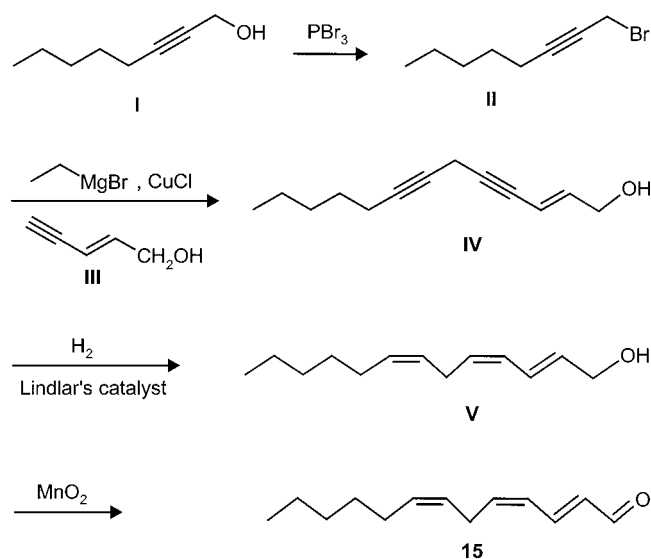
Gas Chromatography–Olfactometry (GC–O). This was performed with a Carlo Erba Mega 2 gas chromatograph (Fisons Instruments, via Brechbühler, Schlieren, Switzerland) equipped with a cold on-column injector and a flame ionization detector (FID). Helium (80 kPa) was used as carrier gas. Fused silica capillary columns of low (DB-5), medium (DB-1701), and high (DB-Wax, DB-FFAP) polarity were used, all 30 m × 0.32 mm with a film thickness of 0.25 μm (J&W Scientific, Folsom, CA). A splitter (Gerstel, Mülheim, Germany) was attached to the end of the capillary column to split the effluent 1:1 into the FID and sniffing port, both held at 230 °C, using deactivated and uncoated fused silica capillaries (50 cm × 0.32 mm). The splitter was flushed with nitrogen (5 mL/min) to accelerate the gas flow. Just prior to the sniffing port, the GC effluent was mixed with humidified air (10 mL/min). Chromatographic conditions were used as described earlier (14). Linear retention indices (RI) were calculated according to van den Dool and Kratz (15).

Aroma Extract Dilution Analysis (AEDA). The sensory significance of each odorant was evaluated by AEDA and expressed as the flavor dilution (FD) factor (16, 17). The FD factors were determined by stepwise diluting the original extract with Et₂O to obtain dilution factors of 2²–2¹⁴ until no odor-active region could be detected by GC–O, which was performed on the DB-1701 capillary column by injecting 0.5 μL. The FD factors were obtained by two trained assessors who injected the sample twice.

Gas Chromatography–Mass Spectroscopy (GC–MS). This was performed on a Finnigan MAT 8430 mass spectrometer (Bremen, Germany). Electron impact (EI) mass spectra were generated at 70 eV. Chemical ionization (MS–CI) was performed at 150 eV with ammonia as the reagent gas. Further details of the GC–MS system and chromatographic conditions have been described previously (14). Relative abundances of the ions are given in percent.

Nuclear Magnetic Resonance (NMR) Spectroscopy. The samples for NMR spectroscopy were prepared in WILMAD 528-PP 5 mm Pyrex NMR tubes, using as solvent deuterated

Scheme 1. Synthesis of (*E,Z,Z*)-2,4,7-Tridecatrienal (15)



chloroform (0.7 mL) from sealed vials. The NMR spectra were acquired on a Bruker AM-360 spectrometer, equipped with a quadrinuclear 5-mm probe head, at 360.13 MHz for ¹H and at 75.56 MHz for ¹³C under standard conditions (14). All shifts are cited in ppm from the internal tetramethylsilane (TMS) standard. One-dimensional ¹H NMR, ¹³C NMR, and proton NOE-difference spectra, and two-dimensional COSY and HETCOR spectra were acquired as described earlier (14), with parameters adapted to the characteristics of the individual samples.

Odor Quality Description. A panel of 10 persons including experienced and inexperienced tasters, all working on flavors, was used for organoleptic evaluations. Four aqueous solutions of 15 were submitted to the panel at different concentrations (a, 0.015 mg/L; b, 0.15 mg/L; c, 1.5 mg/L; and d, 15 mg/L). The panel members were asked to describe the odor characteristics of the solutions in the order of a, b, c, and d. The most frequently used descriptors were summarized for each sample.

Odor Threshold Measurement. The odor threshold was approximated by an olfactometric method described by Ullrich and Grosch (7) using (*E*)-2-decenal as internal standard. (*E,E*)-2,4-Decadienal was included in the mixture as a secondary internal standard.

Synthesis of (*E,Z,Z*)-2,4,7-Tridecatrienal (15). The synthesis procedure for obtaining odorant 15 was adapted from the method described by Meijboom and Stroink (18) for the synthesis of (*E,Z,Z*)-2,4,7-decatrienal (Scheme 1). The four-step pathway starts with bromination of 2-octyn-1-ol, followed by copper-catalyzed coupling of the bromide with the Grignard derivative of (*E*)-2-penten-4-yn-1-ol. Partial hydrogenation of the resulting C₁₃-compound with triple bonds in the positions C-4 and C-7 in the presence of Lindlar's catalyst gave rise to (*E,Z,Z*)-2,4,7-tridecatrien-1-ol, which was finally oxidized with activated MnO₂ to the target compound 15.

1-Bromo-2-octyne (II). This was prepared by bromination of 2-octyn-1-ol (I) with PBr₃ according to the procedure described by Kerdesky and co-workers (19). PBr₃ (8.1 g, 2.8 mL, 30 mmol) was added to a solution of I (10.0 g, 79 mmol) in anhydrous Et₂O (100 mL) containing pyridine (0.3 g). The mixture was stirred at room temperature for 2 h, then poured onto ice, and extracted with Et₂O (3 × 100 mL). The extract was washed successively with aqueous Na₂CO₃ (1 M, 2 × 20 mL) and brine (NaCl, 2 × 10 mL). After drying over anhydrous Na₂SO₄ and removing the solvent, II (10.4 g, 67%) was obtained by distillation under vacuum (7 mbar, 65 °C). GC: RI(DB-5) = 1198, RI(DB-OV1701) = 1271, RI(DB-Wax) = 1593, RI(DB-FFAP) = 1599. MS–CI (ammonia) *m/z* (% relative abundance): 109 (100, [M – HBr]⁺).

Scheme 2. Synthesis of Alkatrienals by Aldol-condensation of Acetaldehyde and Alkadienals (R = methyl, ethyl, propyl, or butyl; R' = methyl or ethyl)

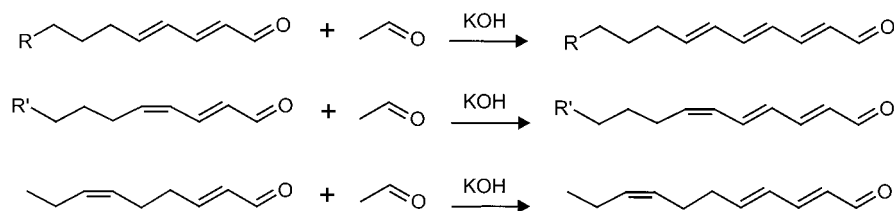


Table 1. Odorants Identified in Autoxidized Arachidonic Acid

| no. | odorant | aroma quality (intensity) ^f | RI OV-1701 | RI DB-5 | FD factor (2 ^g) | previous reports ^g |
|-----|---|--|------------|---------|-----------------------------|---|
| 1 | hexanal ^a | green (3) | 881 | 802 | 9 | (3-6) |
| 2 | 1-octen-3-one ^a | mushroom-like (3) | 1068 | 978 | 11 | (5) |
| 3 | 1-octen-3-ol ^a | mushroom-like (2) | 1082 | 979 | 2 | (5-6) |
| 4 | (Z)-2-octenal ^a | soapy (2) | 1157 | 1046 | 7 | (3) ^{h,i} , (5) ⁱ , (6) |
| 5 | (E)-2-octenal ^a | soapy (2) | 1174 | 1057 | 2 | (3) ^{h,i} , (5) ⁱ , (6) |
| 6 | unknown | sweet, fresh (1-2) | 1220 | | 2 | |
| 7 | (Z)-2-nonenal ^b | soapy (2) | 1260 | 1147 | 4 | (3) ^{h,i} |
| 8 | (E)-2-nonenal ^b | soapy (2) | 1279 | 1161 | 2 | (3) ^{h,i} |
| 9 | (E,Z)-2,4-nonadienal ^b | soapy (2) | 1323 | 1194 | 2 | (3) ^{h,i} |
| 10 | (E,E)-2,4-nonadienal ^a | fatty (2-3) | 1352 | 1215 | 4 | (3) ^{h,i} |
| 11 | (E,Z)-2,4-decadienal ^a | soapy (2-3) | 1433 | 1297 | 11 | (3) ^{h,i} , (5) ⁱ , (6) |
| 12 | (E,E)-2,4-decadienal ^a | fatty (2) | 1460 | 1320 | 10 | (3) ^{h,i} , (5) ⁱ , (6) |
| 13 | 2,5-undecadienal ^{c,d} | soapy (3) | 1477 | 1352 | 7 | (3) ^h |
| 14 | trans-4,5-epoxy-(E)-2-decenal ^a | metallic, green (3) | 1561 | 1381 | 13 | |
| 15 | (E,Z,Z)-2,4,7-tridecatrienal ^{a,e} | egg-white-like, marine (3) | 1737 | 1584 | 11 | (4) ⁱ |
| 16 | (E,E,Z)-2,4,7-tridecatrienal ^a | animal, beefy (2-3) | 1771 | 1630 | 7 | (4) ⁱ |
| 17 | (E,E,E)-2,4,7-tridecatrienal ^a | animal, pig-like (2) | 1791 | 1647 | 7 | (4) ⁱ |
| 18 | unknown | green, metallic (1) | 1873 | 1680 | 4 | |
| 19 | unknown | soapy, geranium-like (1) | 1919 | 1759 | 7 | |

^a Identification based on comparison of odorant and reference compound: retention indices on two capillaries with different polarity, aroma quality, and mass spectra (EI, CI). ^b Identification as described in footnote a, however without mass spectra due to low concentration. ^c Identification based on mass spectra (EI, CI); no reference compound was available. ^d Double bond position and/or configuration deduced from autoxidation mechanism. ^e Structure elucidation by NMR spectroscopy. ^f Intensity perceived at sniffing port, scaled from 1 (low) to 3 (high). ^g Numbering corresponds to references listed in the Literature Cited section. ^h Position of double bond(s) not specified. ⁱ Configuration of double bond(s) not specified.

(E)-2-Tridecen-4,7-diy-1-ol (**IV**). This was prepared by Grignard coupling of (E)-2-penten-4-yl-1-ol and **III** as described for the synthesis of undeca-2,5-diy-1-ol (20). (E)-2-Penten-4-yn-1-ol (3.6 g, 43 mmol) dissolved in anhydrous THF (20 mL) was added dropwise to a solution of ethylmagnesium bromide in Et₂O (1 M, 90 mL) under stirring at 3–5 °C. The solution was further stirred for 2 h at 20 °C. The solution was then cooled to 5 °C and CuCl (2.0 g) was added. After the solution was stirred for 15, **III** (5.1 g, 27 mmol) dissolved in THF (20 mL) was added dropwise. The mixture was refluxed for 24 h, more CuCl (1.0 g) being added after 16 h. H₂SO₄ (2 N, 15 mL) was added to the reaction mixture, which was cooled with ice and then extracted with Et₂O (3 × 50 mL). The extract was washed successively with aqueous solutions of NaHCO₃ (2 N, 2 × 10 mL) and brine (2 × 10 mL). The solvent was removed and **IV** (2.8 g, 28%) was obtained by distillation under vacuum. MS–EI *m/z* (% relative abundance): 190 (10, M⁺), 175 (5), 161 (10), 147 (60), 133 (90), 119 (50), 115 (60), 105 (95), 103 (45), 91 (100), 79 (75), 77 (75), 65 (35), 63 (40), 55 (35), 51 (30). MS–CI (ammonia) *m/z* (% relative abundance): 208 (95, [M + NH₄]⁺), 190 (100, M⁺ or [M + NH₄ – H₂O]⁺), 173 (50, [M + H – H₂O]⁺).

(E,Z,Z)-2,4,7-Tridecatrien-1-ol (**V**). This was prepared by partial hydrogenation of **IV** in CH₃OH at room temperature under normal pressure using Lindlar's catalyst. Monitoring of the reaction by GC–MS indicated **V** as the major compound with some under- and over-hydrogenated byproducts. The product mixture was used in the next step without purification. MS (EI) *m/z* (% relative abundance): 194 (12, M⁺), 163 (17), 151 (5), 137 (21), 123 (46), 119 (29), 109 (29), 107 (28), 105 (51), 95 (50), 93 (77), 91 (90), 81 (76), 79 (100), 77 (45), 67 (67), 65 (22), 55 (52). MS–CI (ammonia) *m/z* (% relative abundance): 212 (48, [M + NH₄]⁺), 194 (97, M⁺ or [M + NH₄ – H₂O]⁺), 177 (100, [M + H – H₂O]⁺).

(E,Z,Z)-2,4,7-Tridecatrienal (**15**). The product mixture (0.8 g) obtained in the previous step containing **V** was oxidized with MnO₂ (8.0 g) in hexane (14). The oxidation, monitored by GC, was complete after 30 min. The target compound **15** was obtained in 80% (GC) purity by CC. The major byproduct was (E,E,Z)-2,4,7-tridecatrienal. GC (**15**): RI (DB-5) = 1584, RI (OV-1701) = 1737, RI (FFAP) = 2130, RI (DB-Wax) = 2117. MS (CI, **15**), ammonia *m/z* (% relative abundance): 210 (98, [M + NH₄]⁺), 193 (100, [M + H]⁺).

Microsynthesis of Alkatrienals. Various alkatrienals were obtained by aldol-condensation of acetaldehyde and the corresponding commercially available alkadienals (Scheme 2) following the general procedure described by Buttery (21). Two drops of aqueous KOH (50%) were added under stirring to an equimolar mixture of acetaldehyde (10 mmol) and an alkadienal (10 mmol) at –5 °C. The mixture was stirred for 15 min at –5 °C and then for 1 h at room temperature. Et₂O (50 mL) was added to dissolve the reaction product. The solution was successively washed with cold aqueous HCl (1N, 3 × 30 mL), saturated NaHCO₃ (2 × 30 mL), and brine (NaCl, 2 × 30 mL). The organic layer was dried over anhydrous Na₂SO₄. The target compounds were tentatively identified by GC–MS.

RESULTS AND DISCUSSION

Aroma Extract Dilution Analysis (AEDA). The aroma extract of autoxidized arachidonic acid revealed the characteristic aroma notes of the original product, i.e., green, metallic, egg-white-like, fatty, and fishy. GC–O of the aroma extract, used as a screening method for odorants, resulted in nineteen odor-active regions (Table 1). AEDA was applied to estimate the sensory contribution of individual odorants to the overall aroma

Table 2. Comparison of the Retention Indices (RI) and RI Increments of Odorant 15 with Those of Selected Alkadienals and Alkatrienals

| no. | odorants | RI values | | | RI increments | | |
|-----|--|-----------|---------|------|---------------|--------------|------------|
| | | SE 54 | OV 1701 | FFAP | OV 1701-SE 54 | FFAP-OV 1701 | FFAP-SE 54 |
| 9 | (<i>E,Z</i>)-2,4-nonadienal ^a | 1195 | 1330 | 1655 | 135 | 325 | 460 |
| 10 | (<i>E,E</i>)-2,4-nonadienal ^a | 1229 | 1366 | 1698 | 137 | 332 | 469 |
| 20 | (<i>E,Z</i>)-2,6-nonadienal ^a | 1155 | 1276 | 1584 | 121 | 308 | 429 |
| 11 | (<i>E,Z</i>)-2,4-decadienal ^a | 1295 | 1435 | 1760 | 140 | 325 | 465 |
| 12 | (<i>E,E</i>)-2,4-decadienal ^a | 1321 | 1459 | 1799 | 138 | 340 | 478 |
| 21 | (<i>E,E</i>)-2,4-undecadienal ^a | 1445 | 1591 | 1931 | 146 | 340 | 486 |
| 22 | (<i>E,E</i>)-2,4-dodecadienal ^a | 1524 | 1673 | 2027 | 149 | 354 | 503 |
| 23 | (<i>E,E,Z</i>)-2,4,6-undecatrienal ^b | 1472 | 1640 | 2078 | 168 | 438 | 606 |
| 24 | (<i>E,E,E</i>)-2,4,6-undecatrienal ^b | 1498 | 1668 | 2106 | 170 | 438 | 608 |
| 25 | (<i>E,E,Z</i>)-2,4,8-undecatrienal ^b | 1413 | 1566 | 1965 | 153 | 399 | 552 |
| 26 | (<i>E,E,Z</i>)-2,4,6-dodecatrienal ^b | 1577 | 1742 | 2183 | 165 | 441 | 606 |
| 27 | (<i>E,E,E</i>)-2,4,6-dodecatrienal ^b | 1600 | 1766 | 2216 | 166 | 450 | 616 |
| 28 | (<i>E,E,E</i>)-2,4,6-tridecatrienal ^b | 1708 | 1883 | 2342 | 175 | 459 | 634 |
| 29 | (<i>E,E,E</i>)-2,4,6-tetradecatrienal ^b | 1806 | 1983 | 2454 | 177 | 471 | 648 |
| 15 | unknown (egg-white-like) | 1584 | 1737 | 2130 | 153 | 393 | 546 |

^a Compounds were commercially available. ^b Compounds were prepared in this work by micro-synthesis.

on the basis of FD factors. The odorants **1**, **2**, **11**, **12**, **14**, and **15** showed high FD factors suggesting a significant contribution to the overall aroma. The aroma qualities of these odorants were green, mushroom-like, fatty, metallic, and egg-white-like, representing well the aroma attributes of autoxidized arachidonic acid. Odorants **4**, **7**, **10**, **13**, **16**, **17**, **18**, and **19** were considered to have medium sensory relevance, whereas odorants **3**, **5**, **6**, **8**, and **9** were of lower aroma impact. On the basis of these results, identification work was focused on odorants having high and medium FD factors.

Identification of Odorants. Sixteen of the nineteen odorants detected by GC-O were identified as shown in Table 1. In general, identification was achieved on the basis of GC retention indices on two capillaries and sensorial characteristics, as well as by comparison of these data with those obtained for the reference compounds (17). The identities of odorants **1**–**5**, **10**–**12**, and **14** were further confirmed by GC-MS (EI and CI). The concentrations of odorants **7**–**9** were too low to obtain unequivocal mass spectra. Their identity, however, was supported by co-injection with the corresponding reference compound. Odorant **13** was tentatively identified on the basis of its chromatographic and mass spectral data (Figure 1A).

The most intensely smelling odorant, *trans*-4,5-epoxy-(*E*)-2-decenal (**14**), was identified for the first time as an oxidative degradation product of arachidonic acid. Its significant contribution is due to its very low odor threshold of 0.0028 ng/L air (22), which means that even extremely low amounts are likely to affect the overall aroma. Odorants **16** and **17** were tentatively identified by comparison of their chromatographic and mass spectral data with those of odorant **15**.

Structure Elucidation of Odorant 15. Special attention was paid to the intense egg-white smelling odorant **15**, showing a high FD factor of 2 (11). Its aroma quality and chromatographic data did not allow a straightforward identification. A single odorant with such a specific note had not yet been reported in the literature.

Retention data. As shown by Anker and co-workers (23), there is a quantitative structure-retention relationship for compounds having the same functional group. Therefore, various alkatrienals were synthesized and their RI values were determined on three capillary columns with different stationary phases. The measured

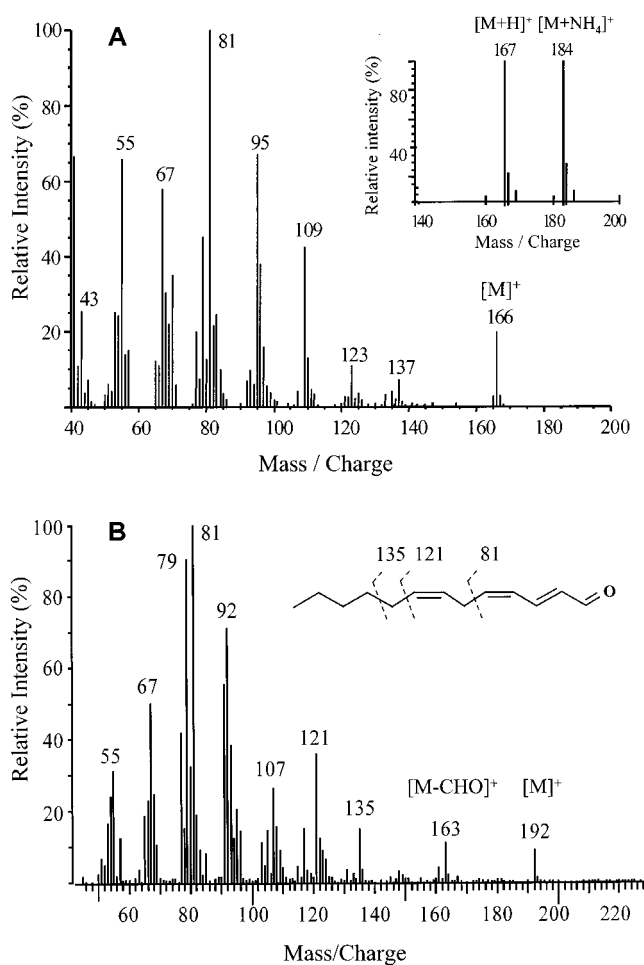


Figure 1. Mass spectra of 2,5-undecadienal (A) and (*E,Z,Z*)-2,4,7-tridecatrienal (B).

RI values and calculated increments (RI differences on two stationary phases) are summarized in Table 2. 2,4-Alkadienals have typical RI increments, which are slightly higher than those of a nonconjugated alkadienal, e.g., (*E,Z*)-2,6-nonadienal (**20**). Also, (*E,E,Z*)-2,4,8-undecatrienal (**25**) showed lower RI increments as compared to **23** and other conjugated alkatrienals. The RI increments of **25** were close to those of the unknown odorant **15**, thus suggesting that the double bonds in **15** are most likely not fully conjugated. However, the

Table 3. ^1H and ^{13}C NMR Data of (*E,Z,Z*)-2,4,7-Tridecatrienal in C^2HCl_3^a

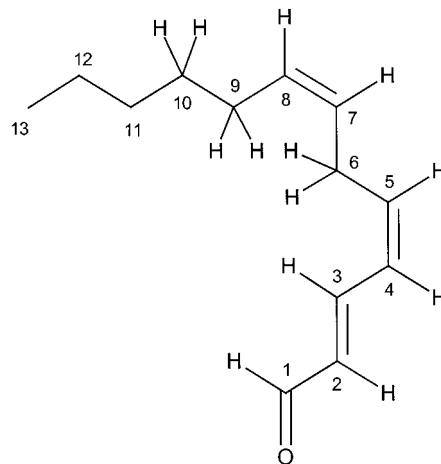
| group | proton NMR ^b | carbon NMR ^c |
|--------------------|---|-------------------------|
| 1-CHO | 9.62, d, 1H, J 7.9 Hz ^d | 193.9, d |
| 2-CH | 6.17, d d, 1H, J 15.2, 8.0 Hz | 132.0, d |
| 3-CH | 7.48, d d d, 1H, J 15.2, 11.5, 1.1 Hz | 146.5, d |
| 4-CH | 6.28, d d t d, 1H, J 11.5, 10.7, 1.6, 0.7 Hz | 126.6, d |
| 5-CH | 5.96, d t t, 1H, J 10.6, 7.9, 0.9 Hz | 141.5, d |
| 6-CH ₂ | 3.09, "t", 2H, J "7.5" Hz | 26.7, t |
| 7-CH | 5.35, d t t, 1H, J 10.6, 7.2, 1.6 Hz | 125.2, d |
| 8-CH | 5.51, d t t, 1H, J 10.7, 7.3, 1.6 Hz | 132.2, d |
| 9-CH ₂ | 2.08, "d t", 2H, J ~6.8, 6.8 Hz | 27.3, t |
| 10-CH ₂ | 1.38, "quintet", 2H, J ~6.8 Hz | 29.2, t |
| 11-CH ₂ | ~1.29 ^e , m, broad, $\geq 2\text{H}^f$ | 31.5, t |
| 12-CH ₂ | ~1.31 ^e , m, broad, $\geq 2\text{H}^f$ | 22.6, t |
| 13-CH ₃ | 0.89, "t", $\geq 3\text{H}^f$, J ~6.9 Hz | 14.1, q |

^a Chemical shift (δ) in ppm from internal TMS. ^b Proton multiplicity abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, d t = doublet of triplets (with decreasing values of coupling constants). Quotes ("...") mean approximate description of the multiplet. The coupling constants are those directly extracted from the spectrum, after moderate Gaussian resolution enhancement, without equalizing the constants belonging to the respective coupling partners. ^c Carbon multiplicity abbreviations: s, d, t, q, denote quaternary, CH-, CH₂- and CH₃-carbons, respectively. ^d Several (up to 8) small doublets with similar coupling constants near the main aldehyde signal, as well as other minor signals in the spectrum, indicated the presence of isomers with different double bond configurations. However, the integral areas of the 11-CH₂, 12-CH₂, and 13-CH₃ signals, which are common to all stereoisomers, indicate that these other isomers together represent $\leq 12\%$ of the (*E,Z,Z*) compound. ^e Signals not resolved in 1-D spectrum; approximate shifts determined from HETCOR traces. ^f Integrals more than integer numbers due to the presence of small amounts of other double bond isomers (see footnote^g).

exact position and stereochemistry could not be determined.

MS. CI measurements suggested a molecular weight of 192, i.e., m/z 193 (100, $[\text{M} + \text{H}]^+$) and m/z 210 (100, $[\text{M} + \text{NH}_4]^+$). Considering 4 double-bond equivalents and the fragment at m/z 163 ($[\text{M} - \text{CHO}]^+$), odorant **15** was proposed to be a tridecatrienal. The position of the double bonds was derived from the fragments at m/z 81, 121, and 135 (Figure 1B). The fragment at m/z 81 indicated the structural element $\text{CH}=\text{CH}-\text{CH}=\text{CH}-\text{CHO}$, which is characteristic for 2,4-alkadienals. Fragments at m/z 121 and 135 suggested the third double bond to be at position C7. On the basis of MS data, odorant **15** was tentatively identified as 2,4,7-tridecatrienal. Taking into account the formation mechanism of odorant **15** (see below) and its relatively small RI increments (Table 2), the configuration of the double bonds was assumed to be (*E,Z,Z*).

NMR Spectroscopy. The ^1H - and ^{13}C NMR spectral data of **15** are given in Table 3. The signal assignment was mainly achieved with the help of two-dimensional COSY and HETCOR spectra, the latter for both direct and multiple-bond C-H correlation. The ^1H NMR data confirm the *E,Z,Z* arrangement of the three C=C double bonds via the typical vicinal double bond coupling constants. One-dimensional NOE-difference spectral data (Table 4) confirmed the *E,Z* configuration of the first two C=C double bonds. However, the NOE of 8-CH on irradiating 7-CH and vice-versa could not be measured because of the proximity of the two signals. The NOE spectral data permitted the predominant spatial arrangement of the molecule to be determined from the through-space polarization enhancement caused by selective irradiation of the individually accessible signals. The magnitudes of the observed NOEs (e.g., 3-H,

**Figure 2.** Predominant stereochemistry of (*E,Z,Z*)-2,4,7-tridecatrienal (**15**) obtained from NOE-difference experiments.

6-H versus 6-H, 9-H) indicate that the molecule probably does not adopt a rigid conformation (Figure 2), but there may be rotational mobility around certain single bonds. The long-range carbon-proton correlations derived from a long-range HETCOR experiment (not given here) confirm the assignments of the carbon resonances, and thus provide an alternative proof of the molecular structure.

Synthesis. (*E,Z,Z*)-2,4,7-Tridecatrienal was synthesized in four steps by analogy to the method for the preparation of (*E,Z,Z*)-2,4,7-decatrienal (**18**). As shown in Scheme 1, bromination of 2-octyn-1-ol (**I**) followed by coupling of the resulting 1-bromo-2-octyne (**II**) with the Grignard derivative of 2-*trans*-penten-4-yn-1-ol (**III**) gave rise to 2-*trans*-tridecen-4,7-diyn-1-ol (**IV**), which has the carbon structure of a tridecatrienal. Partial hydrogenation of **IV** using Lindlar's catalyst was employed to obtain an all *cis*-configuration for C4-C5 and C7-C8 double bonds in the resulting 2,4,7-tridecatrien-1-ol (**V**). The mild and neutral oxidant MnO_2 was used to oxidize **V** to the target compound (**15**) in good yields without changing the *cis*-configuration of the C4-C5 double bond, which is sensitive to acid.

Organoleptic Characteristics. The sensorial characteristics of odorant **15** were evaluated with the substance obtained by synthesis. In diluted aqueous solutions (0.015 mg/L and 0.15 mg/L), it was described as egg-white-like, marine-fresh, fishy, and metallic. It smelled orange-citrus, waxy-fatty, and animal-like in more concentrated solutions (1.5 mg/L and 15 mg/L). The odor threshold in air was estimated to be approximately 0.07 ng/L by GC-O, which is in the same order of magnitude as that of (*E,E*)-2,4-decadienal (**12**) and 1-octen-3-one (**2**).

Formation of Odorants **13** and **15**. The formation of (*E,Z,Z*)-2,4,7-tridecatrienal can be explained by β -cleavage of the corresponding 8-hydroperoxy-5,9,11,14-icosatetraenoic acid (8-HPETE) as shown in Scheme 3. This is in analogy to the formation of (*E,Z,Z*)-2,4,7-decatrienal by autoxidation of linolenic acid (**4**, **18**). Similarly, decomposition of 10-hydroperoxy-5,7,11,14-icosatetraenoic acid (10-HPETE) leads to (*Z,Z*)-2,5-undecadienal, which may be the odorant **13**. (*E,E,Z*)-2,4,7-Tridecatrienal (**16**) and (*E,E,E*)-2,4,7-tridecatrienal (**17**) might be formed by isomerization of odorant **15**.

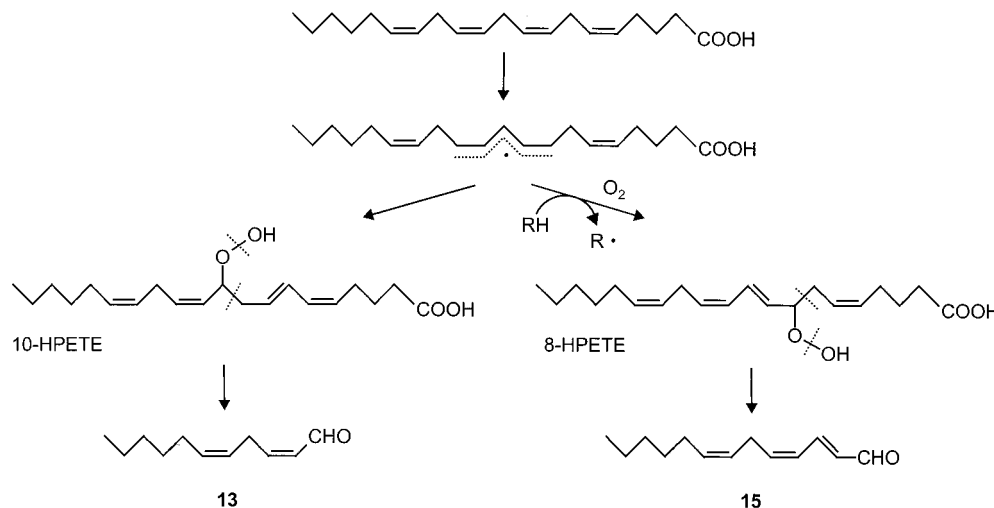
Ellis et al. (3) suggested the formation of 2,4,7-tridecatrienal from 8-HPETE although he could not detect it in autoxidized methyl arachidonate. Badings (4)

Table 4. One-Dimensional NOE-difference Spectral Data of (*E,Z,Z*)-2,4,7-Tridecatrienal

| irradiated proton | NOE response (%) ^a | | | | | | | | | |
|-------------------|-------------------------------|------|-------------|------------|---------------|-------------------|------|------|-------------------|--------------------|
| | 1-CHO | 2-CH | 3-CH | 4-CH | 5-CH | 6-CH ₂ | 7-CH | 8-CH | 9-CH ₂ | 10-CH ₂ |
| 1-CHO | | 2.1 | 9.4 | | | | | | | |
| 2-CH | 2.5 | | 2.0 | | | | | | | |
| 3-CH | 18.8 | ~4.4 | | 1.6 | | 6.3 | | | | |
| 4-CH | 1.3 | | ~2.3 | | ~ 12.8 | | | | | |
| 5-CH | | | | 9.3 | | 1.8 | | | | |
| 6-CH ₂ | | | 18.3 | | 4.3 | | 4.1 | | 4.2 | |
| 7-CH | | | | | | 1.5 | | | | |
| 8-CH | | | | | | | | | 1.5 | 1.2 |
| 9-CH ₂ | | | | | | 3.3 | | 4.9 | | 2.1 |

^a Only NOEs higher than 1% are listed. Estimated error $\pm 0.5\%$. NOEs of signals very close to the irradiation frequency usually can not be detected because of the counteracting direct influence of continuous wave irradiation. Therefore, some NOEs may be missing (e.g., 7-H, 8-H). The responses in bold confirm the *2E,4Z*-configuration. The *7Z* configuration results from the coupling constant $^3J_{7-H,8-H}$ of 10.7 Hz (Table 3).

Scheme 3. Formation of (*E,Z,Z*)-2,4,7-Tridecatrienal and (*Z,Z*)-2,5-Undecadienal by Autoxidation of Arachidonic Acid via the Intermediates 8-Hydroperoxy-5,9,11,14-eicosatetraenoic Acid (8-HPETE, right) and 10-Hydroperoxy-5,7,11,14-eicosatetraenoic Acid (10-HPETE, left), Respectively



tentatively identified the compound in a fraction of autoxidized arachidonic acid, smelling stale egg-like, by GC-MS of its 2,4-dinitrophenylhydrazone derivative but its stereochemistry was not specified. (*E,Z,Z*)-2,4,7-Tridecatrienal was also tentatively identified in cooked chicken (24) by comparing the thin-layer chromatography behaviors and the degree of unsaturation of its dinitrophenylhydrazone derivative with those of a series of aldehydes of different chain lengths and of various degrees of unsaturation. It was supposed to be a breakdown product of arachidonic acid and to contribute to cooked chicken flavor.

In conclusion, characterization of the aroma composition of autoxidized arachidonic acid by GC-O techniques revealed the green-metallic smelling *trans*-4,5-epoxy-(*E*)-decenal as a character impact odorant, which is reported for the first time as an oxidative degradation product of arachidonic acid. (*E,Z,Z*)-2,4,7-Tridecatrienal was unequivocally identified as a degradation product of arachidonic acid, with odor characteristics suggesting a significant contribution to the specific aroma of autoxidized arachidonic acid. The aroma properties of the most potent odorants were in good agreement with the overall aroma of autoxidized arachidonic acid.

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