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Lipoxygenase-1 Inhibition with a Series of Half-Product Analogs

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A new series of sulfur-containing competitive inhibitors for lipoxygenase-1 from soybeans has been synthesized and characterized. The compounds resemble the ω-half of the product of catalysis, and can, therefore, be thought of as half-product analogs. A series of inhibitors differing in the length of the ω-terminal aliphatic substituent was assembled. Lipoxygenase-1 inhibition at pH 9 was greatest for (E)-4-thia-2-undecenal, the compound bearing the n-C_7H_{15} substituent. Longer or shorter aliphatic substituents provided less effective inhibitors. This optimal fit of the inhibitory compounds reflecting the known substrate specificity of the enzyme along with the competitive inhibition kinetics displayed by these substances implicated an active site interaction. The relatively uncomplicated features of the compounds made it possible to explore synthetically for other aspects of the structure favorable for an inhibitory effect. Compounds containing functional groups other than the aldehyde at the 1-position were all less effective inhibitors. In addition to the optimal hydrophobic substituent, an electron-rich region in the molecule was also critical to the inhibitory effect. α,β-Unsaturated aldehydes were about 10 times more effective inhibitors than the saturated analogs. The 4-thia substituent was not absolutely required for inhibition, but electron density at this position was important. γ,δ-Unsaturation replaced the sulfur in this capacity with little effect on the inhibition constant. The electron-rich aldehydes showed no tendency to form hydrates in aqueous solution or Schiff base adducts with the enzyme. Physical evidence for a protein–ligand interaction was sought in a series of ^{1}H NMR spectroscopy experiments. There was clear evidence for a specific interaction between the compounds and the enzyme in these measurements.

Lipoxygenase is a key enzyme in polyunsaturated fatty acid metabolism (*1*). The catalyzed reaction (Scheme 1) involves the incorporation of molecular oxygen into the polyunsaturated system to produce a hydroperoxide product, i.e., catalysis of the autoxidation reaction. The natural substrates for the enzyme are methylene interrupted all Z polyenoic acids. The most extensively studied form of the enzyme, lipoxygenase-1 from soybeans, shows a strong preference for oxygenation of the substrate at the ω-6 position when the reaction is carried out at pH 9 (*2*). Consequently, compounds like linoleic and arachidonic acid are excellent substrates. Increasing the number of carbons in the hydrophobic substituent of the fatty acid drastically reduces the rate of oxygenation. For example, while linoleic acid and 8,11,14-eicosatrienoic acid are excellent substrates, 7,10-octadecadienoic acid and

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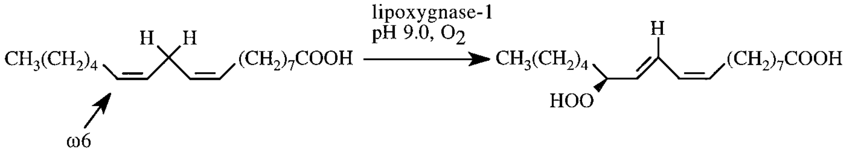

SCHEME 1

8,11,14-docosatrienoic acid are oxygenated at less than 10% of the rate of the compounds with the same number of carbons (*3*). More recently, studies on complete series of synthetic diene and triene substrates have confirmed these earlier findings (*4*). Taken together, these observations have led to the conclusion that lipoxygenase-1 binds its substrate in a hydrophobic pocket with a depth that fixes the polyunsaturated system in position for oxygenation. The recently published crystal structure of the enzyme reveals a long narrow channel in the protein lined with hydrophobic residues that may serve this function (*5*).

The portion of the structure of the substrate on the carboxylic acid side of the polyunsaturated system is much less critical to the oxygenation rate. For example, diene, triene, and tetraene substrates with 18, 20, and 22 total carbon atoms are all oxygenated at comparable rates as long as a product at the ω-6 position can be obtained (*3*). Further, Kyler has demonstrated the regioselective oxygenation of certain nonnatural substrates incorporating an ester function that replaces methylene units in the carboxylate arm (*6*). This shows that the enzyme can tolerate functional group modifications in addition to variations in the chain length on the —COOH side of the substrate.

Lipoxygenase-1 contains a single iron atom in a unique nonheme environment (*7*). When the enzyme is isolated from soybeans (native) the metal cofactor is present as iron(II). Treatment of native lipoxygenase-1 with one equivalent of the product hydroperoxide causes the oxidation of the iron atom to produce the active iron(III) form of the enzyme (*8*). The time course for catalysis initiated with the native enzyme is characterized by a significant lag phase. The rate of the catalyzed reaction increases with time as the iron(III) form of the enzyme is produced (*9*). Redox cycling of the iron atom is probably also involved in the catalytic turnover of the enzyme (*10*). The activating effect of the product on lipoxygenase-1 is specific: simple organic hydroperoxides are ineffective. Further, the concentration dependence of the product activating effect displays saturation behavior in kinetics experiments (*11*). This means that the enzyme has selective binding interactions not only with its substrates, but also with the product. Whether these compounds interact with lipoxygenase-1 at a common site or through distinct sites is not presently known.

The substrate and product binding sites as well as the mechanism of action of lipoxygenase-1 have been probed in a number of studies of the inhibition of catalysis. One class of inhibitors is composed of compounds capable of reducing the iron cofactor (*12–15*). Inhibition was observed as a reduction in the steady state rate, an increase in the lag phase for the enzyme, or both effects. Compounds with this capability are structurally unrelated and include catechols, *N*-alkylhydroxylamines, 2-benzyl-1-naphthols, and a diaryl-*N*-hydroxyurea. It is not clear at present whether this diverse collection of substances gains access to the iron atom through the substrate channel or by some other path. Inhibitory substrate analogs, including product analogs and mechanism based inactivators of lipoxygenase-1, have also been described. Irreversible inactivation of the enzyme by compounds incorporating modifications to the structure of the substrate or product has been observed in a number of studies. For example, acetylenic analogs of the substrate like 5,8,11,14-eicosatetraynoic acid (ETYA) (*16*), 13-thiaarachidonic acid (*17*), (10Z, 13Z)-12-methylidene-10,13-nonadecadienoic acid (*18*), hexanal phenylhydrazone (*19*), and (Z)-12-iodo-9-octadecenoic acid (*20*) all inactivated lipoxygenase-1 without having the carbon chain become covalently attached to the protein. In the studies of ETYA and hexanal phenylhydrazone, it was demonstrated that inactivation was accompanied by oxidation of methionine residues to the corresponding sulfoxide (*21*).

Competitive inhibition of lipoxygenase-1 has been reported for relatively few compounds. Monoenoic fatty acids like oleic and erucic acids, which could be considered unreactive substrate analogs, have this capacity (*22*). Straight chain alcohols with even numbers of carbons (C4–C12) were also found to be competitive inhibitors of lipoxygenase-1 with K_i values in the range of 0.1–50 mM (*23*). The longer n-alcohols were the most effective inhibitors with the C8–C12 compounds having comparable values of K_i. It was concluded that the alcohols inhibited the enzyme by binding an aliphatic substituent to the active site by a hydrophobic interaction. Quite recently chain breaking antioxidants like ascorbic acid were found to be competitive inhibitors of lipoxygenase-1 (*24*). This discovery was unexpected since the compounds bear little structural resemblance to the substrate or product. The most effective inhibitor was 6-palmitoylascorbic acid (K_i, 3 μM), which has a clearly hydrophobic element, but ascorbic acid alone and trolox were also good inhibitors (K_i, 27 μM and 18 μM, respectively). It is not immediately apparent how these compounds could navigate the narrow channel in the published structure of the protein proposed to provide access for the substrate to the vicinity of the iron center without a substantial rearrangement of the amino acid side chains.

We have previously reported on a synthetic product analog for lipoxygenase-1 bearing a sulfur atom at the 13 position, (9Z, 11E)-13-thia-9,11-octadecadienoic acid, which was found to be a competitive inhibitor (K_i, 30 μM) (*25*). This compound was obtained from the Wittig coupling reaction between the triphenylphosphonium salt of 9-bromononanoic acid and (E)-4-thia-2-nonenal. In the course of screening compounds for effects on lipoxygenase catalysis, (E)-4-thia-2-nonenal itself was found to be an effective inhibitor. Expanding on this observation, we report here on the preparation and characterization of a homologous series of aldehydes that can best be described as half-product analogs. This led to the discovery of a new class of competitive inhibitors for lipoxygenase-1 and provided an opportunity to explore more closely the structural features responsible for an enzyme-inhibitor interaction in a readily manipulated set of small molecules.

EXPERIMENTAL

Materials

Lipoxygenase-1 was obtained from soybeans cv. Provar (*26*). The concentration of the enzyme was determined from the absorbance at 280 nm using a molar extinction coefficient of 120,000 liters mol^{-1} cm^{-1} (*27*). Linoleic acid was obtained from Sigma (St. Louis, MO). Acrolein (90%) was obtained from Aldrich (Milwaukee, WI) and used without further purification. (E)-2-Nonenal (97%) and (2E, 4E)-2,4-decadienal (85%) were obtained from Aldrich and were purified by chromatography on silica gel with diethyl ether-hexane mixtures (5 : 95 and 7 : 93, v/v, respectively). 2-Bromopropenal (*28*) and 2-bromopropenal diethyl acetal (*29*) were prepared according to the literature procedures. Distillation of the intermediate 2-bromopropenal is not recommended. This procedure resulted in unpredictable detonation. Therefore the intermediate 2-bromopropenal was used without purification. Ethyl 2-bromopropenoate was prepared immediately before use by treating ethyl acrylate (0.1 mol, 10.5 ml, 9.7 g) dissolved in diethyl ether (50 ml) with bromine (5 ml, 0.1 mol) in a dropwise fashion. The solution was cooled to 0°C and triethylamine (13.5 ml, 0.1 mol) was added gradually. The solution was filtered and the solvent was removed by rotary evaporation. The product was utilized in the syntheses without further purification.

(*E*)*-4-Thia-2-heptenal.* Small pieces of sodium (0.58 g, 25 mmol) were dissolved in ethanol (25 ml) with magnetic stirring under a nitrogen atmosphere. 1-Propanethiol (1.9 g, 25 mmol) was added to the solution and the mixture was stirred for 2 h at 40°C. 2-Bromopropenal diethyl acetal (5.23 g, 25 mmol) was added and the mixture was refluxed for 40 h. The reaction mixture was filtered and the solvent was removed by rotary evaporation. The residue was treated with 10% aqueous HCl (60 ml), w/v, and tetrahydrofuran (120 ml). The hydrolysis reaction was allowed to proceed for 1 h at room temperature. The solvent was removed by rotary evaporation and the aqueous residue was extracted with diethyl ether (3× 20 ml). The organic extracts were washed with saturated aqueous sodium bicarbonate and water. The organic layer was dried over anhydrous sodium sulfate and the solvent was removed by rotary evaporation. The product was purified from the residue by column chromatography on silica gel using a mixture of ether and hexane (15 : 85, v/v). The aldehyde was obtained as a colorless oil (1.52 g, 47%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.0 (t, 3H, $J = 7.3$ Hz, CH_3), 1.70 (tq, 2H, $J = 7.3$ Hz, CH_2CH_3), 2.79 (t, 2H, $J = 7.3$ Hz, CH_2), 6.08 (dd, 1H, $J = 7.7$ Hz, 2-H), 7.54 (d, 1H, $J = 15$ Hz, 3-H), 9.35 (d, 1H, $J = 7.6$ Hz, CHO); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 13.22, 21.74, 34.14, 125.77, 156.62, 189.73. *Anal.* Calcd for $C_6H_{10}OS$: C, 55.35; H, 7.74. Found: C, 55.32; H, 7.72.

(*E*)*-4-thia-2-octenal.* The compound (2.0 g, 55%) was prepared using the same procedure described above using 1-butanethiol. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.95 (t, 3H, $J = 7.6$ Hz, CH_3), 1.47 (tq, 2H, $J = 7.6$ Hz, CH_2CH_3), 1.70 (tt, 2H, $J = 7.6$ Hz, CH_2), 2.85 (t, 2H, 7.6 Hz, SCH_2), 6.14 (dd, 1H, $J = 7.6$ Hz, 2-H), 7.59 (d, 1H, $J = 15$ Hz, 3-H), 9.40 (d, 1H, $J = 7.6$ Hz, CHO); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 13.56, 21.95, 30.33, 31.98, 125.83, 156.74, 189.91. *Anal.* Calcd for $C_7H_{12}OS$: C, 58.25; H, 8.39. Found: C, 57.89; H, 8.66.

(*E*)-*4-thia-2-nonenal.* The compound was prepared on a 0.1 mol scale (13.4 g, 86%) using the same procedure described above using 1-pentanethiol. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.92 (t, 3H, J = 7.0 Hz, CH_3), 1.40 (b, 4H, J = 7.0 Hz, $(CH_2)_2CH_3$), 1.72 (tt, 2H, J = 7.6 Hz, 6-H), 2.85 (t, 2H, 7.6 Hz, SCH_2), 6.15 (dd, 1H, J = 7.5 Hz, 2-H), 7.57 (d, 1H, J = 15 Hz, 3-H), 9.41 (d, 1H, J = 7.7 Hz, CHO); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 13.89, 22.18, 28.03, 30.91, 32.29, 125.85, 156.70, 189.88. *Anal.* Calcd for $C_8H_{14}OS$: C, 60.72; H, 8.92. Found: C, 60.50; H, 9.20.

(*E*)-*4-thia-2-decenal.* The compound (2.7 g, 63%) was prepared using the same procedure described above using 1-hexanethiol. The ether hexane mixture used for the chromatographic purification was 1 : 9, v/v. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.90 (t, 3H, J = 7.0 Hz, CH_3), 1.31 (b, 4H, J = 6.8 Hz, $(CH_2)_2CH_3$), 1.43 (tq, 2H, J = 7.0 Hz, 7-H), 1.70 (tt, 2H, J = 7.6 Hz, 6-H), 2.85 (t, 2H, 7.6 Hz, SCH_2), 6.14 (dd, 1H, J = 7.5 Hz, 2-H), 7.59 (d, 1H, J = 15 Hz, 3-H), 9.40 (d, 1H, J = 7.0 Hz, CHO); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 13.99, 22.48, 28.27, 28.45, 31.24, 32.29, 125.83, 156.75, 189.90. *Anal.* Calcd for $C_9H_{16}OS$: C, 62.75; H, 9.36. Found: C, 62.43; H, 9.55.

(*E*)-*4-Thia-2-undecenal.* The compound (2.98 g, 64%) was prepared using the same procedure described above using 1-heptanethiol. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.89 (t, 3H, J = 7.0 Hz, CH_3), 1.29 (b, 6H, J = 7.6 Hz, $(CH_2)_3CH_3$), 1.42 (tq, 2H, J = 7.6 Hz 7-H), 1.69 (tt, 1H, J = 7.6 Hz, 6-H), 2.85 (t, 2H, J = 7.6 Hz, SCH_2), 6.14 (dd, 1H, J = 7.6 Hz, 2-H), 7.59 (d, 1H, J = 15 Hz, 3-H), 9.41 (d, 1H, J = 7.6 Hz, CHO); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.04, 22.56, 28.33, 28.74, 29.01, 31.62, 32.32, 125.85, 156.69, 189.86. *Anal.* Calcd for $C_{10}H_{18}OS$: C, 64.47; H, 9.74. Found: C, 64.58; H, 10.14.

(*E*)-*4-Thia-2-dodecenal.* The compound (2.65 g, 53%) was prepared using the same procedure described above using 1-octanethiol. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (t, 3H, J = 7.0 Hz, CH_3), 1.26 (b, 8H, J = 7.0 Hz, $(CH_2)_4CH_3$), 1.38 (tq, 2H, J = 7.6 Hz 7-H), 1.67 (tt, 2H, J = 7.6 Hz, 6-H), 2.82 (t, 2H, J = 7.6 Hz, 5-H), 6.15 (dd, 1H, J = 7.4 Hz, 2-H), 7.55 (d, 1H, J = 15.1 Hz, 3-H), 9.37 (d, 1H, J = 7.6 Hz, CHO); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.04, 22.58, 28.30, 28.74, 28.99, 29.05, 31.70, 32.29 125.82, 156.66, 189.81. *Anal.* Calcd for $C_{11}H_{20}OS$: C, 65.95; H, 10.06. Found: C, 66.22; H, 10.56.

(*E*)-*Ethyl 4-Thia-2-nonenoate.* Small pieces of sodium (1.15 g, 50 mmol) were dissolved in ethanol (25 ml) with magnetic stirring under a nitrogen atmosphere. 1-Pentanethiol (6.2 ml, 50 mmol) was added to the solution and the mixture was stirred for 2 h at 40°C. Ethyl 2-bromopropenoate (obtained from 0.1 mol scale preparation) was added to the solution, and the mixture was refluxed for 16 h. The reaction mixture was filtered and the ethanol was removed by rotary evaporation. The residue was treated with water (50 ml) and extracted with diethyl ether (3× 30 ml). The solution was dried over anhydrous sodium sulfate, and the solvent was removed by rotary evaporation. The residue was purified by column chromatography on silica with a mixture of ether and hexane, 1 : 9, v/v. An impure product (1.87 g, 18.5%) was obtained. The compound was further purified in small batchs by HPLC (C18, MeOH : H_2O, 70 : 30, v/v). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, 3H, J = 7.2 Hz, 9-H), 1.24 (t, 3H, J = 7.2 Hz, OCH_2CH_3), 1.33 (b, 4H, J = 7.0 Hz, $(CH_2)_2CH_3$), 1.63 (tt, 2H, J = 7.3 Hz, 6-H), 2.75 (t, 2H, J = 7.5 Hz, 5-H), 4.14 (q, 2H, J = 7.11 Hz, OCH_2CH_3), 5.69 (d, 1H, J = 15.1 Hz, 2-H), 7.65 (d, 1H, J = 15

Hz, 3-H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.84, 14.24, 22.13, 28.23, 30.87, 31.88, 60.10, 113.69, 146.83, 165.33. *Anal.* Calcd for $C_{10}H_{18}O_2S$: C, 59.37; H, 8.97. Found: C, 59.99; H, 9.39.

(*E*)-*Ethyl 4-Thia-2-decenoate.* The same procedure was followed as for the previous compound. Again, an impure product was obtained (1.48 g, 13.7%) that was further purified by HPLC (C18, MeOH : H_2O, 75 : 25, v/v). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.90 (t, 3H, J = 7.0 Hz, 10-H), 1.31 (b, 7H, J = 7.2 Hz, 9-H, 8-H, CH_3), 1.41 (tt, 2H, J = 7.4 Hz, 7-H), 1.68 (tt, 2H, J = 7.3 Hz, 6-H), 2.75 (t, 2H, J = 7.4 Hz, 5-H), 4.18 (q, 2H, J = 7.11 Hz, OCH_2CH_3), 5.74 (d, 1H, J = 15.1 Hz, 2-H), 7.69 (d, 1H, J = 15.1 Hz, 3-H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.00, 14.35, 22.5, 28.47, 28.54, 31.27, 31.97, 60.18, 113.63, 146.93, 165.43.

(*E*)-*4-Thia-2-nonenoic acid.* Sodium hydroxide (1.5 g) was dissolved in 10 ml of boiling methanol. The solution was cooled to room temperature and water (2 drops) was added followed by (E)-ethyl 4-thia-2-noneoate (0.65 g, 3.22 mmol). The reaction was completed in 1 min. The methanol was removed by rotary evaporation. Water (30 ml) was added to the residue, followed by extraction with diethyl ether (3× 20 ml). The ether layers were discarded. The aqueous solution was acidified to pH 2 (HCl) and extracted with diethyl ether (3× 20 ml). The extracts were dried over sodium sulfate and the solvent was removed by rotary evaporation. An impure product (0.55 g, 85%) was obtained. The compound was purified further by HPLC (C18, methanol : water : acetic acid, 54 : 46 : 0.5, v/v/v). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.89 (t, 3H, J = 7.0 Hz, 9-H), 1.36 (b, 4H, J = 6.9 Hz, $(CH_2)_2CH_3$), 1.67 (tt, 2H, J = 7.3 Hz, 6-H), 2.79 (t, 2H, J = 7.5 Hz, 5-H), 5.72 (d, 1H, J = 15.1 Hz, 2-H), 7.50 (d, 1H, J = 15.2 Hz, 3-H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.87, 22.16, 28.17, 30.89, 32.01, 112.57, 150.32, 170.58. *Anal.* Calcd for $C_8H_{14}O_2S$: C, 55.15; H, 8.10. Found: C, 55.28; H, 8.43.

(*E*)-*4-Thia-2-decenoic acid.* The procedure was the same as for the preparation of the previous compound except that the starting material was (E)-ethyl 4-thia-2-decenoate. An impure product (0.45 g, 82%) was obtained. The compound was further purified by HPLC (C18, methanol : water : acetic acid, 70 : 30 : 0.5, v/v/v). ^{1}H NMR (C_6D_6, 400 MHz) 0.82 (t, 3H, J =7.4 Hz, 10-H), 1.00 (b, 4H, J = 6.6 Hz, $(CH_2)_2CH_3$), 1.13 (tt, 2H, J = 7.2 Hz, 7-H), 1.20 (tt, 2H, J = 7.2 Hz, 6-H), 2.13 (t, 2H, J = 6.7 Hz, 5-H), 5.75 (d, 1H, J = 15.1 Hz, 2-H), 7.85 (d, 1H, J = 15.1 Hz, 3-H); ^{13}C NMR (C_6D_6, 100 MHz) 14.13, 22.73, 28.42, 28.50, 31.40, 31.78, 113.35, 150.27, 171.47. *Anal.* Calcd for $C_9H_{16}O_2S$: C, 57.42; H, 8.57. Found: C, 57.85; H, 8.98.

(*E*)-*4-Thia-2-nonen-1-ol.* The sodium borohydride reduction of the (E)-4-thia-2-nonenal was carried out following a literature procedure (*30*). The aldehyde (98%, 0.5 g, 3.2 mmol) was dissolved in a mixture of ethanol (4 ml) and water (2 ml). Sodium borohydride (60 mg, 1.6 mmol) dissolved in a mixture of ethanol (4 ml) and water (4 ml) was added to the solution of the aldehyde at 0°C. The reaction mixture was stirred for 2 h at room temperature. The solution was saturated with sodium chloride, extracted with diethyl ether (3× 30 ml), and the extracts were dried over anhydrous sodium sulfate. The solvent was removed by rotary evaporation, and the residue was purified by chromatography on silica gel (ether : hexane, 30 : 70, v/v). The yield was 0.41 g (82%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, 3H, J = 7.0 Hz, CH_3), 1.33 (b, 4H, J = 7.1 Hz, $(CH_2)_2CH_3$), 1.61 (tt, 2H, J = 7.0 Hz, 6-H), 2.66 (t, 2H, J = 7.4 Hz, 5-H), 4.10 (dd, 2H, J = 5.0 Hz, CH_2OH), 5.67 (dt, 1H, J = 6.5 Hz, 2-H), 6.24 (d, 1H, J = 15.1 Hz, 3-H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.91, 22.20, 28.87, 30.94, 32.12, 63.69, 125.51, 127.62.

(E)-4-Thia-2-decen-1-ol. The procedure was the same as for the previous compound except that the starting material was (E)-4-thia-2-decenal. The yield was 0.34 g (87.8%). ^{1}H NMR (C_6D_6, 400 MHz) δ 0.83 (t, 3H, J = 7.3 Hz, CH_3), 1.18 (b, 6H, J = 7.0 Hz, $(CH_2)_3CH_3$), 1.47 (tt, 2H, J = 7.6 Hz, 6-H), 2.43 (t, 2H, J = 7.4 Hz, 5-H), 2.73 (s, 1H, CH_2OH), 3.93 (d, 2H, J = 5.5 Hz, CH_2OH), 5.61 (dt, 1H, J = 6.5 Hz, 2-H), 6.16 (d, 1H, J = 15.1 Hz, 3-H); ^{13}C NMR (C_6D_6, 100 MHz) δ 14.2, 22.87, 28.77, 29.51, 31.65, 32.28, 63.39, 126.38, 126.95.

(E)-4-Sulfoxyl-2-decenal. (E)-4-Thia-2-decenal (0.178 g, 1.04 mmol) was dissolved in methylene chloride (20 ml) and the solution was cooled in a dry ice-acetone bath. *m*-Chloroperbenzoic acid (0.197 g, 1.1 mmol) was added to the solution under a nitrogen atmosphere. The reaction mixture was allowed to warm to room temperature and was stirred for 2 h. Sodium bicarbonate (0.23 g, 2.5 mmol) in water (30 ml) was added to the mixture. The aqueous layer was extracted with dichloromethane (2× 20 ml), and the combined organic layers were dried over anhydrous sodium sulfate. The solvent was removed by rotary evaporation and the residue was purified by chromatography on silica gel (acetone : dichloromethane : hexane, 5:45 : 50, v/v). The yield was 50 mg (28%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.90 (t, 3H, J = 6.5 Hz, CH_3), 1.33 (b, 4H, J = 3.4 Hz, $(CH_2)_2CH_3$), 1.46 (b, 2H, J = 6.2 Hz 7-H), 1.74–1.84 (dm, 2H, J = 7.0 Hz, 6-H), 2.81–2.94 (dm, 2H, J = 7.0 Hz, 5-H), 6.90 (dq, 1H, J = 7.5 Hz, 2-H), 7.58 (d, 1H, J = 15 Hz, 3-H), 9.80 (d, 1H, J = 7.0 Hz, CHO); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.94, 21.98, 22.36, 28.32, 31.27, 52.90, 134.09, 155.97, 188.58. *Anal.* Calcd for $C_9H_{16}O_2S$: C, 57.42; H, 8.57. Found: C, 57.26; H, 8.93.

(E)-4-Sulfoxyl-2-nonenal. The procedure was the same as for the previous compound except that the starting material was (E)-4-thia-2-nonenal. The yield was 0.42 g (86%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.89 (t, 3H, J = 7.2 Hz, CH_3), 1.33–1.46 (b, 4H, J = 3.4 Hz, $(CH_2)_2CH_3$), 1.71–1.82 (dm, 2H, J = 7.0 Hz, 6-H), 2.77–2.88 (dm, 2H, J = 7.0 Hz, 5-H), 6.87 (dd, 1H, J = 7.0 Hz, 2-H), 7.52 (d, 1H, J = 15.2 Hz, 3-H), 9.77 (d, 1H, J = 7.0 Hz, CHO); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.75, 21.68, 22.19, 30.72, 52.86, 134.07, 155.76, 188.47. *Anal.* Calcd for $C_8H_{14}O_2S$: C, 55.15; H, 8.10. Found: C, 55.28; H, 8.43.

4-Thiadecanal. Sodium hydroxide (0.42 g, 10.5 mmol) was dissolved in boiling ethanol (30 ml). 1-Hexanethiol (1.24 g, 10.5 mmol) was added to the solution at 50°C. The reaction mixture was stirred for 1 h. The solution was cooled to room temperature and 3-chloropropanal diethyl acetal (1.68 ml, 10 mmol) was added. The reaction mixture was refluxed for 2.5 h then filtered. The solvent was removed by rotary evaporation, and the residue was treated with tetrahydrofuran (40 ml) and 10% aqueous HCl (w/v, 25 ml). The mixture was stirred for 1.5 h and water (25 ml) was added. The tetrahydrofuran was removed by rotary evaporation, and the aqueous residue was extracted with diethyl ether (3× 30 ml). The combined ether layers were washed with 5% aqueous $NaHCO_3$ (30 ml) and water (3× 30 ml) and were dried over anhydrous sodium sulfate. The solvent was removed by rotary evaporation, and the residue was purified by chromatography on silica gel