Soybean Lipoxygenase: Substrate Structure and Product Selectivity

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To study the influence of structural properties of the substrate on the selectivity of the dioxygenation catalyzed by soybean lipoxygenase (LOX), a number of synthesized (*Z,Z*)-3,6-dienyl 1-adipates with various distal residues were used and the kinetic parameters, the regioselectivity, and the enantioselectivity of product formation determined. Product analysis comprised the reduction of hydroperoxides, derivatization to their methyl esters, purification by silica gel chromatography, and subsequent HPLC separation of (*Z,E*)-1,3-hydroxydiene methyl esters using both achiral and chiral phases. Enantioseparations were performed by HPLC on chiral phases employing both underivatized products and naphthoylate derivatives. The calculation of the hydrophobicity difference between the distal and the proximal residues of the substrate was confirmed to be a quantitative parameter to predict the positional specificity of the enzymic catalysis. Higher enantiomeric excess was observed only for 7-oxygenated products; 3-oxygenated products were found to be racemic.

Keywords: *Lipoxygenase; selectivity; structure–selectivity relationship; soybean*

INTRODUCTION

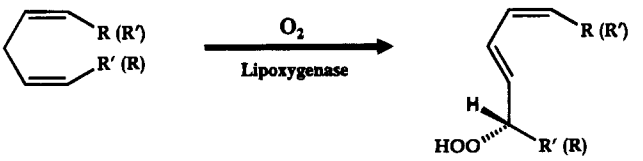
Lipoxygenase (LOX) is a non-heme, iron-containing dioxygenase that catalyzes the regioselective and enantioselective oxidation of unsaturated fatty acids containing one or more (*Z,Z*)-1,4-pentadienoic moieties; for instance, linoleic acid is converted to the (*S*)-13-hydroperoxide by LOX from soybean (Veldink and Vliegenthart, 1984, 1991). The catalytic mechanism (Scheme 1) was proposed to proceed through a free radical intermediate which reacts directly with oxygen or an organoiron intermediate (Corey, 1987). Recently, the three-dimensional protein structure of the native form of LOX isoenzyme L-1 from soybean has been elucidated (Boyington et al., 1993; Minor et al., 1993).

Several hypotheses exist to explain the observed regioselectivity of the enzymic catalysis: (i) the substrate structure and the product selectivity are determined by the normal or "head-to-tail" orientation of the substrate in the active site of LOX (Gardner, 1991); (ii) an optimal fit for the ω-end (distal residue) for the carboxy end (proximal residue) of the substrate might be responsible for the regioselectivity (Kühn et al., 1986); (iii) the difference in hydrophobicity content between the proximal and distal groups of the substrate influences the positional specificity of dioxygenation (Datcheva et al., 1991). The limited number of substrates checked to data (Datcheva et al., 1991; Veldink and Vliegenthart, 1991) together with the insufficient information on kinetics and enantioselectivity (Zhang and Kyler, 1989) stimulated us to study an extended number of synthesized (*Z,Z*)-3,6-dienyl 1-adipates with various distal residues as substrates of LOX catalysis and to determine the kinetic parameters as well as the regioselectivity and enantioselectivity of product formation. In this paper, the results of these studies are reported.

EXPERIMENTAL PROCEDURES

General. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker spectrometers (AC 200; AC 250; WM 400). CIMS spectra were recorded on a Finnigan apparatus (MAT 90 and MAT 8200) using either isobutane or ammonia as reactant gases. EIMS spectra (70 eV) were recorded on a Finnigan MAT 44S spectrometer directly coupled to a Varian gas chromatograph using a J&W DB-5 fused silica column (30 m × 0.25 mm i.d.; df = 0.25 μm) and He as carrier gas. CD spectra were recorded on a CD 6 Jobin Yvon Dichrograph in a 0.5 mm cell using ethanol as solvent. Soybean lipoxygenase isoenzyme L-1 was obtained by purification from soybean according to the method of Axelrod et al. (1981), using slight modifications (Weyd, 1993). All chemicals (analytical grade) were obtained from commercial sources. Molecular modeling studies were performed using the software package SYBYL 5.5 on a ESU 3/32 graphics workstation. Configurations were implemented using MAXIMIN2 software for the minimization of the energies in the TRIPOS force field. Electrostatic and steric forces were visualized using the software 3D-CoMFA.

Scheme 1



General Procedure for Preparation of Substrates. Synthesis was performed as described previously (Carvalho and Prestwich, 1984; Datcheva et al., 1991). To obtain aldehydes that are not commercially available, reduction of the corresponding methyl esters using diisobutylaluminum hydride or oxidation of the corresponding alcohol using pyridinium chlorochromate was performed. The chromatographic and spectral data are summarized in Table 1.

General Procedure for LOX-Catalyzed Dioxygenation of Substrates. To a solution of 123 mg (0.4 mmol) of substrate (for **S1** as an example) in 30 mL of 0.2 M sodium borate buffer (pH 9.0) was added 2 mL of an 18 nM solution (36 pmol) of LOX (L-1) and the mixture stirred at 0 °C under oxygen atmosphere. After 3 h, 30 mL of methanol and 15.9 mg (0.4 mmol = 4 equiv) of sodium borohydride was added, and the reaction mixture was stirred again for 1 h at 0 °C. After acidifying to pH 3, the solution was extracted three times, each with 30 mL of diethyl ether. The combined organic layers were dried over anhydrous sodium sulfate and concentrated in vacuo (rotavapor) to approximately 50 mL. Esterification with diazomethane, separation of the solvent under vacuum (rotavapor), and subsequent flash chromatography on silica gel (pentane–ethyl acetate, 8 + 2) yielded 96 mg (0.28 mmol) of the isomeric 1,3-hydroxydiene methyl esters. Separation of regioisomers was performed by HPLC on a Knauer system equipped with an Eurospher Si 100 column (4.6 × 25

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Table 1. Chromatographic and Spectral Data of Substrates (S1–S13) Used in Lipoxygenase Catalysis

S1 (*Z,Z*)-3,6-dodecadienyl 1-monoadipate ($C_{18}H_{30}O_4$); R_i 2623; EIMS *m/z* (%) 79 (100), 80 (91), 55 (74), 93 (60), 41 (54), 67 (38), 43 (30), 81 (24); DCI-MS (NH_3) *m/z* (%) 328 (100) ($[M + NH_4]^+$), 329 (22); ^1H NMR (200 MHz, $CDCl_3$) δ 0.86 (t, J = 6.5 Hz, 3H), 1.27 (br s, 6H), 1.61–1.69 (m, 4H), 2.04 (q, J = 6.8 Hz, 2H), 2.27–2.43 (m, 6H), 2.77 (t, J = 6.5 Hz, 2H), 4.06 (t, J = 6.9 Hz, 2H), 5.22–5.53 (m, 4H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 14.0, 22.5, 24.0, 24.2, 25.6, 26.8, 27.1, 29.2, 31.4, 33.6, 33.7, 63.7, 124.5, 127.2, 130.5, 131.0, 174.0, 179.4

S2 (*Z,Z*)-3,6-pentadecadienyl 1-monoadipate ($C_{21}H_{36}O_4$); R_i = 2623; EIMS *m/z* (%) 80 (100), 79 (74), 55 (63), 93 (52), 41 (48), 43 (38), 67 (37), 81 (29); DCI-MS (NH_3) *m/z* (%) 370 (100) ($[M + NH_4]^+$), 371 (25); DCI-MS (isobutane) *m/z* (%) 353 (100) ($[M + H]^+$), 354 (25), 206 (9), 147 (6), 113 (6); ^1H NMR (200 MHz, $CDCl_3$) δ 0.87 (t, J = 6.5 Hz, 3H), 1.26 (m, 12H), 1.63–1.72 (m, 4H), 2.04 (q, J = 6.8 Hz, 2H), 2.29–2.44 (m, 4H), 2.37 (q, J = 6.7 Hz, 2H), 2.78 (t, J = 6.5 Hz, 2H), 4.08 (t, J = 6.8 Hz, 2H), 5.24–5.54 (m, 4H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 14.0, 22.6, 24.0, 24.2, 25.7, 26.8, 27.2, 29.3, 29.3, 29.5, 29.6, 31.8, 33.6, 33.8, 63.8, 124.6, 127.3, 130.0, 131.1, 173.3, 179.2

S3 (*Z,Z*)-3,6-octadecadienyl 1-monoadipate ($C_{24}H_{42}O_4$); R_i 2966; EIMS *m/z* (%) 80 (100), 79 (73), 55 (66), 43 (47), 41 (46), 93 (44), 67 (43), 81 (36); DCI-MS (NH_3) *m/z* (%) 412 (100) ($[M + NH_4]^+$), 413 (24), 332 (4); DCI-MS (isobutane) *m/z* (%) 395 (100) ($[M + H]^+$), 396 (24), 161 (12), 248 (11), 147 (7); ^1H NMR (250 MHz, $CDCl_3$) δ 0.87 (t, J = 6.4 Hz, 3H), 1.19–1.30 (m, 18H), 1.63–1.75 (m, 4H), 2.04 (q, J = 6.5 Hz, 2H), 2.29–2.40 (m, 4H), 2.39 (q, J = 6.8 Hz, 2H), 2.79 (t, J = 6.5 Hz, 2H), 4.08 (t, J = 6.8 Hz, 2H), 5.23–5.55 (m, 4H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 14.0, 22.6, 24.0, 24.2, 25.6, 26.8, 27.2, 28.5, 29.3, 29.3, 29.6, 29.6, 29.6, 29.6, 31.9, 33.6, 33.8, 63.8, 124.5, 127.2, 130.6, 131.1, 173.3, 179.4

S4 (*Z,Z*)-3,6-henicosadienyl 1-monoadipate ($C_{27}H_{48}O_4$); R_i 3277; EIMS *m/z* (%) 80 (100), 55 (66), 79 (57), 43 (51), 41 (42), 93 (41), 67 (41), 81 (39); DCI-MS (NH_3) *m/z* (%) 454 (100) ($[M + NH_4]^+$), 374 (83), 455 (32), 375 (19); DCI-MS (isobutane) *m/z* (%) 437 (100) ($[M + H]^+$), 357 (46), 438 (35), 358 (11), 290 (8); ^1H NMR (250 MHz, $CDCl_3$) δ 0.87 (t, J = 6.5 Hz, 3H), 1.23–1.30 (m, 24H), 1.63–1.75 (m, 4H), 2.04 (q, J = 7.0 Hz, 2H), 2.29–2.40 (m, 4H), 2.39 (q, J = 7.1 Hz, 2H), 2.79 (t, J = 6.4 Hz, 2H), 4.08 (t, J = 6.8 Hz, 2H), 5.23–5.55 (m, 4H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 14.1, 22.7, 24.1, 24.3, 25.7, 26.9, 27.3, 29.3, 29.3, 29.6, 29.6, 29.6, 29.7, 29.7, 29.7, 29.7, 29.7, 31.9, 33.6, 33.9, 63.8, 124.6, 127.3, 130.7, 131.1, 173.3, 178.9

S5 9(*R*),13-dimethyl-3(*Z*),6(*Z*),12-tetradecatrienyl 1-monoadipate ($C_{22}H_{36}O_4$); R_i 2858; EIMS *m/z* (%) 41 (100), 69 (86), 55 (79), 81 (48), 67 (41), 79 (40), 95 (33), 80 (29); DCI-MS (NH_3) *m/z* (%) 382 (100) ($[M + NH_4]^+$), 383 (26); DCI-MS (isobutane) *m/z* (%) 365 (100) ($[M + H]^+$), 366 (22), 219 (22), 163 (4); ^1H NMR (250 MHz, $CDCl_3$) δ 0.88 (d, J = 7.1 Hz, 3H), 1.14–1.48 (m, 2H), 1.54 (s, 3H), 1.60–1.71 (m, 7H), 1.87–2.10 (m, 5H), 2.29–2.38 (m, 4H), 2.39 (q, J = 7.1 Hz, 2H), 2.79 (t, J = 6.1 Hz, 2H), 4.08 (t, J = 6.9 Hz, 2H), 5.09 (t, J = 7.0 Hz, 1H), 5.29–5.55 (m, 4H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 17.6, 19.5, 24.1, 24.3, 25.6, 25.7, 25.8, 26.9, 33.0, 33.4, 33.8, 34.5, 36.8, 63.8, 124.6, 124.8, 128.1, 129.1, 131.0, 131.0, 173.3, 178.2

S6 9(*S*),13-dimethyl-3(*Z*),6(*Z*),12-tetradecatrienyl 1-monoadipate ($C_{22}H_{36}O_4$); cf. **S5**

S7 6-cyclohexylidene-(*Z*)-3-hexenyl 1-monoadipate ($C_{18}H_{28}O_4$); R_i 2591; EIMS *m/z* (%) 135 (100), 178 (77), 79 (65), 41 (58), 55 (38), 67 (38), 80 (35), 93 (35); DCI-MS (NH_3) *m/z* (%) 326 (100) ($[M + NH_4]^+$), 327 (21), 314 (20); DCI-MS (isobutane) *m/z* (%) 309 (100) ($[M + H]^+$), 147 (32), 310 (22), 163 (21), 297 (20); ^1H NMR (200 MHz, $CDCl_3$) δ 1.50–1.57 (m, 6H), 1.63–1.72 (m, 4H), 2.00–2.20 (m, 4H), 2.29–2.45 (m, 4H), 2.39 (q, J = 6.8 Hz, 2H), 2.75 (t, J = 7.1 Hz, 2H), 4.08 (t, J = 6.9 Hz, 2H), 5.03 (t, J = 6.7 Hz, 1H), 5.30–5.46 (m, 2H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 24.1, 24.3, 25.5, 26.9, 26.9, 27.7, 28.5, 28.7, 33.4, 33.9, 37.1, 63.9, 119.3, 124.1, 131.8, 133.5, 173.3, 179.4

S8 7-(cyclohex-3-enyl)-(*Z,Z*)-3,6-heptadienyl 1-monoadipate ($C_{19}H_{28}O_4$); R_i 2622; EIMS *m/z* (%), 79 (100), 55 (77), 91 (76), 80 (59), 41 (57), 92 (50), 105 (50), 120 (38); DCI-MS (NH_3) *m/z* (%) 338 (100) ($[M + NH_4]^+$), 339 (21), 164 (10); DCI-MS (isobutane) *m/z* 321 (100) ($[M + H]^+$), 147 (33), 322 (22), 175 (16), 174 (9); ^1H NMR (250 MHz, $CDCl_3$) δ 1.32–1.44 (m, 2H), 1.63–1.84 (m, 6H), 1.99–2.13 (m, 2H), 2.33 (t, J = 6.9 Hz, 2H), 2.34 (t, J = 6.9 Hz, 2H), 2.39 (q, J = 6.9 Hz, 2H), 2.53–2.60 (m, 1H), 2.82 (t, J = 6.7 Hz, 2H), 4.08 (t, J = 6.9 Hz, 2H), 5.20–5.54 (m, 4H), 5.60–5.76 (m, 2H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 24.1, 24.3, 24.7, 25.9, 26.9, 28.9, 31.6, 32.1, 33.6, 33.8, 63.8, 124.7, 126.1, 126.2, 126.9, 131.1, 135.7, 173.2, 179.2

S9 7-cyclohexyl-(*Z,Z*)-3,6-heptadienyl 1-monoadipate ($C_{19}H_{30}O_4$); R_i 2480; EIMS *m/z* (%) 80 (100), 55 (72), 79 (58), 67 (54), 41 (52), 93 (44), 81 (32), 94 (32); DCI-MS (NH_3) *m/z* (%) 340 (100) ($[M + NH_4]^+$), 341 (20); DCI-MS (isobutane) *m/z* (%) 323 (100) ($[M + H]^+$), 324 (22), 116 (11), 113 (10); ^1H NMR (200 MHz, $CDCl_3$) δ 0.95–1.75 (m, 14H), 2.10–2.30 (m, 1H), 2.19–2.41 (m, 4H), 2.40 (q, J = 6.8 Hz, 2H), 2.79 (t, J = 6.6 Hz, 2H), 4.08 (t, J = 6.7 Hz, 2H), 5.13–5.50 (m, 4H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 24.0, 24.3, 25.9, 25.9, 26.0, 26.8, 33.2, 33.2, 33.3, 33.6, 33.8, 36.4, 63.8, 124.5, 125.4, 131.3, 136.6, 173.3, 179.4

S10 8-cyclohexyl-(*Z,Z*)-3,6-octadienyl 1-monoadipate ($C_{20}H_{32}O_4$); R_i 2918; EIMS *m/z* (%) 55 (100), 80 (70), 79 (67), 41 (49), 94 (46), 67 (38), 93 (30); DCI-MS (NH_3) *m/z* (%) 354 (100) ($[M + NH_4]^+$), 355 (21); DCI-MS (isobutane) *m/z* (%) 337 (100) ($[M + H]^+$), 338 (23), 161 (14), 190 (10), 191 (10); ^1H NMR (200 MHz, $CDCl_3$) δ 0.87–0.97 (m, 1H), 1.14–1.71 (m, 14H), 1.94 (t, J = 6.3 Hz, 2H), 2.33 (t, J = 6.8 Hz, 2H), 2.35 (t, J = 6.8 Hz, 2H), 2.38 (q, J = 6.8 Hz, 2H), 2.78 (t, J = 6.3 Hz, 2H), 4.07 (t, J = 6.5 Hz, 2H), 5.31–5.48 (m, 4H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 24.0, 24.2, 25.7, 26.4, 26.4, 26.5, 26.8, 33.2, 33.2, 33.6, 33.8, 35.0, 38.2, 63.8, 124.6, 127.9, 129.1, 131.1, 173.3, 179.3

S11 9-cyclohexyl-(*Z,Z*)-3,6-nonadienyl 1-monoadipate ($C_{21}H_{34}O_4$); R_i 2706; EIMS *m/z* (%) 55 (100), 80 (75), 79 (65), 41 (61), 43 (53), 67 (46), 93 (41), 81 (38); DCI-MS (NH_3) *m/z* (%) 368 (100) ($[M + NH_4]^+$), 369 (21); DCI-MS (isobutane) *m/z* (%) 351 (100) ($[M + H]^+$), 352 (29), 204 (8), 147 (6); ^1H NMR (200 MHz, $CDCl_3$) δ 0.89 (m, 1H), 1.14–1.73 (m, 16H), 2.04 (q, J = 7.1 Hz, 2H), 2.29–2.40 (m, 4H), 2.40 (q, J = 7.2 Hz, 2H), 2.78 (t, J = 6.5 Hz, 2H), 4.08 (t, J = 6.8 Hz, 2H), 5.22–5.54 (m, 4H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 24.1, 24.3, 24.6, 25.7, 26.4, 26.4, 26.7, 26.8, 33.3, 33.3, 33.5, 33.8, 37.2, 37.3, 63.8, 124.6, 127.1, 130.8, 131.1, 173.0, 179.0

S12 10-cyclohexyl-(*Z,Z*)-3,6-decadienyl 1-monoadipate ($C_{22}H_{36}O_4$); R_i 2897; EIMS *m/z* (%) 55 (100), 79 (81), 80 (78), 67 (58), 41 (51), 94 (46), 93 (36), 81 (34); DCI-MS (NH_3) *m/z* (%) 382 (100) ($[M + NH_4]^+$), 383 (27), 218 (5); DCI-MS (isobutane) *m/z* (%) 365 (100) ($[M + H]^+$), 366 (25), 147 (13), 218 (10); ^1H NMR (200 MHz, $CDCl_3$) δ 0.82–0.92 (m, 1H), 1.14–1.71 (m, 18H), 2.02 (q, J = 6.5 Hz, 2H), 2.33 (t, J = 6.8 Hz, 2H), 2.34 (t, J = 6.8 Hz, 2H), 2.36 (q, J = 7.0 Hz, 2H), 2.79 (t, J = 6.5 Hz, 2H), 4.08 (t, J = 6.9 Hz, 2H), 5.24–5.66 (m, 4H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 24.0, 24.3, 25.7, 26.4, 26.4, 26.4, 26.8, 26.9, 27.5, 33.4, 33.4, 33.5, 33.9, 37.1, 37.6, 63.6, 124.6, 127.3, 130.7, 131.1, 173.3, 179.3

S13 11-cyclohexyl-(*Z,Z*)-3,6-undecadienyl 1-monoadipate ($C_{23}H_{38}O_4$); R_i 2955; EIMS *m/z* (%) 55 (100), 80 (69), 79 (57), 41 (51), 67 (48), 81 (42), 83 (36), 94 (33); DCI-MS (NH_3) *m/z* (%) 396 (100) ($[M + NH_4]^+$), 397 (25); DCI-MS (isobutane) *m/z* (%) 379 (100) ($[M + H]^+$), 380 (25), 232 (9), 147 (9); ^1H NMR (250 MHz, $CDCl_3$) δ 0.81–0.89 (m, 1H), 1.08–1.69 (m, 20H), 2.06 (q, J = 6.8 Hz, 2H), 2.33 (t, J = 6.8 Hz, 2H), 2.37 (t, J = 6.8 Hz, 2H), 2.40 (q, J = 6.8 Hz, 2H), 2.78 (t, J = 6.7 Hz, 2H), 4.08 (t, J = 6.8 Hz, 2H), 5.27–5.52 (m, 4H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 24.0, 24.3, 25.5, 26.4, 26.4, 26.4, 26.5, 26.7, 27.3, 29.9, 33.4, 33.4, 33.6, 33.8, 37.4, 37.6, 63.8, 124.6, 127.2, 130.6, 131.1, 173.3, 179.3

Table 2. Chromatographic and Spectral Data of Derivatized Products (P1–P13) Obtained from S1–S13 by Lipoxygenase Catalysis

P1 7(*S*)-hydroxy-(*Z*,*E*)-3,5-dodecadienyl methyl adipate ($C_{19}H_{32}O_5$); R_i 2491; EIMS *m*/*z* (%) 99 (100), 43 (58), 151 (46), 71 (39), 55 (38), 109 (36), 111 (32), 81 (31); DCI-MS (NH_3) *m*/*z* (%) 358 (100) ($[M + NH_4]^+$), 340 (28), 359 (21), 163 (12); DCI-MS (isobutane) *m*/*z* (%) 163 (100), 323 (60) ($[M + H - H_2O]^+$), 324 (13), 164 (11), 217 (6), 161 (7), 340 (6) (M^+); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.7 Hz, 3H), 1.24–1.42 (m, 6H), 1.52 (q, *J* = 6.5 Hz, 2H), 1.63–1.66 (m, 4H), 2.31 (t, *J* = 6.8 Hz, 2H), 2.32 (t, *J* = 6.8 Hz, 2H), 2.52 (br q, *J* = 7.0 Hz, 2H), 3.65 (s, 3H), 4.10 (t, *J* = 6.8 Hz, 2H), 4.16 (q, *J* = 6.3 Hz, 1H), 5.40 (dt, *J* = 6.7/10.8 Hz, 1H), 5.72 (dd, *J* = 6.7/15.1 Hz, 1H), 6.09 (dd, *J* = 11.0/11.0 Hz, 1H), 6.47 (dd, *J* = 11.1/15.1 Hz, 1H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.0, 22.6, 24.4, 24.4, 25.1, 27.4, 31.8, 33.7, 33.9, 37.3, 51.6, 63.5, 72.7, 125.0, 126.7, 130.4, 137.4, 173.3, 173.8

P2 7(*S*)-hydroxy-(*Z*,*E*)-3,5-pentadecadienyl methyl adipate ($C_{22}H_{38}O_5$); R_i 2780; EIMS *m*/*z* (%) 91 (100), 41 (95), 105 (95), 55 (85), 204 (80), 80 (75), 92 (70), DCI-MS (NH_3) *m*/*z* (%) 400 (100) ($[M + NH_4]^+$), 401 (25), 382 (5); DCI-MS (isobutane) *m*/*z* (%) 365 (100) ($[M + H - H_2O]^+$), 205 (91), 366 (22), 206 (18), 382 (7) (M^+), 161 (6); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (t, *J* = 6.5 Hz, 3H), 1.25 (m, 12H), 1.56 (q, *J* = 6.7 Hz, 2H), 1.63–1.72 (m, 4H), 2.29–2.44 (m, 4H), 2.48 (q, *J* = 7.0 Hz, 2H), 3.66 (s, 3H), 4.11 (t, *J* = 6.7 Hz, 2H), 4.17 (q, *J* = 6.8 Hz, 1H), 5.40 (dt, *J* = 6.7/10.4 Hz, 1H), 5.65 (dd, *J* = 6.7/15.1 Hz, 1H), 6.09 (dd, *J* = 10.6/10.6 Hz, 1H), 6.46 (dd, *J* = 11.1/15.1 Hz, 1H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 14.1, 22.7, 24.4, 24.4, 25.4, 27.4, 29.3, 29.5, 29.6, 31.9, 33.7, 33.9, 37.4, 51.6, 63.5, 72.7, 125.0, 126.7, 130.5, 137.4, 173.1, 173.8

P3 7(*S*)-hydroxy-(*Z*,*E*)-3,5-octadecadienyl methyl adipate ($C_{25}H_{44}O_5$); R_i 3036; EIMS *m*/*z* (%) 91 (100) 105 (88), 80 (70), 92 (70), 246 (59), 41 (56), 55 (52), 106 (52); DCI-MS (NH_3) *m*/*z* (%) 442 (100) ($[M + NH_4]^+$), 412 (43), 443 (28), 413 (13); DCI-MS (isobutane) *m*/*z* (%) 407 (100) ($[M + H - H_2O]^+$), 395 (64), 247 (54), 408 (30), 396 (21), 161 (21), 217 (17), 424 (10) (M^+); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.87 (t, *J* = 6.7 Hz, 3H), 1.17–1.45 (m, 18H), 1.52 (m, *J* = 6.7 Hz, 2H), 1.60–1.75 (m, 4H), 2.30–2.40 (m, 4H), 2.51 (q, *J* = 7.0 Hz, 2H), 3.66 (s, 3H), 4.08 (t, *J* = 6.8 Hz, 2H), 4.15 (q, *J* = 6.5 Hz, 1H), 5.36 (dt, *J* = 6.7/10.8 Hz, 1H), 5.71 (dd, *J* = 6.7/15.0 Hz, 1H), 6.08 (dd, *J* = 10.8/10.8 Hz, 1H), 6.46 (dd, *J* = 11.0/15.0 Hz, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 14.1, 22.7, 24.3, 24.3, 25.4, 27.2, 28.2, 29.3, 29.3, 29.6, 29.6, 29.6, 31.9, 33.6, 33.9, 37.6, 51.6, 63.9, 72.6, 124.9, 126.4, 130.4, 137.4, 173.2, 173.9

P4 7(*S*)-hydroxy-(*Z*,*E*)-3,5-henicosadienyl methyl adipate ($C_{28}H_{50}O_5$); R_i > 3200 (RT = 53.14 min); EIMS *m*/*z* (%) 91 (100), 105 (78), 80 (77), 79 (61), 41 (58), 55 (58), 43 (38), 106 (32); DCI-MS (NH_3) *m*/*z* (%) 454 (100), 455 (32), 484 (21) ($[M + NH_4]^+$); DCI-MS (isobutane) *m*/*z* (%) 437 (100), 439 (35), 449 (16) ($[M + H - H_2O]^+$), 469 (14), 289 (12), 466 (1) (M^+); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.87 (t, *J* = 6.7 Hz, 3H), 1.17–1.45 (m, 24H), 1.52 (m, 2H), 1.60–1.75 (m, 4H), 2.30–2.40 (m, 4H), 2.51 (q, *J* = 7.0 Hz, 2H), 3.66 (s, 3H), 4.08 (t, *J* = 6.8 Hz, 2H), 4.15 (q, *J* = 6.5 Hz, 1H), 5.36 (dt, *J* = 6.7/10.8 Hz, 1H), 5.71 (dd, *J* = 6.7/15.0 Hz, 1H), 6.08 (dd, *J* = 10.8/10.8 Hz, 1H), 6.46 (dd, *J* = 11.0/15.0 Hz, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 14.1, 22.7, 24.3, 24.3, 25.4, 27.2, 28.2, 29.3, 29.3, 29.6, 29.6, 29.6, 29.6, 29.7, 29.7, 31.9, 33.6, 33.9, 37.6, 51.6, 63.9, 72.6, 124.9, 126.4, 130.4, 137.4, 173.2, 173.9

P5 9(*R*),13-dimethyl-7(*S*)-hydroxy-3(*Z*),5(*E*),12-tetradecatrienyl methyl adipate ($C_{23}H_{38}O_5$); R_i 2758; EIMS *m*/*z* (%) 41 (100), 55 (100), 173 (77), 105 (72), 91 (72), 69 (56), 131 (49), 216 (47); DCI-MS (NH_3) *m*/*z* (%) 412 (100) ($[M + NH_4]^+$), 413 (28), 396 (11), 394 (6); DCI-MS (isobutane) *m*/*z* (%) 377 (100) ($[M + H - H_2O]^+$), 217 (94), 378 (23), 218 (15), 161 (11), 395 (5) ($[M + H]^+$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.87 (d, *J* = 6.5 Hz, 3H), 1.13–1.52 (m, 4H), 1.59 (s, 3H), 1.60–1.75 (m, 7H), 1.83–2.10 (m, 3H), 2.25–2.35 (m, 4H), 2.38 (q, *J* = 6.3 Hz, 2H), 3.66 (s, 3H), 4.07 (t, *J* = 7.2 Hz, 2H), 4.15 (q, *J* = 5.7 Hz, 1H), 5.08 (t, *J* = 7.0 Hz, 1H), 5.39 (dt, *J* = 6.7/10.8 Hz, 1H), 5.71 (dd, *J* = 6.7/15.0 Hz, 1H), 6.07 (dd, *J* = 11.5/11.5 Hz, 1H), 6.47 (dd, *J* = 11.1/15.0 Hz, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 17.6 19.5, 24.3, 24.3, 25.6, 25.7, 27.3, 33.0, 33.6, 33.9, 36.7, 39.2, 51.5, 63.8, 72.6, 124.6, 124.7, 126.4, 130.4, 131.0, 137.4, 173.3, 173.8

P6 9(*S*),13-dimethyl-7(*S*)-hydroxy-3(*Z*),5(*E*),12-tetradecatrienyl methyl adipate ($C_{23}H_{38}O_5$); cf. P5

P7 6-cyclohexylidenyl-3-hydroxy-4(*E*)-hexenyl methyl adipate ($C_{19}H_{30}O_5$); R_i 2572; EIMS *m*/*z* (%) 160 (100), 91 (52), 131 (50), 117 (48), 118 (43), 55 (37), 119 (31), 132 (30); DCI-MS (NH_3) *m*/*z* (%) 354 (100) ($[M + NH_4 - 2H]^+$), 326 (74), 288 (37), 312 (32), 338 (18) ($[M]^+$), 356 (13) ($[M + NH_4]^+$), 279 (13); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.50–1.57 (m, 6H), 1.63–1.72 (m, 4H), 1.95–2.06 (m, 2H), 1.99–2.16 (m, 4H), 2.29–2.39 (m, 4H), 3.67 (s, 3H), 4.17 (t, *J* = 6.8 Hz, 2H), 4.30 (q, *J* = 6.7 Hz, 1H), 5.65 (dd, *J* = 6.6/15.1 Hz, 1H), 6.11 (d, *J* = 11.4 Hz, 1H), 6.47 (dd, *J* = 11.0/15.0 Hz, 1H)

P8 7-(cyclohex-3-enyl)-7(*S*)-hydroxy-(*Z*,*E*)-3,5-heptadienyl methyl adipate ($C_{20}H_{30}O_5$); R_i 2823; EIMS *m*/*z* (%) 81 (100), 109 (76), 79 (58), 55 (53), 41 (39), 111 (36), 80 (35); DCI-MS (NH_3) *m*/*z* (%) 368 (100) ($[M + NH_4]^+$), 350 (25), 369 (22), 333 (10); DCI-MS (isobutane) *m*/*z* (%) 333 (100) ($[M + H - H_2O]^+$), 173 (66), 334 (24), 161 (9), 174 (9), 223 (8), 350 (7) (M^+); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.13–1.31 (m, 2H), 1.57–1.73 (m, 6H), 1.99–2.07 (m, 3H), 2.24–2.31 (m, 4H), 2.48 (q, *J* = 7.0 Hz, 2H), 3.63 (s, 3H), 3.97 (t, *J* = 6.5 Hz, 1H), 4.07 (t, *J* = 6.8 Hz, 2H), 5.36 (dt, *J* = 6.7/10.8 Hz, 1H), 5.64 (m, 2H), 5.70 (dd, *J* = 6.7/15.1 Hz, 1H), 6.06 (dd, *J* = 10.9/10.9 Hz, 1H), 6.43 (dd, *J* = 11.1/15.2 Hz, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 24.2, 24.2, 24.7, 24.9, 27.1, 27.2, 33.5, 33.8, 39.7, 51.5, 63.4, 76.5, 125.9, 126.2, 126.6, 126.8, 130.4, 135.7, 173.2, 173.7

P9 7-cyclohexyl-7(*S*)-hydroxy-(*Z*,*E*)-3,5-heptadienyl methyladipate ($C_{20}H_{32}O_5$); R_i 2692; EIMS *m*/*z* (%) 174 (100), 92 (80), 55 (50), 91 (46), 41 (35), 79 (33), 175 (23), 93 (23); DCI-MS (NH_3) *m*/*z* (%) 370 (100) ($[M + NH_4]^+$), 352 (39), 371 (24); DCI-MS (isobutane) *m*/*z* (%) 335 (100) ($[M + H - H_2O]^+$), 175 (61), 336 (19), 176 (9), 161 (9), 352 (5) (M^+); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.89–1.38 (m, 10H), 1.60–1.67 (m, 4H), 1.80–1.87 (m, 1H), 2.26–2.35 (m, 4H), 2.51 (q, *J* = 6.7 Hz, 2H), 3.66 (s, 3H), 3.90 (t, *J* = 6.6 Hz, 1H), 4.10 (t, *J* = 6.8 Hz, 2H), 5.38 (dt, *J* = 6.7/10.8 Hz, 1H), 5.71 (dd, *J* = 7.1/15.1 Hz, 1H), 6.09 (dd, *J* = 10.9/10.9 Hz, 1H), 6.44 (dd, *J* = 11.0/15.1 Hz, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 24.3, 24.3, 26.0, 26.1, 26.5, 27.3, 28.5, 28.8, 33.6, 33.9, 43.9, 51.5, 63.5, 77.1, 125.9, 126.5, 130.5, 136.0, 173.2, 173.8

P10 8-cyclohexyl-7(*S*)-hydroxy-(*Z*,*E*)-3,5-octadienyl methyl adipate ($C_{21}H_{34}O_5$); R_i 2879; EIMS *m*/*z* (%) 55 (100), 91 (100), 92 (99), 106 (86), 41 (68), 79 (68), 188 (68), 105 (62); DCI-MS (NH_3) *m*/*z* (%) 384 (100) ($[M + NH_4]^+$), 385 (20), 366 (7); DCI-MS (isobutane) *m*/*z* (%) 349 (100) ($[M + H - H_2O]^+$), 189 (95), 350 (22), 190 (17), 366 (9) (M^+); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.87–0.99 (m, 1H), 1.09–1.70 (m, 14H), 1.84 (t, *J* = 6.7 Hz, 2H), 2.27–2.35 (m, 4H), 2.51 (q, *J* = 6.7 Hz, 2H), 3.65 (s, 3H), 4.09 (t, *J* = 6.8 Hz, 2H), 4.16 (t, *J* = 5.9 Hz, 1H), 5.38 (dt, *J* = 7.7/10.7 Hz, 1H), 5.70 (dd, *J* = 6.7/15.1 Hz, 1H), 6.07 (dd, *J* = 11.1/11.1 Hz, 1H), 6.44 (dd, *J* = 11.1/15.1 Hz, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 24.3, 24.3, 26.1, 26.3, 27.3, 33.0, 33.1, 33.6, 33.9, 38.3, 45.1, 51.5, 63.5, 70.1, 124.7, 126.6, 130.5, 137.8, 173.2, 173.8

P11 9-cyclohexyl-7(*S*)-hydroxy-(*Z*,*E*)-3,5-nonadienyl methyl adipat ($C_{22}H_{36}O_5$); R_i 2783; EIMS *m*/*z* (%) 106 (100), 55 (77), 91 (62), 41 (60), 202 (41), 79 (26), 105 (24), 119 (23); DCI-MS (NH_3) *m*/*z* (%) 398 (100) ($[M + NH_4]^+$), 399 (24), 381 (2); DCI-MS (isobutane) *m*/*z* (%) 203 (100), 363 (90) ($[M + H - H_2O]^+$), 364 (22), 204 (16), 380 (8) (M^+), 161 (4); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.87 (m, 1H), 1.14–1.34 (m, 12H), 1.42 (s, 1H (OH)), 1.55 (q, *J* = 6.9 Hz, 2H), 1.58–1.69 (m, 4H), 2.27–2.35 (m, 4H), 2.51 (q, *J* = 7.1 Hz, 2H), 3.66 (s, 3H), 4.10 (t, *J* = 6.8 Hz, 2H), 4.14 (q, *J* = 6.5 Hz, 1H), 5.39 (dt, *J* = 7.6/10.8 Hz, 1H), 5.70 (dd, *J* = 6.7/15.1 Hz, 1H), 6.08 (dd, *J* = 10.8/10.8 Hz, 1H), 6.45 (dd, *J* = 10.9/15.1 Hz, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 24.3, 24.3, 25.1, 26.3, 26.3, 27.3, 33.2, 33.3, 33.6, 33.8, 34.7, 36.2, 37.7, 51.5, 63.5, 72.9, 125.0, 126.6, 130.5, 137.4, 172.3, 173.8

Table 2 (Continued)

P12 10-cyclohexyl-7(*S*)-hydroxy-(*Z,E*)-3,5-decadienyl methyl adipate ($C_{23}H_{38}O_5$); R_i 3037; EIMS *m/z* (%) 136 (100), 55 (82), 91 (78), 41 (58), 79 (37), 216 (31), 92 (29), 105 (29); DCI-MS (NH_3) *m/z* (%) 412 (100) ($[M + NH_4]^+$), 413 (27), 394 (7); DCI-MS (isobutane) *m/z* (%) 377 (100) ($[M + H - H_2O]^+$), 217 (65), 378 (23), 218 (13), 394 (9) (M^+), 161 (7); ^1H NMR (250 MHz, $CDCl_3$) δ 0.86 (m, 1H), 1.16–1.70 (m, 20H), 2.27–2.35 (m, 4H), 2.51 (q, J = 7.0 Hz, 2H), 3.66 (s, 3H), 4.10 (t, J = 6.6 Hz, 2H), 4.15 (q, J = 5.9 Hz, 1H), 5.38 (dt, J = 7.7/10.8 Hz, 1H), 5.71 (dd, J = 6.7/15.0 Hz, 1H), 6.07 (dd, J = 11.1/11.1 Hz, 1H), 6.45 (dd, J = 11.1/15.0 Hz, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 24.3, 24.3, 26.3, 26.4, 26.4, 26.7, 27.3, 33.3, 33.4, 33.4, 33.6, 33.8, 37.5, 37.6, 51.6, 63.5, 72.6, 124.9, 126.6, 130.4, 137.4, 173.3, 173.8

P13 11-cyclohexyl-7(*S*)-hydroxy-(*Z,E*)-3,5-undecadienyl methyl adipate ($C_{24}H_{40}O_5$); R_i 3178; EIMS *m/z* (%) 55 (100), 91 (90), 41 (80), 150 (70), 79 (54), 67 (47), 81 (46), 80 (37); DCI-MS (NH_3) *m/z* (%) 426 (100) ($[M + NH_4]^+$), 427 (25), 408 (6); DCI-MS (isobutane) *m/z* 391 (100) ($[M + H - H_2O]^+$), 231 (74), 392 (27), 232 (13), 408 (10) (M^+), 161 (8); ^1H NMR (250 MHz, $CDCl_3$) δ 0.86 (m, 1H), 1.01–1.70 (m, 22H), 2.24–2.33 (m, 4H), 2.49 (q, J = 7.0 Hz, 2H), 3.64 (s, 3H), 4.08 (t, J = 6.8 Hz, 2H), 4.13 (q, J = 6.0 Hz, 1H), 5.37 (dt, J = 7.7/10.8 Hz, 1H), 5.69 (dd, J = 6.7/14.8 Hz, 1H), 6.06 (dd, J = 11.1/11.1 Hz, 1H), 6.44 (dd, J = 11.1/15.0 Hz, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 24.3, 24.3, 26.3, 26.3, 26.4, 26.4, 26.7, 27.3, 33.3, 33.4, 33.4, 33.6, 33.8, 37.3, 37.5, 51.5, 63.4, 72.5, 124.8, 126.5, 130.4, 137.4, 173.2, 173.7

cm; 5 μm; Knauer) employing pentane–ethanol (99 + 1) at 1.0 mL min^{-1}, monitoring at 234 nm and measuring the UV spectra by photodiode array detection. Quantification was carried out by integration of peak areas. The chromatographic and spectral data are outlined in Table 2 (cf. also products **P1–P13** in Tables 4 and 5).

Separation of enantiomers (without derivatization) was performed by HPLC using either Chiralcel OB-H or Chiralcel OD 5 μm columns (Daicel) eluting with 2-propanol–hexane (8 + 92) at 0.8 mL min^{-1} and monitoring at 234 nm. Naphthoylates (Kühn et al., 1987) were separated by HPLC using a Pirkle type column with ionically bound dinitrobenzoylphenylglycine (DNBPG) (5 μm, Baker) eluting with 2-propanol–hexane (1 + 99) at 0.8 mL min^{-1} and monitoring at 222 nm.

RESULTS

The synthesized monomeric adipates of (*Z,Z*)-3,6-dien-1-ols used as substrates (**S1–S13**) of LOX catalysis are represented in Figure 1. The kinetic parameters measured at pH 9 in air-saturated buffer are summarized in Table 3. From the kinetic data it is obvious that the elongation of the distal residue (**S1–S3**) led to an increase of K_m and a decrease of V_{max}. Comparing the kinetic data of **S7–S13**, each bearing an cyclohexyl group in the ω-position, highest substrate affinity was found for **S10**. For **S7–S9** (shorter chain lengths) and for **S11–S13** (longer chain lengths) an increase of K_m values was observed.

The steps to analyze the reaction products comprised the reduction of hydroperoxides formed, derivatization to their methyl esters, purification by silica gel flash chromatography, and subsequent HPLC separation of (*Z,E*)-1,3-hydroxydiene methyl esters using both achiral and chiral phases. The distribution of regioisomers formed during LOX catalysis using linoleic acid (**LA**), linolenic acid (**LnA**), and **S1–S13** is represented in Table 4. In Figure 2, the HPLC separation of oxygenated products formed from **S9** is outlined as a representative example. As shown from Table 4, elongation of the distal residue (**S1–S4**; **S9–S13**) resulted in a decrease of the corresponding 7-oxygenated isomers. Both the introduction of a double bond in the cyclohexyl group in the ω-position (**S8**) and the insertion of a chiral center in β-position to the C6/C7 double bond (**S5**, **S6**) did not affect the regioselectivity. The substrate **S7** showed a different behavior; as major isomer (99%) the 3-oxygenated product was formed. This result can be explained by the presence of an additional alicyclic C–C bond at the C6/C7 double bond, favoring the attack of oxygen at the C3-position.

The percentual ratios of enantiomeric oxygenation products are listed in Table 5. Enantioseparations were performed by HPLC on chiral phases using both underivatized products and naphthoylate derivatives. The absolute configuration of products was assigned by CD spectroscopy (Nakanishi et al., 1994). As shown from Table 5, higher enantiomeric excess (ee) was only observed for 7-oxygenation (**P1–P13**); racemic mixtures were obtained for 3-oxygenated products. The occurrence of racemic mixtures for the *E,E*-configured products (yields, cf. Table 4) indicates chemical isomerization caused by autoxidation.

Table 3. Kinetic Parameters Measured for Linoleic Acid (LA) and Synthesized Monomeric Adipates of (*Z,Z*)-3,6-Dien-1-ols (S1–S13) Used in LOX Catalysis

substrate	K_m (mM)	V_{max} (μmol min^{-1})	k_{cat} (min^{-1})	k_{cat}/K_m (mM^{-1} min^{-1})
S1	0.05	58.4	5840	116800
S2	0.13	13.4	1335	10269
S3	2.50	1.4	141	56
S4	nd[a]	nd	nd	nd
S5	0.37	2.6	258	697
S6	nd	nd	nd	nd
S7	0.15	1.4	140	940
S8	0.20	1.5	152	760
S9	0.13	1.4	142	1092
S10	0.04	2.3	232	5800
S11	0.10	1.6	163	1630
S12	0.73	1.5	148	203
S13	1.00	0.7	74	74
LA	0.03	74.2	7416	247200

[a] nd, not determined.

Table 4. Percentual Ratio of Regioisomeric Oxygenated Products Formed from Linoleic Acid (LA), Linolenic Acid (LnA), and Synthesized Monomeric Adipates of (*Z,Z*)-3,6-Dien-1-ols (S1–S13) Used in LOX Catalysis (Cf. Experimental Procedures)

		regioisomeric oxygenated products				
substrate	products	7-OH-(*Z,E*) (%)	7-OH-(*E,E*) (%)	3-OH-(*E,Z*) (%)	3-OH-(*E,E*) (%)	7:3 (%:%)
S1	**P1**	85	4	10	1	89:11
S2	**P2**	54	4	10	1	63:37
S3	**P3**	40	14	36	10	54:46
S4	**P4**	32	3	55	10	35:65
S5	**P5**	38	3	48	11	41:59
S6	**P6**	34	4	50	12	38:62
S7	**P7**	1	0	99	0	1:99
S8	**P8**	72	4	23	1	76:24
S9	**P9**	75	5	18	2	80:20
S10	**P10**	79	3	17	1	82:18
S11	**P11**	62	7	26	5	69:31
S12	**P12**	50	6	36	8	56:44
S13	**P13**	37	6	50	7	43:57
LA[a]		94	1	3	2	95:5
LnA[a]		83	4	9	4	87:13

[a] For the oxygenation products of LA and LnA the 7-position corresponds to the C13-position and the 3-position to the C9-position.

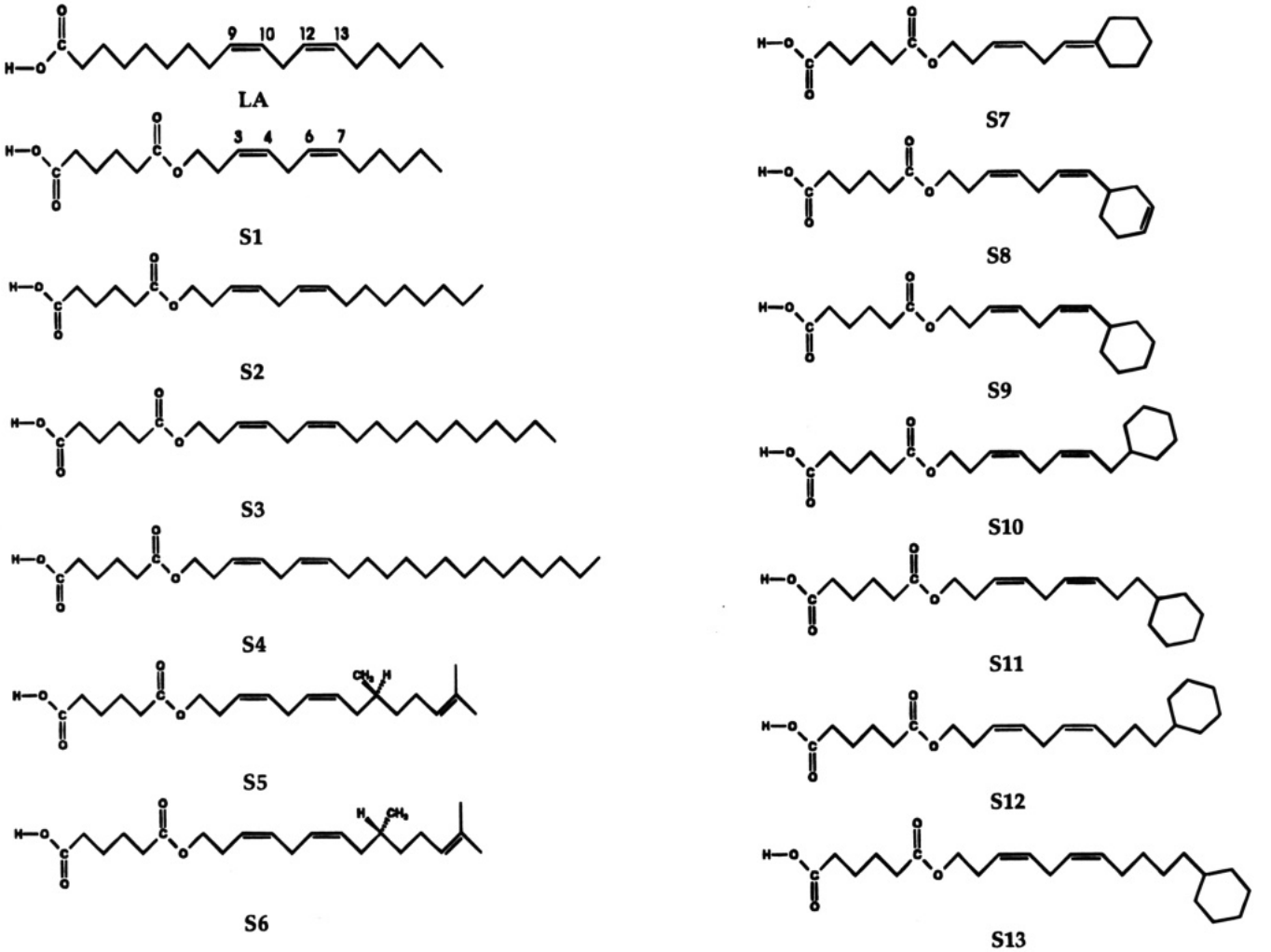

Figure 1. Substrates for lipoxygenase catalysis: linoleic acid (**LA**) and monomeric adipates of (*Z,Z*)-3,6-dien-1-ols (**S1**−**S13**).

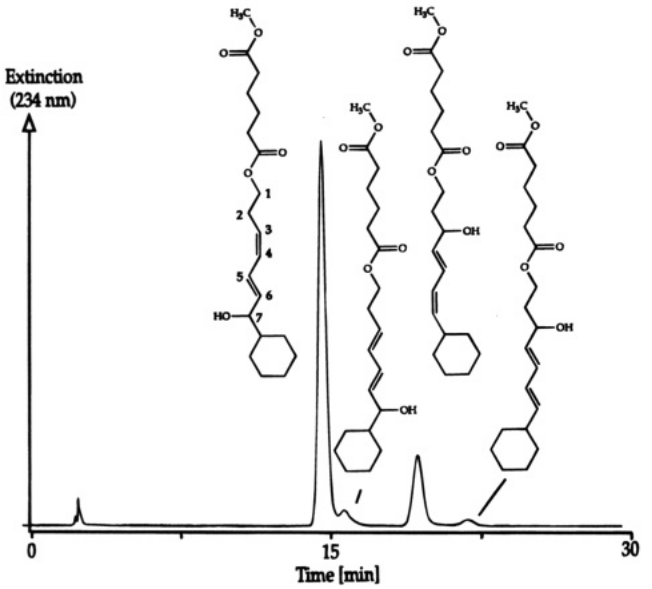

Figure 2. HPLC separation of regioisomeric oxygenation products. Column, Eurospher Si 100; flow, 1 mL/min; detection, 234 nm; eluent, pentane−ethanol (99 + 1).

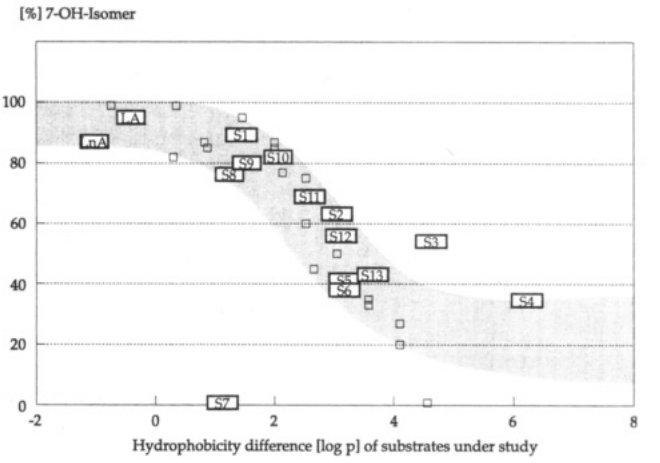

Figure 3. Relation between the percentage of 7-oxygenated products and the hydrophobicity difference between the proximal and distal residue of the substrates. **LA**, linoleic acid; **LnA**, linolenic acid; **S1**−**S13**, substrates under study; □, data evaluated from published substrates (Datcheva et al., 1991).

DISCUSSION

As to the regioselectivity observed during LOX-catalyzed dioxygenation of **S1**−**S13** (Table 4), similar results have been reported previously (Datcheva et al., 1991). To correlate both the data from earlier published substrates and our actually determined data with the structures of substrates, a calculation of hydrophobicity using the hydrophobic fragmental constants (*f* values) was performed. The *f* values represent the hydrophobicity contribution of a constituent part of a molecular structure of the total hydrophobicity (Rekker, 1977). In Figure 3 the relation between the percentage of 7-oxygenated products and the hydrophobicity difference

Table 5. Percentual Ratio of Enantiomeric Oxygenated Products Formed from Linoleic Acid (LA) and Synthesized Monomeric Adipates of (Z,Z)-3,6-Dien-1-ols (S1–S13) Used in LOX Catalysis (Cf. Experimental Procedures)

	enantiomeric product											
	7-OH-(Z,E)			7-OH-(E,E)			3-OH-(E,Z)			3-OH-(E,E)		
substrate	S (%)	R (%)	ee (%)	S (%)	R (%)	ee (%)	S (%)	R (%)	ee (%)	S (%)	R (%)	ee (%)
S1	94	6	88[a]	51	49	2[b]	52	48	4[a]	49	51	2[a]
S2	59	41	18[a]	49	51	2[b]	47	53	6[a]	50	50	0[a]
S3	64	36	28[a]	49	51	2[a]	54	46	8[b]	51	49	2[b]
S4	55	45	10[b]	50	50	0[b]	51	49	2[a]	50	50	0[b]
S5			nd[d]			nd	52	48	4[b]	51	49	2[b]
S6			nd			nd	51	49	2[b]	52	48	4[b]
S7							51	49	2[b]			
S8	94	6	88[b,c]	54	46	8[b]	50	50	0[b]	52	48	4[b]
S9	88	12	76[b,c]	51	49	2[b]	48	52	4[a]	51	49	2[b]
S10	80	20	60[b,c]	46	54	8[b]	47	53	6[a]	48	52	4[b]
S11	90	10	80[b,c]	49	51	2[a]	48	52	4[a]	49	51	2[b]
S12	96	4	92[b,c]	51	49	2[a]	49	51	2[a]	50	50	0[b]
S13	99	1	98[b,c]	49	51	2[b]	51	49	2[a]	49	51	2[b]
LA[e]	99	1	98[a,c]	50	50	0[a]	52	48	4[a]	48	52	4[a]

[a] Separation on Chiracel OB-H (Daicel). [b] Separation on Chiralcel OD (Daicel). [c] Separation on DNBPG (Baker) after derivatization into naphthoylates. [d] nd, not determined. [e] For the oxygenation products of LA the 7-position corresponds to the C13-position, the 3-position to the C9-position.

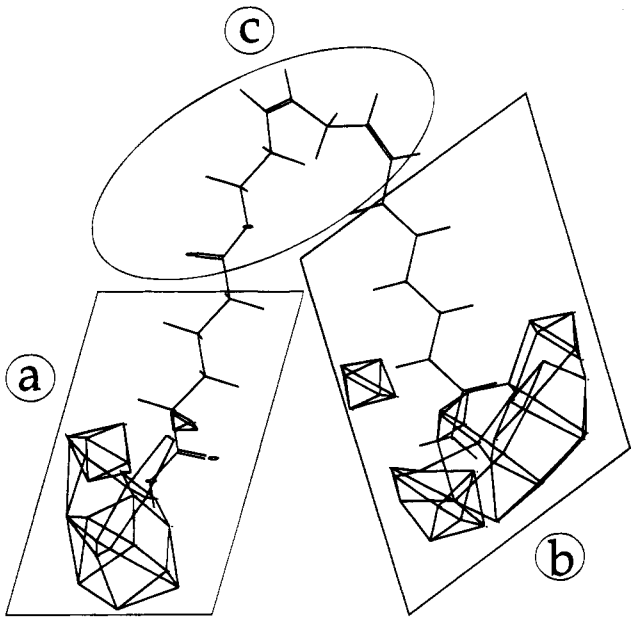

Figure 4. "Horseshoe"-like configuration of the lipoxygenase substrates: (a) proximal residue; (b) distal residue; (c) pentadienoic moiety.

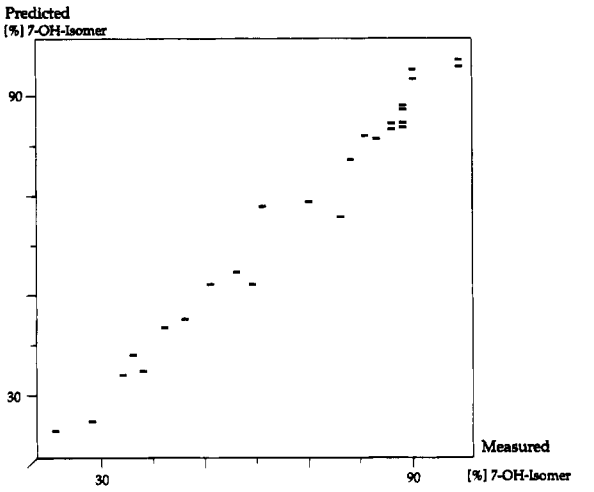

Figure 5. Correlation between the measured and predicted percentual ratio of 7-oxygenated products in lipoxygenase catalysis using SYBYL 5.5 (r^2 = 0.852 and σ = 0.097). The prediction of the level of 7-oxygenated products was performed using molecular structures of substrates in the horseshoe-like configuration and the observed regioisomer ratio (cf. Figure 3; **S3/S4** not considered) as data set. For each individual substrate structure the product ratio was calculated after elimination of the observed values for this substrate using the remaining data set. The predicted level was then compared with the observed ratio of regioisomers employing the cross-validation method.

between the proximal and distal residues of the substrates is graphically outlined. Except for **S3** and **S7** a distinct influence of substance hydrophobicity on the regioselectivity was observed; i.e., increasing hydrophobicity differences between the proximal and distal residues resulted in a decrease of selectivity. While for **S7** and for 7,11,15-trimethyl-(Z,Z,E)-hexatetraenyl 1-adipate [percentual ratio of 1% for the 7-oxygenated regioisomer, according to Datcheva et al. (1991)] (spot at log p = 4.6) the divergent behavior can be explained by its particular structural properties blocking the 7-position, the lack of data for substrates with hydrophobicity differences >4 does not allow us to interpret sufficiently the observed high amount of the 7-oxygenated isomer of **S3**. We find that a sigmoid plot with a broadening end at higher hydrophobicity differences fits best for the relation between the hydrophobicity difference and the observed regioselectivity (an also possible hyperbolic relation would suggest a 100% selectivity for the 3-oxygenated products at higher hydrophobicity differences, which is not given).

Considering electrostatic and steric interactions, computer-assisted calculations of published (Datcheva et al., 1991) and measured data (cf. Table 4) confirmed "horseshoe"-like configuration with planar pentadienoic moiety of the substrates (Figure 4). The correlation pattern obtained for the measured and predicted values is represented in Figure 5. The evaluations, i.e. hydrophobicity calculations and molecular modeling, reveal the importance of both residues (Figure 4a,b) attached to the central pentadienoic moiety (Figure 4c). Thus, to predict the regioselectivity of a substrate of LOX catalysis, the calculation of hydrophobicity differences can be used as a quantitative parameter (Datcheva et al., 1991; Hatanaka et al., 1993).

The observed enantioselectivity during LOX-catalyzed dioxygenation did not allow us to derive a definite structure–selectivity relationship. Only the 7-oxygenated Z,E-configured dioxygenation products showed an

enrichment of the *S*-isomer (cf. Table 5); the other regioisomers were found to be racemic. The influence of the elongation of aliphatic chains in the distal residue was more significant than that of the ω-standing cyclohexyl residues. There was no relation between the ratio of the enantiomers and the measured kinetic parameters. Thus, substrate–protein interactions seem to be more important than simple autoxidation. With the three-dimensional protein structure in hand, a more detailed interpretation of the observed data can be performed.

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LITERATURE CITED

Axelrod, B.; Cheesbrough, T. M.; Laakso, S. Lipoxygenase from soybeans. *Methods Enzymol.* **1981**, *71*, 441–451.

Boyington, J. C.; Gaffney, B. J.; Amzel, M. The three-dimensional structure of an arachidonic acid 15-lipoxygenase. *Science* **1993**, *260*, 1482–1486.

Carvalho, J. F.; Prestwich, G. D. Synthesis of ω-tritiated and ω-fluorinated analogs of the trail pheromone of subterrainean termites. *J. Org. Chem.* **1984**, *49*, 1251–1258.

Corey, E. J. Mechanism of enzymic lipoxygenation of arachidonic acid. In *Stereochemistry of Organic and Bioorganic Transformations*; Bartmann, W., Sharpless, K. B., Eds.; VCH Publishers: Weinheim, 1987; pp 1–12.

Datcheva, V. K.; Kiss, K.; Solomon, L.; Kyler, K. S. Asymmetric hydroxylation with lipoxygenase: the role of group hydrophobicity on regioselectivity. *J. Am. Chem. Soc.* **1991**, *113*, 270–274.

Gardner, H. W. Recent investigations into the lipoxygenase pathway of planes. *Biochim. Biophys. Acta* **1991**, *1084*, 221–239.

Hatanaka, A.; Kajiwara, T.; Matsui, K. Reaction specificity of lipoxygenase and hydroperoxide lyase. In *Progress in Flavour Precursor Studies*; Schreier, P., Winterhalter, P., Eds.; Allured: Carol Stream, IL, 1993; pp 151–174.

Kühn, H.; Schewe, T.; Rapaport, S. M. The stereochemistry of the reactions of lipoxygenases and their metabolites. Proposed nomenclature of lipoxygenases and related enzymes. *Adv. Enzymol.* **1986**, *58*, 273–311.

Kühn, H.; Wiesner, R.; Lankin, V. Z.; Nekrasov, A.; Alder, L.; Schewe, T. Analysis of the stereochemistry of lipoxygenase-derived hydropolyenic fatty acids by means of chiral phase high-pressure liquid chromatography. *Anal. Biochem.* **1987**, *160*, 24–34.

Minor, W.; Steczko, J.; Bolin, J. T.; Otwinowski, Z.; Axelrod, B. *Crystallographic* determination of the active site iron and its ligands in soybean lipoxygenase L-1. *Biochemistry* **1993**, *32*, 6320–6323.

Nakanishi, K., Berova, N., Woody, R. W., Eds. *Circular Dichroism—Principles and Applications*; VCH Publishers: New York, 1994.

Rekker, R. F. *The Hydrophobic Fragmental Constant. Its Derivation and Application*; Elsevier: Amsterdam, 1977.

Veldink, G. A.; Vliegenthart, J. F. G. Lipoxygenases, nonheme iron-containing enzymes. *Adv. Inorg. Biochem.* **1984**, *6*, 139–164.

Veldink, G. A.; Vliegenthart, J. F. G. Substrates and products of lipoxygenase catalysis. *Stud. Nat. Prod. Chem.* **1991**, *9*, 559–589.

Weyd, S. Studies on the isolation of lipoxygenase isoenzymes for soybean and their characterization. Doctoral Thesis, Universität Würzburg, 1993.

Zhang, P.; Kyler, K. S. Enzymatic asymmetric hydroxylation of pentadienols using soybean lipoxygenase. *J. Am. Chem. Soc.* **1989**, *111*, 9241–9242.

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