

	CYP71D351	CYP71D12
<b>FD661166</b>	100%	91%
<b>FD660753</b>	99%	91%
<b>FD661225</b>	100%	96%
<b>FD662166</b>	99%	91%

**Supplemental Table 1.** Nucleic acid sequence identity of the four T16H-annotated EST identified within the *C. roseus* epidermome EST database with *CYP71D351* and *CYP71D12* (Murata et al., 2008).

<b>Compound</b>	<b>Formula</b>	<b>Calculated (m/z, [M+H])</b>	<b>Measured (m/z, [M+H])</b>	<b>Error (ppm)</b>
16-hydroxytabersonine	C <sub>21</sub> H <sub>24</sub> N <sub>2</sub> O <sub>3</sub>	353,1860	353,1850	2.74
desacetoxyvindorosine	C <sub>22</sub> H <sub>28</sub> N <sub>2</sub> O <sub>3</sub>	369,2173	369,2176	0.90
deacetylvindorosine	C <sub>22</sub> H <sub>28</sub> N <sub>2</sub> O <sub>4</sub>	385,2122	385,2120	0.48
vindorosine	C <sub>24</sub> H <sub>30</sub> N <sub>2</sub> O <sub>5</sub>	427,2227	427,2227	0.11
desacetoxyvindoline	C <sub>23</sub> H <sub>30</sub> N <sub>2</sub> O <sub>4</sub>	399,2278	399,2280	0.42
deacetylvindoline	C <sub>23</sub> H <sub>30</sub> N <sub>2</sub> O <sub>5</sub>	415,2227	415,2229	0.36

**Supplemental Table 2.** Measured masses of the intermediates of vindoline/vindorosine biosynthetic pathways. Tabersonine and vindoline were identified according to standards.

**Supplemental Table 3.** Primers used in this study.

Primers annotated T16H and 2T16H were used for amplification of the CYP71D12 and CYP71D351 sequences, respectively. “q” primers were used for qPCR.

Primers	Sequence	Application
T16H-BglII	GCAGATCTGATGGAATTCTATTATTTTCTCTACTTGGCC	<i>cDNA cloning</i>
T16H-SpeI	GCACTAGTAGCAGGAGAAGAGGAAGAAAAATTA	
CrT16H-RT01	GCTTCATCCACCAGTTCCAT	
CrT16H-RT02	CCGGACATATCCTTCTTCCA	
2T16H-A	AAGAGAATGGGGAACAAAACAAGGATC	
2T16H-51	ATACCTTTCTTACCTCTTCTTGTC	
2T16H-52	CACTGTCGTTGACGATGTCTCAGTT	
2T16H-YFPfor	GCAGATCTATGGAGTTGTATTATTTTCCACCTTTGC	<i>Full length ORF cloning , YFP fusion construct- riboprobes</i>
2T16H-YFPrev	GCACTAGTATATTTACCTTTGAGAGAAGAAGCAGAAT	
T16H-BglII	GCAGATCTGATGGAATTCTATTATTTTCTCTACTTGGCC	<i>Yeast expression</i>
T16H-Bgl2stop	GCAGATCTCAAGCAGGAGAAGAGGAAGAAAAATTA	
2T16H-Bgl2-stop	GCAGATCTCTAATATTTACCTTTGAGAGAAGAAGCAGAAT	
qT16H-up	GCCAAAAACGCCAATATTCAAACC	<i>qPCR analysis in periwinkle organs and cultivars</i>
qT16H-down	ATGTGATGAGTATGGCCACCGC	
q2T16H-up	GATCAACTCACAGTGGCAGTC	
q2T16H-down	GACTTGAGGACTTGTGATTGGC	
qG10H-for	CATTATTAGGCGACCAACC	
qG10H-rev	GAACTTCTTTCGCCATTGTT	
qOMT-for	AATGGGCATTTCTCTTAAGGA	
qOMT-rev	CCGTAATACAAATTGGGTACAA	
qNMT-for	GGCTATCAACCTTATAAATGCA	
qNMT-rev	ATCTTCCGAACGGAATTTAACT	
qDAT-for2	GGTTCAATTTATTTCTCACGTAC	
qDAT-rev2	AACTATCAGAAAGGTAAGCATCGA	
qD4H-for	ATAGTTAATCATGGGATTCCACAAGATGTT	
qD4H-rev	GTTTCATGAAACTTACGAACTCCATCTAC	
qMAT-for	GATACTGTCAATATCGAACAGG	
qMAT-rev	GATGTCAACGTTTTCCATATCG	
qRPS9-for	TTACAAGTCCCTTCGGTGGT	
qRSP9-rev	TGCTTATTCTTCATCCTCTTCATC	
qR60S-for	TCTTAGTTGGAATGTTTCAGCACCTG	
qR60S-rev	TCTTAGTTGGAATGTTTCAGCACCTG	
2T16H fw	TGATTGGAAACTTGCCAGTGATGA	<i>VIGS constructs</i>
2T16H rev	ATGGATTAGCAACGAAATCTTCCGTA	
q2T16H fw	GATCAACTCACAGTGGCAGTC	<i>qPCR in silenced plants</i>
q2T16H rev	GACTTGAGGACTTGTGATTGGC	

CYP71D12	.....ATGGAATTCTATTATTTCTCTACTTGGC	1
CYP71D351	AGGCTTGATTTTGGTCAACTTTCTCTACCTCATGGAGTTGTATTATTTTCCACCTTTGC	1
CYP71D12	CTTCCTTCTTTCTGCTTCATTTTATCAAAAACCACAAAGAAATTTGGCCAAAACAGCCA	30
CYP71D351	CTTCCTTCTTTCTGCTTCATTTTAGCCAAAACCTCTAAAGAAATCTGGCCAATCAAATCT	61
CYP71D12	ATATTCAAACCATGATGAGCTACCTCCGGGGCTCCCCAAATTCCTATATTAGGAAATGC	90
CYP71D351	TA.....AGCTGCCTCTGGGGCTCCCCAATTCCTATATTAGGAAATGC	121
CYP71D12	CCATCAACTTAGCGGTGGCCATACTCATCACATTC TAAGAGATTTGGCCAAAAATATGG	150
CYP71D351	CCATCAACTTATAGGTGGCCATACTCATCACATTC TAAGAGATTTGGCCAAAAATATGG	166
CYP71D12	GCCGTTGATGCACCTAAAGATTGGTGAAGTTCAACCATTGTTGCATCTCACCACAAAT	210
CYP71D351	ACCGTTGATGCACCTAAAGACTGGTGAAGTTCAACCATTGTTGCATCCTCACCAGAAAT	226
CYP71D12	TGCTGAAGAGATTTT TAGAACGCATGATATTCTTTTGCCGATAGACCCTCAAATCTTGA	270
CYP71D351	TGCTGAAGAGATGTTTAAACACATGATGTTCTCTTTGCCGACAGACCCTCAAATATTGT	286
CYP71D12	GTCTTTTAAATCGTGTCTTATGATTTTTCAGATATGGTTGTTAGTCCATATGGTAATTA	330
CYP71D351	TGCCTTCAAATCTTGTCTTATGATTTTTCAGATATGGTTGTTAGTCCATATGGCAATTA	346
CYP71D12	TTGGAGACAACCTTCGTAAAATTAGCATGATGGAACCTCTTAGCCAAAAGAGTGTCCAATC	390
CYP71D351	TTGGAGACAACCTTCGTAAAATTAGCATGATGAGACTTTTAGCCAAAAGAGTGTCCAATC	406
CYP71D12	TTTTAGATCAATTAGAGAAGAGGAAGTATTAATTTTATTAATCAATTGGTTCCAAGA	450
CYP71D351	TTTTAGATCAATTAGAGAAGAGGAAGTATTAATTTTATTAATCAATTGGTTCCGAGAGA	466
CYP71D12	GGGTACAAGAATTAATCTCAGCAAAGAGATATCGTTACTTATTTATGGAATTACTACGGC	510
CYP71D351	GGGTACAAAAATTAATCTTAGCAAGGAAATATCGTTACTTATTTATGGAATTACTACGGC	526
CYP71D12	TGCTGCTTTTGGAGAGAAAAATAAGAATACAGAAGATTTATTCGTCCTTCTTGATCAACT	570
CYP71D351	TGCTGCTTTTGGAGAAAAATAAGAATACAGAAGATTTATTCGTCCTTCTTGATCAACT	586
CYP71D12	TACAAAGGCAGTAGCGGAACCTAACATTGCAGATATGTTCCCTTCTCTCAAATTTCTTCA	630
CYP71D351	CACAGTGGCAGTCGCGGAACCTAACATTGCAGATATGTTCCCTCTATCAATTTCTTAA	646
CYP71D12	ATTGATTAGTACATCAAAATATAAGATTGAGAAAAACACAAACAATTTGATGTTATAGT	690
CYP71D351	ATTAATTAGTAGATCGAAATATAAGATTGAGAAAAACACAAAAATTTGATGCCATAGT	706
CYP71D12	TGAAACTATTCTCAAAGGCATAAGGAGAAAAATCA.....ACAAGCCCTTAAGTC...A	750
CYP71D351	TCAAACTATTCTCAACCATCATAAGGATAGATTAGCCAATCACAAGTCTCAAGTCATGA	766
CYP71D12	AGAGAATGGAGAAAAAAGGAGGACCTTGTGATGTGCTACTCAATATTCAACGACGTAA	801
CYP71D351	AGAGAATGGGGAACAAAACAAGGATCTTGTGATGTGCTACTCAATATTCAACAACGTGG	826
CYP71D12	TGACTTTGAAGCCCCACTGGGGGATAAAAACATCAAAGCCATAATCTTCAACATATTAG	861
CYP71D351	TGATTTTGATACACCCTAGGTGATCGCAGCGTCAAAGCAGTAATTTTAAACATATTAG	886
CYP71D12	TGCCGGCACTGAGACATCGTCAACAACAGTCGATTGGGCAATGTGCGAAATGATAAAAAA	921
CYP71D351	TGCCGGCACTGAGACATCGTCAACGACAGTGGATTGGGCCATGTGTGAAATGATAAAAAA	946
CYP71D12	TCCAACGGTAATGAAAAAGGCACAAGAAGAGGTAAGAAAGGTATTTAATGAAGAAGGAAA	981
CYP71D351	TCCAACGATAATGAAAAAGGCACAAGAAGAGGTAAGAAAGGTATATAATGAAGAAGGAAA	1006

CYP71D12	TGTTGATGAAACAAAACCTTCATCAACTAAAATATTTACAAGCAGTGATTAAGAAACATT	1041
CYP71D351	TGTTAATGAAACAAAACCTTCATCAGCTAAAATATTTAAAAGCAGTGATTAAGAAACATT	1066
CYP71D12	AAGGCTTCATCCACCAGTTCCATTACTACTTCCAAGAGAATGTCGAGAACAATGTAAGAT	1101
CYP71D351	AAGGCTTCATCCACCAGTTCCATTACTACTTCCAAGAGAATGTCGAGAACAATGTTGAGAT	1126
	<i>CrT16H-RT01</i> →	
CYP71D12	TAAAGGGTACACAATACCATCCAAATCTAGAGTTATAGTCAATGCATGGGCTATCGGAAG	1161
CYP71D351	TAAAGGGTACACAATACCATCCAAATCTAGAGTTATAGTCAATGCATGGGCTATCGGAAG	1186
CYP71D12	GGATCCAAATTACTGGATTGAACCTGAGAAATTTAACCCGGATAGATTTCTTGAATCAAA	1221
CYP71D351	GGATCCGAATTACTGGATTGAACCTGAAAATTTAACCCGGAGAGATTTCTTGAATCAGA	1246
	← <i>CrT16H-RT02</i>	
CYP71D12	AGTTGATTTTAAAGGAAATTCATTTCGAGTATCTACCATTTGGTGGTGGGAAGAAGGATATG	1281
CYP71D351	AGTTGATTTTAAAGGAAACTCATTCGAGTATCTGCCGTTTGGTGGTGGGAAGAAGGATATG	1306
CYP71D12	TCCGGGCATAACATTTGCTTTGGCTAATATAGAATTGCCATTAGCACAACTTTTGTCCA	1341
CYP71D351	TCCGGGCATAACATTTGCTTTGGCTAATATAGAACTGCCATTAGCACAACTTTTGTCCA	1366
CYP71D12	TTTTGATTGGCAAT.....CAAATACTGAGAAATTAATATGAAAGAGAG	1401
CYP71D351	TTTTGATTGGAAACTTGCCAGTGATGAAACAAATATTGATAAATTAGACATGACGGAGAG	1426
CYP71D12	TAGAGGGGTAACAGTTAGGCGAGAAGATGATTTGTATTTGACTCCAGTTAATTTTCTTC	1446
CYP71D351	TAGAGGGGTAACAGTTAGAAGAGAAGATGATTTGTGTCTGATCCATTTTCCTTATCTGTC	1486
CYP71D12	CTCTTCTCTGCTTGAAAATCTTTACCG.....AAACAATTCAAGACATG.	1506
CYP71D351	TTCTTCTCTCAAAGGTAAATATTAGATGGAGACAACATCACCAAATAATTGAAGACCAAG	1546
CYP71D12	...GTAGTCCATGAAGGATTTTTTTTTTTTTTTCTTTCTTCCTTTTCTTACGAGAAGTCC	1551
CYP71D351	ATTGTAGGTCATGAATAGTTGTTCGGTCAAGAAATGGTGGCAAAAATTGCTACGGGAAGATT	1606
CYP71D12	ATGAAGGATTAAGTTTGTGGATTGTTAGTTTGCCTTTGGTAACAAGTTTGTGGATTGTT	1608
CYP71D351	TCGTTGCTAATCCAT.....TGTAATTTTTT	1666
CYP71D12	TTGTATCTAAATGAATAAAGCAATGATTGCCTCATCAAAAAAAA	1668
CYP71D351	TTGTATA.....	1692

**Supplemental Figure 1.** Nucleic acid sequence alignment of *CYP71D12* and *CYP71D351*. Arrows designated the primers used for amplification of the partial *CYP71D351* or *CYP71D12* cDNAs. Initiation and termination codons are indicated by blue and pink highlighting, respectively. The yellow highlighting delimits the silencing fragment used in VIGS assays. All other regions of the coding sequences display high identity leading to the production of identical 21 to 23 mers siRNA. A single 23 mers siRNA (red bar) is common to the *CYP71D12* and the *CYP71D351* selected region.

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CYP71D351 1 MELYYFSTFAFLLCFILAAKTLKKSGGSS-----NLKLEPLGPPPIPILGNAHQIIGGHTHHILRDLAKKYGPLMHLKTGEV
CYP71D12 1 MEFYYFLYLAFLLCFILSKTKKFGONSQYSNHDELPPGPPQIPILGNAHQISGGHTHHILRDLAKKYGPLMHLKIGEV

CYP71D351 76 STIVASSPEIAEENFKTHDVLFADRPSNIVAFKILSYDMSDVISPYGNYWRQLRKISMMELFSQRSVQSFRSIREEEVL
CYP71D12 81 STIVASSPQIAEENFRTHDILFADRPSNLESFKIVSYDFSDMVVISPYGNYWRQLRKISMMELLSQRSVQSFRSIREEEVL

CYP71D351 156 NFIKSIGSREGTRINLSKEISLLIYGITTRAAFGEKNKNTEEFIRLLDQLTVAVAEPNIADMFPSINFLKLISRSSKYKIE
CYP71D12 161 NFIKSIGSREGTRINLSKEISLLIYGITTRAAFGEKNKNTEEFIRLLDQLTKAVAEPNIADMFPSIKFLQLISTSKYKIE

CYP71D351 236 KIHKNFDALVQTILNNHKKDRLANHKSSSSEENGEQNKDLVDVLLNIQQRGDFDTPLGDRSVKALIFNIFSAGTETSSTTV
CYP71D12 241 KIHKCFDVIVETILLKGHKEKINKPLS---QENGEKKEDLVDVLLNIQRRNDFEAPLGDANIKALIFNIFSAGTETSSTTV

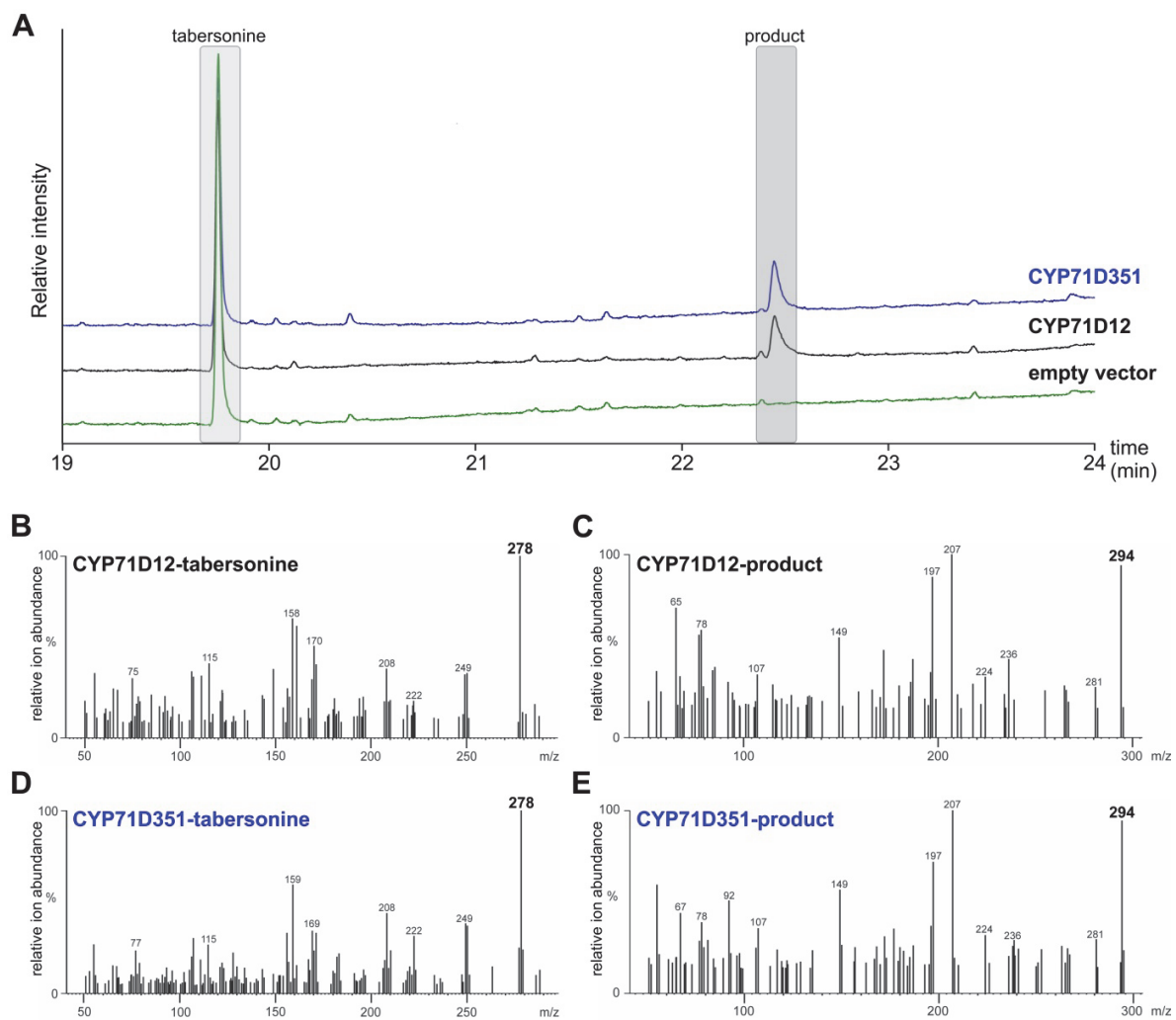
CYP71D351 316 DWAMCEMIKNPTIMKKAQEEVRKVNEEGNVNETKLHQLKYLKAVIKETLRLHPPVPLLLPRECREQCETIKGYTIPSKSR
CYP71D12 318 DWAMCEMIKNPTIMKKAQEEVRKVNEEGNVNETKLHQLKYLQAVIKETLRLHPPVPLLLPRECREQCETIKGYTIPSKSR

CYP71D351 396 VIVNAWAIGRDPNYWIEPENFNFPRFLESEVDFKGNSFEYLPFGGRRICPGITFALANIELPLAQLLFHFDWKLASDET
CYP71D12 398 VIVNAWAIGRDPNYWIEPEKFNPRFLESKVDFKGNSFEYLPFGGRRICPGITFALANIELPLAQLLFHFDW-----QS

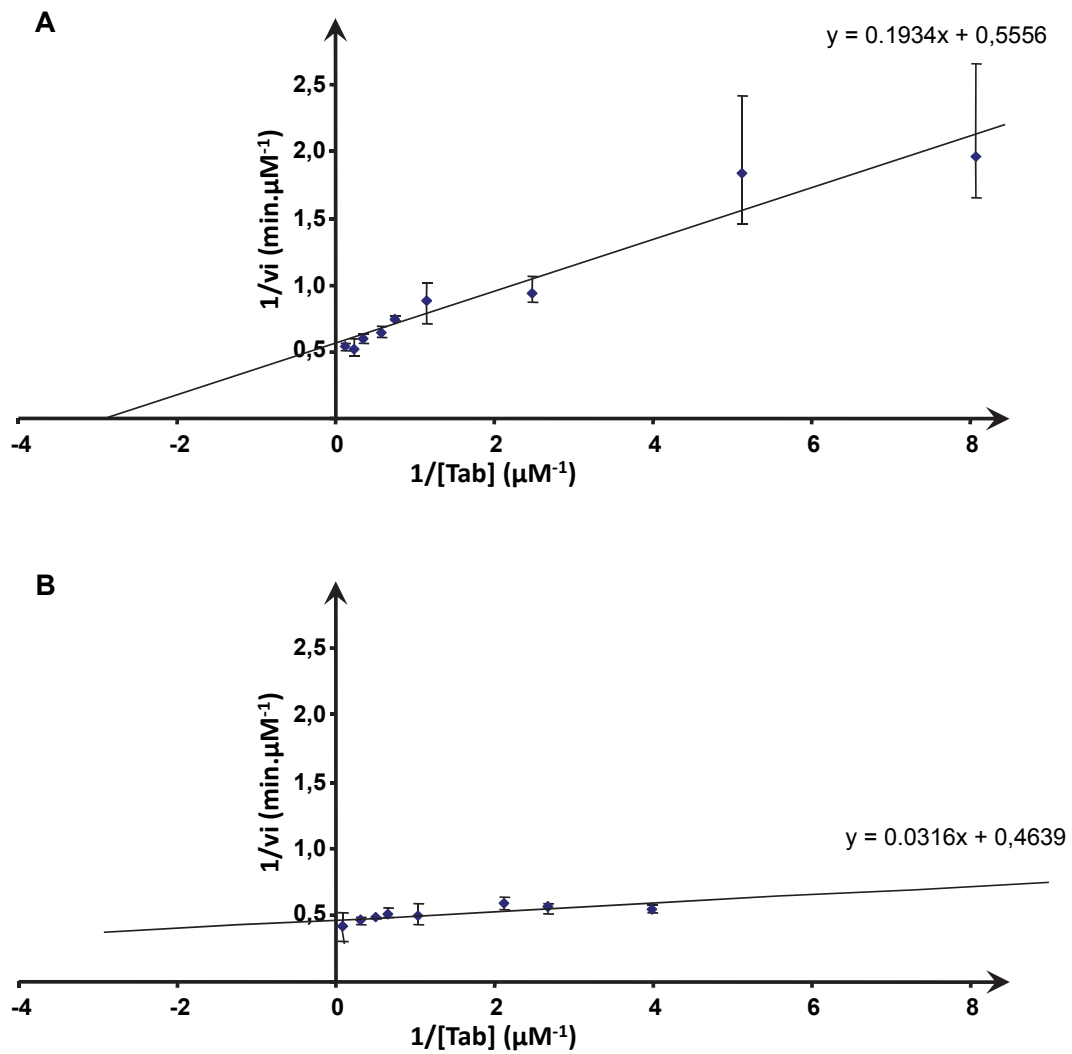
CYP71D351 476 NIKKLDMTESRGVTVRREDDLCLIPFPSSASSLKGKY
CYP71D12 473 NTEKLNMTESRGVTVRREDDLCLIPVNSSSSPA---

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**Supplemental Figure 2.** Amino-acid sequence alignment of CYP71D351 and CYP71D12. Sequence identity and similarity are highlighted by black and grey shading, respectively. Red bars denote the position of the predicted transmembrane helix as described in Guirimand *et al.*, (2009).

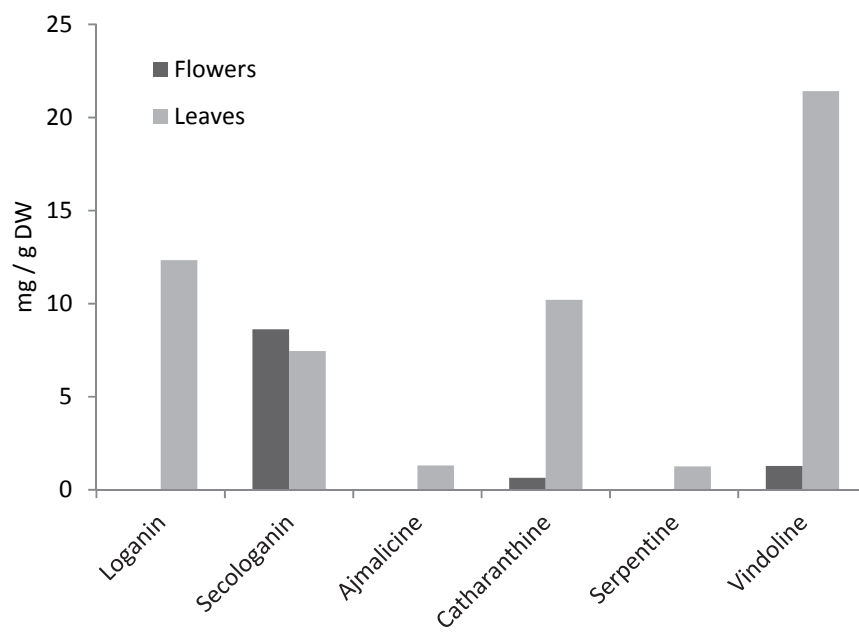


**Supplemental Figure 3.** Characterization of the reaction product of CYP71D351, CYP71D12 and empty vector yeast microsomes incubated with tabersonine (100  $\mu$ M) and NADPH (600  $\mu$ M). (A) GC chromatograms and (B-E) corresponding MS spectra of tabersonine and reaction product.

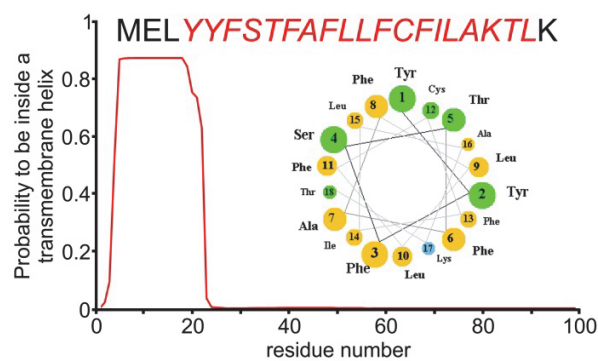


**Supplemental Figure 4.** Effect of tabersonine concentration on T16H activity of CYP71D12 (A) and CYP71D351 (B). Lineweaver-Burk plots ( $[\text{NADPH}] = 1\text{mM}$ ).





**Supplemental Figure 5.** HPLC comparative analysis of MIAs accumulation in flowers (dark grey) and young leaves (light grey) of *C. roseus* (Pacifica Pink cultivar) according to Guirimand et al. (2010).



**Supplemental Figure 6.** Detection of a putative transmembrane helix at the N-terminal end of CYP71D351 (T16H2). The probability of a residue to be inside a transmembrane helix has been calculated for the 100-first residues of CYP71D351 with a Markov model by the TMHMM server (<http://www.cbs.dtu.dk/services/TMHMM/>). The inset shows the predicted sequence of the transmembrane helix and a projection of the predicted helical wheel represented as a cross sectional view of the axis using a device available at (<http://cti.itc.virginia.edu/~cmg/Demo/wheel/wheelApp.html>). Non polar, polar and basic residues are represented in yellow, green and blue, respectively.