**Supplemental material**

An allene oxide and 12-oxophytodienoic acid are key intermediates in jasmonic acid biosynthesis by *Fusarium oxysporum*

**Ernst H. Oliw and Mats Hamberg**

**Fig. I**

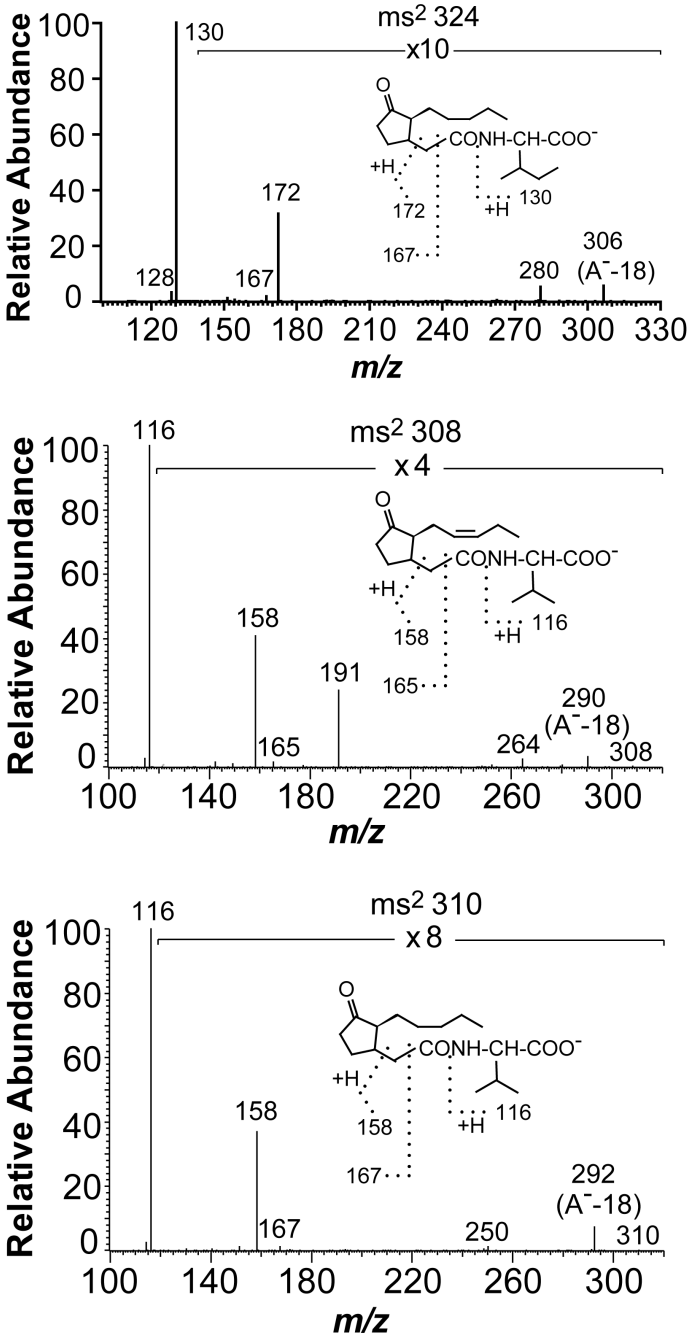



**Fig. I.** $MS^2$ spectrum of 9,10-dihydro-JA-Ile, JA-Val, and 9,10-dihydro-JA-Val. The spectra are magnified 10, 4, and 8 times as shown by the inserts.

**Fig. II**

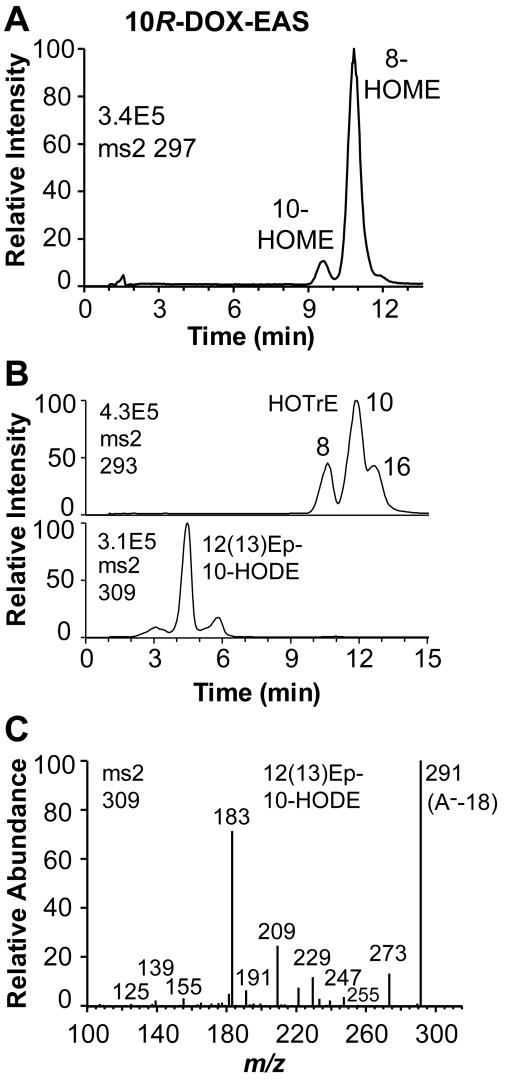



**Fig. II.** Oxidation of 18:1*n*-9 and 18:3*n*-3 by recombinant 10*R*-DOX-EAS *of F. oxysporum*. A. LC-MS analysis of formation of HOME from 18:1*n*-9. 8-HOME was the main product along with small amounts of 10-HOME. B. Chromatograms from LC-MS analysis of products formed from 18:3*n*-3. Top. LC-MS analysis with separation of of 8-, 10- and 16-HOTrE (as indicated by the numbers of the peaks). Steric analysis showed that 16-HOTrE was racemic (data not shown). Bottom. MS/MS analysis of formation of epoxy alcohols and dihydroxy metabolites. The main product was 12(13)Ep-10-HODE. C. $MS^2$ spectrum of 12(13)Ep-10-HODE. See Fig. 5 for formation of major ions.

**Fig. III**

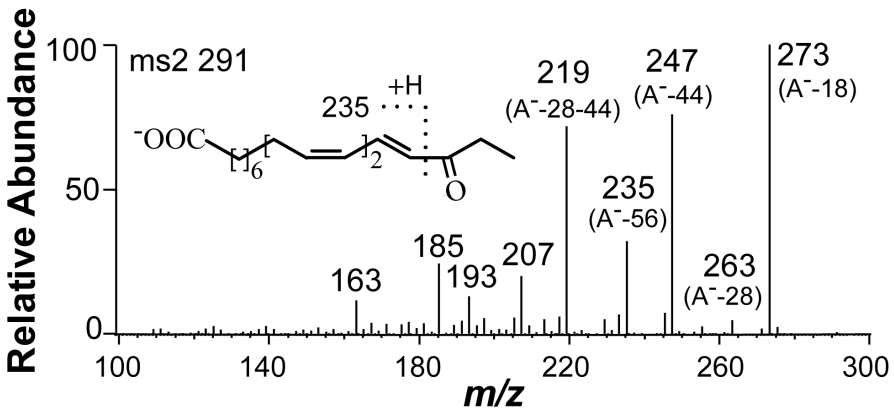
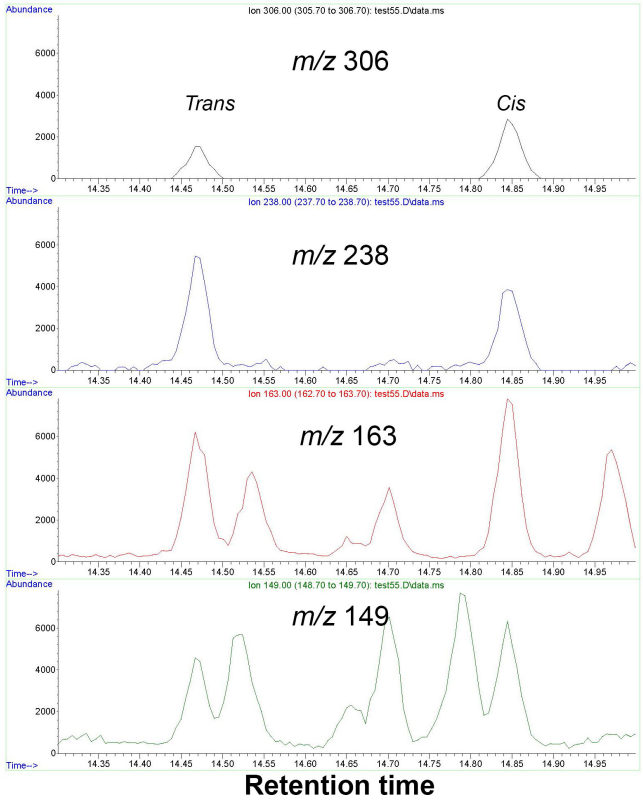



**Fig. III**. $MS^2$ spectrum (*m/z* 291--> full scan) of 16-KOTrE. Many fragments are formed by loss of water ($A^-$ -18), CO ($A^-$ -28), $CO_2$ ($A^-$ -44), and OC=CH-$CH_3$ ($A^-$ -56) as indicated by the labels and the insert. Hydrogenation supported the structure. The $MS^3$ spectrum of 16-HPOTrE (*m/z* 309-->291-->full scan) was identical to the $MS^2$ spectrum of 16-KOTrE.

**Fig. IV**

A




B

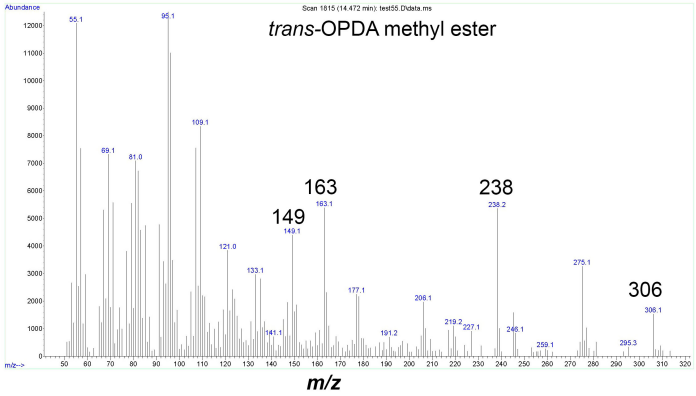



**Fig. IV.** GC-MS analysis of 12-OPDA generated by *F. oxysporum*. A, selected monitoring of mass-spectral ions typical for the methyl esters of 12-OPDA side chain *cis* and *trans* isomers. B, electronic impact mass spectrum recorded at 12.47 min, *i.e.* the methyl ester of *trans*-12-OPDA, showing *m/z* 306 ($M^+$), 275 ($M^+$ - $OCH_3$), 238 ($M^+$ - 68; rearrangement followed by loss of $C_5H_8$), 206 (238 - $CH_3OH$), 163, and 149.

**Fig. V**

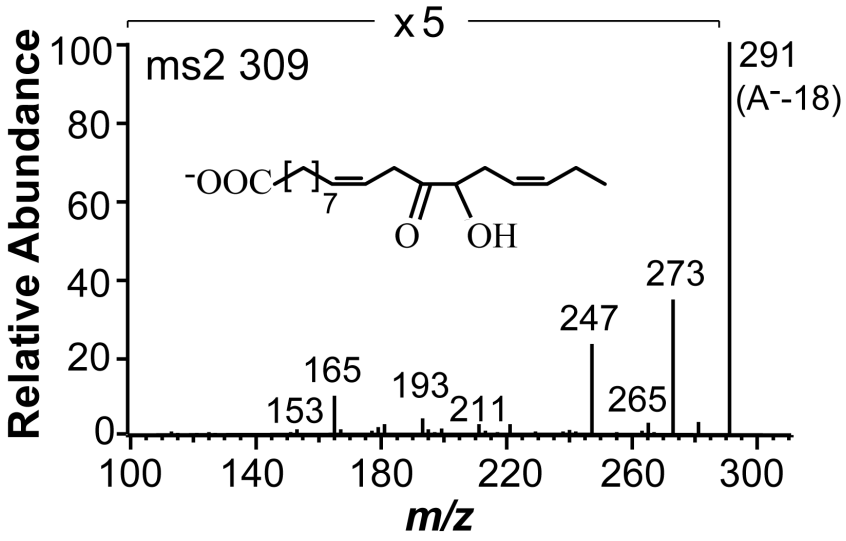



**Fig. V**. $MS^2$ spectrum of the α-ketol (12-oxo-13*R*-hydroxy-9*Z*,15*Z*-octadecadienoic acid). The ions at *m/z* 291, 273 and 247 dominated the spectrum, whereas the signals at *m/z* 153 and 165 were much less intense (<2% of the base peak). The spectrum is magnified x5 as shown by the insert.

**Fig. VI**

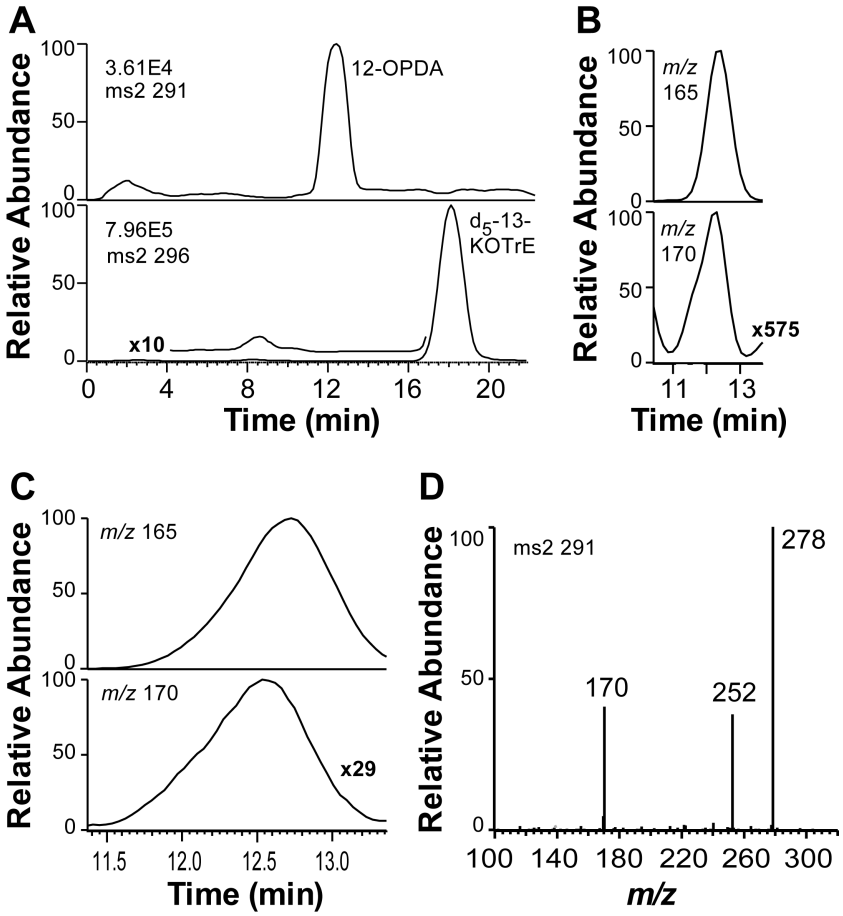



**Fig. VI.** RP-HPLC-MS/MS analysis of endogenous biosynthesis of 12-OPDA and transformation of $d_5$-13*S*-HPOTrE by mycelia of Fot in 1 h. A. The top chromatogram shows formation of 12-OPDA from endogenous 18:3*n*-3 and the bottom chromatogram shows that 100 μM $d_5$-13*S*-HPOTrE is transformed to $d_5$-13-KOTrE, but not to significant amounts of $d_5$-12-OPDA. B. Selected ion chromatograms show that the signal intensities of *m/z* 165 are about 575 times stronger than the signal at *m/z* 170 (possibly due to $d_5$-12-OPDA). C and D. Incubation with 1 mM $d_5$-13*S*-HPOTrE increased the biosynthesis to significant amounts of $d_5$-12-OPDA as judged from the signal intensity of *m/z* 170 (C) and the $MS^2$ spectrum (D).

**Fig. VII**

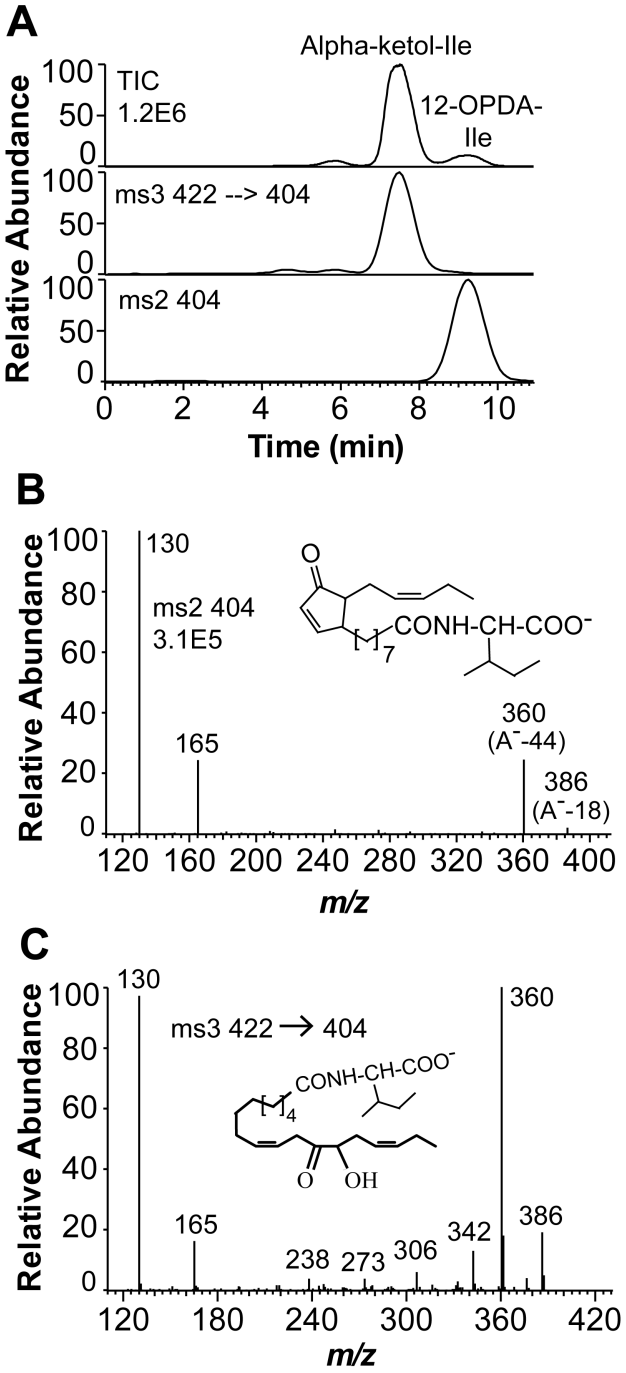



**Fig. VII**. RP-HPLC-MS/MS analysis of the transformation of 13*S*-HPOTrE-Ile by acetone powder of flaxseed (AOS). A. RP-HPLC-MS/MS analysis of the two major products, which were identified as 12-keto-13-hydroxy-octadecadienoyl-Ile (α-ketol-Ile; 90%) and 12-OPDA-Ile (10%). TIC, total ion current. The RP-HPLC column was eluted with 70% methanol. B. $MS^2$ spectrum of 12-OPDA-Ile. C. $MS^3$ spectrum of α-ketol-Ile.