Supporting Information

Cytochrome P450 and *O*-methyltransferase catalyze the final steps in the biosynthesis of the anti-addictive alkaloid ibogaine from *Tabernanthe iboga*

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Table S1: Primers used for Full-length Gene amplification and colony PCR

Gene	Strand	In-Fusion Site	Primer Sequence (5'-3')
I10H	Forward	ACCCTCACTAAAGGGCGGCCGCAACC	ATGGAGCTGATCGTCTCCTTC
I10H	Reverse	GTCATCCTTGTAATCCATCGATAC	TGCTTCATCCATCATTACC
P450-2	Forward	ACCCTCACTAAAGGGCGGCCGCAACC	ATGGAGTTAGTCACTGCC
P450-2	Reverse	GTCATCCTTGTAATCCATCGATAC	CACAGAAGAATGACTATAAGGAATTG
P450-3	Forward	ACCCTCACTAAAGGGCGGCCGCAACC	GAGCTCATCTTCTTTGTCTG
P450-3	Reverse	GTCATCCTTGTAATCCATCGATAC	TTGTAGATAAGAACGGGAGTG
P450-4	Forward	ACCCTCACTAAAGGGCGGCCGCAACC	ATGGAGCTCATCTTTCTCTTCTG
P450-4	Reverse	GTCATCCTTGTAATCCATCGATAC	GATCGCTTTTTTCAAAGAAGAAC
P450-5	Forward	ACCCTCACTAAAGGGCGGCCGCAACC	ATGGAGTTCATTCTTTTCCTTTGCATTC
P450-5	Reverse	GTCATCCTTGTAATCCATCGATAC	CTCCAGTTTTAGGAAAGAACC
N10OMT	Forward	AAGTTCTGTTTCAGGGC	CCGGACGCGAAATCTGCCGAAC
N10OMT	Reverse	ATGGTCTAGAAAGCTTTA	AGGATACACTTCAATGAGACTCC
OMT-2	Forward	AAGTTCTGTTTCAGGGC	CCGGCAATGGTTGAGAAATCTGC
OMT-2	Reverse	ATGGTCTAGAAAGCTTTA	AGGATAGACCTCAATGAGAC
OMT-3	Forward	AAGTTCTGTTTCAGGGC	CCGGGAGAAGCCCAGGCTCAG
OMT-3	Reverse	ATGGTCTAGAAAGCTTTA	AGGGTAGGCTTCAATGACAG
OMT-4	Forward	AAGTTCTGTTTCAGGGC	CCGAGTGTAGCTTTGAATGGTG
OMT-4	Reverse	ATGGTCTAGAAAGCTTTA	ATAATAAACCTCAATAAGAGATCTC
OMT-5	Forward	AAGTTCTGTTTCAGGGC	CCGGCAGGAGAAGAGGAAGCTTG
OMT-5	Reverse	ATGGTCTAGAAAGCTTTA	TTTAAGCAATTCCATAATCCAACTGTTG
OMT-6	Forward	AAGTTCTGTTTCAGGGC	CCGGATTCTTCCCCCACAATCC
OMT-6	Reverse	ATGGTCTAGAAAGCTTTA	CTTGTAGAACTCCATGATCCAC
		Primers used for Colony PCR	
Name	Strand	Vector	Primer Sequence (5'-3')
GAL10-F	Forward	pESC-leu2Δ	GGTGGTAATGCCATGTAATATG
GAL10-R	Reverse	pESC-leu2Δ	GGCAAGGTAGACAAGCCGACAAC
T7-F	Forward	pOPINF	TAATACGACTCACTATAGGG
pOPINF-R	Reverse	pOPINF	TAGCCAGAAGTCAGATGCT

Table S2: Primers used for RT-qPCR

Gene	Strand	Primer Sequence (5'-3')
I10H	Forward	AGGCCTCCCTCACTGTGTCCT
I10H	Reverse	TGGATGGTCTGTCGGCAAAGA
N10OMT	Forward	AAGTGCGCTTACGATGCT
N10OMT	Reverse	TCTTCATTCTGGAACCACTCAC
N2227	Forward	GTGAACGTGACCAGTGCTATAA
N2227	Reverse	CAAGCAGGTGGACTCTCTTTAC

Table S3: NMR spectra of Coronaridine

Number	This Study		Reference (22)	
	^{1}H (*J*/H)	^{13}C	^{1}H (*J*/Hz)	^{13}C
2	-	138.5	-	136.6
3	2.79 (2H, ddd, 8.5, 1.7, 1.7), 2.94 (2H, ddd, 2.5, 3.7, 8.5)	54.3	2.81 (1H, brd), 2.89-2.92 (1H,m)	51.6
5	3.40 (1H, m), 3.00-3.16 (1H, m)	54.7	3.36-3.42 (1H, m), 3.15-3.23 (1H, m)	53.1
6	3.00-3.16 (2H, m)	22.8	2.98-3.04 (1H, m), 3.15-3.23 (1H, m)	22.1
7	-	110.7	-	110.3
8	-	137.6	-	128.8
9	7.40 (1H, ddd, 7.8, 1.0, 1.0)	118.7	7.47 (1H, brd)	118.4
10	7.02 (1H, ddd, 7.6, 7.6, 1.2)	122.2	7.08 (1H, ddd)	119.2
11	6.96 (1H, ddd, 7.5, 7.5, 1.1)	119.5	7.14 (1H, ddd)	121.9
12	7.24 (1H, ddd, 7.9,1.0, 1.0)	111.5	7.24 (1H, dd)	110.3
13	-	129.6	-	135.5
14	1.85 (1H, m)	29.0	1.88 (brs)	27.4
15	1.12 (1H, m), 1.76 (1H, m)	33.2	1.13 (1H, ddt), 1.73 (1H, m)	32.0
16	-	56.4	-	55.1
17	1.92 (1H, m), 2.72 (1H, ddd, 13.1, 2.1, 2.1)	37.0	1.9 (1H, ddd), 2.58 (1H, dd)	36.5
18	0.91 (3H, t, 7.4)	12.1	0.9 (3H,t)	11.6
19	1.34-1.49 (1H, m), 1.50-1.62 (1H, m)	28.0	1.4-1.47 (1H, m), 1.53-1.60 (1H, m)	26.7
20	1.34-1.49 (1H, m)	40.1	1.32-1.35 (1H, m)	39.1
21	3.62 (1H, brs)	57.9	3.56 (1H, brs)	57.5
22	-	176.3	-	175.7
CO_2CH_3	3.69 (3H, s)	52.9	3.71 (1H, s)	52.5

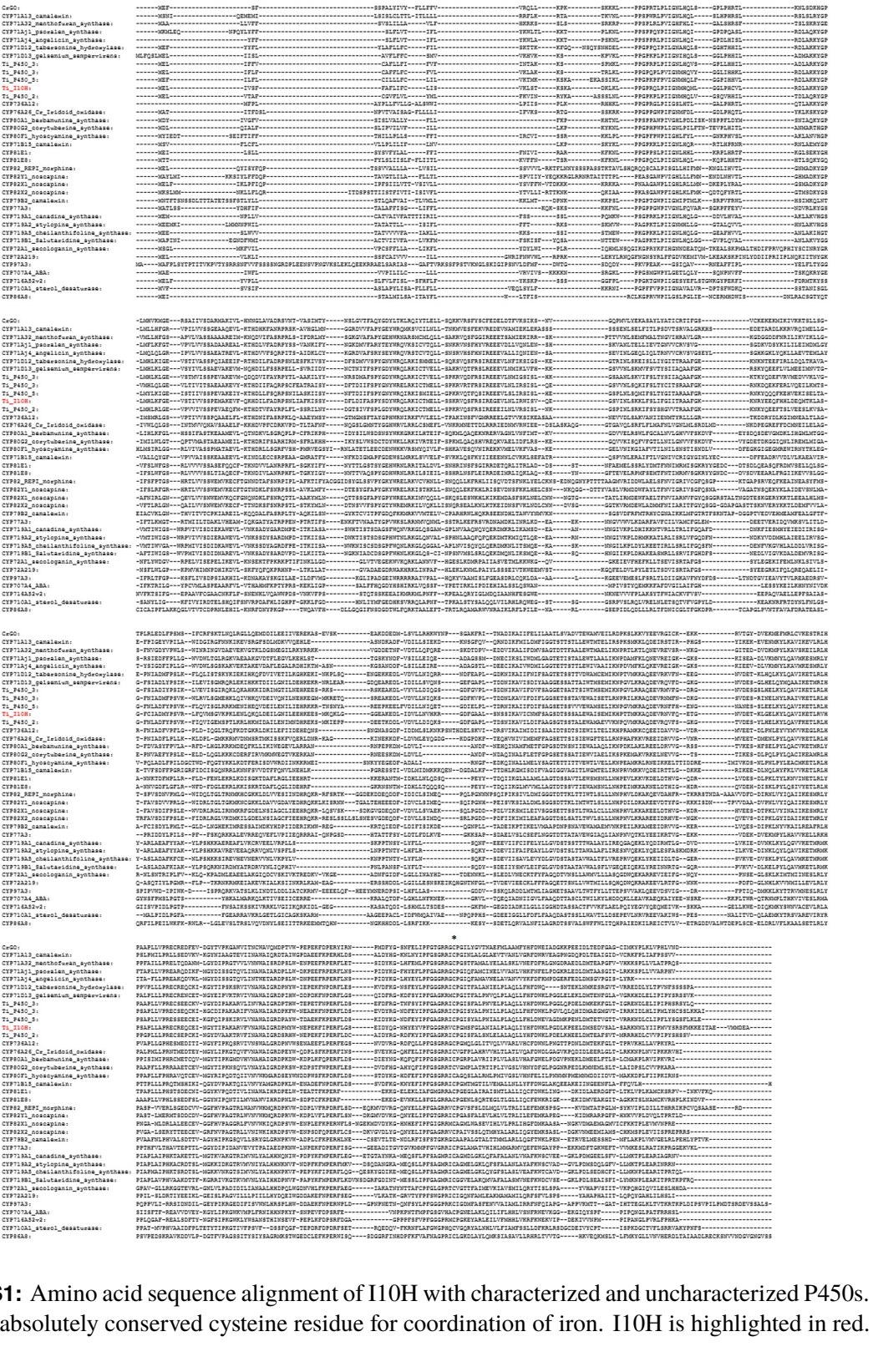

Figure S1: Amino acid sequence alignment of I10H with characterized and uncharacterized P450s. Asterisk indicate absolutely conserved cysteine residue for coordination of iron. I10H is highlighted in red.

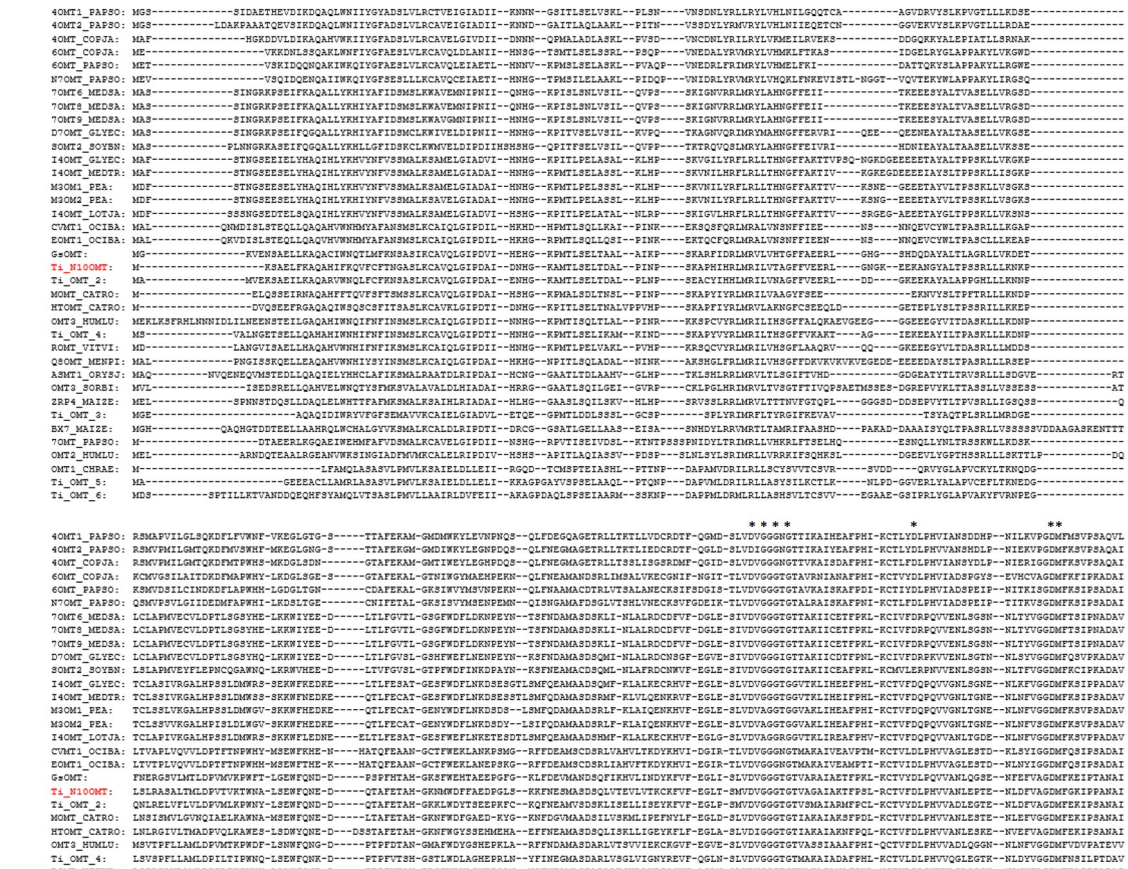

Figure S2: Amino acid sequence alignment of N10OMT with characterized and uncharacterized OMTs. asterisks indicate conserved residues for SAM binding. N10OMT is highlighted in red.

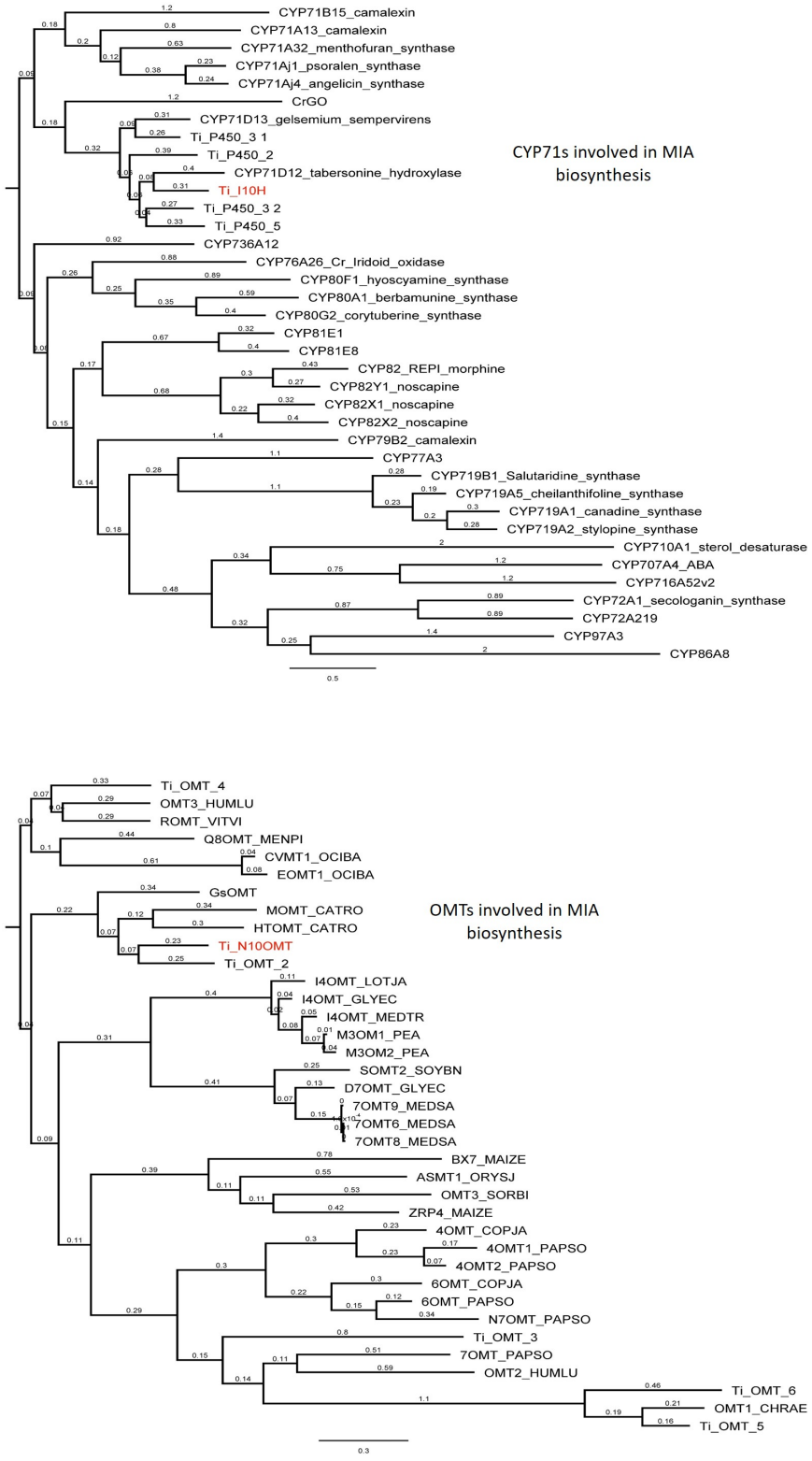

Figure S3: Phylogenetic trees of I10H (TOP) and N10OMT(Bottom) with characterized and uncharacterized proteins. CYP71D12 *C.roseus* tabersonine hydroxylase, T16H; HTOMT CATRO, *C.roseus* 16OMT. I10H and N10OMT are highlighted in red.

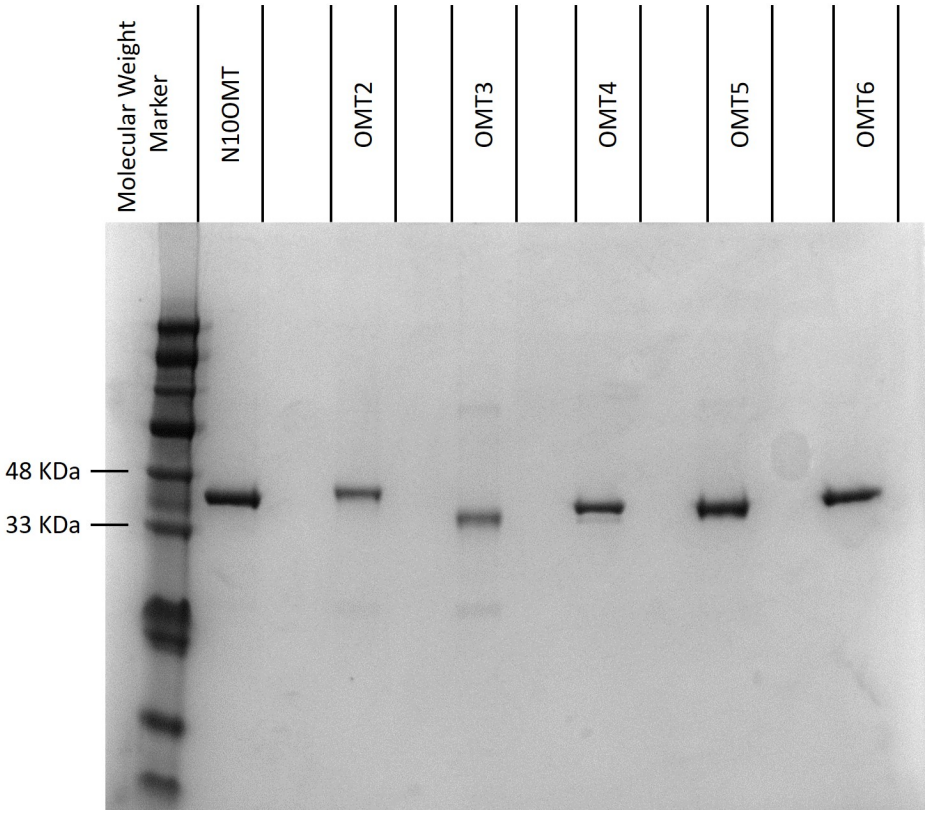

Figure S4: SDS-PAGE of recombinant OMTs produced in *Escherichia coli*. The left lane contains molecular weight protein markers and corresponding sizes are indicated to the left of the panel. All other lanes feature purified protein from *E. coli* strain soluBL21. Purification of polyhistidine-tagged recombinant proteins was achieved using a Nickel-affinity column and size exclusion chromatography. Visualization was achieved using Coomassie blue staining.