Supporting Information

Soybean Lipoxygenase-Mediated Oxygenation of Monounsaturated Fatty Acids to Enones

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Representative procedure for the oxidation of monoolefins: 12(*Z*)-octadecenoic acid (5). To a beaker containing a teflon-coated stir bar, and 50 mg of melted 12(*Z*)-octadecenoic acid was added 50 mL of chilled borate buffer (0.1 M, pH 9.0, 4 °C) with vigorous stirring. The turbid mixture was placed in a pressure chamber (Parr Instrument Co., Moline IL) chilled to 4 °C, and 250 mg of SBLO-1 was added in a single portion with continued stirring. The chamber was then sealed and was pressurized to a constant 18 atm of oxygen. After 24 h of stirring at 4 °C, the oxygen supply was disconnected and the pressure chamber was slowly decompressed to avoid excess foaming of the reaction mixture. The reaction was removed from the chamber and the pH was adjusted to ~3 by the dropwise addition of glacial acetic acid. After filtration through a plug of Celite 545, the eluent was extracted three times with 50-mL portions of diethyl ether. The organic fractions were combined, rinsed with brine, then were dried over Na_2SO_4. After filtration and removal of the solvent *in vacuo*, column chromatography utilizing a 1:10:0.2 ethyl acetate/hexane/acetic acid mobile phase yielded 21.8 mg (41%) of **6** as a colorless solid and 3.1 mg (6%) of **7** as a colorless solid.

13-oxo-11(*E*)-octadecenoic acid (6): ^{1}H NMR ($CDCl_3$) δ 6.83 (1 H, dt, J = 15.9 Hz, J = 6.9 Hz), 6.08 (1 H, d, J = 15.9 Hz), 2.52 (2 H, t, J = 7.5 Hz), 2.34 (2 H, t, J = 7.5 Hz), 2.19 (2 H, q, J = 6.8 Hz), 1.60 (6 H, m), 1.66 – 1.20 (14 H, m), 0.89 (3 H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 201.2, 179.3, 147.4, 130.3, 40.0, 33.9, 32.4, 31.5, 29.2, 29.1, 29.1, 29.0, 28.1, 24.6, 24.0, 22.5, 13.9; Anal. Calcd for $C_{18}H_{32}O_3$: C, 72.93; H 10.88. Found: C, 72.69; H, 10.86. Methylation

with diazomethane followed by reduction with H_2 over 5% Pd on $BaSO_4$ gave a product with mass spectroscopic properties consistent with methyl 13-oxooctadecanoate: CI MS(isobutane) m/z 313 (M+1); EI MS - Figure 1. Further reduction of this material with $NaBH_4$ followed by treatment with bis(trimethylsilyl)trifluoroacetamide gave a product with an EI MS (Figure 2) consistent with methyl 13-trimethylsilyloxyoctadecanoate.

11-oxo-12(*Z*)-octadecenoic acid (7): ^{1}H NMR ($CDCl_3$) 6.10 (2 H, m), 2.60 (2 H, q, J = 6.9 Hz), 2.43 (2 H, t, J = 7.4 Hz), 2.34 (2 H, t, J = 7.5 Hz), 1.60 (6 H, m), 1.28 (14 H, m), 0.88 (3 H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) 202.1, 178.9, 148.6, 126.6, 44.2, 33.8, 31.5, 29.4, 29.3, 29.2, 29.2, 29.1, 29.0, 28.9, 24.6, 24.0, 22.5, 14.0. The chemical shifts of the vinyl protons are consistent with a *Z*-enone (see refs 12 and 13 in the text). Treatment with diazomethane followed by reduction of the double bond gave a product with a mass spectrum identical to that in Figure 3.

Additional Mass Spectra.

Figure 3: EI MS of **4**.

Figure 4: EI mass spectrum of product obtained by methylation of **4** followed by hydrogenation. Identical spectra were obtained on substances produced by methylation/hydrogenation of **7** and **8**.

Procedure for competition experiments. Each experiment contained 6.7 μM lipoxygenase and 6.7 μM 13-HPOD in 1.2 mL of 50 mM borate, pH 9.0. Experiments 1–3 contained approximately 75 μM **3** and 75 μM **5**; experiments 4 and 5 contained 100 μM **3** and 50 μM **5**. Substrates were added at t = 0, and a 0.50-mL aliquot was immediately withdrawn, treated with 15(Z)-octadecenoic acid (final concentration = 75 μM) as an internal standard and acidified (pH < 3) with 1 M HCl. After 60 min, a second 0.50-mL aliquot was withdrawn and treated in the same manner. Each acidified aliquot was applied to a 0.5-mL Bakerbond C18 solid phase extraction column that had been previously washed with 10 mL of methanol and 5 mL of

deionized water. After application of the sample, the column was washed with 10 mL of deionized water, and the fatty acids were eluted with 6 mL of methanol, The methanol eluent was concentrated to dryness, and the residue was dissolved in 25 μL of methanol and treated with about 200 μL of ethereal diazomethane, prepared in a Aldrich MNNG diazomethane generator (Cat # Z41,173-6). The solvents and excess diazomethane were removed under nitrogen, and the derivatized samples were dissolved in 50 μL of methanol. Aliquots (2 μL) of these solutions were analyzed on a Hewlett Packard 5880A gas chromatograph using a 12m x 0.2 mm HP1 capillary column with methylsilicone (0.33 μm film thickness) as stationary phase and a helium flow rate of about 1.5 mL/min. The temperature program was as follows: initial temperature 150 °C for 2 min followed by an increase of 8 °C/min up to 250 °C followed by elution at 250 °C for 3 min. Under these conditions, the methyl esters of **3**, **5** and the internal standard were cleanly separated; in a typical run the retention times were 12.05, 12.18 and 12.39 min, respectively.

Results. R_0 refers to the ratio of the peak areas of the methyl esters of **5** and **3** in the aliquot taken at t = 0, and R_{60} refers to the ratio of the same peaks in the 60-min aliquot. The fraction of compound **5** remaining after 60 min (1-f), was calculated from the ratios of the peak areas of the methyl ester of **5** and the methyl ester of the internal standard in the t = 60 and t = 0 aliquots. This quantity was corrected for a small decrease in the concentration of **5** in control experiments from which enzyme was omitted.

Expt.	R_0	R_{60}	1-f	$\frac{(k_{cat}/K_m)_5}{(k_{cat}/K_m)_3}$
1	1.12	1.94	.495	4.6
2	1.11	1.79	.561	5.7
3	1.12	3.6	.236	5.2
4	2.34	9.66	.140	3.6
5	2.27	13.7	.096	4.3
				Ave = 4.7 ± 0.8

Figure 1

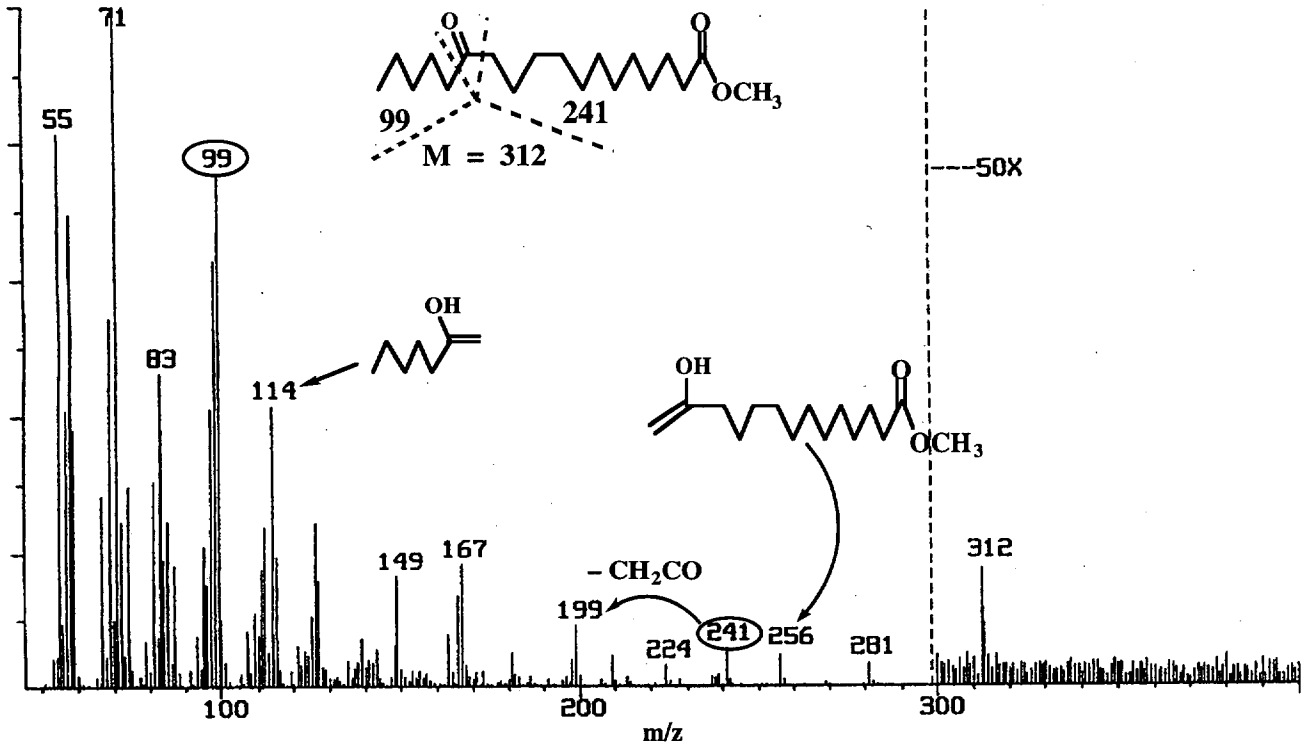

Figure 2

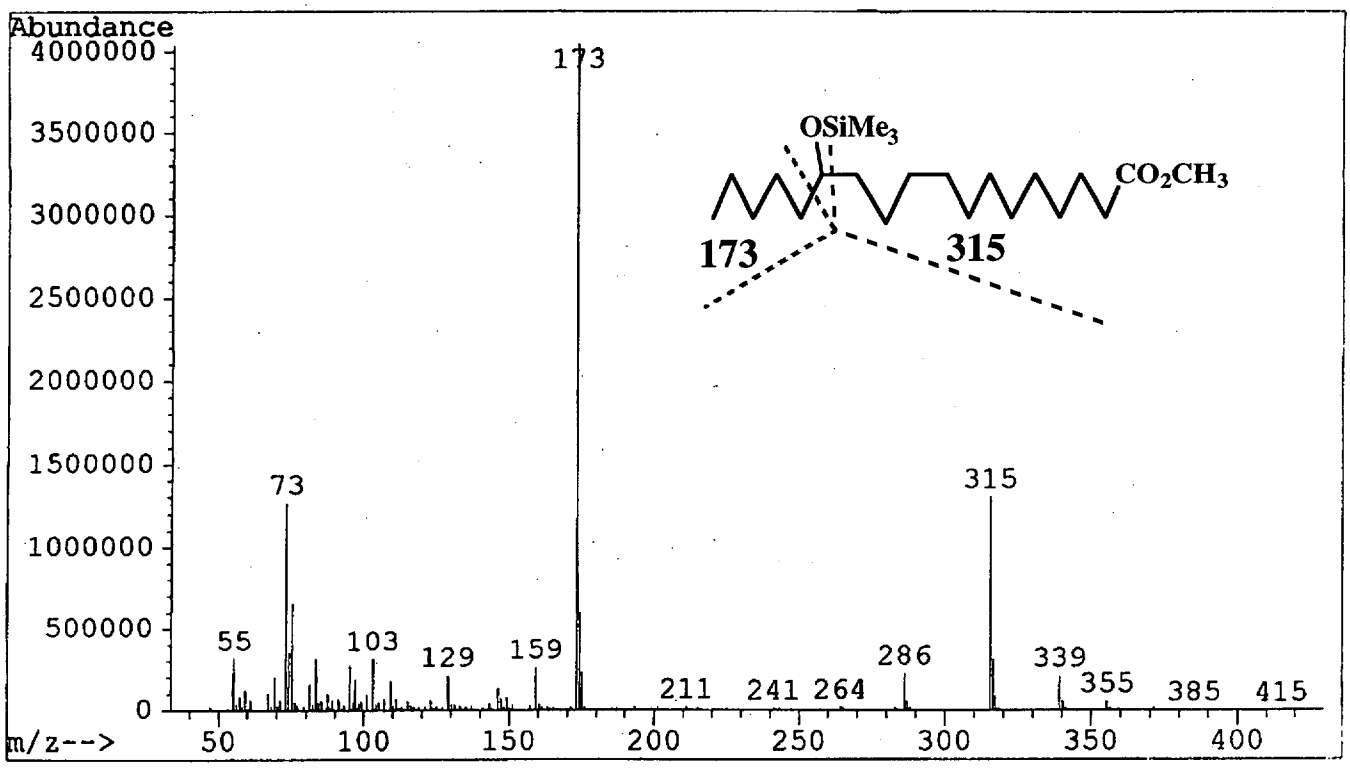
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Figure 3

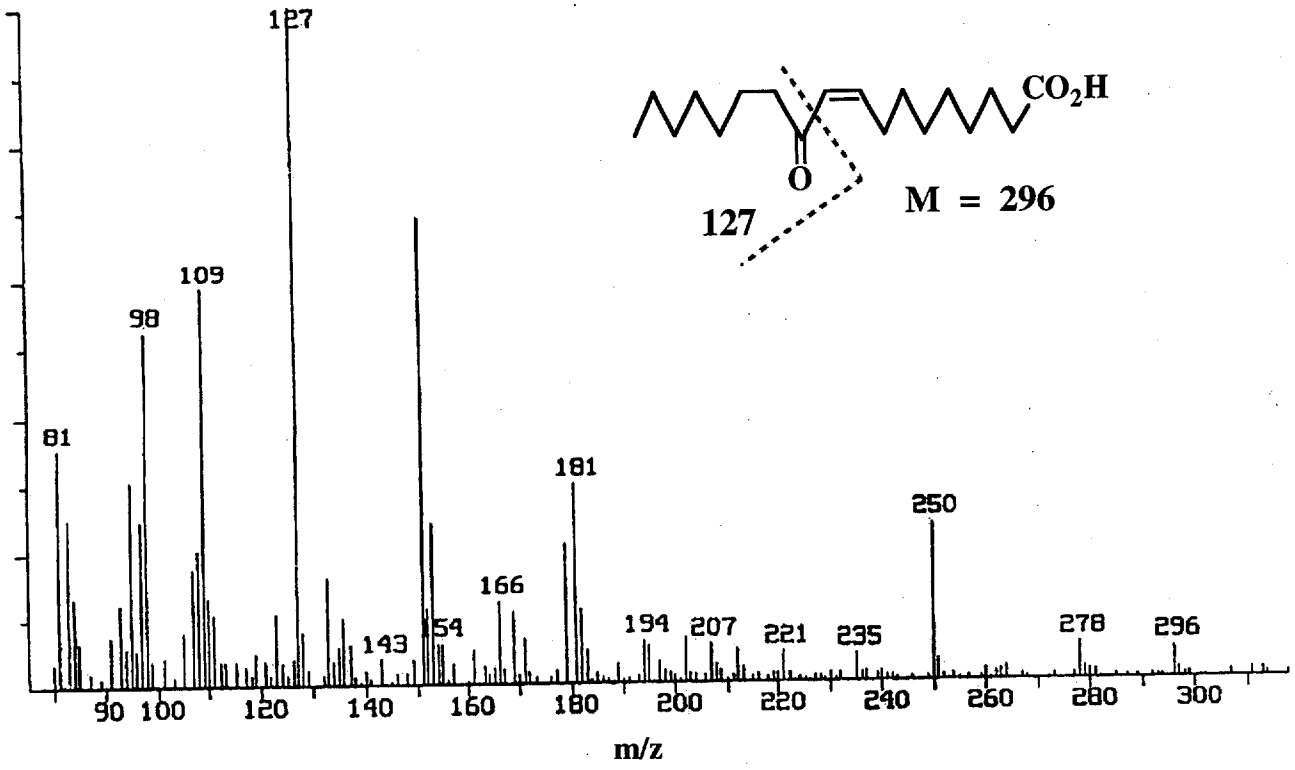
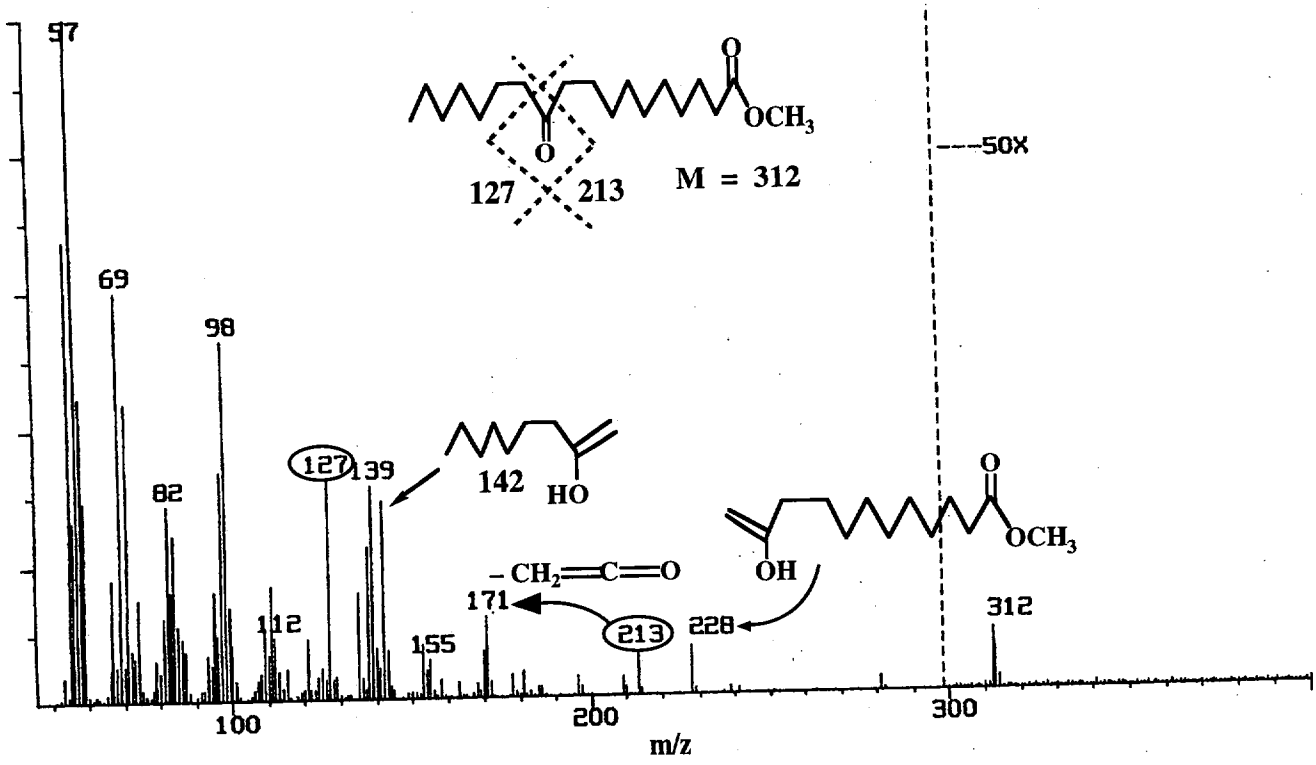


Figure 4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