Supporting Information for:

Characterization of CYP76AH4 clarifies phenolic diterpenoid biosynthesis in the Lamiaceae

Jiachen Zi and Reuben J. Peters

Dept. Biochem., Biophys. & Mol. Biol., Iowa State University, Ames, IA 50011 USA

Materials and Methods

General

Unless otherwise noted, chemicals were purchased from Fisher Scientific and molecular biology reagents from Invitrogen. Sequence analyses were carried out with the CLC Main Workbench 6.9 software package (CLCbio). HPLC separations were carried out with an Agilent 1100 series instrument equipped with fraction collector and diode array detector. Gas chromatography with mass spectrometric detection (GC-MS) analyses were performed using a Varian (Palo Alto, CA, USA) 3900 instrument with HP-5ms column and Saturn 2100 ion trap mass spectrometer in electron ionization (70 eV) mode. Samples (1 µL) were injected in splitless mode at 50 °C and, after a 3 min. hold, the temperature raised at 14 °C/min. to 300 °C, where it was held for 3 min. MS data was collected from *m/z* from 90 to 600, starting 12 min. after the injection until the end of the run. GC with flame ionization detection was carried out using an Agilent (Santa Clara, CA, USA) 6890 GC with HP-5ms column, and the same protocol utilized as for GC-MS analyses.

Discovery of CYP76AH4-7

BLAST searches of the rosemary transcriptome (http://www.medicinalplantgenomics.msu.edu) using CYP76AH1 (GenBank JX422213)[1] as the query sequence yielded a number of hits. At least five appeared to be full-length and fall within the CYP76AH sub-family (i.e., the translated amino acid sequences were > 55% identical to CYP76AH1). Although three of these were annotated as a single locus (roa_locus_3663), they shared less than 97% aa sequence identity (using isotigs 5, 8 & 9 as representative), suggesting that they might be distinct loci. The other two were more clearly distinct (roa_locus_4381 and roa_locus_31503). These were submitted to Prof. David Nelson (Univ. Tenn. Health Sci. Center) for determination of CYP nomenclature, which was assigned as follows: CYP76AH4 (roa_locus_3663_iso_9), with the other two isotigs from this locus differing essentially only in a 54 aa stretch in the middle these were suggested to be isoforms/variants CYP76AH5v1 (roa_locus_3663_iso_8) and CYP76AH5v2 (roa_locus_3663_iso_5), along with the more distinct CYP76AH6 (roa_locus_4381), and CYP76AH7 (roa_locus_31503).

Synthesis and cloning of CYP76AH1 and 4-7 for recombinant expression

Completely recoded genes for CYP76AH1 and CYP76AH4-7, including both isoforms of CYP76AH5, as well as the CYP reductase (CPR) from Danshen (DsCPR1, GenBank FR693803),[1] were synthesized (Genscript) with optimized codon usage for expression in *E. coli* and, in the case of the CYP, also modified N-termini[2] (see sequences below). These were amplified via PCR, inserted into pENTR/SD/D-TOPO vectors, and each construct verified by full gene sequencing. DsCPR1 was sub-cloned into the multiple cloning site (MSC) 2 of pCDFDuet (Novagen) using BglII and KpnI restriction sites, which were introduced at the 5' and 3' ends (respectively) of the synthetic gene during sub-cloning. CYP76AH1 & 4-7 were sub-cloned into the MCS1 of pETDuet using the EcoRI and HindIII restriction sites, which were introduced at the 5' and 3' ends (respectively) of the synthetic genes during sub-cloning. Finally, to enable co-production of miltiradiene (**1**), the previously described DsKSL[3] was sub-cloned into the EcoRI and HindIII restriction sites of the MSC1 of a pCDFDuet vector carrying either DsCPR1 or a previously cloned[4] CPR from *Arabidopsis thaliana* (AtCPR1[5, 6]), which were inserted into MCS2 using NdeI and KpnI restriction sites.

Characterization of CYP76AH1 and 4-7 via metabolic engineering

The ability of CYP76AH1 & 4-7 to react with **1** (actually, as eventually realized, **3**) was investigated using a previously described modular metabolic engineering system.[7] Briefly, *E. coli* strain OverExpress C41 (Lucigen) was transformed with pGGnC, harboring a geranylgeranyl diphosphate synthase and copalyl diphosphate synthase, the pCDFDuet/CPR/DsKSL and pETDuet/CYP76AHx constructs described above, as well as a previously described pIRS vector overexpressing key enzymes in the upstream isoprenoid precursor supply pathway.[8] The resulting recombinant strains were grown at 37 °C in 50 mL TB (terrific broth) with appropriate antibiotics to an OD_{600} of 0.4–0.6, when the temperature was lowered to 16 °C, the cultures were supplemented with *δ*-aminolevulinic acid (75 mg/L) and riboflavin (3 mg/L), and after one hour equilibration time induced with 1 mM IPTG (isopropyl *β*-D-thiogalacto-pyranoside). After fermentation for an additional 72 h at 16 °C, the cultures were extracted with an equal volume of hexanes, which was dried under nitrogen gas (N_2), and resuspended in 200 μL hexanes for analysis by GC-MS. From these studies, of CP76AH4-7, CYP76AH4 gave the best yield of ferruginol (**2**), with co-expression of AtCPR1 or DsCPR1 making no difference (the data shown is here from co-expression with AtCPR1).

Characterization of CYP76AH1 & 4 via in vitro reactions

CYP76AH1 or CYP76AH4 constructs were co-expressed with DsCPR in *E. coli* strain OverExpress C43, and in vitro reactions were carried out with the resulting clarified lysates as described previously.[9] This enabled kinetic assays, which were carried out much as previously described.[4] Serial concentrations of abietatriene (5 μM, 10 μM, 15 μM, 20 μM, 30 μM, 40 μM, 60 μM, 80 μM and 120 μM) together with 0.4 mM NADPH were assays in 500 μL enzyme reactions. The resulting solutions were extracted twice with 1 mL hexanes, the extracts dried under N_2, with the residue resuspended in 150 μL hexanes for quantification by GC analysis.

Isolation of miltiradiene, abietatriene and ferruginol

To obtain miltiradiene, 3 L of recombinant *E. coli* C41 (Table S1) transformed with pGGnC, pIRS and pDEST14/DsKSL was grown as described previously.[3] These were extracted with an equal volume of hexanes, which was dried using a rotovap, and the residue resuspended in 3 mL of hexanes, which was subjected to flash chromatography over a 4 g-silica column, with elution by hexanes. After being dried under N_2, 15 mg miltiradiene (**1**) was obtained. Abietatriene (**3**) was produced by putting an open glass vial containing 3 mg of **1** upside down on an UV light plate, with the sample repeatedly dissolved in hexanes and evaporated under N_2 every other hour. After 6 h, **1** was completely converted into **3** (2.6 mg). Ferruginol (**2**) was obtained from *Salvia* *miltiorrhiza* hairy root cultures, which we have described previously.[1] After induction by yeast extract (100 μg/mL) and silver nitrate (30 μM), the cultures were grown for another week, and a total of 62 g tissues were frozen in liquid N_2, ground into powder that was resuspended in 200 mL methanol, and extracted with 200 mL hexanes. The hexane phase was dried using a rotovap, and the residue was resuspended in 2 mL of a 1:1 mixture of acetonitrile and water and separated by reversed-phase HPLC over an Agilent Eclipse XDB-C8 column (5μm, 9.4 × 250 mm), with elution via a linear gradient of 67%-100% acetonitrile in water to afford ferruginol (2.3 mg). The isolated ferruginol (**2**) and abietatriene (**3**) were confirmed by NMR analysis (Table S1 and Figure S2). Briefly, the purified compound was dried under a gentle stream of N_2, and then dissolved in 0.5 mL deuterated chloroform ($CDCl_3$; Sigma-Aldrich), with this evaporation-resuspension process repeated once more to completely remove the protonated acetonitrile solvent. This sample was placed in NMR microtubes (Shigemi, Allison Park, PA) for analyses, and chemical shifts were referenced using known chloroform-d (^{13}C 77.23, ^{1}H 7.24 ppm) signals offset from TMS. Structural analysis was performed using 1D ^{1}H, and 2D DQF-COSY, HSQC, HMQC, HMBC, and NOESY experiment spectra acquired at 700 MHz, and 1D ^{13}C and DEPT135 spectra (174 MHz) using standard experiments from the Bruker TopSpin v1.4 software.

Phytochemical analyses

To verify the presence of **1** – **3**, rosemary aerial tissue (5.3 g) or induced *S. miltiorrhiza* hairy roots (3.8 g) were frozen in liquid nitrogen and grinded to a fine powder. This material was extracted with 2 × 50 mL hexanes and then 50 mL methanol, which was re-extracted with an additional 50 mL of hexanes. The hexanes extracts were pooled and evaporated under N_2, with the resulting residues resuspended in 50 μL hexanes for analysis by GC-MS.

References

1.J. Guo, Y. J. Zhou, M. L. Hillwig, Y. Shen, L. Yang, Y. Wang, X. Zhang, W. Liu, R. J. Peters, X. Chen, Z. K. Zhao and L. Huang, Proc Natl Acad Sci U S A, 2013, 110, 12108-12113.
2.E. E. Scott, M. Spatzenegger and J. R. Halpert, Arch. Biochem. Biophys., 2001, 395, 57-68.
3.W. Gao, M. L. Hillwig, L. Huang, G. Cui, X. Wang, J. Kong, B. Yang and R. J. Peters, Org. Lett., 2009, 11, 5170-5173.
4.D. Morrone, X. Chen, R. M. Coates and R. J. Peters, Biochem. J., 2010, 431, 337-344.
5.P. Urban, C. Mignotte, M. Kazmaier, F. Delorme and D. Pompon, J. Biol. Chem., 1997, 272, 19176-19186.
6.M. Mizutani and D. Ohta, Plant Physiol., 1998, 116, 357-367.
7.A. Cyr, P. R. Wilderman, M. Determan and R. J. Peters, J. Am. Chem. Soc., 2007, 129, 6684-6685.
8.D. Morrone, L. Lowry, M. K. Determan, D. M. Hershey, M. Xu and R. J. Peters, Appl. Microbiol. Biotechnol., 2010, 85, 1893-1906.
9.Y. Wu, M. L. Hillwig, Q. Wang and R. J. Peters, FEBS Lett., 2011, 585, 3446-3451.

Figure S1. Alignment of CYP76AH1 (Danshen) with CYP76AH4-7 (rosemary).

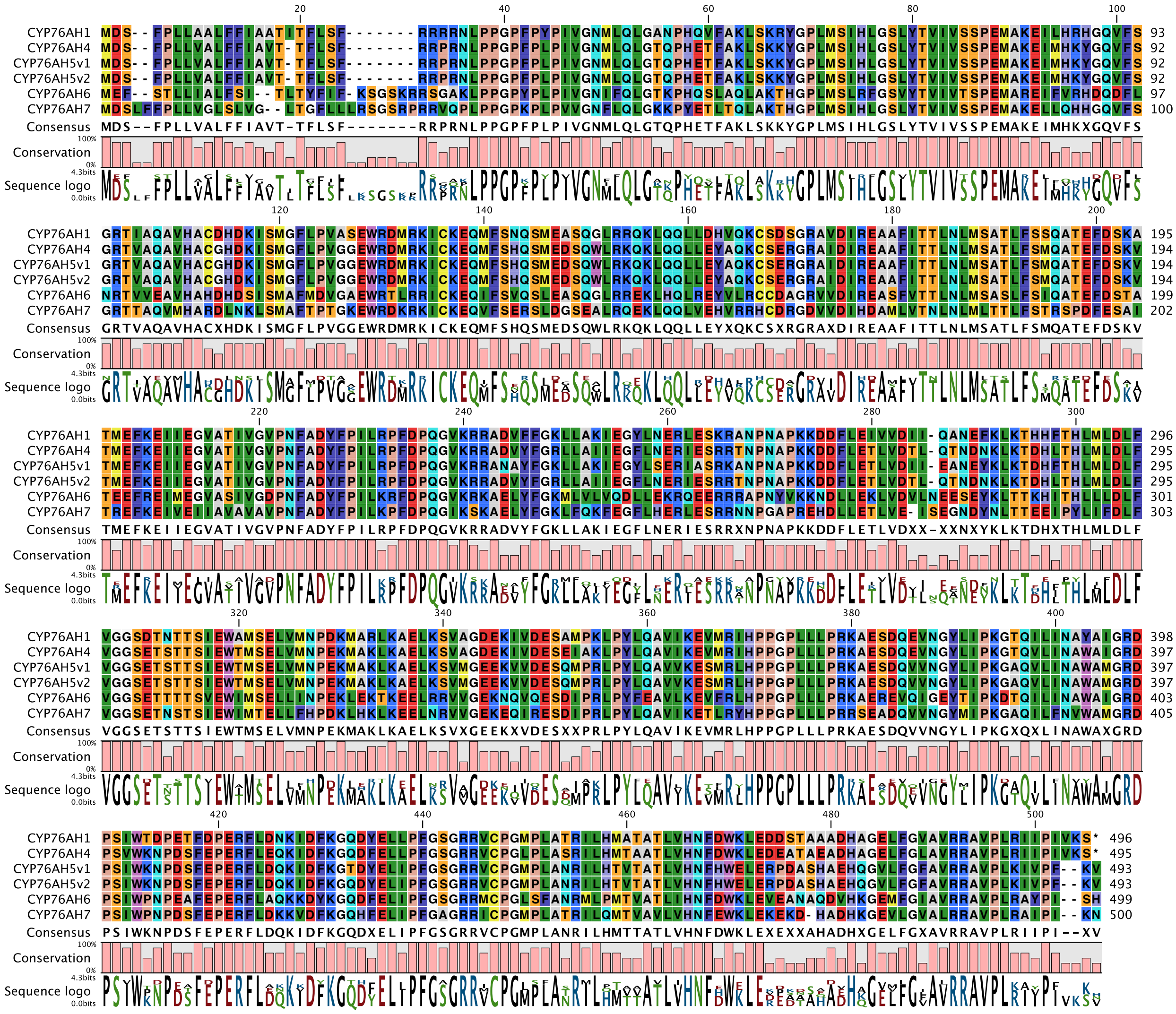
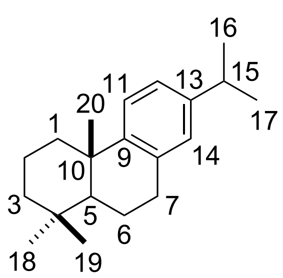
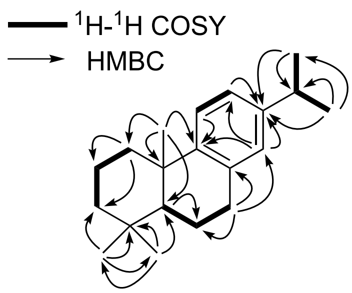


Figure S2. Numbering and selected ^{1}H-^{1}H COSY and HMBC correlations of abietatriene (**3**)



Abietatriene (**3**)



Abietatriene (**3**)

Figure S3. ^{1}H spectrum of Abietatriene (**3**)

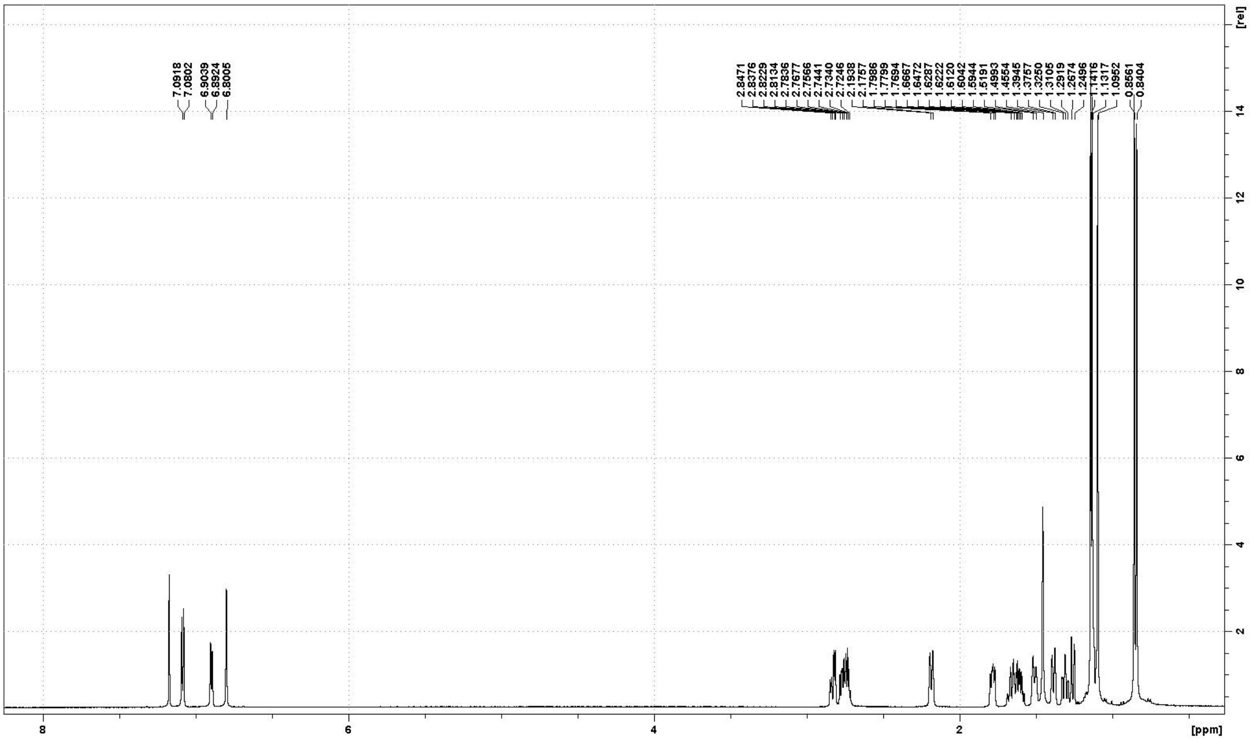


Figure S4. ^{13}C spectrum of Abietatriene (**3**)

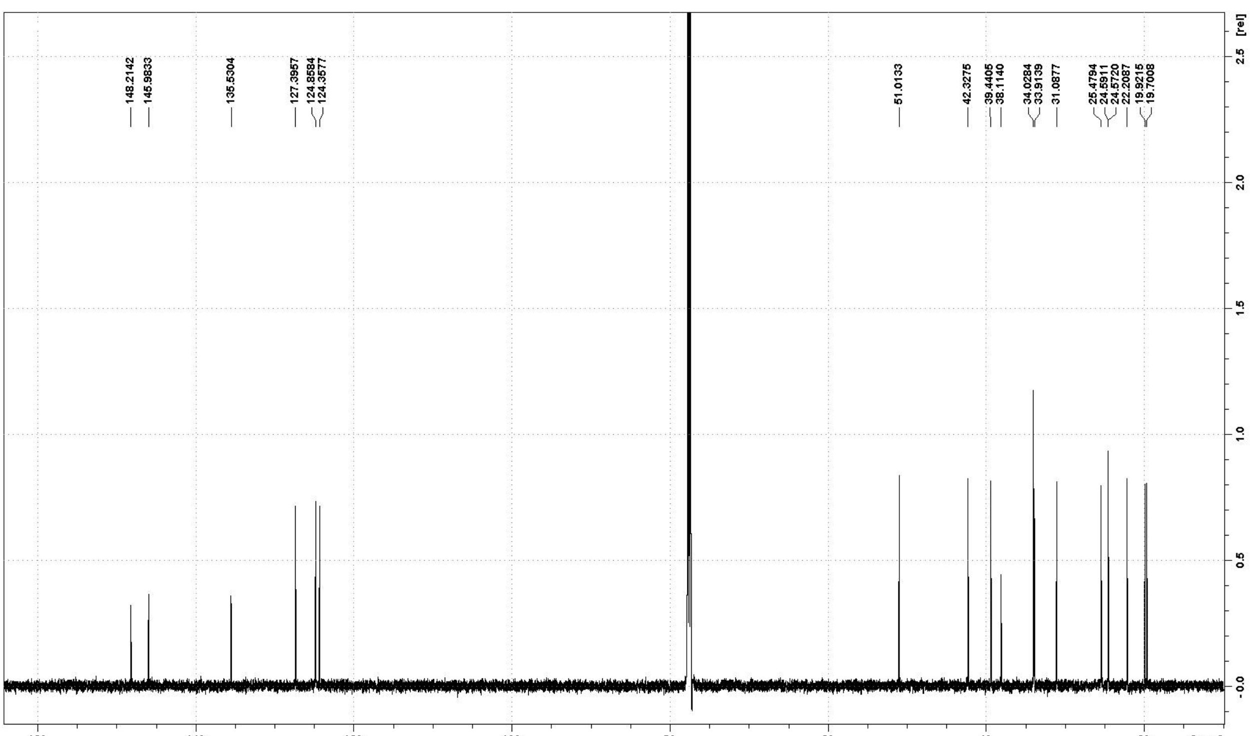


Figure S5. HSQC spectrum of Abietatriene (**3**)

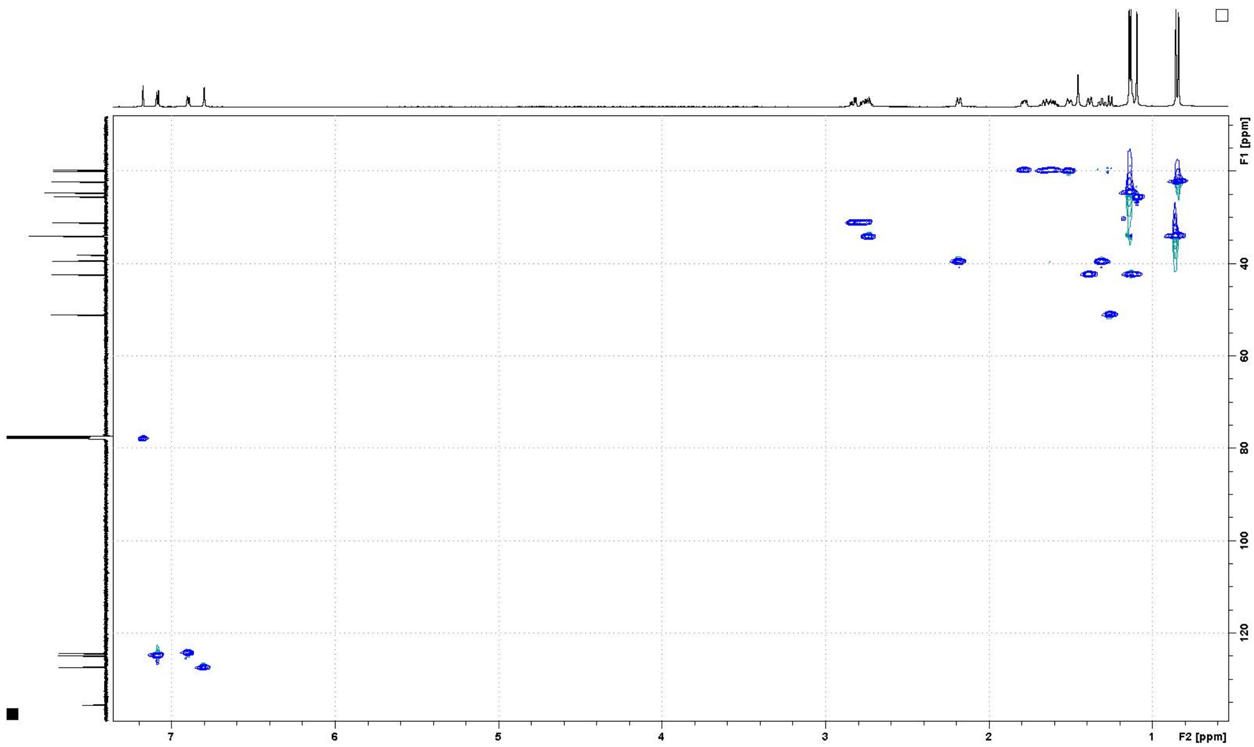


Figure S6. HMBC spectrum of Abietatriene (**3**)

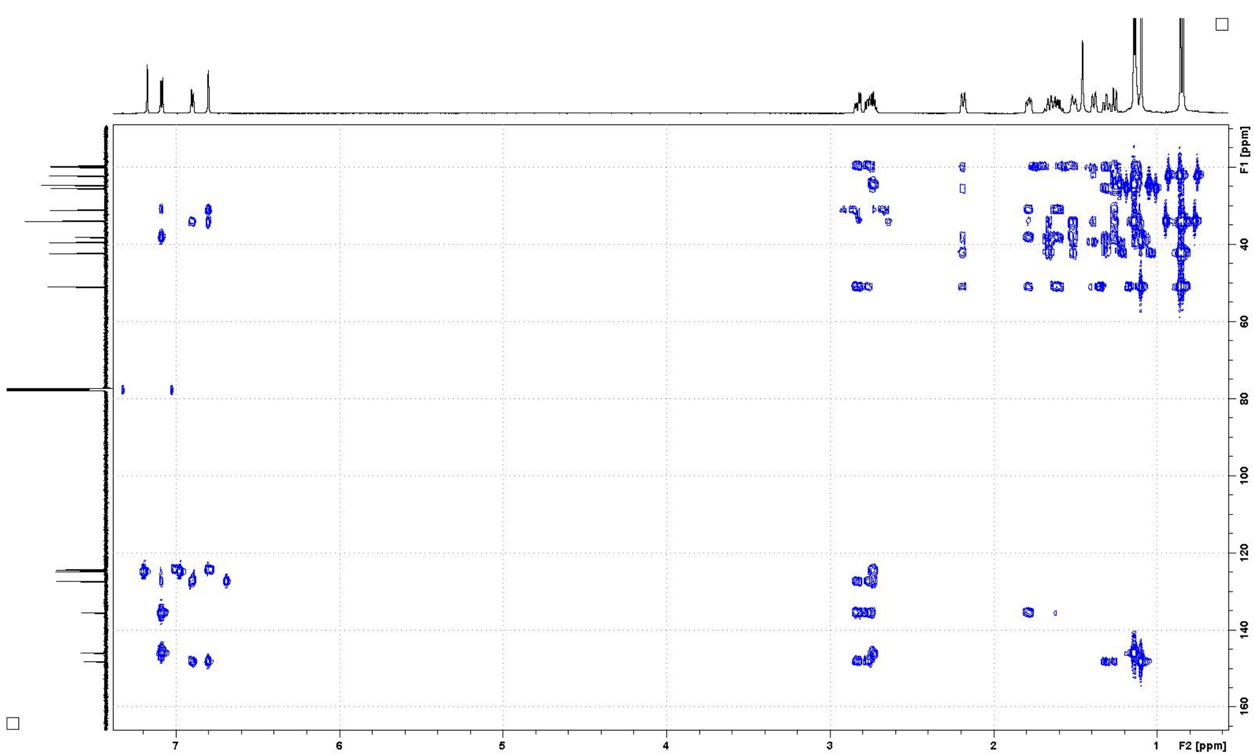


Figure S7. NOESY spectrum of Abietatriene (**3**)

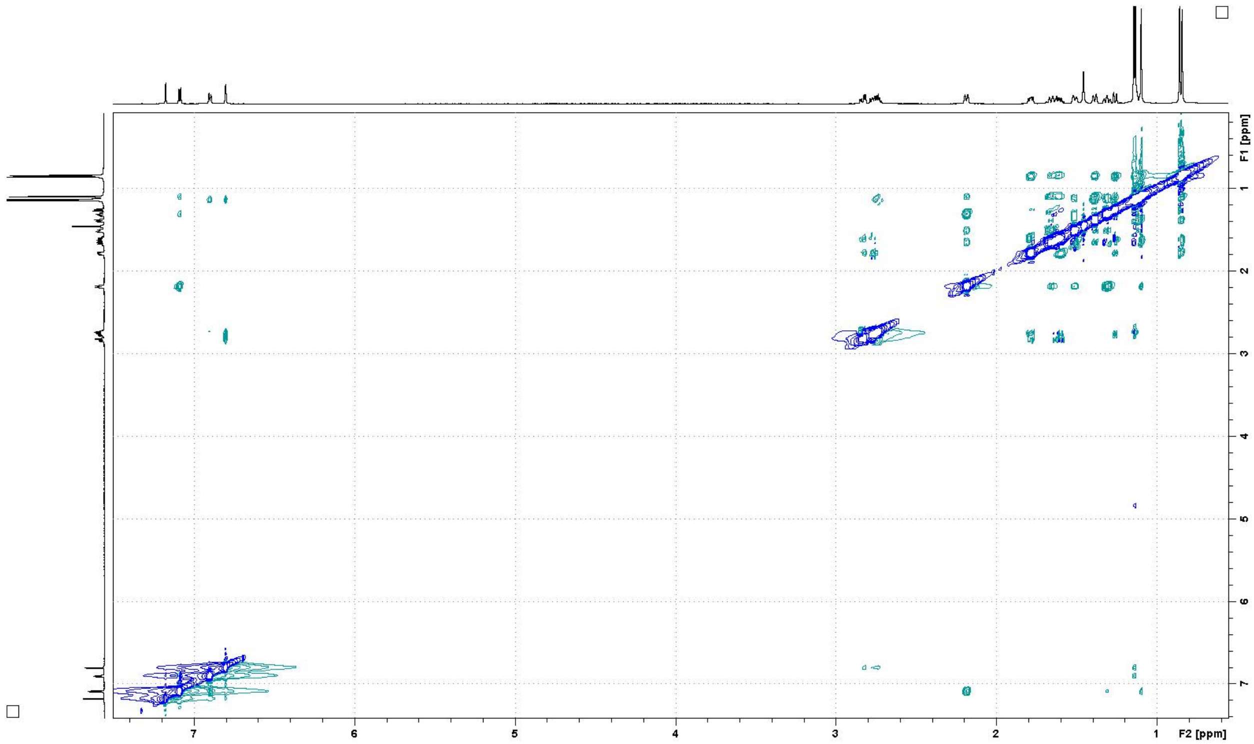


Table S1. NMR Data (in CD_3Cl)

Position	Abietatriene (**3**)		Ferruginol (**2**)	
	δ_H	δ_C	δ_H	δ_C
1 a	2.18 (1H, br.d, *J* = 12.5 Hz)	39.4	2.15 (1H, br.d, *J* = 12.4 Hz)	39.1
b	1.31 (1H, br.d, *J* = 12.5 Hz)		1.36 (1H, br.d, *J* = 12.4, 3.4 Hz)	
2 a	1.66 (1H, m)	19.9	1.71 (1H, m)	19.5
b	1.51 (1H, m)		1.57 (1H, m)	
3 a	1.38 (1H, d, *J* = 13.2 Hz)	42.3	1.45 (1H, br.d, *J* = 13.1 Hz)	41.9
b	1.12 (1H, m)		1.18 (1H, m)	
4		34.0		33.5
5	1.26 (1H, d, *J* = 12.3 Hz)	51.0	1.30 (1H, dd, *J* = 12.4, 1.8 Hz)	50.6
6 a	1.78 (1H, dd, *J* = 13.1, 7.6)	19.7	1.83 (1H, m)	19.4
b	1.62 (1H, m)		1.64 (1H, m)	
7 a	2.83 (1H, dd, *J* = 17.2, 6.6 Hz)	31.1	2.84 (1H, dd, *J* = 16.5, 6.4 Hz)	30.0
b	2.78 (1H, m)		2.75 (1H, ddd, *J* = 16.5, 11.4, 7.6 Hz)	
8		135.5		127.5
9		148.2		148.9
10		38.1		37.7
11	7.09 (1H, d, *J* = 8.2 Hz)	124.9	6.61 (1H, s)	111.2
12	6.90 (1H, br.d, *J* = 8.2 Hz)	124.4		150.9
13		146.0		131.5
14	6.80 (1H, br.s)	127.4	6.81 (1H, s)	126.8
15	2.74 (1H, m)	34.0	3.08 (1H, m)	27.0
16	1.14 (3H, d, *J* = 6.9 Hz)	24.57	1.22 (3H, d, *J* = 7.0 Hz)	22.8
17	1.14 (3H, d, *J* = 6.9 Hz)	24.59	1.20 (3H, d, *J* = 7.0 Hz)	22.9
18	0.86 (3H, s)	33.9	0.92 (3H, s)	33.5
19	0.84 (3H, s)	22.2	0.89 (3H, s)	21.8
20	1.09 (3H, s)	25.5	1.15 (3H, s)	25.0

Figure S8. **1** is spontaneously converted to **3**. GC-MS chromatogram of extract from *E. coli* expressing only enzymes for the production of **1** (i.e., no CYP or CPR).

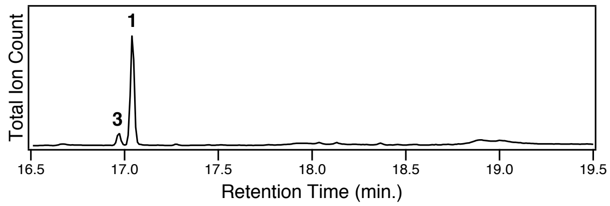


Figure S9. CYP76AH1 also only hydroxylates **3**. GC-MS chromatograms of in vitro reactions with (A) **1** (selected ions *m/z* = 272 + 286) or (B) **3** (selected ions *m/z* = 255 + 286).

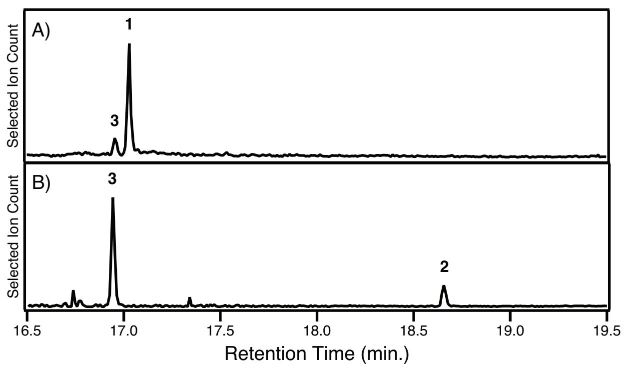


Figure S10. GC-MS analysis of **1** incubated in phosphate buffer, pH 7.5 for 3 days at room temperature. (A) In the presence of O_2. (B) In the absence of O_2 (removed by bubbling N_2 through solution for 15 min.).

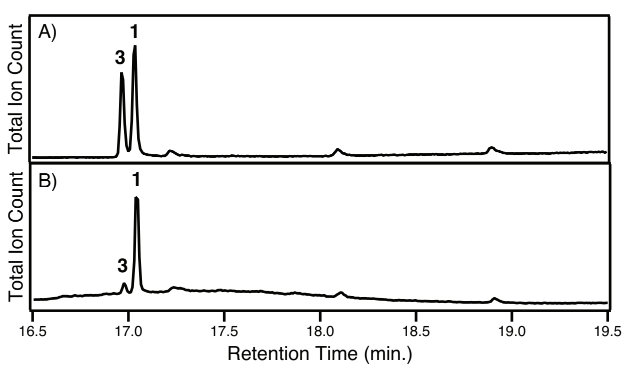
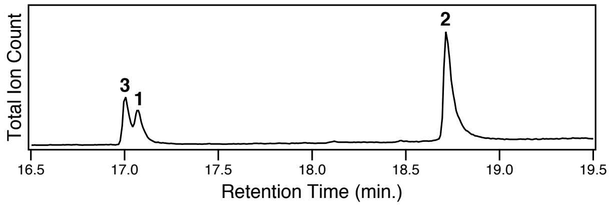


Figure S11. GC-MS analysis of *S. miltiorrhiza* hairy root extract.



Synthetic gene sequences

CYP76AH1

ATGGCTAAGAAAACCTCGTCAAAGGGTAAACTGCCGCCGGGTCCGTTCCCGTATCCGATTGTGGGTAACATGCTGCAACTGGGTGC
TAACCCGCATCAGGTGTTTGCAAAACTGTCGAAGCGTTATGGCCCGCTGATGAGTATTCACCTGGGTTCCCTGTACACGGTCATCG
TGAGCTCTCCGGAAATGGCCAAAGAAATTCTGCATCGTCACGGCCAGGTGTTTAGCGGCCGTACCATTGCCCAAGCAGTCCATGCA
TGCGATCACGACAAAATCTCTATGGGCTTTCTGCCGGTTGCTAGTGAATGGCGTGATATGCGCAAAATCTGTAAGGAACAGATGTT
CAGCAATCAATCTATGGAAGCATCTCAGGGCCTGCGTCGCCAAAAGCTGCAGCAACTGCTGGATCATGTGCAGAAATGCTCAGATT
CGGGTCGTGCTGTTGACATTCGCGAAGCGGCCTTTATCACCACGCTGAACCTGATGTCGGCCACCCTGTTTAGTTCCCAAGCAACG
GAATTTGATAGCAAAGCTACGATGGAATTTAAGGAAATTATCGAAGGCGTGGCGACCATTGTTGGTGTCCCGAATTTTGCCGATTA
TTTCCCGATCCTGCGTCCGTTCGACCCGCAGGGTGTTAAACGTCGCGCAGATGTCTTTTTCGGCAAACTGCTGGCTAAGATTGAAG
GTTACCTGAACGAACGTCTGGAAAGTAAACGCGCTAACCCGAATGCGCCGAAAAAGGATGACTTTCTGGAAATCGTGGTTGATATT
ATCCAGGCGAATGAATTTAAACTGAAGACCCATCACTTCACGCATCTGATGCTGGACCTGTTTGTCGGCGGTTCAGACACCAACAC
CACGTCGATTGAATGGGCGATGAGCGAACTGGTTATGAATCCGGATAAAATGGCCCGCCTGAAAGCAGAACTGAAGTCCGTGGCCG
GCGATGAAAAAATCGTTGACGAATCAGCGATGCCGAAGCTGCCGTATCTGCAGGCCGTCATTAAAGAAGTGATGCGTATCCACCCG
CCGGGTCCGCTGCTGCTGCCGCGCAAGGCGGAATCTGACCAGGAAGTGAACGGCTATCTGATTCCGAAAGGCACCCAAATTCTGAT
CAATGCTTACGCGATTGGCCGTGATCCGAGTATCTGGACCGATCCGGAAACGTTTGACCCGGAACGCTTCCTGGATAACAAAATTG
ACTTTAAGGGCCAGGATTACGAACTGCTGCCGTTCGGCAGCGGTCGTCGCGTGTGTCCGGGTATGCCGCTGGCAACGCGTATCCTG
CACATGGCCACCGCAACGCTGGTTCACAATTTTGACTGGAAACTGGAAGATGACTCCACCGCAGCTGCGGATCATGCCGGCGAACT
GTTCGGTGTGGCTGTTCGTCGCGCGGTCCCGCTGCGTATTATCCCGATTGTGAAATCATAA

CYP76AH4

ATGGCTAAGAAAACCTCGTCCAAGGGTAAACTGCCGCCGGGTCCGTTCCCGCTGCCGATTGTGGGTAACATGCTGCAACTGGGTAC
GCAGCCGCATGAAACGTTCGCCAAACTGTCTAAAAAGTATGGCCCGCTGATGTCTATTCACCTGGGTAGTCTGTACACCGTGATCG
TTAGCTCTCCGGAAATGGCCAAAGAAATTATGCATAAGTATGGCCAGGTGTTTTCGGGTCGTACCGTTGCGCAAGCCGTCCATGCA
TGCGGCCACGATAAAATTAGCATGGGTTTCCTGCCGGTTGGCGGTGAATGGCGTGACATGCGCAAAATCTGTAAGGAACAGATGTT
TAGTCACCAAAGCATGGAAGATTCTCAGTGGCTGCGTAAACAAAAGCTGCAGCAACTGCTGGAATACGCTCAGAAATGCAGTGAAC
GTGGCCGCGCGATTGATATCCGCGAAGCGGCCTTTATCACCACGCTGAATCTGATGTCCGCTACCCTGTTCTCAATGCAAGCGACG
GAATTTGATAGCAAAGTCACGATGGAATTTAAGGAAATTATCGAAGGCGTGGCCACCATTGTCGGTGTGCCGAATTTTGCAGACTA
TTTCCCGATCCTGCGCCCGTTTGATCCGCAGGGTGTTAAACGTCGCGCTGACGTCTACTTCGGCCGTCTGCTGGCGATTATCGAAG
GTTTTCTGAACGAACGCATTGAATCCCGTCGCACGAACCCGAATGCGCCGAAAAAGGATGACTTCCTGGAAACCCTGGTGGATACC
CTGCAGACGAACGACAATAAACTGAAGACCGATCATCTGACGCACCTGATGCTGGACCTGTTTGTGGGCGGTTCAGAAACGTCGAC
CACGAGCATCGAATGGACCATGAGTGAACTGGTTATGAACCCGGAAAAAATGGCTAAACTGAAGGCGGAACTGAAATCTGTGGCAG
GCGATGAAAAGATTGTTGACGAAAGTGAAATCGCCAAACTGCCGTATCTGCAGGCAGTGATTAAGGAAGTTATGCGTATCCATCCG
CCGGGTCCGCTGCTGCTGCCGCGCAAAGCGGAAAGCGATCAGGAAGTTAACGGCTACCTGATTCCGAAAGGCACCCAAGTCCTGAT
TAATGCATGGGCTATCGGCCGTGACCCGTCGGTGTGGAAAAACCCGGATAGCTTTGAACCGGAACGCTTCCTGGAACAGAAAATTG
ATTTTAAGGGCCAAGACTTCGAACTGCTGCCGTTTGGCTCCGGTCGTCGCGTCTGTCCGGGTCTGCCGCTGGCTTCACGTATCCTG
CACATGACCGCAGCTACGCTGGTTCACAATTTCGATTGGAAACTGGAAGACGAAGCGACCGCCGAAGCAGATCACGCCGGCGAACT
GTTTGGTCTGGCAGTCCGTCGCGCAGTGCCGCTGCGTATTATCCCGATTGTTAAAAGCTAA

CYP76AH5v1

ATGGCTAAGAAAACCTCGTCAAAGGGTAAACTGCCGCCGGGTCCGTTCCCGCTGCCGATTGTGGGTAACATGCTGCAACTGGGTAC
GCAGCCGCATGAAACCTTTGCCAAACTGAGCAAAAAGTATGGCCCGCTGATGTCTATTCACCTGGGTAGTCTGTACACGGTGATCG
TTAGCTCTCCGGAAATGGCTAAGGAAATCATGCATAAGTACGGCCAGGTGTTTTCGGGTCGTACCGTTGCACAAGCTGTCCATGCC
TGCGGCCACGATAAAATTAGCATGGGTTTTCTGCCGGTTGGCGGTGAATGGCGTGACATGCGCAAAATCTGTAAGGAACAGATGTT
CAGTCACCAAAGCATGGAAGATTCCCAGTGGCTGCGTAAACAAAAGCTGCAGCAACTGCTGGAATACGCTCAGAAATGCTCAGAAC
GTGGCCGCGCGATTGATATCCGCGAAGCGGCCTTTATCACCACGCTGAATCTGATGTCCGCCACGCTGTTTTCAATGCAAGCAACC
GAATTTGACTCTAAGGTGACGATGGAATTTAAGGAAATCATCGAAGGCGTTGCCACCATTGTCGGTGTGCCGAATTTTGCAGATTA
TTTCCCGATCCTGCGTCCGTTTGACCCGCAGGGTGTGAAACGTCGCGCGAACGCCTATTTCGGCAAACTGCTGGCAAAGATTGAAG
GTTACCTGTCGGAACGTATCGCAAGCCGCAAAGCTAACCCGAATGCGCCGAAAAAGGATGACTTTCTGGAAACCCTGGTTGATATT
ATCGAAGCGAATGAATACAAACTGAAGACGGATCATTTTACCCACCTGATGCTGGACCTGTTCGTCGGCGGTTCAGAAACCTCGAC
CACGAGCATTGAATGGACGATGAGTGAACTGGTCATGAACCCGGAAAAAATGGCCAAACTGAAGGCAGAACTGAAATCCGTGATGG
GCGAAGAAAAGGTGGTTGATGAATCACAGATGCCGCGCCTGCCGTATCTGCAAGCGGTCGTGAAAGAATCTATGCGTCTGCATCCG
CCGGGTCCGCTGCTGCTGCCGCGCAAAGCCGAAAGTGACCAGGTTGTCAACGGCTACCTGATTCCGAAAGGTGCACAAGTTCTGAT
CAATGCATGGGCTATGGGCCGTGATCCGTCTATTTGGAAAAACCCGGACAGTTTTGAACCGGAACGCTTCCTGGATCAGAAAATTG
ACTTTAAGGGCACCGATTATGAACTGATCCCGTTCGGCTCGGGTCGTCGCGTGTGTCCGGGTATGCCGCTGGCTAATCGTATTCTG
CATACCGTTACGGCGACCCTGGTCCATAACTTTCACTGGGAACTGGAACGCCCGGATGCGTCACATGCCGAACACCAGGGCGTTCT
GTTTGGTTTCGCTGTGCGTCGCGCGGTTCCGCTGAAAATCGTCCCGTTCAAGGTGTAA

CYP76AH5v2

ATGGCTAAGAAAACCTCCTCCAAGGGTAAACTGCCGCCGGGTCCGTTCCCGCTGCCGATTGTGGGTAACATGCTGCAACTGGGTAC
GCAGCCGCATGAAACCTTTGCGAAACTGTCTAAAAAGTATGGCCCGCTGATGTCTATTCACCTGGGTAGTCTGTACACCGTGATCG
TTAGCTCTCCGGAAATGGCGAAAGAAATTATGCATAAGTATGGCCAGGTGTTTTCCGGTCGTACCGTTGCACAAGCTGTCCATGCC
TGCGGCCACGATAAAATTTCAATGGGTTTTCTGCCGGTTGGCGGTGAATGGCGTGACATGCGCAAAATCTGTAAGGAACAGATGTT
CAGTCACCAAAGCATGGAAGATTCTCAGTGGCTGCGTAAACAAAAGCTGCAGCAACTGCTGGAATACGCACAGAAATGCAGTGAAC
GTGGCCGCGCTATTGATATCCGCGAAGCGGCCTTTATCACCACGCTGAATCTGATGTCCGCGACGCTGTTTTCAATGCAGGCCACC
GAATTTGACAGCAAAGTCACGATGGAATTTAAGGAAATTATCGAAGGCGTGGCGACCATTGTCGGTGTGCCGAATTTTGCCGACTA
TTTCCCGATCCTGCGTCCGTTCGATCCGCAGGGTGTGAAACGTCGCGCAGACGTTTACTTTGGCCGTCTGCTGGCTATTATCGAAG
GTTTCCTGAACGAACGCATTGAAAGCCGTCGCACCAACCCGAATGCACCGAAAAAGGATGACTTTCTGGAAACCCTGGTGGATACG
CTGCAGACCAACGACAATAAACTGAAGACGGATCATCTGACCCACCTGATGCTGGACCTGTTTGTTGGCGGTTCAGAAACCTCGAC
CACGAGCATCGAATGGACGATGAGCGAACTGGTTATGAACCCGGAAAAAATGGCGAAACTGAAGGCCGAACTGAAATCTGTCATGG
GCGAAGAAAAGGTGGTTGATGAAAGTCAGATGCCGCGCCTGCCGTATCTGCAAGCCGTCGTGAAAGAATCGATGCGTCTGCATCCG
CCGGGTCCGCTGCTGCTGCCGCGCAAAGCAGAAAGCGACCAGGTTGTCAACGGCTACCTGATTCCGAAAGGTGCTCAAGTGCTGAT
CAATGCGTGGGCGATGGGCCGTGACCCGTCGATTTGGAAAAACCCGGATAGCTTTGAACCGGAACGCTTCCTGGATCAGAAAATTG
ACTTTAAGGGCCAAGATTATGAACTGATCCCGTTCGGCTCCGGTCGTCGCGTGTGTCCGGGTATGCCGCTGGCAAATCGTATTCTG
CATACCGTTACGGCCACCCTGGTCCATAACTTTCACTGGGAACTGGAACGCCCGGATGCAAGTCATGCTGAACACCAGGGCGTTCT
GTTTGGTTTCGCAGTGCGTCGCGCTGTTCCGCTGAAAATCGTCCCGTTCAAGGTGTAA

CYP76AH6

ATGGCTAAGAAGACGTCCTCAAAGGGTAAACTGCCGCCGGGTCCGTATCCGCTGCCGATTGTGGGTAACATTTTTCAACTGGGTAC
GAAACCGCACCAGTCCCTGGCACAACTGGCAAAGACCCACGGCCCGCTGATGAGCCTGCGCTTTGGTTCTGTTTATACCGTGATTG
TTACGTCACCGGAAATGGCCCGCGAAATCTTTGTTCGTCACGATCAGGACTTCCTGAACCGCACCGTGGTTGAAGCAGTCCATGCT
CACGATCATGACAGCATTTCTATGGCATTTATGGATGTGGGCGCTGAATGGCGTACGCTGCGTCGCATTTGCAAAGAACAGATCTT
CTCAGTGCAATCGCTGGAAGCATCGCAGGGTCTGCGTCGCGAAAAACTGCATCAACTGCGCGAATACGTTCTGCGTTGCTGTGATG
CCGGTCGCGTCGTGGACATTCGTGAAGCAAGCTTTGTCACCACGCTGAACCTGATGAGTGCGTCCCTGTTTTCGATCCAGGCCACC
GAATTTGATAGCACCGCAACGGAAGAATTTCGCGAAATTATGGAAGGCGTGGCTTCTATCGTTGGTGATCCGAATTTTGCGGACTA
TTTCCCGATTCTGAAACGCTTTGATCCGCAGGGCGTTAAGCGTAAGGCCGAACTGTACTTCGGTAAAATGCTGGTCCTGGTGCAGG
ACCTGCTGGAAAAGCGTCAAGAAGAACGTCGCCGTGCACCGAACTATGTGAAAAAGAATGATCTGCTGGAAAAACTGGTTGACGTC
CTGAACGAAGAAAGCGAATACAAACTGACCACGAAGCACATTACCCATCTGCTGCTGGACCTGTTTGTCGGCGGTAGTGAAACCAC
GACCACGTCCGTGGAATGGATTATGTCAGAACTGCTGATCAACCCGGAAAAACTGGAAAAAACCAAGGAAGAACTGCGCCGTGTTG
TCGGCGAAAAGAACCAGGTTCAAGAATCTGATATCCCGCGCCTGCCGTATTTGAAGCTGTCCTGAAAGAAGTGTTCCGTCTGCAC
CCGCCGGGTCCGCTGCTGCTGCCGCGCAAAGCGGAACGTGAAGTGCAGATTGGTGAATACACCATCCCGAAGGATACGCAAATTCT
GATCAACGCGTGGGCCATTGGTCGCGACCCGAGTATCTGGCCGAATCCGGAAGCTTTTGAACCGGAACGTTTCCTGGCGCAGAAAA
AGGATTATAAAGGCCAAGACTTTGAACTGATTCCGTTCGGCTCTGGTCGCCGTATGTGTCCGGGTCTGAGTTTTGCCAATCGCATG
CTGCCGATGACCGTGGCAACGCTGATCCACAACTTCGATTGGAAACTGGAAGTGGAAGCAAATGCTCAGGACGTTCATAAGGGCGA
AATGTTTGGTATTGCAGTTCGCCGTGCCGTCCCGCTGCGTGCATACCCGATCTCCCATTAA

CYP76AH7

ATGGCTAAGAAAACCTCGTCAAAGGGTAAACTGCCGCCGGGTCCGAAACCGCTGCCGGTCGTGGGTAACTTCCTGCAACTGGGTAA
AAAACCGTATGAAACCCTGACGCAGCTGGCTAAGACCCACGGCCCGCTGATGAGTATTCACCTGGGTTCCCTGTACACGGTGATCG
TTAGCTCTCCGGAAATGGCAAAAGAACTGCTGCAGCATCACGGCCAAGTGTTTAGCGGTCGTACCACGGCCCAGGTTATGCATGCA
CGCGATCTGAATAAACTGTCTATGGCCTTTACCCCGACGGGCAAGGAATGGCGTGATAAACGCAAGATTTGCAAAGAACAGGTGTT
CTCAGAACGTTCGCTGGACGGTAGCGAAGCGCTGCGCCAAGAAAAACTGCAGCAACTGGTCGAACATGTGCGTCGCCACTGTGATC
GTGGCGACGTGGTTGATATCCACGACGCCATGCTGGTGACCAACCTGAATCTGATGCTGACCACGCTGTTTAGCACCCGTTCTCCG
GATTTCGAAAGCGCAATTACGCGCGAATTTAAAGAAATCGTTGAAATTATCGCGGTCGCCGTGGCAGTTCCGAACTTCGCTGATTA
TTTTCCGATTCTGAAACCGTTTGACCCGCAGGGTATCAAATCTAAGGCGGAACTGTACTTCGGCAAACTGTTTCAAAAGTTCGAAG
GTTTTCTGCATGAACGTCTGGAAAGTCGTCGCAACAATCCGGGTGCACCGCGCGAACACGATCTGCTGGAAACCCTGGTCGAAATT
TCCGAGGGTAACGACTATAATCTGACCACGGAAGAAATTCCGTACCTGATCTTCGACCTGTTTGTGGGCGGTAGCGAAACCAATAG
TACGTCCATTGAATGGATCATGACCGAACTGCTGTTTCATCCGGATAAGCTGCACAAACTGAAGGAAGAACTGAACCGTGTCGTGG
GCGAAAAAGAACAGATTCGTGAATCAGACATCCCGCGCCTGCCGTATCTGCAAGCTGTGATTAAAGAAACCCTGCGCTACCATCCG
CCGGGTCCGCTGCTGCTGCCGCGTCGCTCGGAAGCGGATCAGGTTGTCAACGGCTATATGATTCCGAAAGGTGCTCAAATCCTGTT
TAATGTTTGGGCGATGGGCCGTGATCCGTCAATTTGGCCGAACCCGGACTCGTTCGAACCGGAACGCTTTCTGGATAAAAAGGTCG
ACTTCAAAGGCCAGCATTTTGAACTGATTCCGTTCGGTGCAGGTCGTCGCATCTGCCCGGGTATGCCGCTGGCAACCCGTATCCTG
CAGATGACGGTCGCAGTGCTGGTTCACAATTTCGAATGGAAGCTGGAAAAAGAAAAGGATCATGCCGACCACAAAGGCGAAGTTCT
GGGTGTCGCTCTGCGTCGCGCGGTTCCGCTGCGTGCAATTCCGATCAAAAACTAA