Supplemental Data

to

Biochemical Characterization of Oxygenation of Unsaturated Fatty Acids by the Dioxygenase and Hydroperoxide Isomerase of *Pseudomonas aeruginosa* 42A2

Eriel Martínez, Mats Hamberg, Montse Busquets, Pilar Díaz, Angeles Manresa, and Ernst H. Oliw

1. Authentic standards: 10*S*-HPOME, 10*S*-HOME, and 7*S*,10*S*-DiHOME.

Metabolites were purified from the supernatant of a 14 h culture of *P.aeruginosa* 42A2 with oleic acid. After extractive isolation with ethyl acetate, the organic phase was then evaporated to dryness. The lipids were resolved by silicic acid chromatography (20 x 1 cm; silicic acid (Sigma)). The column was eluted with hexane/diethyl ether/acetic acid, 75/15/10, and an aliquot of each fraction was analyzed by TLC. This yielded mg amounts of all the three metabolites, which were characterized by mass spectroscopy and NMR in agreement with previous data (9,10,18).

2. Effect of pH, temperature and substrate concentration on the reaction rate of diol synthase.

The effects of pH, temperatures, and substrate concentration on the reaction rate are summarized in Fig. S1 A, B and C, respectively. Data were obtained by LC-MS/MS analysis. The K_m was estimated to be 1.7 mM.

Fig. 1S

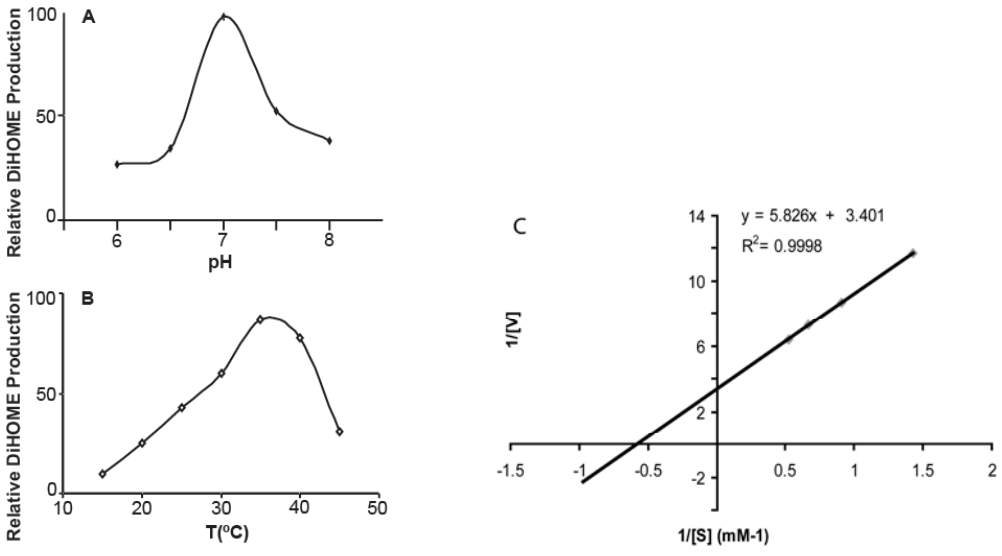

Fig. S1. Effect of temperature, pH, and substrate concentration on the catalytic activity of the diol synthase of *P. aeruginosa*. A, Effect of pH. B, Effect of temperature. C, Estimation of K_m by Michaelis-Menten reciprocal plot.

2. Preparatation and isomerization of hydroperoxides of 9*Z*-18:1.

10*R*-HPOME - The 9- and 10-hydroperoxides of 9*Z*-18:1 were obtained by photooxidation and separated by CP-HPLC with MS/MS detection, as shown in Fig. S2-A. 10*S*-HPOME eluted before 10*R*-HPOME (Reprosil Chiral NR, eluted with hexane/isopropanol/acetic acid, 98.7/1.2/0.1, at 0.3 ml/min). 10*R*-HPOME was not isomerized by the diol synthase, as judged from TLC analysis (Fig. S2-B). The MS/MS spectra are shown in Fig. S3.

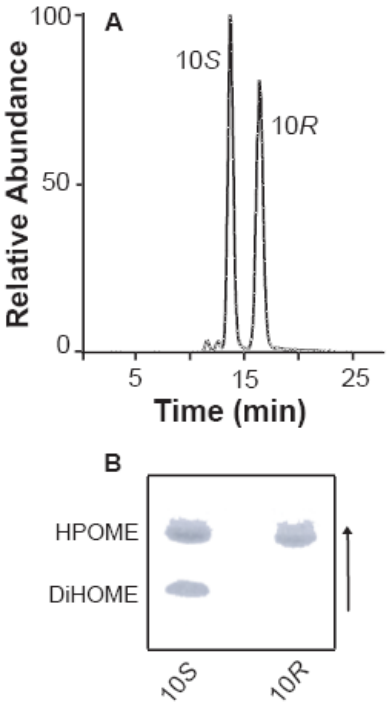

Fig. S2 Separation of 10*R*- and 10*S*-HPOME by CP-HPLC and bioconversion of 11*S*-HPOME. A, CP-HPLC-MS/MS analysis of 10-HPOME. The *S* stereoisomer eluted before the *R* stereoisomer. The figure shows the reconstructed ion chromatogram of *m/z* 155. The two isomers of 9-HPOME were also separated on this column (data not shown). B, TLC

analysis of products formed from 10*S*- and 10*R*-HPOME; only the former was converted to the 7,10-diol.

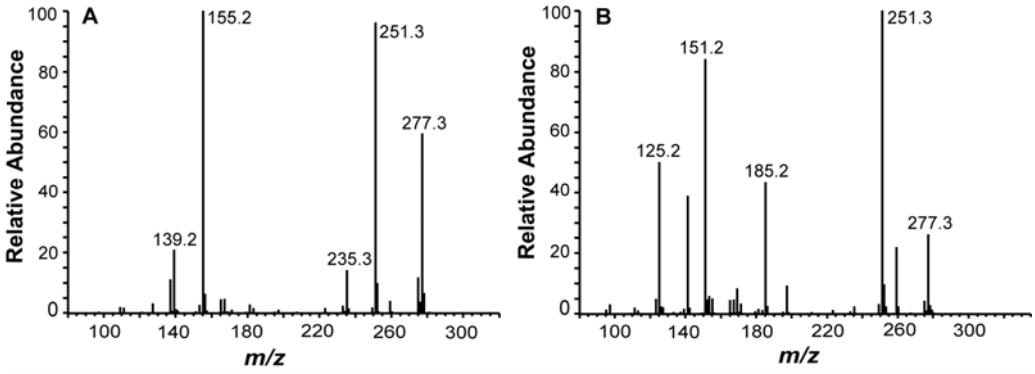

Fig. S3. MS^3 spectra of 9-HPOME and 10-HPOME obtained by photooxidation. A, MS^3 spectrum (*m/z* 313→295→full scan) of 10-HPOME. A characteristic signal was observed at *m/z* 155. B, MS^3 spectrum (*m/z* 313→295→full scan) of 9-HPOME.

[$^{18}O_2$]10*S*-HPOME - [$^{18}O_2$]10*S*-HPOME (>95% $^{18}O_2$) was isolated from an incubation of *P. aeruginosa* under oxygen-18 atmosphere and purified by RP-HPLC. LC-MS/MS analysis confirmed that hydroperoxides was transformed to [$^{18}O_2$]7*S*,10*S*-DiHOME by the diol synthase activity of *P. aeruginosa*; a detailed analysis of the MS/MS spectrum is reported elsewhere[2].

3. Oxygenation of linoleic and vaccenic acids

Linoleic acids - Linoleic and U[^{13}C]linoleic acids were transformed by the diol synthase and 10*S*-DOX activities to 7,10-DiHODE, 10-HODE, and 8-HODE, which were identified by LC-MS/MS. The MS/MS spectrum of 7,10-DiHODE and U[^{13}C]7,10-DiHODE are shown for comparison in Fig. S4. This metabolite was also identified by GC-MS analysis (TMS ether methyl ester derivative[2]).

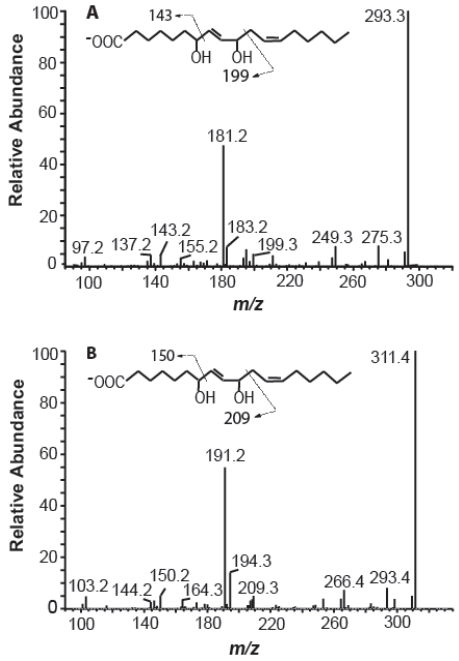

Fig. S4. MS/MS spectra of 7,10-DiHODE (top; *m/z* 311→full scan) and U[^{13}C]7,10-DiHODE (bottom; *m/z* 329→full scan).

The mechanism of hydrogen abstraction at C-8 was investigated with [8*R*-^{2}H]linoleic acid as a substrate for the 10*S*-DOX activity of the 7,10-diol synthase and for the arachidonate 15-lipoxygenase (21) of this preparation. The MS/MS spectra of 10-HODE and 13-HODE are shown for comparison in Fig. S5. The deuterium label was lost in 10-HODE (Fig. 5S-A), but retained in the lipoxygenase product (Fig. 5S-B).

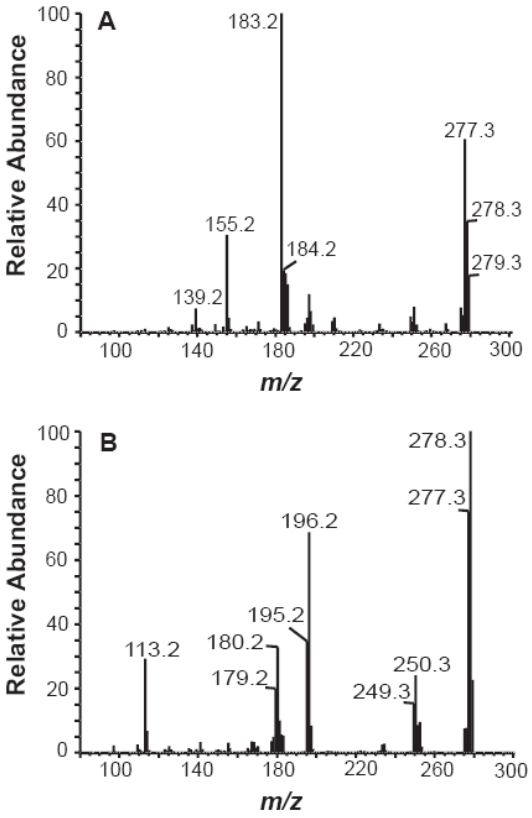

Fig. S5. MS/MS spectra of 10-HODE and [8*R*-^{2}H]13-HODE obtained from an incubation of the diol synthase and lipoxygenase activity of *P. aeruginosa* with [8*R*-^{2}H] 18:2 (64% ^{2}H). The deuterium label was not retained in 10-HODE (A) but present in 13-HODE (B), as shown by the four pairs of doublet signals.

The mechanism of hydrogen abstraction at C-7 was investigated with [7*R*-^{2}H]linoleic acid as a substrate by MS/MS analysis of 7,10-DiHODE. The results showed that the label was retained as judged from distinct doublet signals, *inter alia,* at *m/z* 293 (100%, base peak) and *m/z* 294 (75%), *m/z* 181 (58%) and *m/z* 182 (32%). These results supported suprafacial oxygenation at C-7.

Vaccenic acid, 11- and 12-HPOME. Vaccenic acid was transformed to hydroperoxides and diols as shown in Fig. S6. The hydroperoxides were a mixture of 11- and 12-HPOME, and this was confirmed by comparison with authentic standards. The latter were obtained by photooxidation of vaccenic acid and by separation of the hydroperoxides by CP-HPLC, essentially as described above for 9- and 10-HPOME above (Figs. S2 and S3).

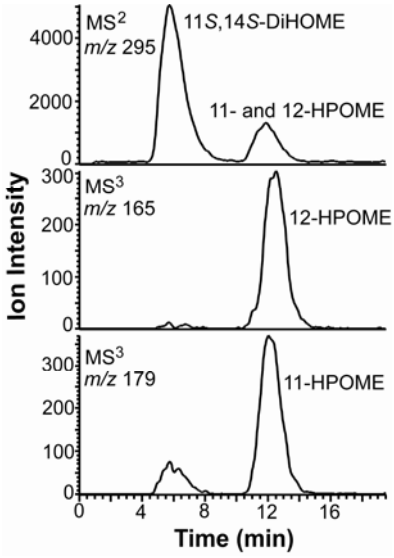

Fig. S6. LC-MS/MS analysis of products formed from *cis* vaccenic acid (11*Z*-81:1). The polar metabolite was identified as 11,14-DiHOME and the second peak contained a mixture of 11- and 12-HPOME, as shown by the reconstructed ion chromatograms (*m/z* 165 and *m/z* 179, cf. Fig. S7).

The MS^3 spectra of 11- and 12-HPOME are shown in Fig. S7. Characteristic signals in the MS^3 spectrum of 12-HPOME were noted by a weak signal at *m/z* 183 (155+28, cf. Fig. S3) and a strong signal at *m/z* 165 (183-18). The corresponding spectrum of 11-HPOME was similar to the MS^3 spectrum of 9-HPOME and showed signals, *inter alia*, at *m/z* 179 (151+28, cf. Fig. S3) and *m/z* 153 (125+28).

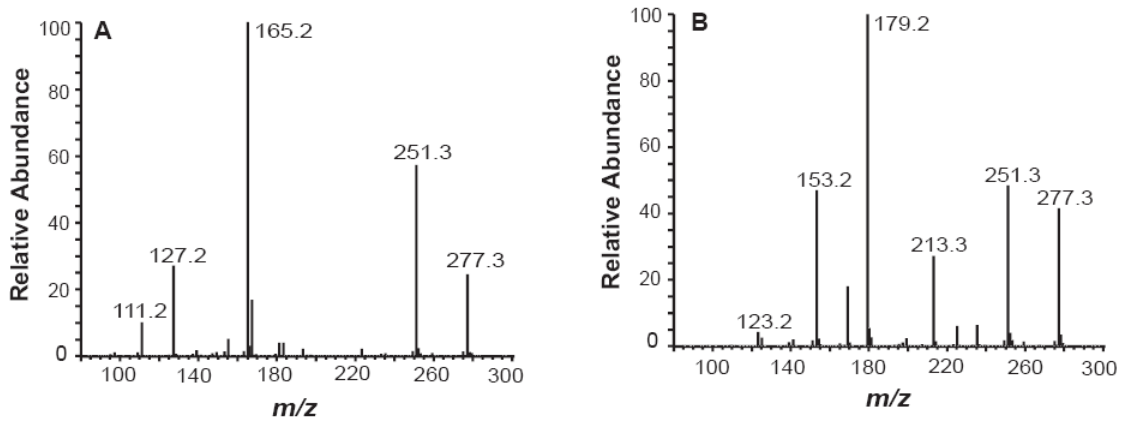

Fig. S7. MS^3 spectra of 11-HPOME and 12-HPOME obtained by photooxidation and separated by CP-HPLC. A, MS^3 spectrum (*m/z* 313→295→full scan) of 12-HPOME. B, MS^3 spectrum (*m/z* 313→ 295→full scan) of 11-HPOME.