Aroma Properties of a Homologous Series of 2,3-Epoxyalkanals and *trans*-4,5-Epoxyalk-2-enals

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A few odor-active epoxyaldehydes, formed during lipid peroxidation, have recently been reported as intense aroma compounds in foods. However, very little is known about their flavor properties in general. Syntheses of homologous *trans*-2,3-epoxyalkanals (C_6-C_{12}) and *trans*-4,5-epoxy-(*E*)-2-alkenals (C_7-C_{12}) followed by structural characterization using mass spectrometry (MS/EI; MS/CI) and ^{1}H NMR measurements were performed. An evaluation of their odor qualities and odor thresholds by gas chromatography−olfactometry revealed the following: within the *trans*-2,3-epoxyalkanals, the odor quality changed from grassy for the compounds with six and seven carbon atoms to citrus-like or soapy for aldehydes with eight and more carbon atoms. The odor thresholds lay in the range of 3−15 ng/L (in air) and were nearly identical within the series; however, a slight minimum was measured for *trans*-2,3-epoxyoctanal to *trans*-2,3-epoxydecanal. In the series of the *trans*-4,5-epoxyalk-(*E*)-2-enals the C_{10} compound was characterized by the lowest odor threshold of 0.6−2.5 pg/L of air. However, all *trans*-4,5-epoxy-alk-(*E*)-2-enals smelled intensely metallic.

Keywords: *trans-3-Alkyloxirane carboxaldehydes; 3-(trans-3-alkyloxiranyl)-(E)-2-propenals; structure/odor activity; odor threshold; odor quality*

INTRODUCTION

A few volatile epoxyaldehydes have been identified as lipid degradation products in model systems (*1*−*4*) and also in some foods (*5*−*12*). Until now, four compounds have been reported, namely, 4,5-epoxy-(*E*)-hept-2-enal (*1*, *5*), 4,5-epoxy-(*E*)-non-2-enal (*7*), and 4,5-epoxy-(*E*)-dec-2-enal (*2*−*4*, *6*−*10*) as well as 2,3-epoxyoctanal (*11*).

trans-4,5-Epoxy-(*E*)-hept-2-enal was the first member of this group of compounds reported as a constituent of oxidized butterfat (*5*). Although the compound did not contribute significantly to the off-flavor, its identification was an important step in understanding epoxyaldehyde formation by oxidative processes in foods. The authors proposed *trans*-15,16-epoxy-12-hydroperoxyoctadeca-(*Z*,*E*)-9,13-dienoic acid, an oxidation product of linolenic acid, as its precursor (*5*). Later it was shown (*1*) that the thermal decomposition of methyl linoleate hydroperoxides yielded 4,5-epoxyhept-2-enal.

Selke et al. (*2*) tentatively identified the homologous 4,5-epoxy-(*E*)-dec-2-enal as a volatile compound in trilinolein heated in air. The compound was proposed to originate from 12,13-epoxy-9-hydroperoxyoctadec-10-enoic acid by an α-cleavage (cf. Figure 1), a proposal that was later confirmed by the same author (*3*). Further studies showed that, besides 13-hydroperoxy-9,11-octadecadienoic acid, the 9-hydroperoxy-10,12-octadecadienoic acid is also an effective precursor of the epoxyaldehyde via 2,4-decadienal as the key intermediate (cf. Figure 2) (*13*). The first report on the identification of 4,5-epoxy-(*E*)-2-decenal in a food was in wheat bread crumb (*6*). In addition, *trans*-4,5-epoxy-(*E*)-2-decenal has been reported as contributor to the aroma of several foods, for example, stored soybean oil (*7*), warmed-over flavor in meat (*8*), orange juice (*9*), or rye bread crumb (*10*).

The homologous *trans*-4,5-epoxy-(*E*)-2-nonenal has been reported as an aroma compound in stored soybean oil (*7*). Its formation can be explained as shown for the respective epoxydecenal in Figure 2, but via 2,4-nonadienal as the precursor.

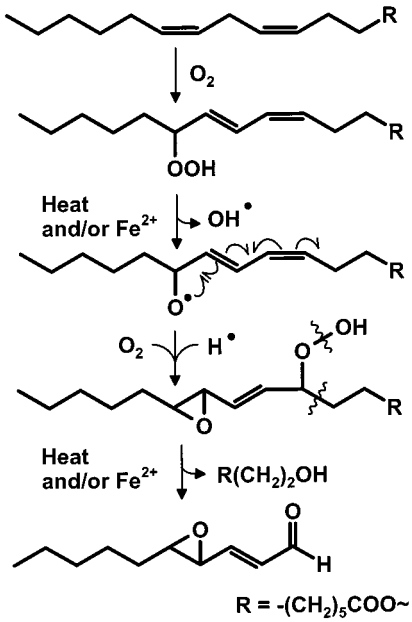

Figure 1. Proposed pathway for the formation of *trans*-4,5-epoxy-(*E*)-dec-2-enal from linoleic acid [according to Gardner and Selke (*3*)].

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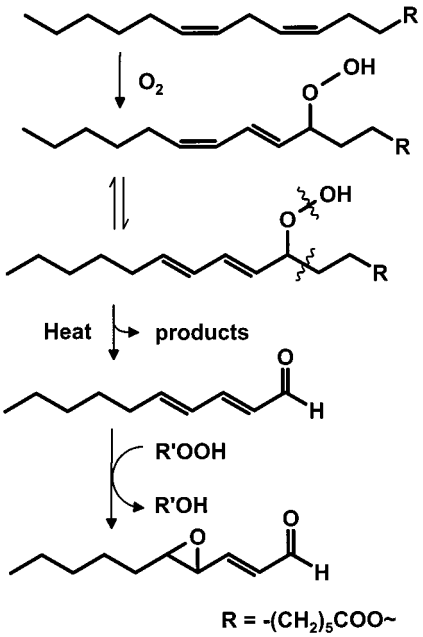

Figure 2. Formation of *trans*-4,5-epoxy-(*E*)-dec-2-enal by peroxidation of (*E*,*E*)-deca-2,4-dienal [according to Gassenmeier and Schieberle (*13*)].

Guth and Grosch (*11*) identified *trans*-2,3-epoxyoctanal as a contributor to the aroma of extruded oat flour. Its origin has not yet been studied; however, the oxidation of the double bond in (*E*)-oct-2-enal is the most probable way of forming this compound.

Besides the four epoxyaldehydes discussed above, no report on homologous compounds can be found in the food-related literature. However, the formation pathways displayed in Figures 1 and 2 suggest that others might also occur in foods but are as yet unidentified. Therefore, the aim of the present work was to elaborate analytical and sensory data on a homologous series of expoxyaldehydes with the 2,3-epoxyalkanal or the 4,5-epoxy-(*E*)-2-enal structure. For volatility reasons, only compounds with 6−12 carbons were investigated.

MATERIALS AND METHODS

Chemicals. The following chemicals were purchased from the suppliers given in parentheses: (*E*)-hex-2-enal, (*E*)-hept-2-enal, (*E*)-oct-2-enal, (*E*)-non-2-enal, (*E*)-dec-2-enal, (*E*,*E*)-deca-2,4-dienal, (*E*,*E*)-nona-2,4-dienal, (*E*,*E*)-octa-2,4-dienal, and (*E*,*E*)-hepta-2,4-dienal (Aldrich, Steinheim, Germany); (*E*)-undec-2-enal and (*E*)-dodec-2-enal (Alfa, Karlsruhe, Germany); (*E*,*E*)-undeca-2,4-dienal (ICN, Eschwege, Germany); (*E*,*E*)-dodeca-2,4-dienal (Lancaster, Mühlheim, Germany).

Syntheses. *trans-2,3-Epoxyalkanals.* The target compounds were synthesized according to the method described by Guth and Grosch (*11*), by oxidation of the corresponding (*E*)-alk-2-enals with alkaline hydrogen peroxide.

trans-4,5-Epoxy-(E)-alk-2-enals. The compounds were synthesized according to the method described by Schieberle and Grosch (*6*), by oxidation of the corresponding (*E*,*E*)-alka-2,4-dienals with 3-chloroperbenzoic acid.

The compounds were purified by column chromatography on silica gel using a pentane/diethyl ether gradient (*14*). For ^{1}H NMR measurements, the compounds were purified by preparative gas chromatography as described recently (*14*).

High-Resolution Gas Chromatography—Olfactometry (HRGC-O) and HRGC—Mass Spectrometry (HRGC-MS). HRGC was performed by means of a type 8000 gas chromatograph (Fisons Instruments, Mainz, Germany), using the following capillary columns: FFAP (30 m × 0.32 mm i.d. fused silica capillary, free fatty acid phase FFAP, 0.25 μm; Chrompack, Mühlheim, Germany); DB-5 (30 m × 0.32 mm i.d. fused silica capillary DB-5, 0.25 μm; J&W Scientific, Fisons Instruments); and DB-1701 (30 m × 0.32 mm fused silica capillary DB-1701, 0.25 μm; Chrompack). The samples were introduced via the on-column injection technique at 35 °C. After 2 min, the temperature of the oven was raised at 40 °C/min to 50 °C (SE-54, OV-1701) or 60 °C (FFAP), respectively, held for 2 min isothermally, then raised at 6 °C/min to 180 °C, and finally raised at 10 °C/min to 230 °C and held for 10 min. The flow rate of the helium carrier gas was 2.5 mL/min. At the end of the capillary, the effluent was split 1:1 (by volume) into an FID and a sniffing port using two deactivated but uncoated fused silica capillaries (50 cm × 0.32 mm i.d.). The FID and the sniffing port were held at 220 and 240 °C, respectively. Linear retention indices (RI) of the compounds were calculated as previously described (*15*). MS analysis was performed with a MAT-95 S (Finnigan, Bremen, Germany) in tandem with the capillary columns described above. Mass spectra in the electron impact mode (MS/EI) were generated at 70 eV and in the chemical ionization mode (MS/CI) at 115 eV with isobutane as the reactant gas.

Proton Magnetic Resonance Spectra (^{1}H NMR). ^{1}H NMR spectra were recorded with a Bruker AM 360 spectrometer operating at 360 MHz.

Determination of Odor Qualities and Odor Thresholds in Air. Thresholds in air were determined by GC-O as described by Ulrich and Grosch (*16*) using (*E*)-dec-2-enal (2.7 ng/L in air) instead of hexanal as the internal standard. For the sniffing experiments, (*E*)-dec-2-enal (1 mg) and the respective odorants under investigation (amounts between 100 and 0.01 mg depending on the odor threshold) were dissolved in 10 mL of diethyl ether. Serial dilutions (1:1) were prepared, and 0.5 μL of these solutions was analyzed by GC-O. On the basis of flavor dilution factors determined for (*E*)-dec-2-enal and the synthesized odorants, respectively, the odor thresholds were calculated in relation to the odor threshold of (*E*)-dec-2-enal.

RESULTS AND DISCUSSION

Epoxyalkanals. A homologous series of seven *trans*-2,3-epoxyalkanals (2,3-epoxyhexanal to 2,3-epoxydodecanal) was synthesized by epoxidation of the corresponding (*E*)-2-alkenals. The compounds were purified by column chromatography and were characterized by mass spectrometry in the MS/EI and MS/CI modes as well as by their retention indices on two GC stationary phases of different polarities. The results of the mass spectral measurements and the determination of the retention indices are summarized in Table 1. As expected, the retention indices on a nonpolar stationary phase increased by a factor of ∼100 with increasing carbon atoms from 6 to 12. The differences between the retention indices on the polar FFAP and DB-5 columns lay in a narrow range, slightly increasing from 448 (C_6) to 467 (C_{12}).

In the mass spectra obtained by chemical ionization, the base peak was $M^+ + 1$, thereby confirming the molecular weight in all homologues. Fragmentation by electron impact ionization gave *m*/*z* 71 as the base fragment, and even for the 2,3-epoxydodecanal no other fragment with an intensity >10% appeared in the mass range above *m*/*z* 71 (Table 1). These results clearly indicate that, solely on the basis of MS/EI, the identification of epoxyalkanals, for example, in a food flavor extract, would be impossible. The *trans*-configuration of the epoxy group was confirmed by ^{1}H NMR measurements, and the data agreed with results published for the C_8−C_{10} epoxyaldehydes (*17*).

Solutions of each compound were then analyzed by GC-O, and the odor thresholds were determined in

Table 1. Retention Indices (RI) and Mass Spectral Data (MS/CI and MS/EI) of Homologous *trans*-2,3-Epoxyalkanals

odorant	RI on FFAP	RI on DB-5	*m/z* (relative abundance in %) MS-CI (isobutane)	MS-EI
trans-2,3-epoxyhexanal	1340	892	115 (100, $M^+ + 1$), 99 (37), 97 (6)	71 (100), 41 (30), 39 (10), 43 (9), 55 (9), 57 (8), 113 (1)
trans-2,3-epoxyheptanal	1446	992	129 (100, $M^+ + 1$), 113 (42), 111 (18)	71 (100), 41 (23), 55 (19), 43 (17), 57 (12), 39 (9), 42 (8), 81 (5), 127 (1)
trans-2,3-epoxyoctanal	1547	1089	143 (100, $M^+ + 1$), 125 (45, $M^+ + 1 - H_2O$), 97 (40), 107 (32)	71 (100), 41 (3), 43 (14), 55 (12), 57 (11), 69 (8), 141 (1)
trans-2,3-epoxynonanal	1653	1194	157 (100, $M^+ + 1$), 121 (52), 139 (36, $M^+ + 1 - H_2O$)	71 (100), 41 (27), 55 (25), 43 (19), 57 (13), 67 (5), 155 (1)
trans-2,3-epoxydecanal	1756	1296	171 (100, $M^+ + 1$), 153 (90, $M^+ + 1 - H_2O$), 135 (30)	71 (100), 41 (18), 55 (18), 43 (17), 57 (13), 69 (8), 81 (7), 67 (4), 169 (1)
trans-2,3-epoxyundecanal	1859	1396	185 (100, $M^+ + 1$), 167 (80, $M^+ + 1 - H_2O$), 149 (45)	71 (100), 41 (24), 57 (22), 55 (21), 43 (21), 69 (17), 81 (9), 95 (8), 183 (1)
trans-2,3-epoxydodecanal	1967	1500	199 (100, $M^+ + 1$), 200 (12)	71 (100), 43 (21), 55 (21), 57 (21), 41 (20), 69 (13) 95 (10), 109 (4), 197 (1)

Table 2. Odor Qualities and Odor Thresholds of Homologous *trans*-2,3-Epoxyalkanals

odorant	odor quality	threshold in air ng/L	threshold in air nmol/L	previously identified in
trans-2,3-epoxyhexanal	grassy	6.3	0.055	nr[a]
trans-2,3-epoxyheptanal	grassy	6.9	0.054	nr
trans-2,3-epoxyoctanal	citrus-like, fatty	2.6	0.018	oatmeal (*7*, *12*)
trans-2,3-epoxynonanal	citrus-like, fatty	3.5	0.022	nr
trans-2,3-epoxydecanal	citrus-like, soapy	3.7	0.022	nr
trans-2,3-epoxyundecanal	citrus-like, soapy	6.1	0.033	nr
trans-2,3-epoxydodecanal	citrus-like, soapy	15.4	0.077	nr

[a] nr, not yet reported in foods.

Table 3. Retention Indices (RI) and Mass Spectral Data (MS/CI and MS/EI) of Homologous *trans*-4,5-Epoxy-(*E*)-alk-2-enals

odorant	RI on FFAP	RI on DB-5	RI on DB-1701	*m/z* (relative abundance in %) MS-CI (isobutane)	MS-EI
trans-4,5-epoxy-(*E*)-hept-2-enal	1698	1080	1228	127 (100, $M^+ + 1$), 109 (8)	68 (100), 41 (52), 81 (40), 110 (10)
trans-4,5-epoxy-(*E*)-oct-2-enal	nd[a]	1181	1330	141 (100, $M^+ + 1$), 123 (10)	68 (100), 41 (30), 81 (32), 124 (8)
trans-4,5-epoxy-(*E*)-non-2-enal	nd[a]	1279	1434	155 (100, $M^+ + 1$), 137 (20)	68 (100), 41 (20), 81 (42), 138 (10)
trans-4,5-epoxy-(*E*)-dec-2-enal	2006	1381	1538	169 (100, $M^+ + 1$), 151 (18)	68 (100), 41 (16), 81 (41), 152 (6)
trans-4,5-epoxy-(*E*)-undec-2-enal	2100	1483	1644	183 (100, $M^+ + 1$), 167 (20)	68 (100), 41 (12), 81 (28), 166 (4)
trans-4,5-epoxy-(*E*)-dodec-2-enal	2210	1590	1760	197 (100, $M^+ + 1$), 179 (40)	68 (100), 41 (34), 81 (43), 180 (2)[b]

[a] nd, not determined. [b] Data from ref *19*.

Table 4. Odor Qualities and Odor Thresholds of Homologous *trans*-4,5-Epoxy-(*E*)-alk-2-enals

odorant	odor quality	threshold in air ng/L	threshold in air nmol/L	previously identified in
trans-4,5-epoxy-(*E*)-hept-2-enal	metallic	>1000	>8	butterfat (*5*)
trans-4,5-epoxy-(*E*)-oct-2-enal	metallic	7−28	0.05−0.2	nr[a]
trans-4,5-epoxy-(*E*)-non-2-enal	metallic	0.07−0.28	0.0005−0.002	soyabean oil, rapeseed oil (*7*)
trans-4,5-epoxy-(*E*)-dec-2-enal	metallic	$(6-25) \times 10^{-4}$	4.10×10^{-5}	wheat bread crumb (*6*), soy bean oil, rapeseed oil (*7*), beef meat (*8*), orange juice (*9*)
trans-4,5-epoxy-(*E*)-undec-2-enal	metallic	$(1.5-5) \times 10^{-2}$	$(8-30) \times 10^{-5}$	nr
trans-4,5-epoxy-(*E*)-dodec-2-enal	metallic	6×10^{-3}	4×10^{-5}	nr

[a] nr, not reported as a food constituent.

comparison to (*E*)-2-decenal for which an odor threshold of 2.7 ng/L in air has been reported (*18*). The results (Table 2) showed that the odor thresholds were quite low but differed only slightly from those of *trans*-2,3-epoxyhexanal to *trans*-2,3-epoxydodecanal. In general, the odor thresholds are comparable to those of (*E*)-2-alkenals, like (*E*)-2-octenal (*18*).

The determination of the odor qualities, determined at the sniffing port, however, showed clear differences among the seven epoxyaldehydes. Whereas the C_6 and C_7 compounds smelled grassy at a level of 30 ng/L in air, the higher homologues gave citrus-like, soapy odor impressions at the same concentration level.

***trans*-4,5-Epoxy-(*E*)-2-alkenals.** As confirmed by ^1H NMR measurements, the epoxides synthesized showed a *trans*-double bond and a *trans*-epoxide. The retention indices on a nonpolar stationary phase also showed a linear increase by a factor of ~100 in each successive homologue. The differences in the retention indices between a polar stationary phase (FFAP) and the DB-5 were quite consistent and lay between 617 (C_{11}) and 625 (C_{10}). Compared with the *trans*-2,3-epoxyalkanals, the difference was higher by ~170 units, which is due to the presence of the double bond.

In the MS/CI, the base peak was always the $M^+ + 1$ ion, but also an elimination of water was observed ($M^+ + 1 - 18$). The mass spectral data obtained by MS/EI gave two main fragments, *m/z* 68 (base peak) and *m/z* 81, as the most intense ions in all homologues. As already observed for the epoxyalkanals, no intense fragment with intensities >10% was observable in the higher mass range (> *m/z* 81). Thus, the MS/EI alone

will not give sufficient information to clearly identify these compounds, for example, in a food extract.

A determination of the odor thresholds in air confirmed a clear minimum for *trans*-4,5-epoxy-(*E*)-dec-2-enal, eliciting an odor threshold of ~0.1 pg/L in air. However, the odor qualities of all homologues were identical when evaluated at a level of ~10 times the odor threshold.

It is interesting to note that the introduction of an epoxy group into an α,*β*-unsaturated aldehyde clearly changed the odor quality from, for example, fatty, tallowy [(*E*)-non-2-enal] to citrus-like, soapy in the *trans*-2,3-epoxynonanal. In general, the odor impression of the epoxyalkanals much more resembled the odor quality of the respective saturated aldehyde, for example, nonanal. In contrast to the *trans*-4,5-epoxy-(*E*)-alk-2-enals, which all elicited a distinct metallic odor, no metallic odor quality was found for any of the *trans*-2,3-epoxyalkanals.

In particular, the data for the *trans*-2,3-epoxyaldehydes clearly show that the odor quality may change with chain length, thereby illustrating that odor qualities cannot be predicted from chemical structures but have to be investigated on the basis of sensory studies with reference compounds.

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