Supporting information for:

Optimization of recombinant expression enables discovery of novel cytochrome P450 activity in rice diterpenoid biosynthesis

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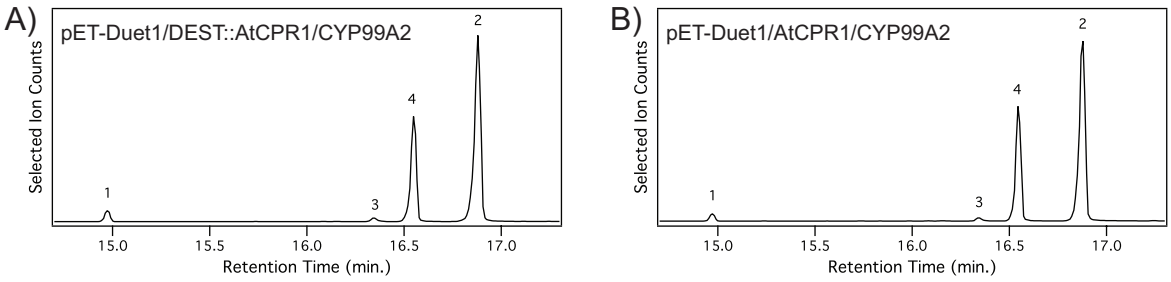
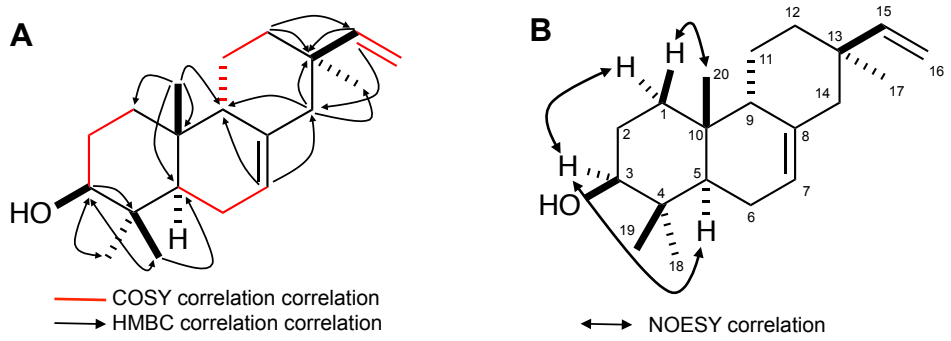
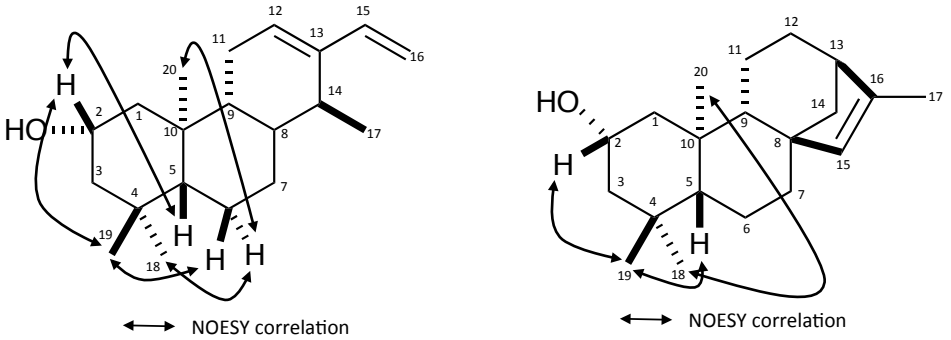


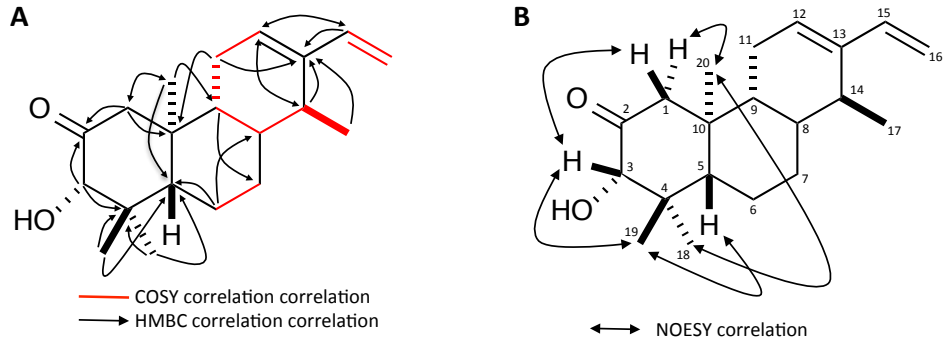
Supplemental Figure S1. Comparison of CYP99A2 expression vectors in our metabolic engineering system. Either (A) pET-Duet1/DEST::AtCPR1/CYP99A2 or (B) pET-Duet/AtCPR1/CYP99A2 were co-expressed along with pGG*s*C and pCDF-Duet1/DEST::OsKLS4/AtCPR1, to produce the *syn*-pimaradiene precursor (**1**) that was further sequentially transformed by CYP99A2 to *syn*-pimaradien-19-ol (**2**), *syn*-pimaradien-19-al (**3**), and then *syn*-pimaradien-19-oic acid (observed here as the methyl ester, **4**).



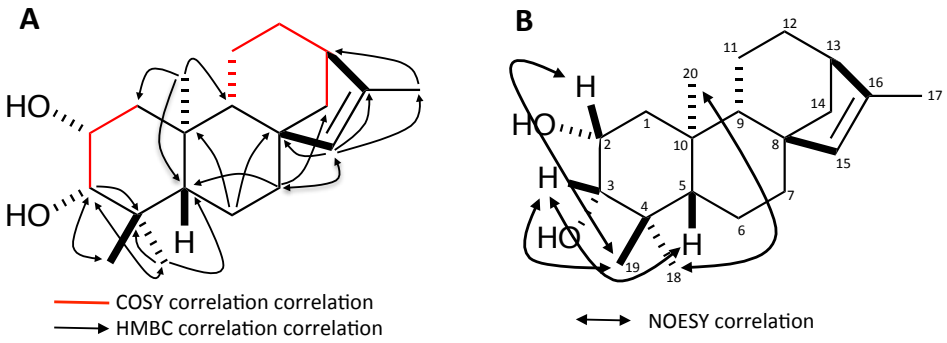
Supplemental Figure S2. Key COSY, HMBC and NOESY correlations observed for *syn*-pimaradien-3β-ol.



Supplemental Figure S3. Key NOESY correlations observed for 2α-hydroxy-*ent*-cassadiene and 2α-hydroxy-*ent*-isokaurene.



Supplemental Figure S4. Key COSY, HMBC and NOESY correlations observed for 3α-hydroxy-*ent*-cassadien-2-one.



Supplemental Figure S5. Key COSY, HMBC and NOESY correlations observed for 2α,3α-dihydroxy-*ent*-isokaurene.

Supplemental Table S1. ^{1}H-NMR and ^{13}C-NMR assignments for *syn*-pimaradien-3β-ol.

position	δ_C (ppm)	δ_H (ppm)	multiplicity
1	34.8	α 1.50	m
		β 1.16	m
2	28.0	1.58	m
		1.55	m
3	80.2	3.12	dd (*J* = 7.6, 7.6 Hz)
4	39.4	-	
5	43.8	1.13	m
6	24.0	1.93	m
		1.88	m
7	120.1	5.24	d (*J* = 5.3 Hz)
8	137.3	-	
9	53.6	1.28	m
10	35.6	-	
11	25.7	1.61	m
		1.14	m
12	38.3	1.42	m
		1.36	ddd (*J* = 13.2, 13.2, 4.2 Hz)
13	39.2	-	
14	48.4	1.92	m
		1.75	dd (*J* = 12.1, 2.3 Hz)
15	150.9	5.74	dd (*J* = 17.5, 10.8 Hz)
16	109.8	4.84	d (*J* = 17.5 Hz)
		4.78	d (*J* = 10.8 Hz)
17	22.4	0.802	s
18	16.2	0.799	s
19	28.9	0.93	s
20	22.8	0.85	s

Supplemental Table S2. ^{1}H-NMR and ^{13}C-NMR assignments for 2α-hydroxy-*ent*-cassadiene.

position	δ_C (ppm)	δ_H (ppm)	multiplicity
1	45.6	α 1.38	m
		β 1.76	brd (*J* = 12.0 hz)
2	68.3	4.15	brs
3	47.1	α 1.66	m
		β 1.45	m
4	32.9	-	
5	54.3	0.97	m
6	21.8	α 1.41	m
		β 1.66	m
7	31.1	α 1.66	m
		β 1.38	m
8	35.0	1.61	m
9	45.0	1.30	m
10	36.8	-	
11	25.4	α 2.00	m
		β 2.10	m
12	128.7	5.60	brs
13	141.9	-	
14	32.2	2.39	m
15	138.9	6.20	dd (*J* = 17.6, 11.6 hz)
16	109.7	5.06	d (*J* = 17.6 hz)
		4.88	d (*J* = 11.6 hz)
17	14.8	0.93	s
18	24.8	1.06	s
19	34.1	0.92	s
20	16.5	1.09	s

Supplemental Table S3. ^{1}H-NMR and ^{13}C-NMR assignments for 3α-hydroxy-*ent*-cassadien-2-one.

position	δ_C (ppm)	δ_H (ppm)	multiplicity
1	52.0	α 2.42	d (*J* = 12.9 Hz)
		β 2.21	d (*J* = 12.9 Hz)
2	211.5	-	
3	83.3	3.92	s
4	46.3	-	
5	54.4	1.54	m
6	31.0	1.72	m
		1.46	m
7	21.9	α 1.46	m
		β 1.77	m
8	35.1	1.55	m
9	45.2	1.61	m
10	44.4	-	
11	25.6	α 1.96	m
		β 2.05	m
12	127.8	5.57	m
13	142.5	-	
14	32.3	2.43	m
15	138.9	6.20	dd (*J* = 17.6, 10.8 Hz)
16	110.6	5.057	d (*J* = 17.6 Hz)
		4.882	d (*J* = 10.8 Hz)
17	15.1	0.929	d (*J* = 7.0 Hz)
18	29.8	1.062	s
19	17.3	0.917	s
20	14.8	1.091	s

Supplemental Table S4. ^{1}H-NMR and ^{13}C-NMR assignments for 2α,3α-dihydroxy-*ent*-isokaurene.

position	δ_C (ppm)	δ_H (ppm)	multiplicity
1	44.5	2.26	dd (*J* = 14.9, 2.8 Hz)
		1.03	m
2	71.2	4.08	m
3	78.5	3.18	dd (*J* = 7.0, 4.1 Hz)
4	38.2	-	
5	54.8	0.80	m
6	18.9	1.31	m
		1.30	m
7	39.3	1.53	m
		1.56	m
8	49.0	-	
9	49.2	0.87	m
10	38.9	-	
11	18.9	1.55	m
		1.31	m
12	25.0	1.48	m
		1.48	
13	44.8	2.29	m
14	43.6	2.05	m
		1.30	
15	135.4	5.02	
16	142.9	-	
17	15.3	1.68	s
18	29.7	0.974	s
19	29.7	0.966	s
20	18.9	1.32	s