| | CYP71D351 | CYP71D12 |
|---|---|---|
| **FD661166** | 100% | 91% |
| **FD660753** | 99% | 91% |
| **FD661225** | 100% | 96% |
| **FD662166** | 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).

| Compound | Formula | Calculated (m/z, [M+H]) | Measured (m/z, [M+H]) | Error (ppm) |
|---|---|---|---|---|
| 16-hydroxytabersonine | $C_{21}H_{24}N_2O_3$ | 353,1860 | 353,1850 | 2.74 |
| desacetoxyvindorosine | $C_{22}H_{28}N_2O_3$ | 369,2173 | 369,2176 | 0.90 |
| deacetylvindorosine | $C_{22}H_{28}N_2O_4$ | 385,2122 | 385,2120 | 0.48 |
| vindorosine | $C_{24}H_{30}N_2O_5$ | 427,2227 | 427,2227 | 0.11 |
| desacetoxyvindoline | $C_{23}H_{30}N_2O_4$ | 399,2278 | 399,2280 | 0.42 |
| deacetylvindoline | $C_{23}H_{30}N_2O_5$ | 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 | |
| T16H-SpeI | GCACTAGTAGCAGGAGAAGAGGAAGAAAAATTAACT | |
| CrT16H-RT01 | GCTTCATCCACCAGTTCCAT | |
| CrT16H-RT02 | CCGGACATATCCTTCTTCCA | ***cDNA cloning*** |
| 2T16H-A | AAGAGAATGGGGAACAAAACAAGGATC | |
| 2T16H-51 | ATACCTTTCTTACCTCTTCTTGTGCC | |
| 2T16H-52 | CACTGTCGTTGACGATGTCTCAGTT | |
| 2T16H-YFPfor | GCAGATCTATGGAGTTGTATTATTTTTCCACCTTTGC | ***Full length ORF cloning , YFP fusion construct- riboprobes*** |
| 2T16H-YFPrev | GCACTAGTATATTTACCTTTGAGAGAAGAAGCAGAAT | |
| T16H-BglII | GCAGATCTGATGGAATTCTATTATTTTCTCTACTTGGCC | |
| T16H-Bgl2stop | GCAGATCTTCAAGCAGGAGAAGAGGAAGAAAAATTAACT | ***Yeast expression*** |
| 2T16H-Bgl2-stop | GCAGATCTCTAATATTTACCTTTGAGAGAAGAAGCAGAAT | |
| qT16H-up | GCCCAAAACAGCCAATATTCAAACC | |
| qT16H-down | ATGTGATGAGTATGGCCACCGC | |
| q2T16H-up | GATCAACTCACAGTGGCAGTC | |
| q2T16H-down | GACTTGAGGACTTGTGATTGGC | |
| qG10H-for | CATTTATTAGGCGACCAACC | |
| qG10H-rev | GAACTTCTTTCGCCATTGTT | |
| qOMT-for | AATGGGCATTTCTCTTTAAGGA | |
| qOMT-rev | CCGTAATACAAATTGGGTACAA | |
| qNMT-for | GGCTATCAACCTTATAAATGCA | |
| qNMT-rev | ATCTTCCGAACGGAATTTAACT | ***qPCR analysis in periwinkle organs and cultivars*** |
| qDAT-for2 | GGTTTCAATTTATTTCTCACGTAC | |
| qDAT-rev2 | AACTATCAGAAAGGTAAGCATCGA | |
| qD4H-for | ATAGTTAATCATGGGATTCCACAAGATGTT | |
| qD4H-rev | GTTCATGAAACTTACGAACTCCATCTAC | |
| qMAT-for | GATACTGTCAATATCGAACAGG | |
| qMAT-rev | GATGTCAACGTTTTCCATATCG | |
| qRPS9-for | TTACAAGTCCCTTCGGTGGT | |
| qRSP9-rev | TGCTTATTCTTCATCCTCTTCATC | |
| qR60S-for | TCTTAGTTGGAATGTTCAGCACCTG | |
| qR60S-rev | TCTTAGTTGGAATGTTCAGCACCTG | |
| 2T16H fw | TGATTGGAAACTTGCCAGTGATGA | ***VIGS constructs*** |
| 2T16H rev | ATGGATTAGCAACGAAATCTTCCGTA | |
| q2T16H fw | GATCAACTCACAGTGGCAGTC | ***qPCR in silenced plants*** |
| q2T16H rev | GACTTGAGGACTTGTGATTGGC | |


CYP71D12 ATGGAATTCTATTATTTTCTCTACTTGGC 1
CYP71D351 AGGCTTGATTTTGGTCAACTTTCTCTACCTCATGGAGTTGTATTATTTTTCCACCTTTGC 1
CYP71D12 CTTCCTTCTTTTCTGCTTCATTTTATCAAAAACCACAAAGAAATTTGGCCAAAACAGCCA 30
CYP71D351 CTTCCTTCTTTTCTGCTTCATTTTAGCCAAAACTCTAAAGAAATCTGGCCAATCAAATCT 61
CYP71D12 ATATTCAAACCATGATGAGCTACCTCCGGGGCCTCCCCAAATTCCTATATTAGGAAATGC 90
CYP71D351 TA.....AGCTGCCTCTGGGGCCTCCCCCAATTCCTATATTAGGAAATGC 121
CYP71D12 CCATCAACTTAGCGGTGGCCATACTCATCACATTCTAAGAGATTTGGCCAAAAAATATGG 150
CYP71D351 CCATCAACTTATAGGTGGCCATACTCATCACATTCTAAGAGATTTGGCCAAAAAATATGG 166
CYP71D12 GCCGTTGATGCACTTAAAGATTGGTGAAGTTTCAACCATTGTTGCATCTTCACCACAAAT 210
CYP71D351 ACCGTTGATGCACTTAAAGACTGGTGAAGTTTCAACCATTGTTGCATCCTCACCAGAAAT 226
CYP71D12 TGCTGAAGAGATTTTTAGAACGCATGATATTCTTTTTGCCGATAGACCCTCAAATCTTGA 270
CYP71D351 TGCTGAAGAGATGTTTAAAACACATGATGTTCTCTTTGCCGACAGACCCTCAAATATTGT 286
CYP71D12 GTCTTTTAAAATCGTGTCTTATGATTTTTCAGATATGGTTGTTAGTCCATATGGTAATTA 330
CYP71D351 TGCCTTCAAAATCTTGTCTTATGATTATTCGGATGTTGTCATTAGTCCATATGGCAATTA 346
CYP71D12 TTGGAGACAACTTCGTAAAATTAGCATGATGGAACTTCTTAGCCAAAAGAGTGTCCAATC 390
CYP71D351 TTGGAGACAACTTCGTAAAATTAGCATGATGGAGCTTTTTAGCCAAAGGAGTGTTCAATC 406
CYP71D12 TTTTAGATCAATTAGAGAAGAGGAAGTATTAAATTTTATTAAATCAATTGGTTCCAAAGA 450
CYP71D351 TTTTAGATCAATTAGAGAAGAGGAAGTATTGAATTTTATTAAATCAATTGGTTCGAGAGA 466
CYP71D12 GGGTACAAGAATTAATCTCAGCAAAGAGATATCGTTACTTATTTATGGAATTACTACGCG 510
CYP71D351 GGGTACAAAAATTAATCTTAGCAAGGAAATATCGTTACTTATTTATGGAATTACTACGCG 526
CYP71D12 TGCTGCTTTTGGAGAGAAAAATAAGAATACAGAAGAATTTATTCGTCTTCTTGATCAACT 570
CYP71D351 TGCTGCTTTTGGAGAAAAAAATAAGAATACAGAAGAATTTATTCGTCTTCTTGATCAACT 586
CYP71D12 TACAAAGGCAGTAGCGGAACCTAACATTGCAGATATGTTCCCTTCTCTCAAATTTCTTCA 630
CYP71D351 CACAGTGGCAGTCGCGGAACCTAACATTGCAGATATGTTCCCCTCTATCAATTTTCTTAA 646
CYP71D12 ATTGATTAGTACATCAAAATATAAGATTGAGAAAATACACAAACAATTTGATGTTATAGT 690
CYP71D351 ATTAATTAGTAGATCGAAATATAAGATTGAGAAAATACACAAAAATTTTGATGCCATAGT 706
CYP71D12 TGAAACTATTCTCAAAGGGCATAAGGAGAAAATCA.....ACAAGCCCTTAAGTC...A 750
CYP71D351 TCAAACTATTCTCAACCATCATAAGGATAGATTAGCCAATCACAAGTCCTCAAGTCATGA 766
CYP71D12 AGAGAATGGAGAAAAAAAGGAGGACCTTGTTGATGTGCTACTCAATATTCAACGACGTAA 801
CYP71D351 AGAGAATGGGGAACAAAACAAGGATCTTGTTGATGTTCTACTCAATATTCAACAACGTGG 826
CYP71D12 TGACTTTGAAGCCCCACTGGGGGATAAAAACATCAAAGCCATAATCTTCAACATATTCAG 861
CYP71D351 TGATTTTGATACACCACTAGGTGATCGCAGCGTCAAAGCAGTAATTTTTAACATATTCAG 886
CYP71D12 TGCCGGCACTGAGACATCGTCAACAACAGTCGATTGGGCAATGTGCGAAATGATAAAAAA 921
CYP71D351 TGCCGGAACTGAGACATCGTCAACGACAGTGGATTGGGCCATGTGTGAAATGATAAAAAA 946
CYP71D12 TCCAACGGTAATGAAAAAGGCACAAGAAGAGGTAAGAAAGGTATTTAATGAAGAAGGAAA 981
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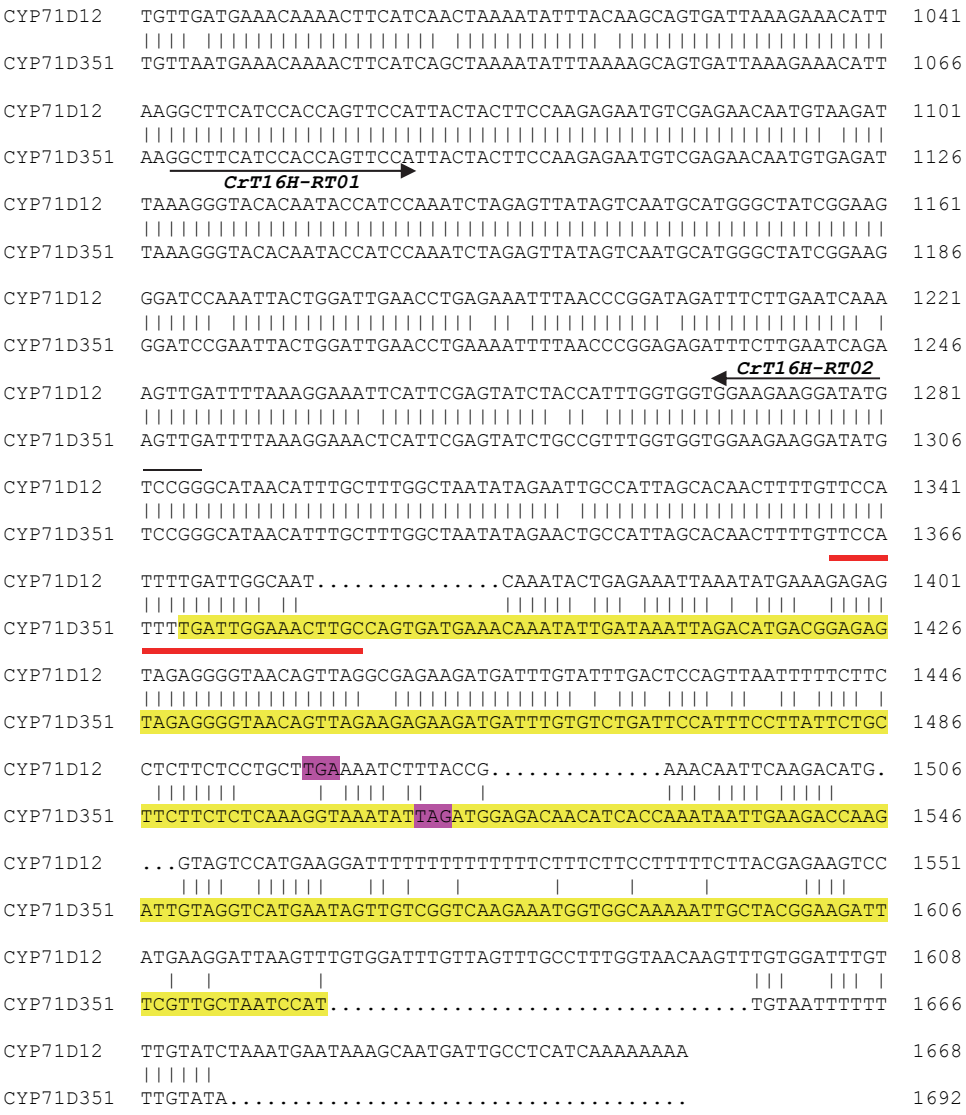
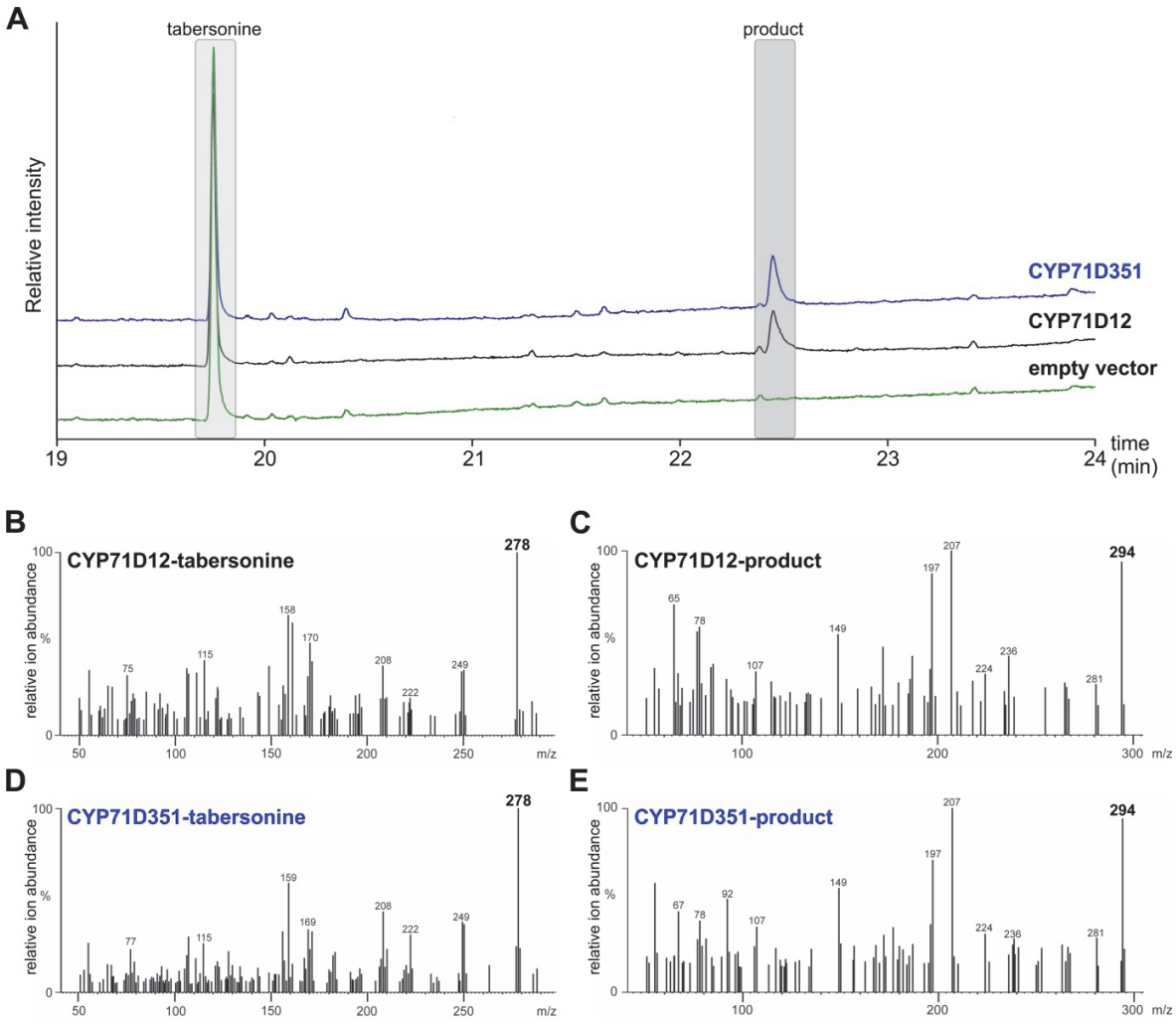
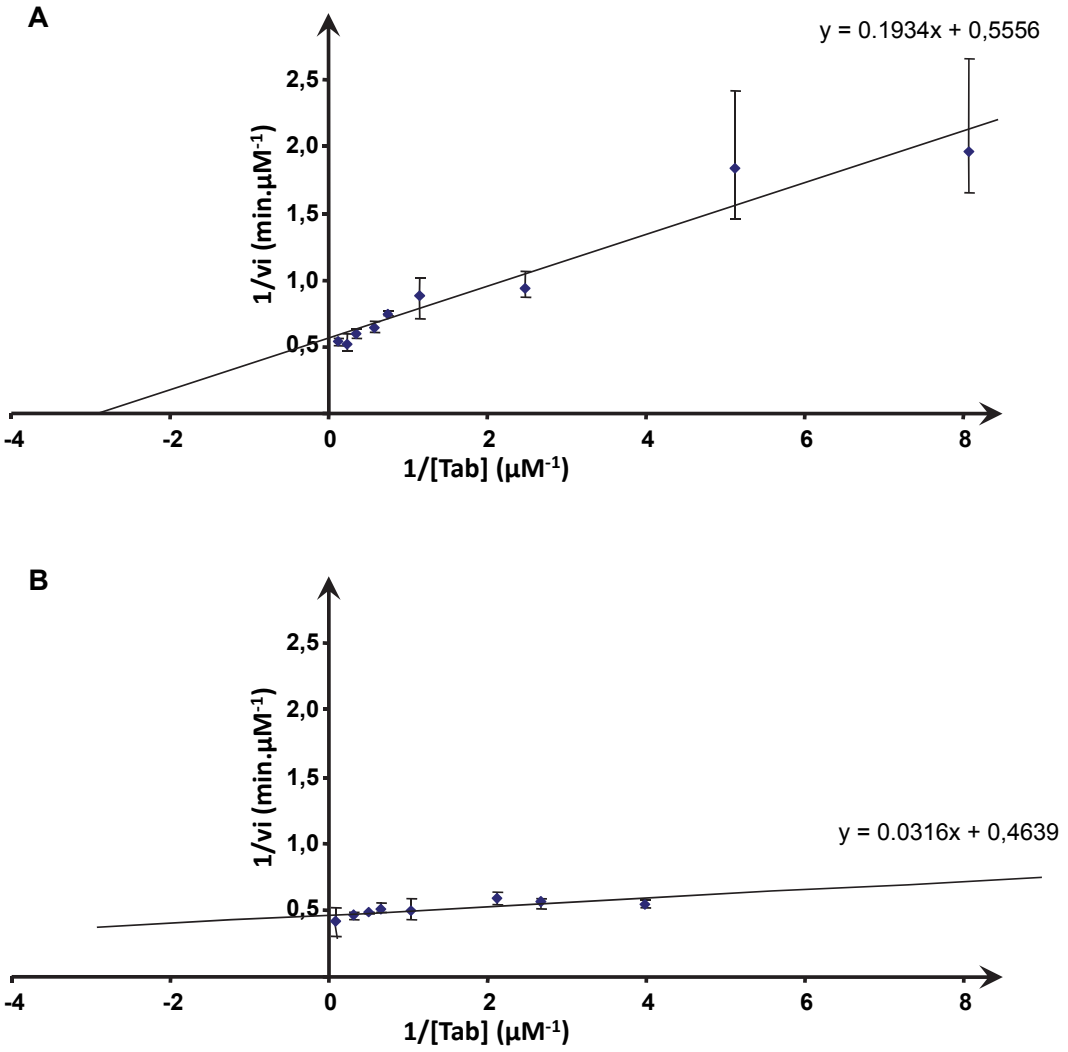



**Supplemental Figure 1.** Nucleic acid sequence alignment of *CYP71D12* and *CYP71D351*. Arrows designated the primers used for amplification of the partial *CYP71D351* or *CYP71D12* cDNAs. Initiation and termination codons are indicated by blue and pink highlighting, respectively. The yellow highlighting 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.

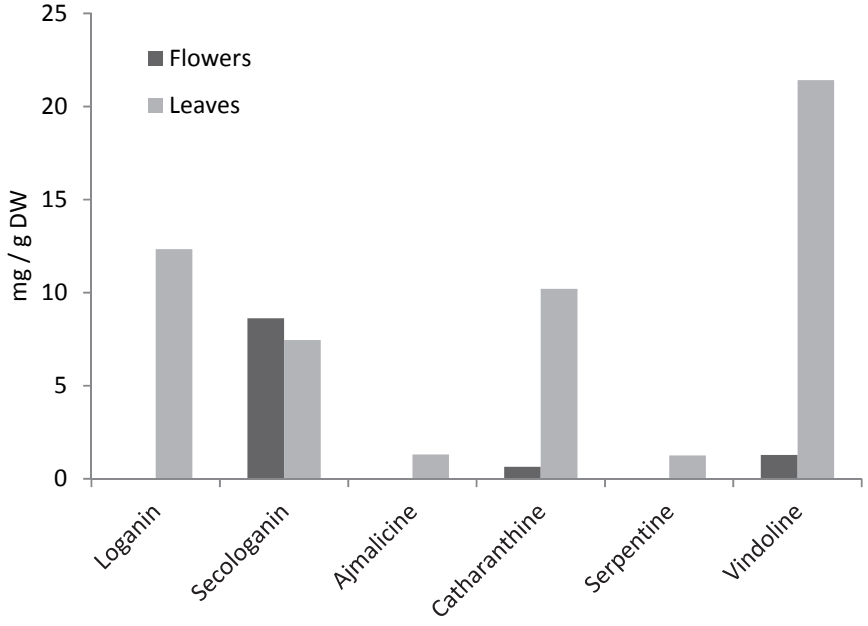

**Supplemental Figure 2.** Amino-acid sequence alignment of CYP71D351 and CYP71D12. Sequence identity and similarity are highlighted by black and grey shading, respectively. Red bars denote the position of the predicted transmembrane helix as described in Guirimand et *al*., (2009).



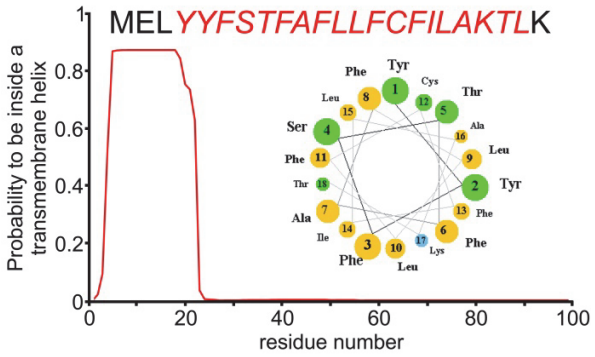

**Supplemental Figure 3.** Characterization of the reaction product of CYP71D351, CYP71D12 and empty vector yeast microsomes incubated with tabersonine (100 µM) and NADPH (600 µM). (A) GC chromatograms and (B-E) corresponding MS spectra of tabersonine and reaction product.




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




**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.