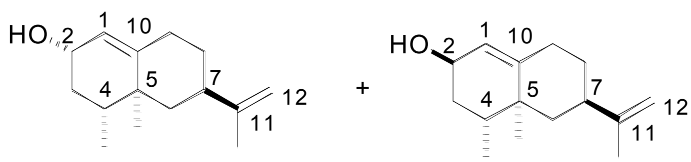
Table of Contents for Supplemental Data, Tables and Figures

Supplemental Data 1 **Analytical and NMR Information**

General Information for Chemical Structure Analysis – ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ (^{1}H, 7.26; ^{13}C, 77.0) and benzene-d_6 with Varian U500 spectrometers at the University of Illinois. Chemical shifts are in ppm and coupling constants are in Hertz. Infrared (IR) spectra were obtained using a Perkin Elmer Spectrum BX spectrophotometer referenced to polystyrene standard. Data are presented as frequency of absorption (cm^{-1}). Optical rotations were acquired on a JASCO P-1020 Digital Polarimeter. TLC analyses were performed on silica gel 60 F254 precoated, 250-μm plates. All R_f values are on silica gel TLC plates until otherwise noted. TLC visualizations were performed with 5% phosphomolybdic acid (0.2 M in 2.5% conc. H_2SO_4/EtOH (v/v)), I_2, or UV.



nootkatol (β-nootkatol) 2-epinootkatol (α-nootkatol)

Reference Standards of Nootkatol and 2-Epinootkatol

Reduction of nootkatone (115 mg, 0.53 mmol) with $LiAlH_4$ (16 mg, 0.40 mmol) in ether (3 ml) was conducted in a manner similar to literature procedures (S1, S2). Purification by flash chromatography on silica gel (5:1 hexane/ethyl acetate) provided pure samples of nootkatol and 2-epinootkatol.

A search of the Chemical Abstracts data base found 46 references to the nootkatols in numerous patents and journal articles describing ways to obtain the isomers by chemical and biological oxidations of valencene and by chemical and biological reductions of nootkatone, their occurrence as natural products, and their physical properties and practical applications. Although the physical characterization data below appear to be in rather good agreement with the literature data for the nootkatols prepared by hydride reductions of nootkatone (eg ^{1}H NMR data, refs 1-5) and the relative stereochemical assignments for the allylic hydroxyl groups are consistent, the names given to the compounds are often different and inconsistent. We prefer the derivative names nootkatol and 2-epinootkatol to avoid the potential confusion associated with α and β designations that commonly refer to relative configurations.

Data for nootkatol (β-nootkatol, 2α-hydroxyvalencene, (*2R, 4R, 5S, 7R*)-Eremophila-1(10), 11(12)-dien-2-ol): yield 77 mg (66%), oil, TLC R_f 0.20 (4:1 hexane/ethyl acetate); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.30 (app q, 1H, J_{app} = 1.5 Hz, vinyl *H*), 4.66 (m, 2H, vinyl *H*), 4.21 (ddt, 1H, *J* = 9.8, 6.2, 2.1 Hz, C*H*OH), 2.31 (tdt, 1H, *J* = 14.2, 4.4, 2.0 Hz, allyl C*H₂*), 2.23 (tt, 1H, *J* = 12.5, 3.2 Hz, allyl C*H*), 2.09 (ddd, 1H, *J* = 14.1, 4.2, 2.9 Hz, allyl C*H₂*), 1.84 (dt, 1H, *J* = 12.5, 2.4

Hz, CH_2), 1.79 (m, 1H, CH_2CHOH), 1.74 (m, 1H, CH_2CHOH), 1.69 (quintet, 3H, J = 1.0 Hz, CH_3), 1.49 (dqd, 1H, J = 14.1, 7.2, 2.4 Hz, CHCH$_3$), 1.35 (td, 1H, J = 12.7, 10.0 Hz, CH_2), 1.19 (dtd, 1H, J = 13.6, 12.4, 4.0 Hz, CH_2), 1.27 (dd, 1H, J = 13.3, 7.6 Hz, CH_2), 0.98 (s, 3H, CH_3), 0.87 (d, 3H, J = 7.0 Hz, CH_3); ^{13}C NMR (126 MHz, CDCl$_3$) δ 150.5, 146.1, 124.6, 108.8, 68.1, 44.7, 40.8, 39.4, 38.3, 37.3, 32.9, 30.0, 21.0, 18.4, 15.6.

Data for 2-epinootkatol (α-Nootkatol, 2β-hydroxyvalencene, (*2S, 4R, 5S, 7R*)-Eremophila-1(10), 11(12)-dien-2-ol): yield 2.8 mg (3.5%); oil, lit[S4] crystalline solid, mp 77-79 °C; TLC R_f 0.23 (4:1 hexane/ethyl acetate); ^{1}H NMR (CDCl$_3$, 500 MHz) δ 5.50 (m, 1H, vinyl *H*), 4.69 (m, 2H, vinyl *H*), 4.07 (m, 1H, C*H*OH), 2.31 (tdt, 1H, J = 13.9, 4.1, 1.9 Hz, allyl CH_2), 2.24 (tt, 1H, J = 12.0, 2.4 Hz, allyl C*H*), 2.14 (ddd, 1H, J = 14.2, 3.9, 2.6 Hz, allyl CH_2), 1.89 (dt, 1H, J = 12.8, 2.7 Hz, CH_2), 1.80 (m, 1H, CH_2CHOH), 1.71 (m, 3H, J = 1.0 Hz, CH_3), 1.69 (m, 1H, CH_2CHOH), 1.62 (td, 1H, J = 12.7, 4.1 Hz, CH_2), 1.57 (dqd, 1H, J = 14.0, 3.1, 1.7 Hz, CHCH$_3$), 1.24 (dtd, 1H, J = 13.4, 12.4, 4.1 Hz, CH_2), 1.01 (t, 1H, J = 12.7 Hz, CH_2), 0.90 (s, 3H, CH_3), 0.89 (d, 3H, J = 7.1 Hz, CH_3).

Data for solavetivol (ca 2 mg): TLC TLC R_f = 0.24 (4:1 hexane/ethyl acetate); $[\alpha]^{25}_D$ +8.3 (c = 0.29, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.33 (qt, 1H, J = 2.4, 1.2 Hz, =CH), 4.71 (m, 1H, =CH$_2$), 4.69 (m, 1H, =CH$_2$), 4.18 (tdd, 1H, J = 6.3, 2.4, 1.8 Hz, C*H*OH), 2.50 (tt, 1H, J = 11.5, 7.7 Hz, H7), 1.86 (ddd, 1H, J = 6.3, 2.6, 1.2 Hz, H7 or H8), 1.83 (ddd, 1H, J = 6.0, 2.4, 1.2 Hz, H3α), 1.76 (dd, 1H, J = 7.1, 1.8 Hz, H3β), 1.74 (s, 3H, CH$_3$), 1.73 (s, 3H, CH$_3$), 1.73~1.74 (m, 1H, H4), 1.71 (dd, 1H, J = 7.0, 2.3 Hz, H9), 1.60 (tt, 1H, J = 8.0, 1.9 Hz, H7 or H8), 1.57 (dd, 1H, J = 2.1, 1.5 Hz, H7 or H8), 1.56 (m, 1H, H7 or H8), 1.34 (ddd, 1H, J = 12.8, 10.4, 7.9 Hz, H9), 1.34 (s, 1H, OH), 1.00 (d, 3H, J = 6.8 Hz, CH$_3$); COSY (500 MHz, CDCl$_3$) (5.33) δ 4.18; (4.18) δ 5.33, 1.83, 1.76, 1.34; (2.50) δ 1.86, 1.60, 1.57, 1.56; (1.00) δ 1.73~1.74; ^{1}H NMR NOE (500 MHz, CDCl$_3$): Irrad. δ 4.18, obs. 5.33 (3.9%), 1.86 (3.8%), 1.76 ~ 1.74 (3.9%); ^{13}C NMR (126 MHz, CDCl$_3$) δ 148.2, 144.7, 124.9, 108.3, 67.2, 49.0, 47.8, 40.7, 38.0, 37.9, 33.2, 32.9, 21.3, 20.5, 17.4; FTIR (neat film) ν 3339 (br), 2953, 2872, 1645, 1451, 1376, 1033, 885.

Data for 2β(OH)EA (3.1 mg): TLC R_f = 0.22 (4:1 hexane/ethyl acetate); $[\alpha]^{25}_D$ -11 (c = 0.31, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.64 (tt, 1H, J = 6.6, 1.8 Hz, =CH), 4.70 (m, 1H, =CH$_2$), 4.67 (m, 1H, =CH$_2$), 3.77 (tt, 1H, J = 11.4, 5.0 Hz, C*H*OH), 2.33 (ddd, 1H, J = 12.3, 5.3, 2.0 Hz, H3α), 2.26 (tddd, 1H, J = 11.4, 2.8, 1.9, 0.9 Hz, H1β), 2.18 (tt, 1H, J = 12.4, 3.5 Hz, H7), 2.01 (ddddd, 1H, J = 16.2, 6.8, 4.2, 2.6, 0.9 Hz, H8), 1.89 (td, 1H, J = 12.8, 5.2 Hz, H3β), 1.81 (tdd, 1H, J = 11.5, 3.9, 2.0 Hz, H8), 1.76 (dt, 1H, J = 15.8, 3.0 Hz, H6α), 1.73 (s, 3H, CH$_3$), 1.67 (dd, 1H, J = 5.2, 2.1 Hz, H1α), 1.64-1.67 (m, 1H, H4), 1.34 (s, 1H, OH), 1.30 (t, 1H, J = 13.6 Hz, H6β), 1.00 (d, 3H, J = 7.2 Hz, CH$_3$); COSY (500 MHz, CDCl$_3$): (5.64) δ 2.01, 2.26; (4.70) and (4.67) δ 1.73; (3.77) δ 1.67, 1.89, 2.33; (1.81) δ 2.01, 2.18, 2.26; (1.30) δ 2.18, 1.76; (1.00) δ 1.64; ^{13}C NMR (126 MHz,

$CDCl_3$) δ 150.0, 137.9, 132.1, 128.6, 108.4, 67.6, 42.7, 41.7, 41.3, 39.6, 31.7, 30.0, 29.9, 21.0, 18.4; FTIR (neat film) ν 3339 (br), 2927, 2873, 1459, 1375, 1246, 1057, 887, 747.

Data for 2β(OH)EE (210 μg): ^{1}H NMR (C_6D_6, 500 MHz) δ 5.42 (dd, 1H, *J* = 3.3, 1.9 Hz, vinyl *H*), 4.83 (dq, 1H, *J* = 1.4, 0.7 Hz, vinyl *H*), 4.81 (dq, 1H, *J* = 2.9, 1.5 Hz, vinyl *H*), 4.02 (app ddd, 1H, *J* = 9.1, 5.5, 3.3 Hz, C*H*OH), 2.45 (dtdd, 1H, *J* = 9.5, 7.3, 3.2, 2.2 Hz, allyl CH_2), 2.10 (qd, 1H, *J* = 7.3, 6.8 Hz, allyl C*H*), 1.90 (ddd, 1H, *J* = 13.6, 7.6, 5.9 Hz, allyl CH_2), 1.78 (dd, 1H, *J* = 13.5, 7.8 Hz, CH_2), 1.64 (quintet, 3H, *J* = 0.6 Hz, CH_3), 1.56 (dddd, 1H, *J* = 12.2, 5.5, 2.5, 1.4 Hz, CH_2CHOH), 1.54-1.52 (m, 1H, CH_2), 1.52 (dd, 1H, *J* = 13.3, 6.5 Hz, CH_2), 1.30 (ddd, 1H, *J* = 12.0, 7.1, 2.6 Hz, C*H*CH_3), 1.27 (dd, 1H, *J* = 13.3, 7.6 Hz, CH_2), 1.15 (td, 1H, *J* = 12.1, 9.1 Hz, CH_2CHOH), 1.01 (s, 3H, CH_3), 0.79 (d, 3H, *J* = 7.0 Hz, CH_3); COSY (500 MHz, C_6D_6) δ 5.42 (4.02, 2.45, 1.56), 4.83 (2.10, 1.64), 4.81 (1.64), 4.02 (1.56, 1.15), 2.45 (1.90), 1.78 (1.52), 1.56 (1.15), 1.52 (1.27), 1.30 (0.79).

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S3. Sakamaki, H.; Itoh, K.-I.; Taniai, T.; Kitanaka, S.; Takagi, Y.; Chai, W.; Horiuchi, C. A. (2005) Journal of Molecular Catalysis B: Enzymatic, **32**, 103-106.

S4. Kaspera, R.; Krings, U.; Nanzad, T.; Berger, R. G. (2005) *Appl. Microbiol. Biotechnol.* **67**, 477-483.

S5. Weyerstahl, P.; Marschall, H.; Splittgerber, U.; Wolf, D. (2000) *Flavour and Fragrance Journal*, **15**, 153-173.

Supplemental Table 1 **Primers for the construction of EAH mutants.**

*Substrate recognition site (SRS) in which mutation was introduced.

**The codon changed to make the desired mutation was shown in red boldface.

EAH mutation	SRS	Template*	Primers used for mutagenesis**
S368V	5	EAH	5'-GACTTCATCCACCG**GT**TCCACTTTTGGTCC-3'
			5'-GGACCAAAAGTGGA**AC**CGGTGGATGAAGTC-3'
S482V	6	EAH	5'-GACTTAGACTTGACCGAATTA**GT**GGGAATAACTATTGCTAGAAAGGG-3'
			5'-CCCTTTCTAGCAATAGTTATTCCC**AC**TAATTCGGTCAAGTCTAAGTC-3'
I484V	6	EAH	5'-GACTTGACCGAATTATCGGGA**G**TAACTATTGCTAGAAAGGG-3'
			5'-CCCTTTCTAGCAATAGTTA**C**TCCCGATAATTCGGTCAAGTC-3'
I486A	6	EAH	5'-CGAATTATCGGGAATAACT**GC**TGCTAGAAAGGGTGGCC-3'
			5'-GGCCACCCTTTCTAGCA**GC**AGTTATTCCCGATAATTCG-3'
S368V/S482V	5+6	EAH-S368V	5'-GACTTAGACTTGACCGAATTA**GT**GGGAATAACTATTGCTAGAAAGGG-3'
			5'-CCCTTTCTAGCAATAGTTATTCCC**AC**TAATTCGGTCAAGTCTAAGTC-3'
S368V/I484V	5+6	EAH-S368V	5'-GACTTGACCGAATTATCGGGA**G**TAACTATTGCTAGAAAGGG-3'
			5'-CCCTTTCTAGCAATAGTTA**C**TCCCGATAATTCGGTCAAGTC-3'
S368V/I486A	5+6	EAH-S368V	5'-CGAATTATCGGGAATAACT**GC**TGCTAGAAAGGGTGGCC-3'
			5'-GGCCACCCTTTCTAGCA**GC**AGTTATTCCCGATAATTCG-3'

Supplemental Table 2 **Primers for the construction of HPO mutants.**

*Substrate recognition site (SRS) in which mutation was introduced.

**The codon changed to make the desired mutation was shown in red boldface.

HPO mutation	SRS*	Template	Primers used for mutagenesis**
V366S	5	HPO	5'-GACTCCATCCACCA**TC**TCCACTTTTGGTCC-3'
			5'-GGACCAAAAGTGGA**GA**TGGTGGATGGAGTC -3'
V480S	6	HPO	5'-GACTTGGATTTGACAGAATTG**AG**TGGAGTAACTGCTGCCAGAAAGAG-3'
			5'-CTCTTTCTGGCAGCAGTTACTCCA**CT**CAATTCTGTCAAATCCAAGTC-3'
V482I	6	HPO	5'-GACAGAATTGGTTGGA**A**TAACTGCTGCCAGAAAGAG-3'
			5'-CTCTTTCTGGCAGCAGTTA**T**TCCAACCAATTCTGTC-3'
A484I	6	HPO	5'-GAATTGGTTGGAGTAACT**AT**TGCCAGAAAGAGTGATC-3'
			5'-GATCACTCTTTCTGGCA**AT**AGTTACTCCAACCAATTC-3'
V480S/V482I	6	HPO	5'-GACTTGGATTTGACAGAATTG**AG**TGGA**A**TAACTGCTGCCAGAAAGAGTG-3'
			5'-CACTCTTTCTGGCAGCAGTTA**T**TCCA**CT**CAATTCTGTCAAATCCAAGTC-3'
V480S/A484I	6	HPO	5'-GACTTGGATTTGACAGAATTG**AG**TGGAGTAACT**AT**TGCCAGAAAGAGTG-3'
			5'-CACTCTTTCTGGCA**AT**AGTTACTCCA**CT**CAATTCTGTCAAATCCAAGTC-3'
V482I/A484I	6	HPO	5'-GACTTGGATTTGACAGAATTGGTTGGA**A**TAACT**AT**TGCCAGAAAGAGTG-3'
			5'-CACTCTTTCTGGCA**AT**AGTTA**T**TCCAACCAATTCTGTCAAATCCAAGTC-3'
V480S/V482I/A484I	6	HPO	5'-GACTTGGATTTGACAGAATTG**AG**TGGA**A**TAACT**AT**TGCCAGAAAGAGTG-3'
			5'-CACTCTTTCTGGCA**AT**AGTTA**T**TCCA**CT**CAATTCTGTCAAATCCAAGTC-3'
V366S/V480S	5+6	HPO-V366S	5'-GACTTGGATTTGACAGAATTG**AG**TGGAGTAACTGCTGCCAGAAAGAG-3'
			5'-CTCTTTCTGGCAGCAGTTACTCCA**CT**CAATTCTGTCAAATCCAAGTC-3'
V366S/V482I	5+6	HPO-V366S	5'-GACAGAATTGGTTGGA**A**TAACTGCTGCCAGAAAGAG-3'
			5'-CTCTTTCTGGCAGCAGTTA**T**TCCAACCAATTCTGTC-3'
V366S/A484I	5+6	HPO-V366S	5'-GAATTGGTTGGAGTAACT**AT**TGCCAGAAAGAGTGATC-3'
			5'-GATCACTCTTTCTGGCA**AT**AGTTACTCCAACCAATTC-3'
V366S/V480S/V482I	5+6	HPO-V366S	5'-GACTTGGATTTGACAGAATTG**AG**TGGA**A**TAACTGCTGCCAGAAAGAGTG-3'
			5'-CACTCTTTCTGGCAGCAGTTA**T**TCCA**CT**CAATTCTGTCAAATCCAAGTC-3'
V366S/V480S/A484I	5+6	HPO-V366S	5'-GACTTGGATTTGACAGAATTG**AG**TGGAGTAACT**AT**TGCCAGAAAGAGTG-3'
			5'-CACTCTTTCTGGCA**AT**AGTTACTCCA**CT**CAATTCTGTCAAATCCAAGTC-3'
V366S/V482I/A484I	5+6	HPO-V366S	5'-GACTTGGATTTGACAGAATTGGTTGGA**A**TAACT**AT**TGCCAGAAAGAGTG-3'
			5'-CACTCTTTCTGGCA**AT**AGTTA**T**TCCAACCAATTCTGTCAAATCCAAGTC-3'
V366S/V480S/V482I/A484I	5+6	HPO-V366S	5'-GACTTGGATTTGACAGAATTG**AG**TGGA**A**TAACT**AT**TGCCAGAAAGAGTG-3'
			5'-CACTCTTTCTGGCA**AT**AGTTA**T**TCCA**CT**CAATTCTGTCAAATCCAAGTC-3'

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71D4    (23) 5'-ATGCAATTCTTGAGCTTGGCTTCCATCTTCCTTTTTCTATCTTTTCTGTTTTTGTTAAGGAAATGGAA
71D6     (1) 5'----CCAAAATGCAA-TTGATTTCCATTTTTCTTTTCATATGTTTCCTCTTTTTGTTAAGGAAATGGAA
71D7     (1) 5'---------ATGCAA-TTGGTTTCCATTTTCCTTTTCATATCTTTCCTCTTTTTGTTAAGGAAATGGAA
71D16    (1) 5'-ATGCAGTTCTTCAACTTCTTTTCCCTTTTCCTTTTTGTGTCATTTCTCTTTTTATTTAAGAAATGGAA
71D20    (1) 5'-ATGCAATTCTTCAGCTTGGTTTCCATTTTCCTATTCCTATCTTTCCTATTTCTGTTGAGGAAATGGAA
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Forward primers F1 F2 F3

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71D4    (1464) GAGTAACTGCTGCCAGAAAAGATGACCTTATTTTGGTTGCCACTCCTTATGAACCACCTCGACAATGA-3'
71D6    (1453) GAATGTCTGCGGCTAGTAAGGACGATCTTTACTTGATTGCCACTCCTTATCAACCTTAATGGCATTTA-3'
71D7    (1445) GAATTACTGCTGCTAGAAAGGGTGATCTTTACTTAATTGCCACTCCTCATCAACCTTAA         -3'
71D16   (1433) GAGCAGCTTGTGCCAGAAAGAATGACCTACACTTGATCGCTACTGCTTATCAACACTGTGAAGAGTGA-3'
71D20   (1448) GAATAACTATTGCTAGAAAGGGTGGCCTTTACTTAAATGCCACTCCTTATCAACCTTCTCGAGAGTAA-3'
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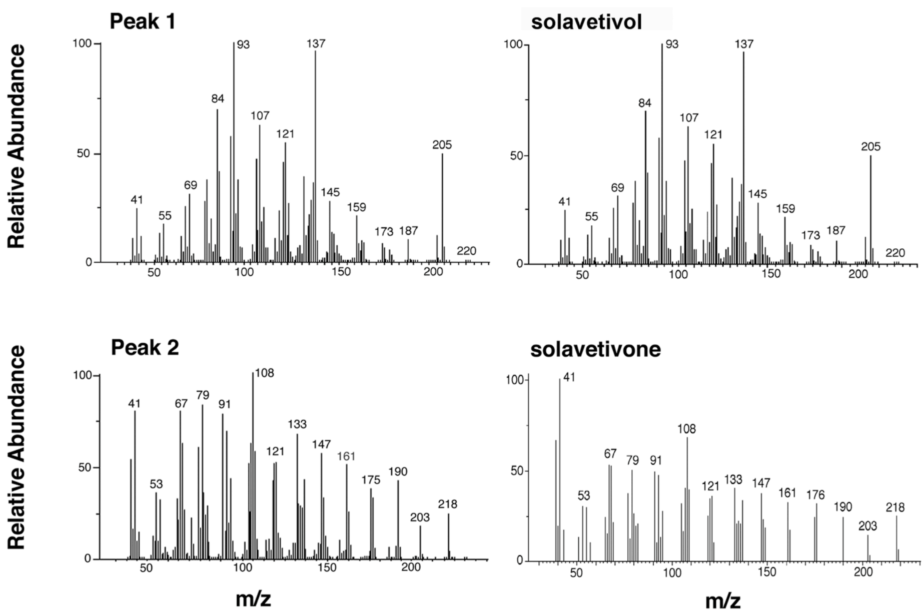
Reverse primers R1 R2 R3

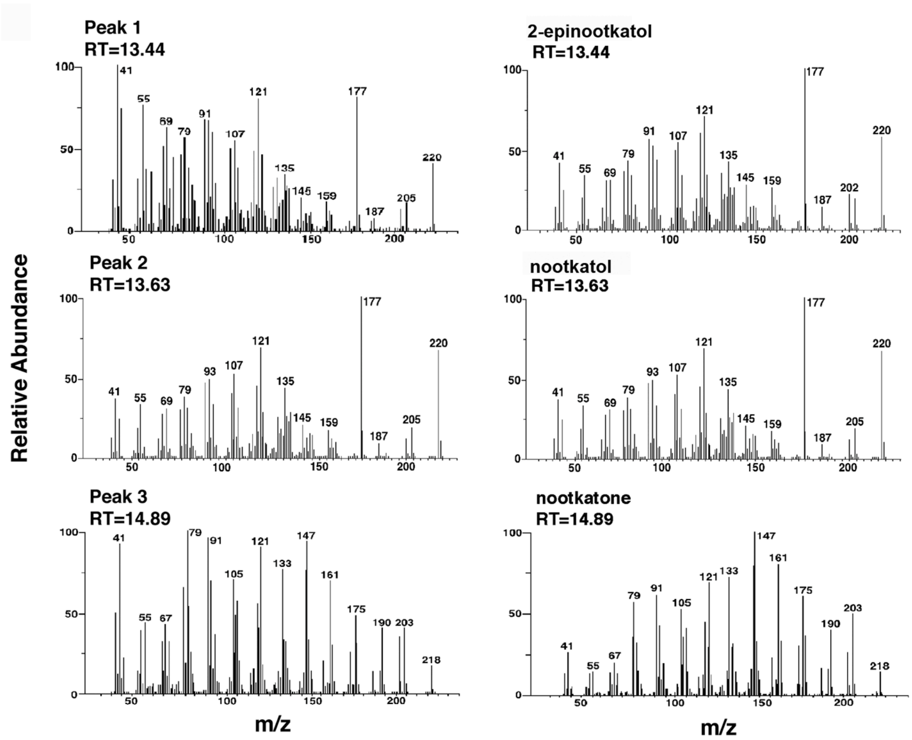
Supplemental Fig.1 **Alignment of 71D subfamily genes and the primers used for cloning of premnaspirodiene oxygenase from *Hyoscyamus muticus.*** Four 71D subfamily genes similar to EAH (71D20) were selected and aligned using vector NTI. Accession number are as follows: CYP71D4 (AJ296346), CYP71D6 (U48434)(ref. Y), CYP71D7 (U48435)(ref. Y), CYP71D16 (AF166332)(35) and CYP71D20 (EAH; AF368376)(25). Arrow shows the position of the primers.

F1 primer, 5'-ATGCAATTCTTCAGCTTGGTTTCC-3';
F2 primer, 5'-TTGGYTTCCATYTTCCTWTT-3';
F3 primer, 5'-TTTYTGTTRAGGAAATGGAA-3';
R1 primer, 5'-TTACTCTCGAGAAGGTTGATAAGG-3';
R2 primer, 5'-AGAAGGTTSATAAGGAGT-3';
R3 primer, 5'-AAGGTTSATAAGGAGTGGCA-3'.

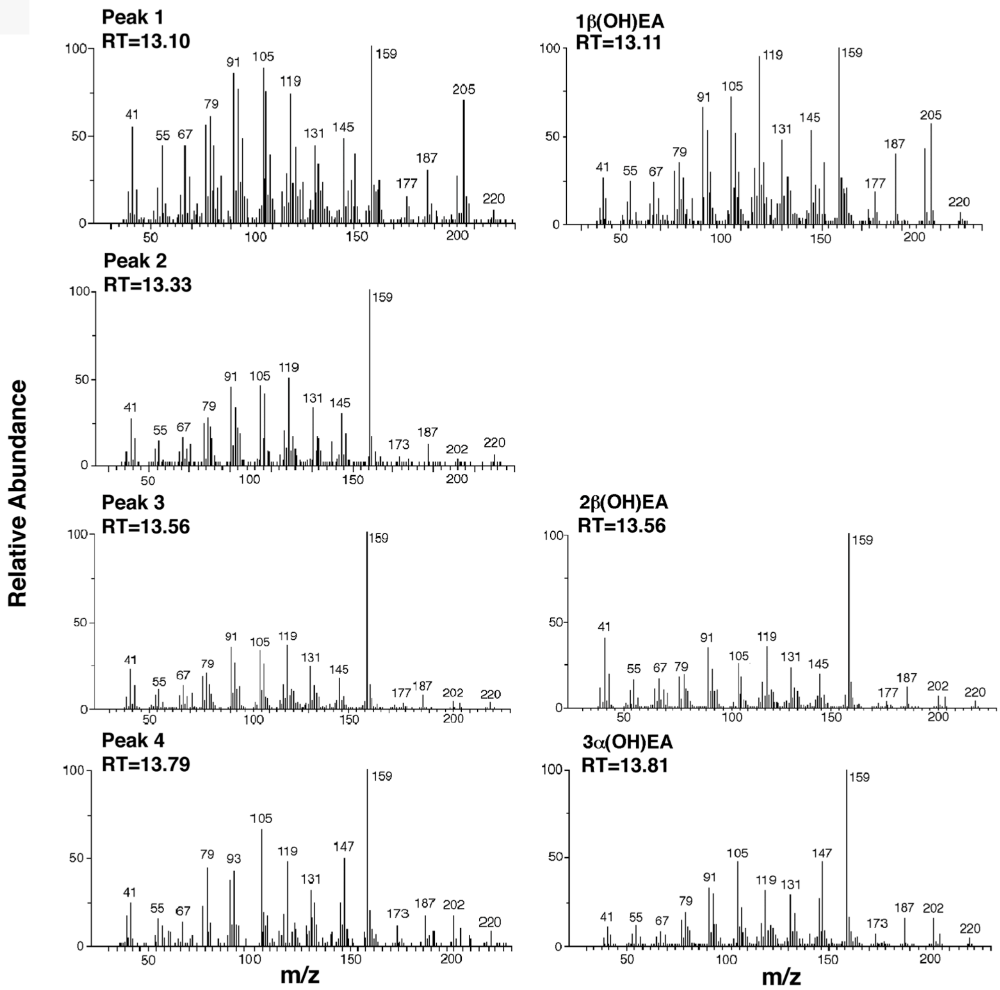
The F1 and R1 primers designed upon the EAH sequence successfully amplified the cDNA fragment from H. muticus RNA of similar size to that of EAH. The fragment was cloned into pGEM-T Easy vector (Promega, WI, USA) and sequenced according to standard procedures. Subsequent studies were performed to isolate other similar P450 genes using additional sets of degenerate PCR primers: forward (F2 and F3) and reverse (R2 and R3). PCR fragments obtained from the primer sets of F2-R1 and F2-R2 were cloned into pGEM-T Easy vector and all together 8 clones were sequenced. All of the cDNA sequences were identical to that obtained with the F1-R1 primer set, which represented a full-length cDNA from the indicated start codon (5' ATG) to the stop codon (3' TGA).



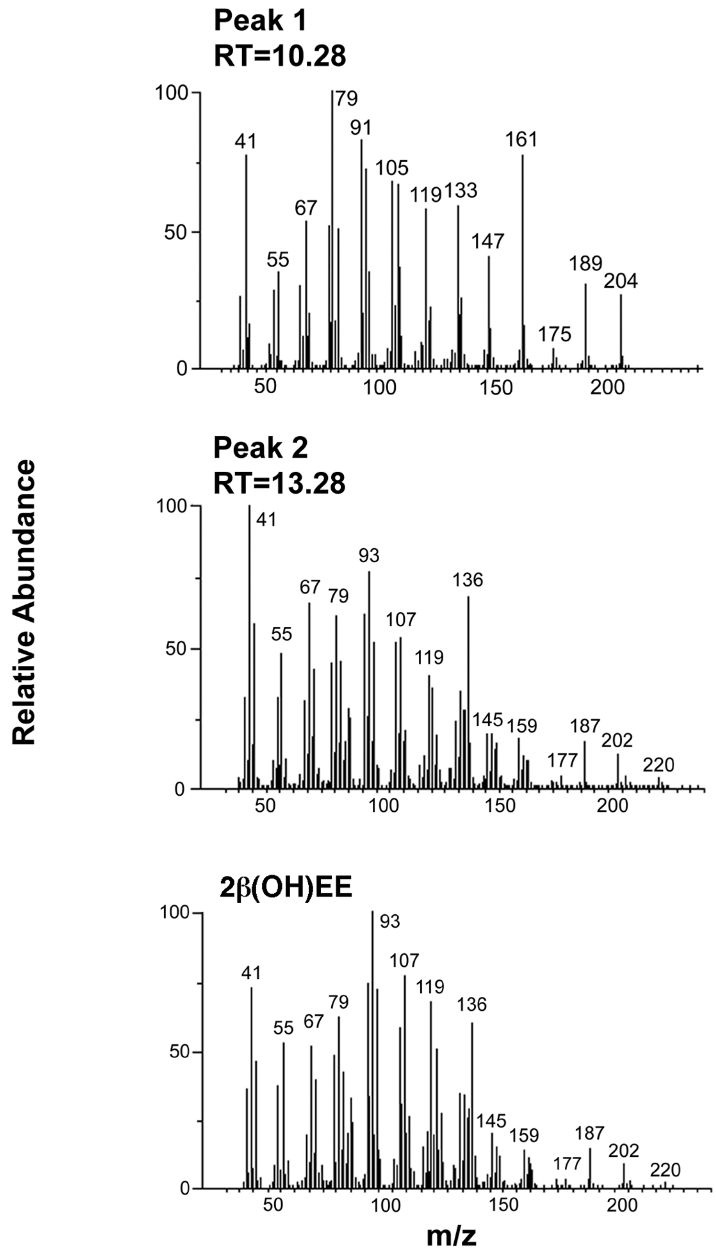
Supplemental Fig. 2 **Mass spectra for reactions products generated by HPO incubated with premnaspirodiene.** Microsmes isolated from yeast over-expressing the HPO cDNA were incubated with 40 μM of premnaspirodiene for 30 min before profiling the reaction products by GC/MS. The MS for peaks 1 and 2 noted in Fig. 2A are compared to those for purified solavetivol, and to the NIST library data for solavetivone, respectively.



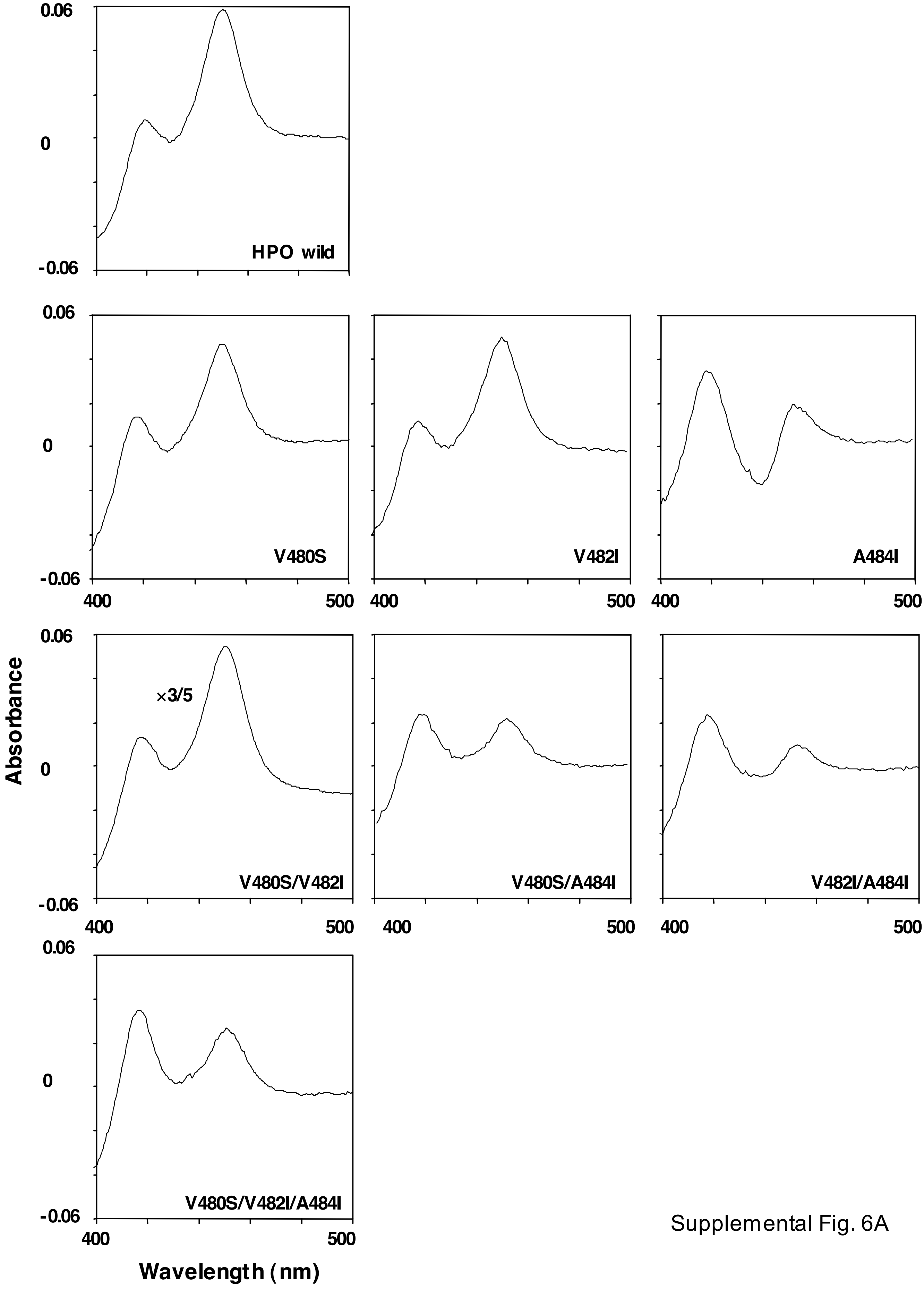
Supplemental Fig. 3 **Mass spectra for reactions products generated by HPO incubated with valencene.** Microsmes isolated from yeast over-expressing the HPO cDNA were incubated with 40 μM of premnaspirodiene for 10 min before profiling the reaction products by GC/MS. The MS for peaks 1-3 noted in Fig. 3A are compared to those for authentic α-nootkatol, β-nootkatol, and nootkatone.



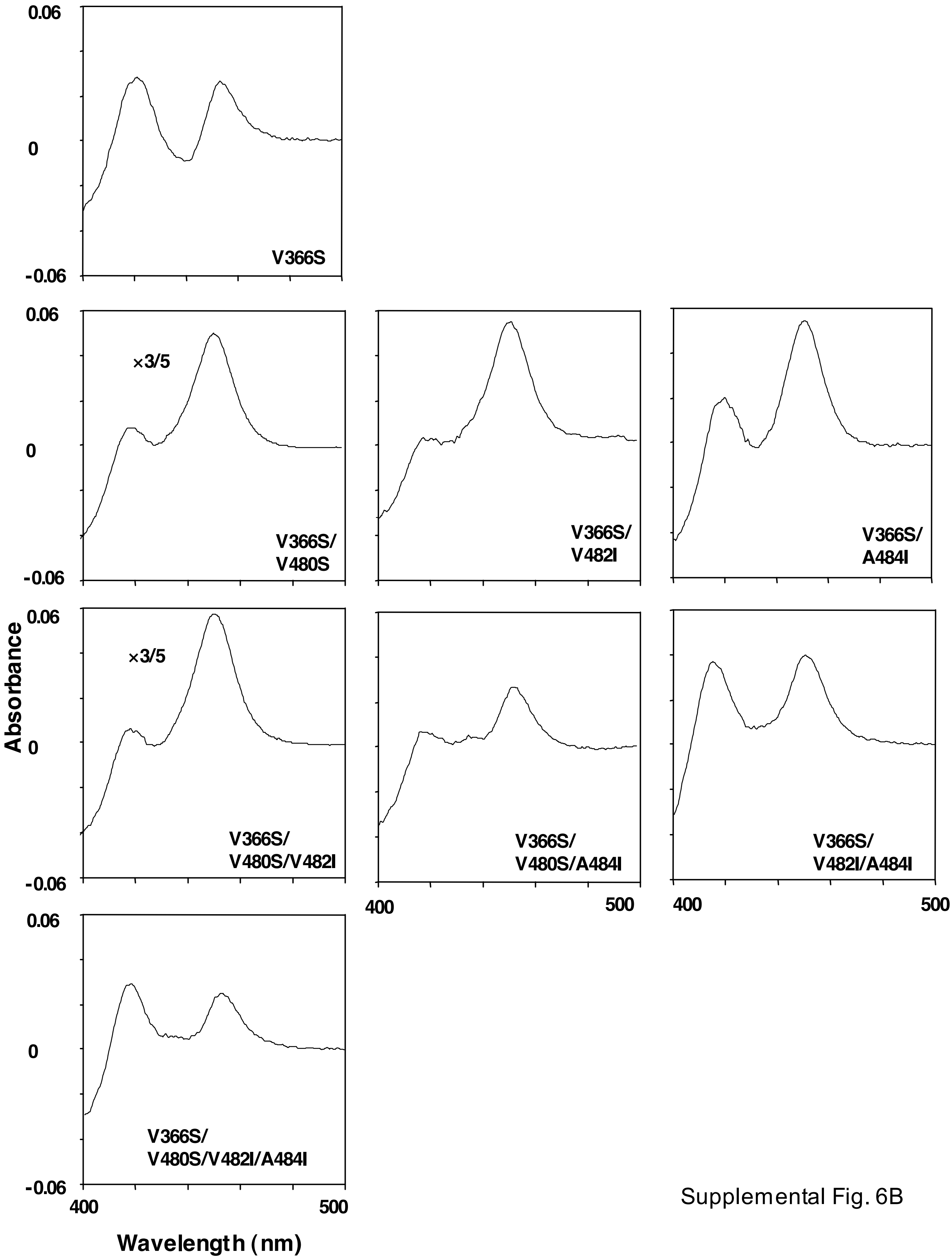
Supplemental Fig. 4 **Mass spectra for reactions products generated by HPO incubated with EA.** Microsmes isolated from yeast over-expressing the HPO cDNA were incubated with 40 μM of EA for 10 min before profiling the reaction products by GC/MS. The MS for peaks 1-4 noted in Fig. 4A are compared to those for authentic 1β(OH)EA, 2β(OH)EA and 3α(OH)EA.



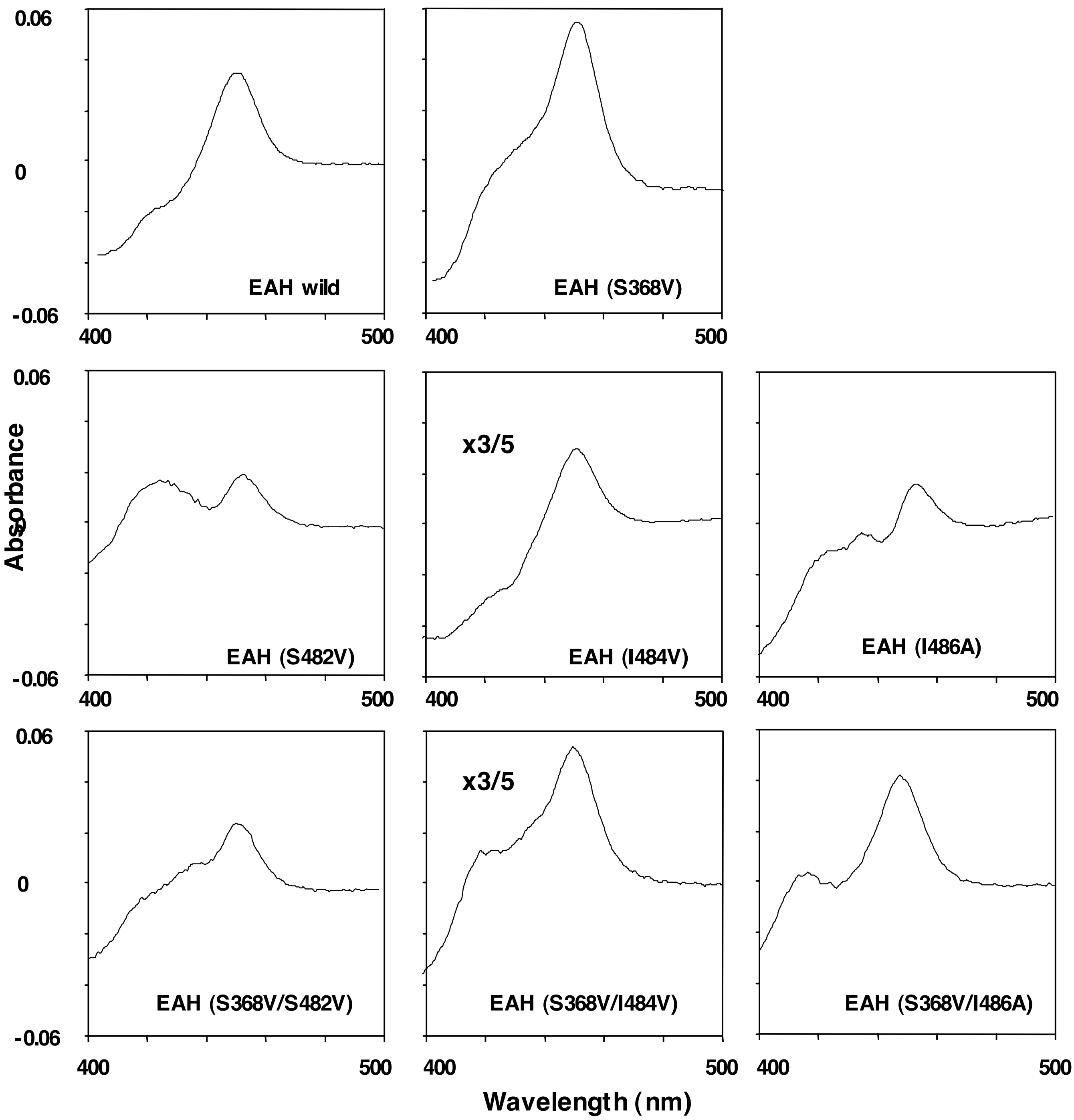
Supplemental Fig. 5 **Mass spectra for reactions products generated by HPO incubated with EE.** Microsmes isolated from yeast over-expressing the HPO cDNA were incubated with the mixture of 30 µM EE (peak 1 in Fig. 5) and 10 µM of a double bond isomer of EE (*, Fig. 5) for 10 min before profiling the reaction products by GC/MS. The MS for peaks 1 and 2 noted in Fig. 5 are compared to that for 2β(OH)EE.



Supplemental Fig. 6A

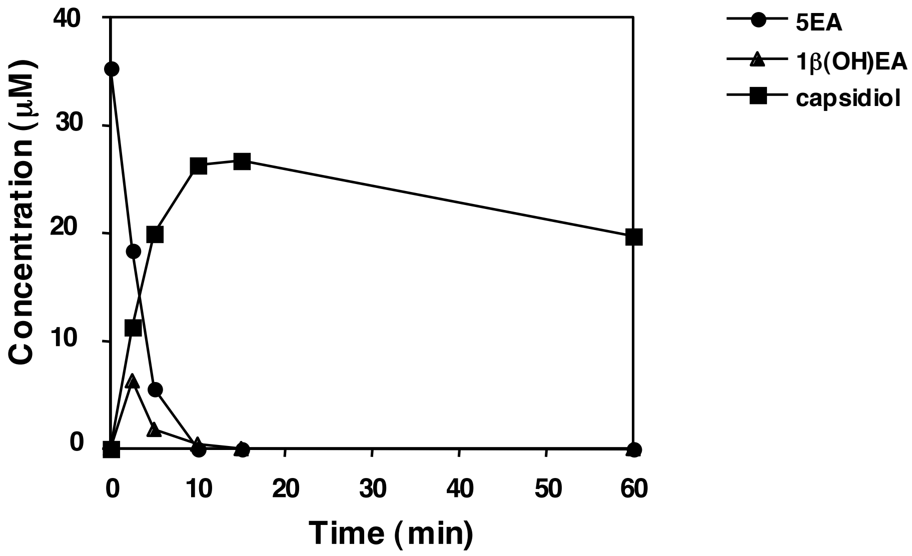


Supplemental Fig. 6B



Supplemental Fig. 6C

Supplemental Fig. 6 **CO difference spectra for the wild type and mutant enzymes.** Reciprocal mutations between EAH and HPO were introduced in SRS 5 and 6 as described in the EXPERIMENTAL PROCEDURES. The mutant genes were expressed in yeast, and the extracted microsomes were used for CO difference spectroscopy to quantify and normalize the amount of properly folded CYP enzymes used in subsequent enzyme assays. CO difference spectra for mutations in SRS 6 of HPO (A), mutations in SRS 5 and 6 of HPO (B), and mutations in SRS 5 and 6 of EAH (C).



Supplemental Fig. 7 **Time-dependent consumption of substrate and accumulation of EAH reaction products.** EAH was incubated with 40 μM EA (●) for 2, 5, 10, 15 and 60 min, and the ethyl acetate-extractable products were analyzed by GC/MS. 1β(OH)EA (▲) and capsidiol (■) were the only NADPH-dependent products detected.