Supplementary Information

Sarpagan bridge enzyme has substrate-controlled cyclization and aromatization modes

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[#]These authors contributed equally to this work

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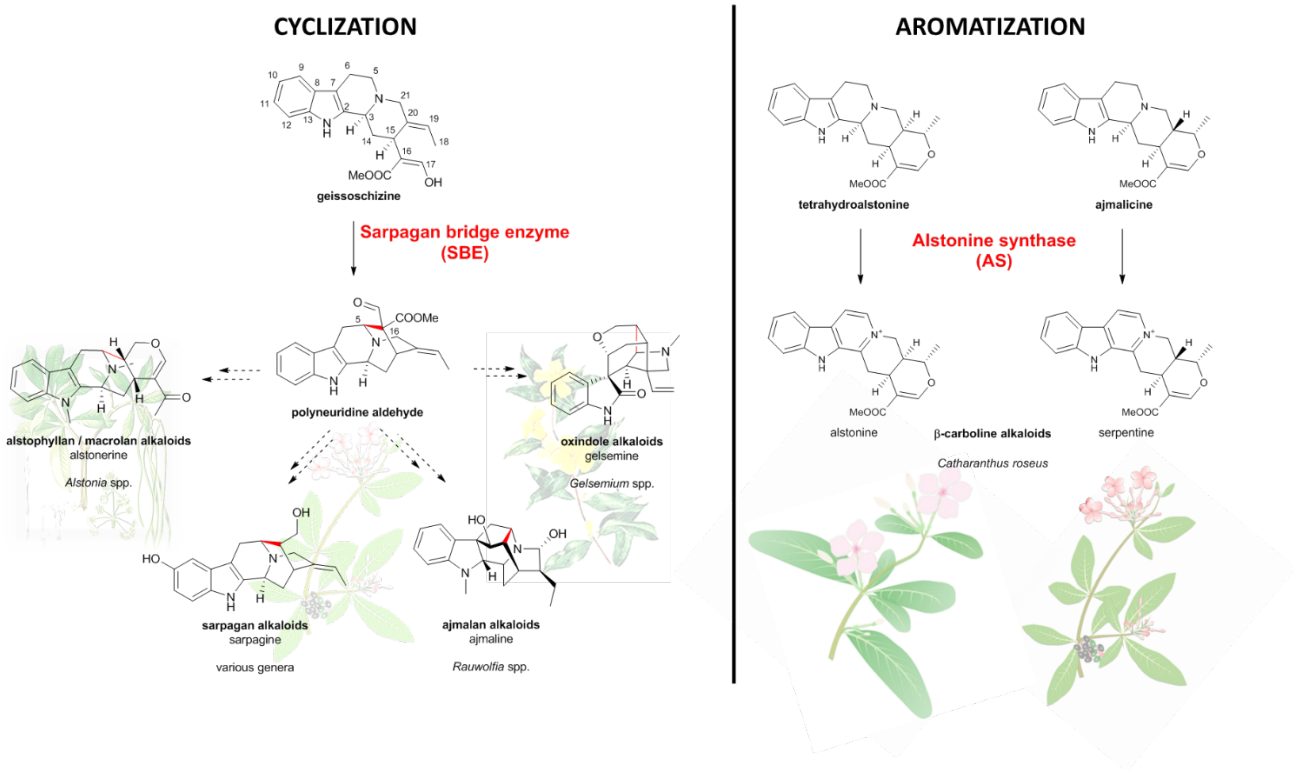
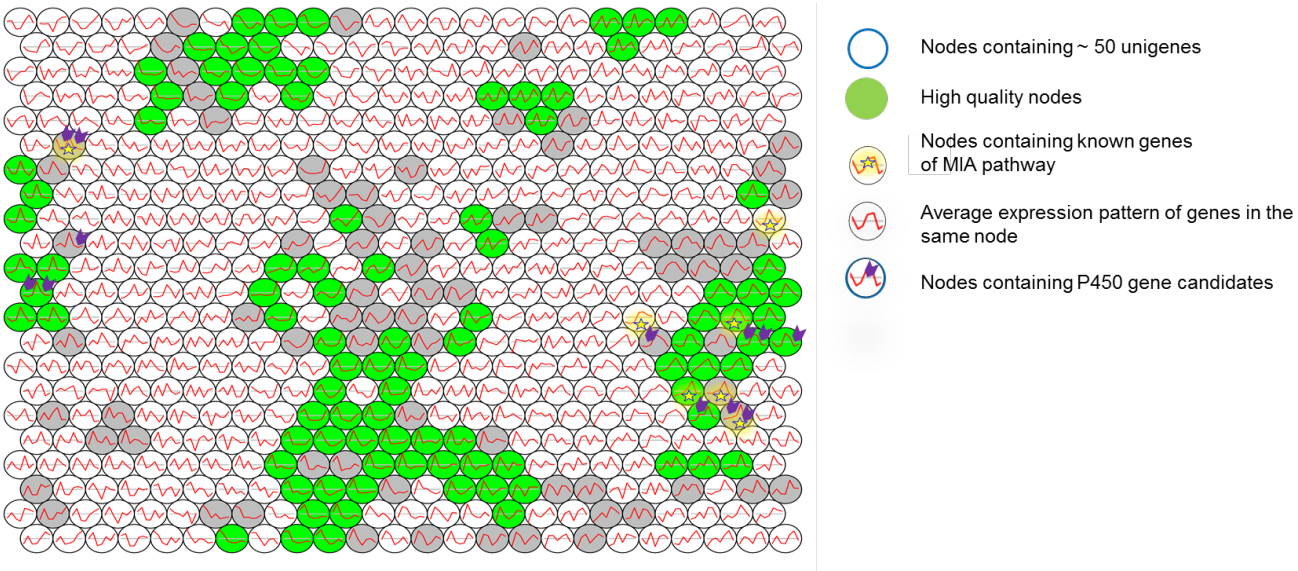
Table of Contents

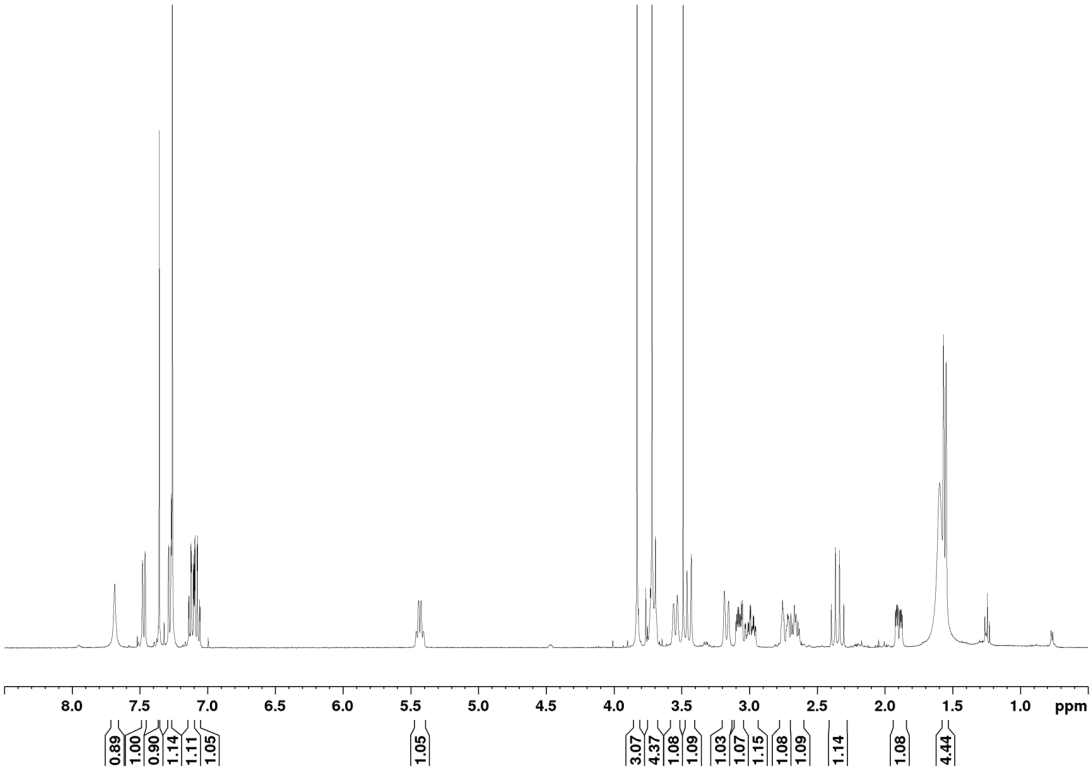
Supplementary Figures



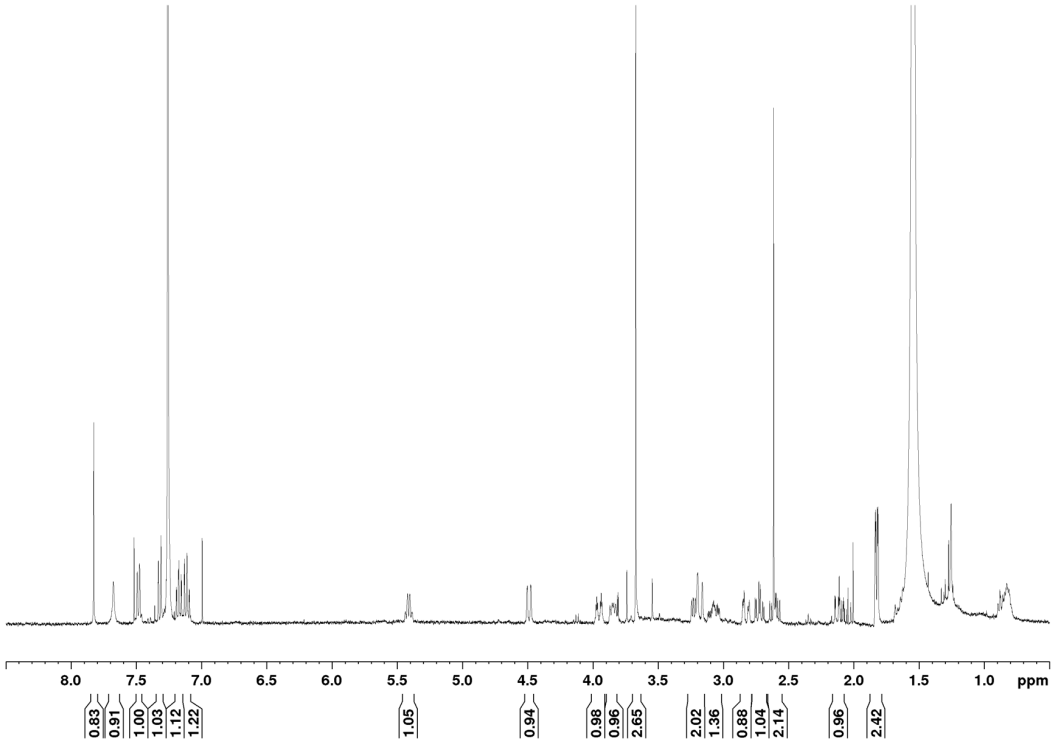
Supplementary Figure 1. Sarpagan bridge enzyme and its homologue alstonine synthase. The formation of polyneuridine aldehyde by sarpagan bridge enzyme is a central step in the biosynthetic pathways to a wide variety of monoterpene indole alkaloids scaffolds. In this work, we identify sarpagan bridge enzyme and show that its homologue alstonine synthase catalyzes aromatization to β-carbolines.



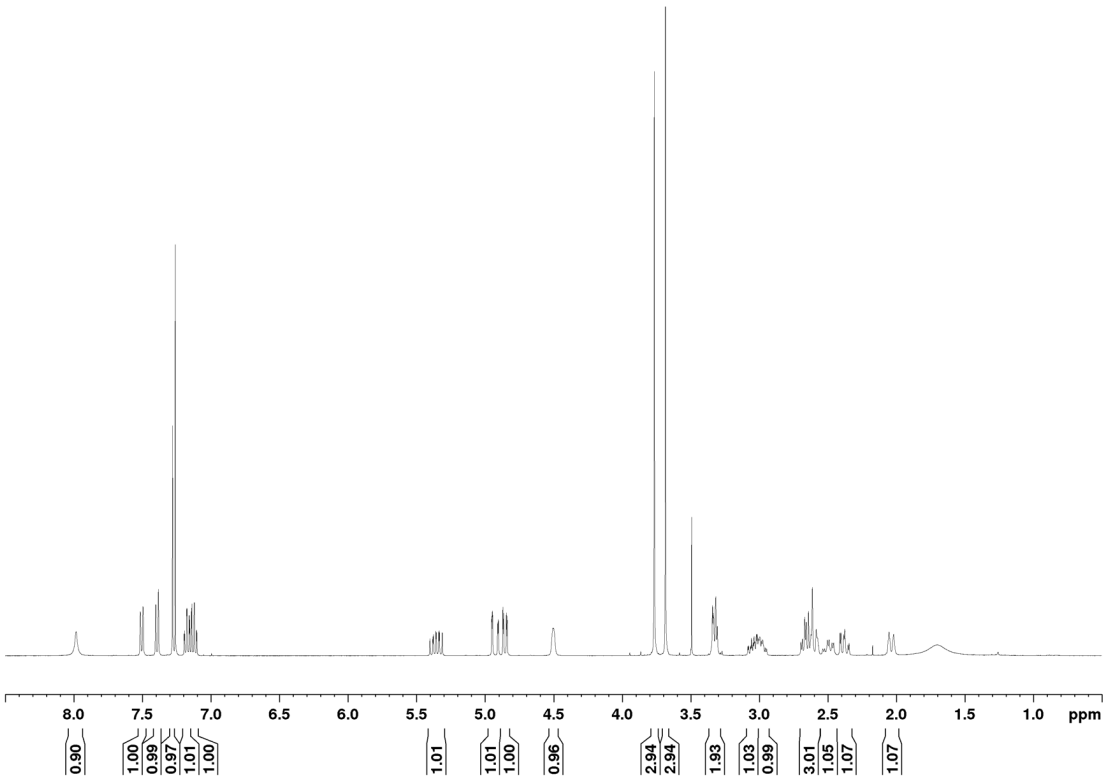
Supplementary Figure 2. Candidate selection based on a self-organizing map using *R. serpentina* transcriptomic data. Each node represents approximately 50 unigenes with the most similar expression profile. The reported MIA pathway genes are represented by yellow stars. Purple arrows represent CYP candidates; some nodes contain more than one candidate. Green nodes signify the highest quality nodes, grey and white nodes are of lower quality. See methods for a detailed description of the quality metrics.



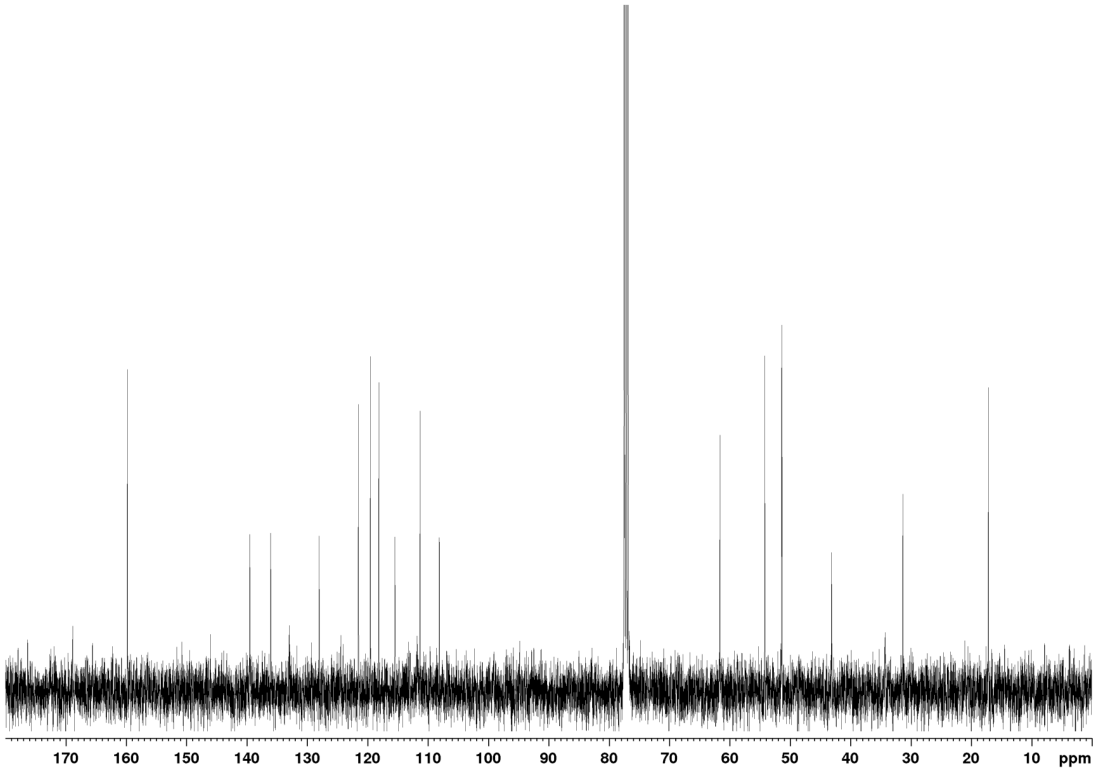
Supplementary Figure 3a. ^{1}H NMR spectrum of isolated geissoschizine methyl ether ($CDCl_3$, 298 K, 400 MHz). The compound was characterized by NMR when isolated for the first time. A compound with identical retention time, HRMS and MSMS was also obtained from a second batch of plant material.



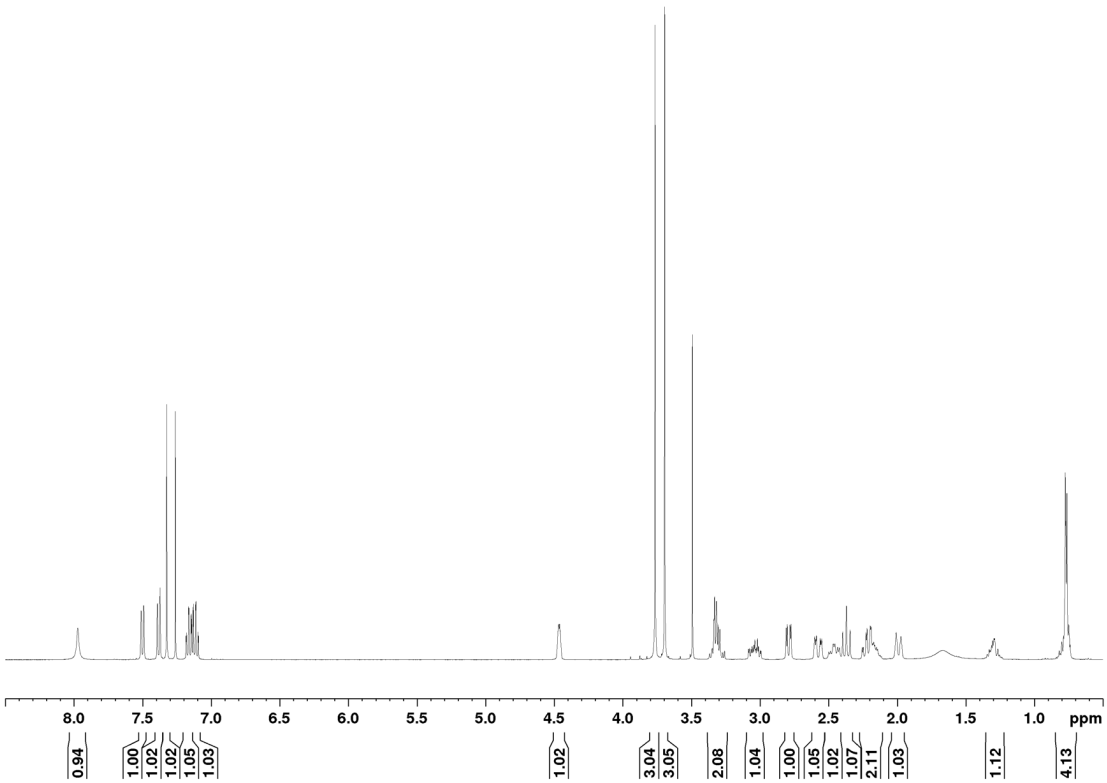
Supplementary Figure 3b. ^{1}H NMR spectrum of semisynthetic geissoschizine ($CDCl_3$, 298 K, 400 MHz). The compound was characterized by NMR when synthesized for the first time. A compound with identical retention time, HRMS and MSMS was obtained from a second experiment.



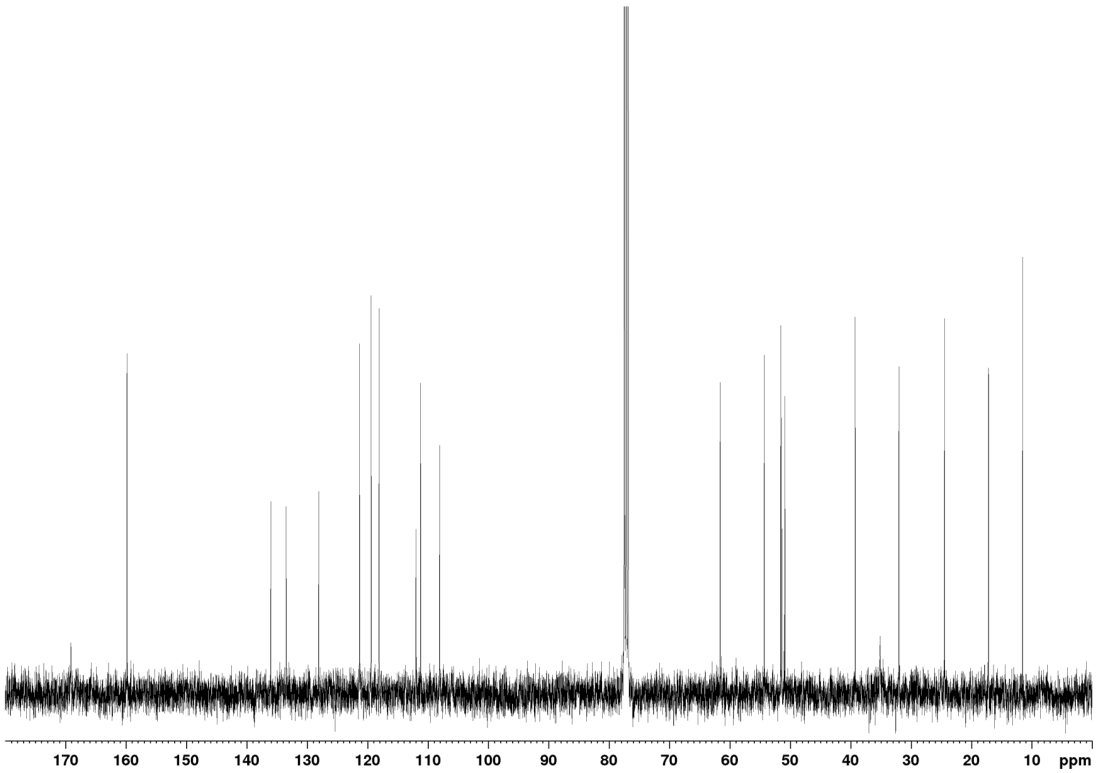
Supplementary Figure 3c. ^{1}H NMR spectrum of isolated hirsuteine ($CDCl_3$, 298 K, 400 MHz). The compound was characterized by NMR when isolated for the first time. A compound with identical retention time, HRMS and MSMS was also obtained from a second batch of plant material.



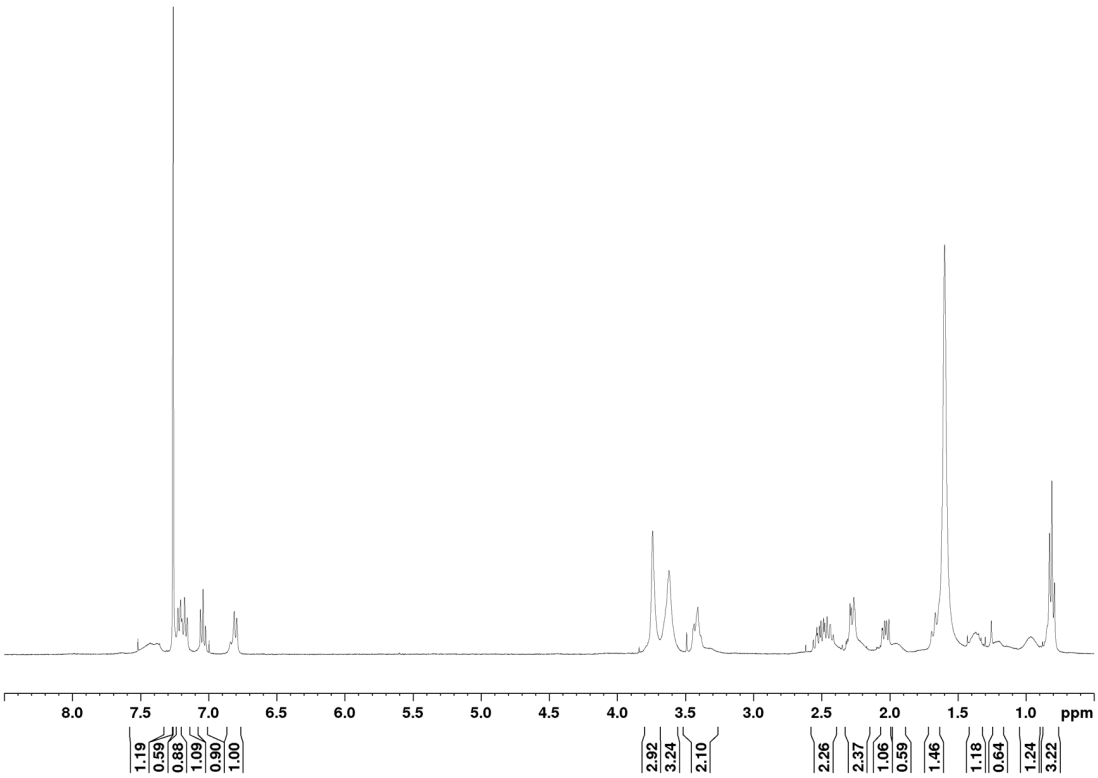
Supplementary Figure 3d. ^{13}C NMR spectrum of isolated hirsuteine ($CDCl_3$, 298 K, 100 MHz). The compound was characterized by NMR when isolated for the first time. A compound with identical retention time, HRMS and MSMS was also obtained from a second batch of plant material.



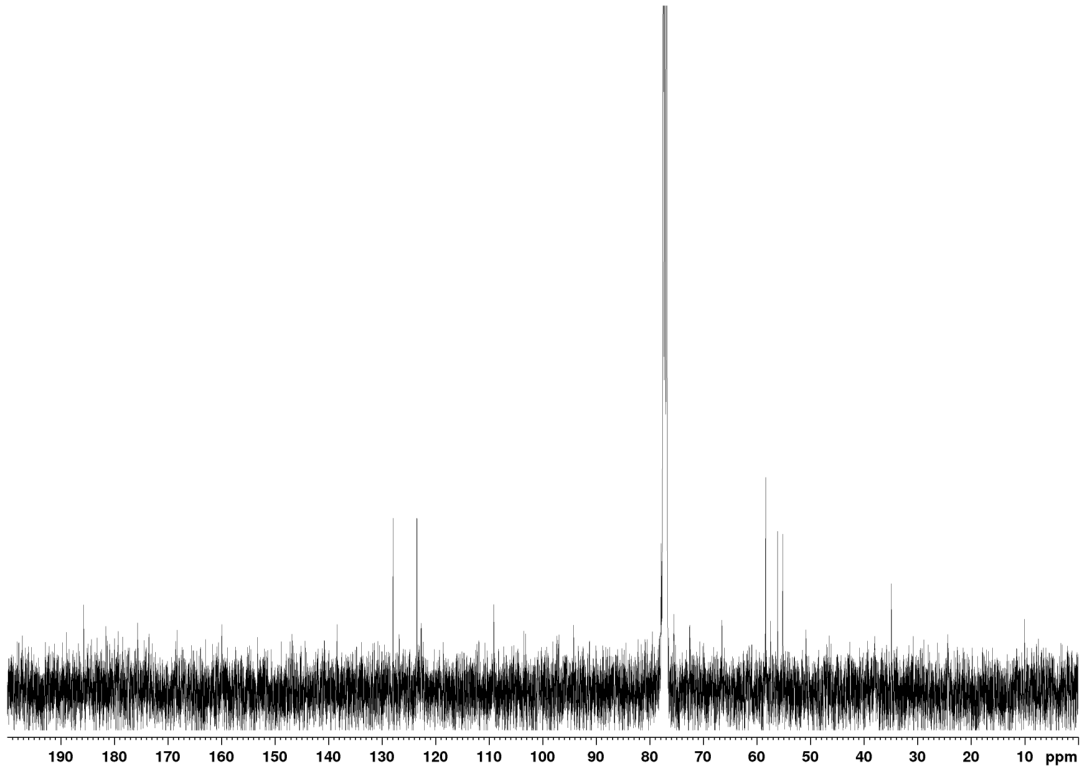
Supplementary Figure 3e. ^{1}H NMR spectrum of isolated hirsutine ($CDCl_3$, 298 K, 400 MHz). The compound was characterized by NMR when isolated for the first time. A compound with identical retention time, HRMS and MSMS was also obtained from a second batch of plant material.



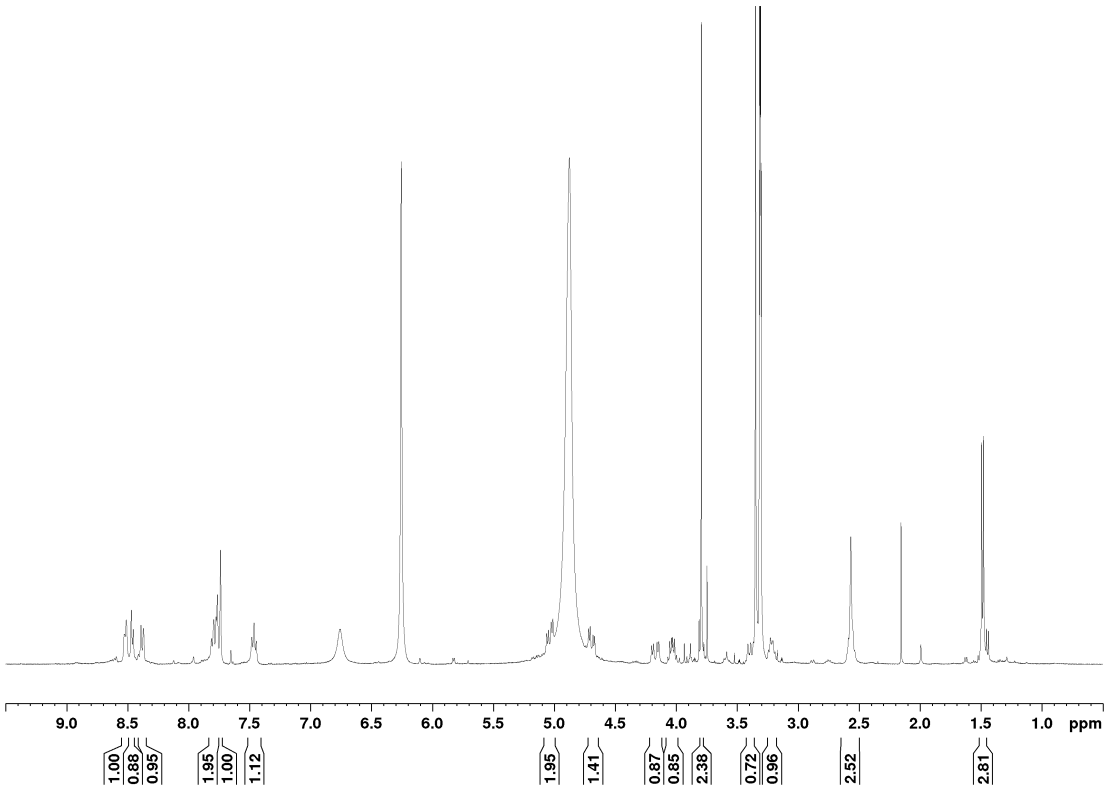
Supplementary Figure 3f. ^{13}C NMR spectrum of isolated hirsutine ($CDCl_3$, 298 K, 100 MHz). The compound was characterized by NMR when isolated for the first time. A compound with identical retention time, HRMS and MSMS was also obtained from a second batch of plant material.



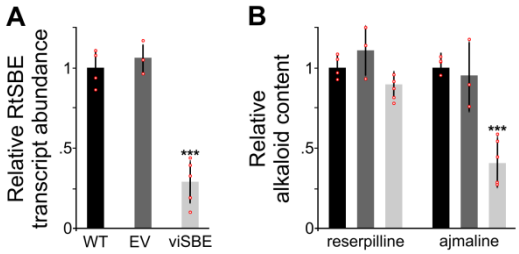
Supplementary Figure 3g. ^{1}H NMR spectrum of isolated rhynchophylline ($CDCl_3$, 298 K, 400 MHz). The compound was characterized by NMR when isolated for the first time. A compound with identical retention time, HRMS and MSMS was also obtained from a second batch of plant material.



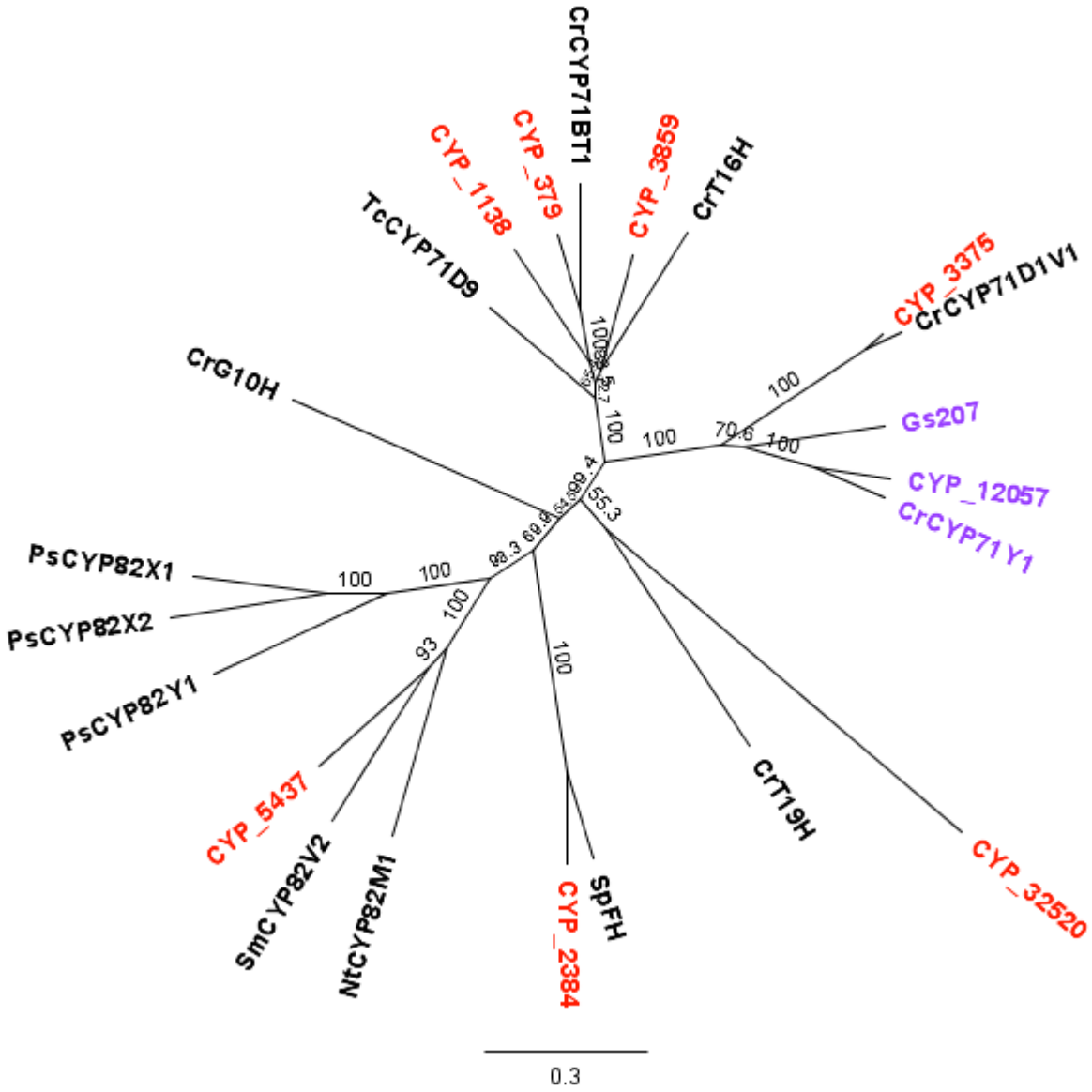
Supplementary Figure 3h. ^{13}C NMR spectrum of isolated rhynchophylline ($CDCl_3$, 298 K, 100 MHz). The compound was characterized by NMR when isolated for the first time. A compound with identical retention time, HRMS and MSMS was also obtained from a second batch of plant material.



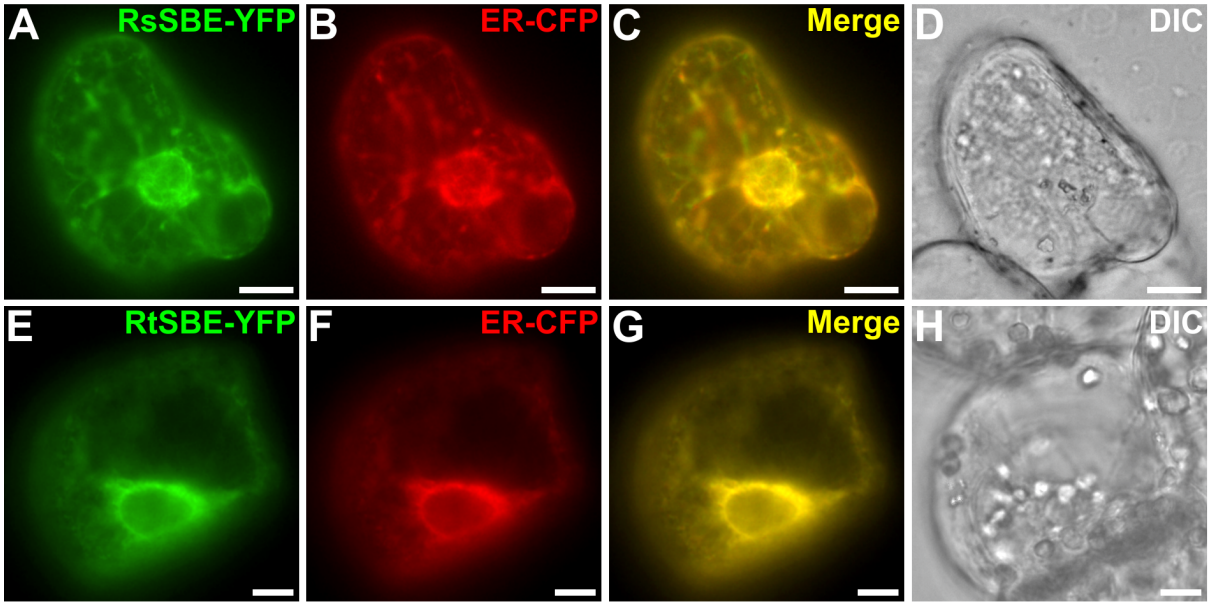
Supplementary Figure 3i. ^{1}H NMR spectrum of synthetic alstonine ($CDCl_3$, 298 K, 400 MHz). The compound was synthesized according to literature. NMR data was in agreement with reported data (see Supplementary Table 2).



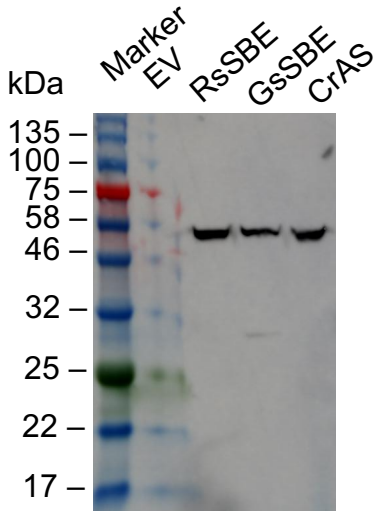
Supplementary Figure 4. Silencing of the *Rauwolfia tetraphylla* ortholog of SBE led to a specific decrease of the ajmaline content. Relative expression of SBE (A) and relative amount of reserpilline and ajmaline (B) in leaves of *R. tetraphylla* wild type plants (WT, black bars), plants transformed with pTRV2- empty vector (EV, dark grey bars) and plants transformed with pTRV2-RtSBE (viSBE, light grey). Each red dot represents a datapoint that is the mean of three technical replicates of one plant. Thus, red dots indicate individual average gene expression, reserpilline or ajmaline content. The silencing experiment was performed on four WT, three EV and five viSBE plants, with error bars indicating the mean ± SE for these biological (individual plant) replicates. Asterisks denote statistical significance (***$P < 0.001$, Two-sided Student's t-test, gene expression $P=3.02 \times 10^{-5}$; ajmaline $P=3.15 \times 10^{-4}$). Experiment was repeated in duplicate.



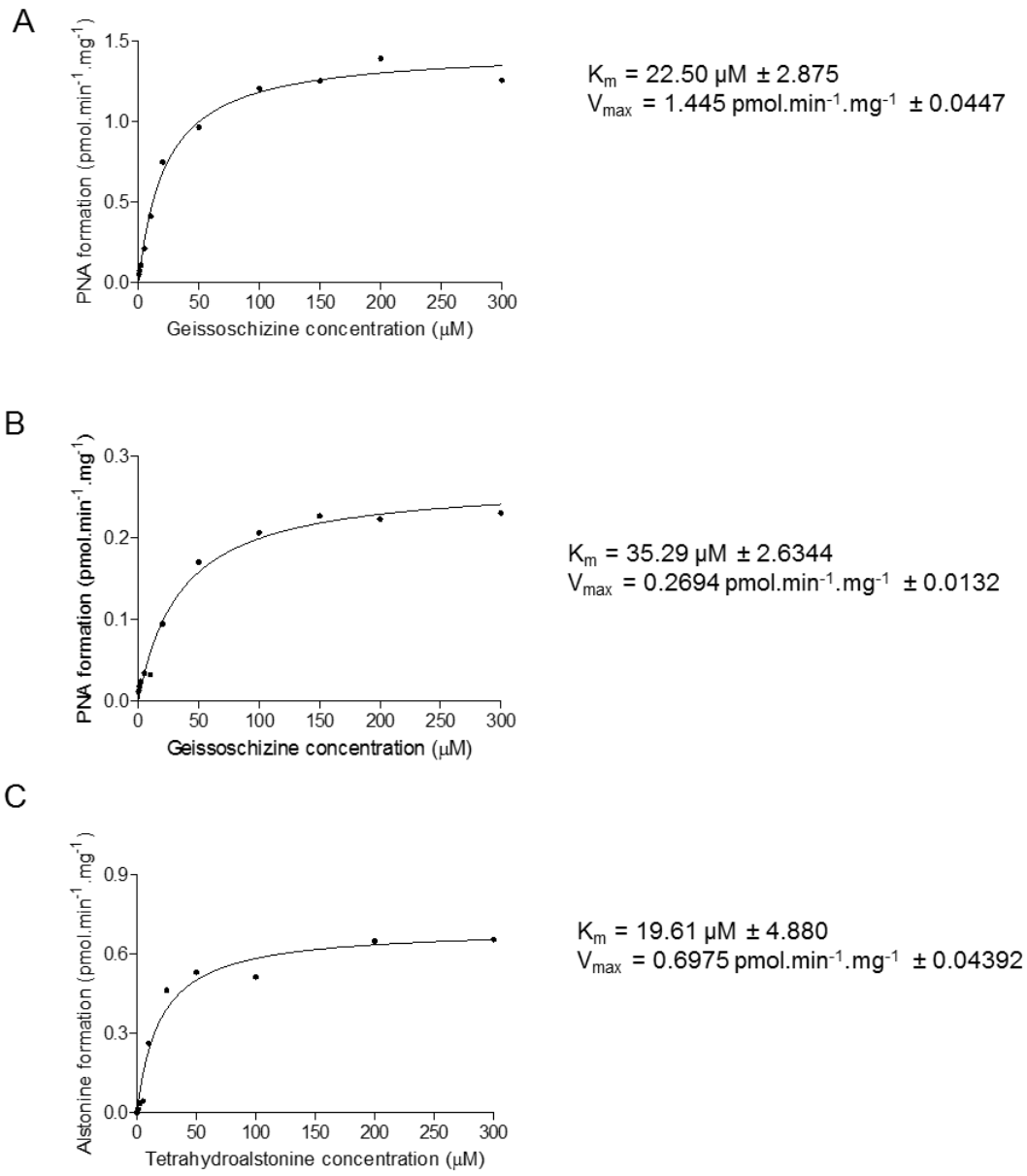
Supplementary Figure 5. Unrooted neighbour-joining phylogenetic tree for CYP candidates from this study (red and purple) and previously reported CYPs from other organisms. Active sarpagan bridge enzymes are shown in purple. Bootstrap frequencies for each clade were based on 1000 iterations. Phylogenetic tree was built using the Geneious Tree Builder program in the Geneious software package (Geneious 8.1.8, Biomatters).



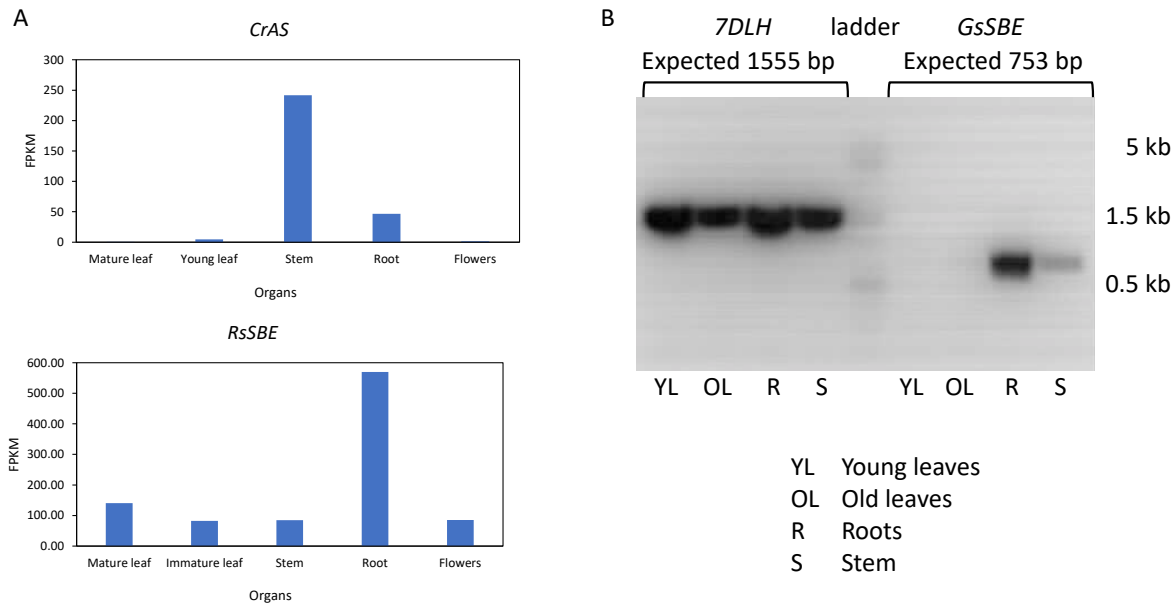
Supplementary Figure 6. Subcellular localization of SBE (*R. serpentina*, Rs; *R. tetraphylla*, Rt). (A-D) Subcellular localization of RsSBE YFP fusion protein in *C. roseus* cells. Cells were transiently co-transformed with plasmids expressing RsSBE-YFP (A), RtSBE (E) or an endoplasmic reticulum CFP marker (ER-CFP, B, F). Colocalization of the fluorescence signals appears in yellow when merging the two individual (green/red) false color images (C, G). Cell morphology is observed with differential interference contrast (DIC) (D, H). Bars, 10 µm. Transformation was repeated three times and for each more than 100 transformed cells were observed.



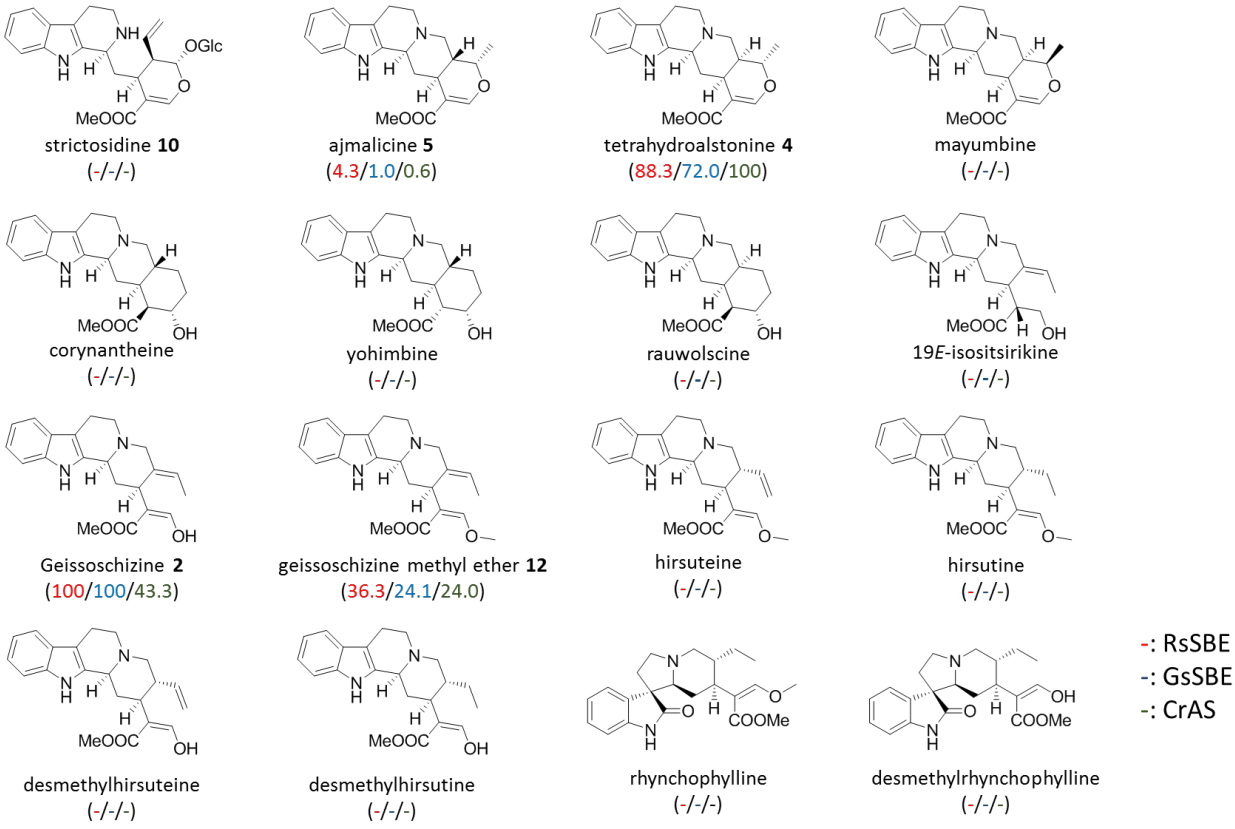
Supplementary Figure 7. Western blot showing the expression of RsSBE, GsSBE and CrAS (A) in *Saccharomyces cerevisiae* harbouring pESC-Leu2d::CPR (EV) and pESC-Leu2d::CYP/CPR. Protein expression was induced by adding galactose. Recombinant P450 proteins were detected using α-FLAG antibodies. Experiment were repeated two times independently with similar results.



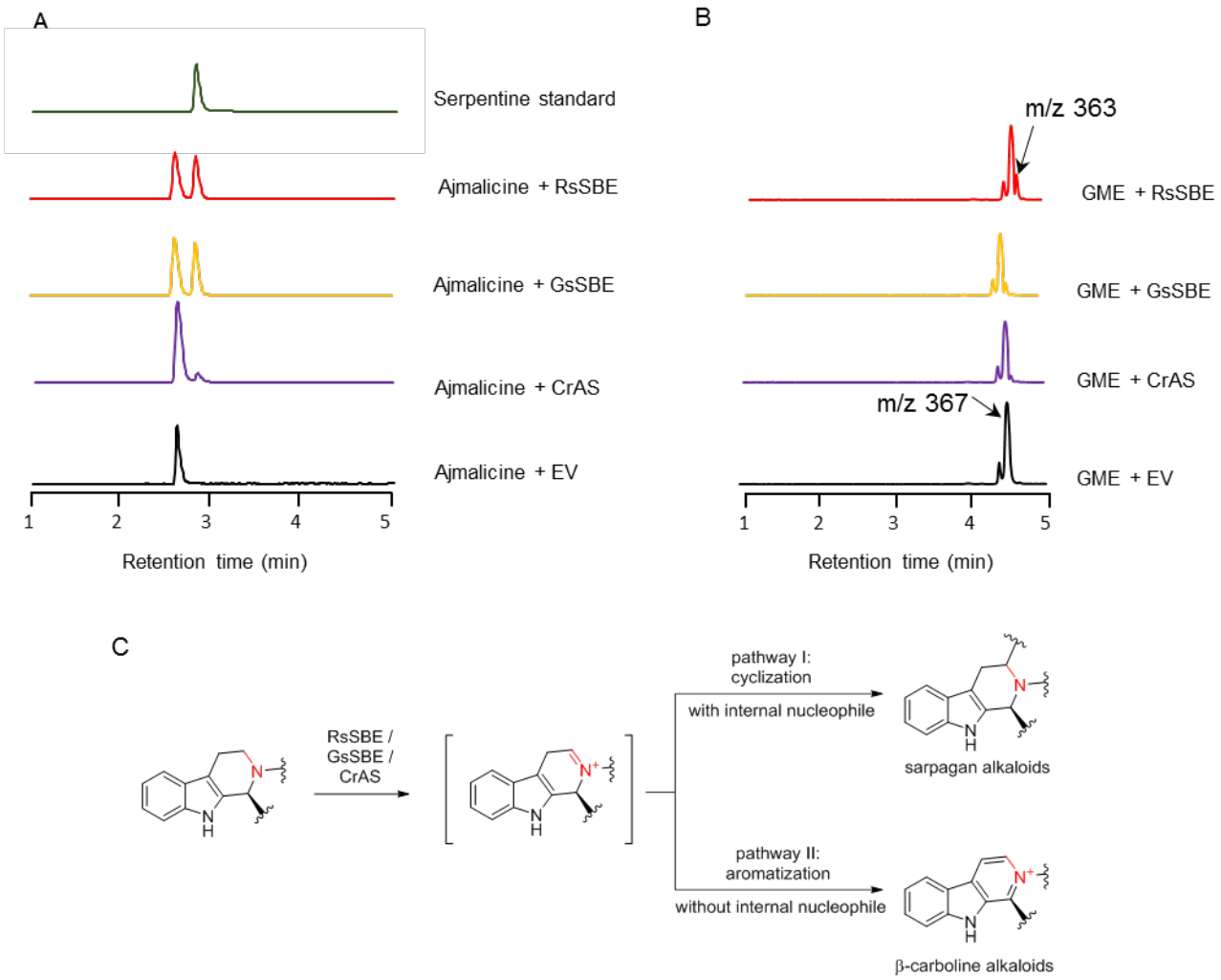
Supplementary Figure 8. Steady-state enzyme kinetics of identified enzymes in total microsomal protein extracts of *S. cerevisiae*. A. RsSBE with geissoschizine. B. GsSBE with geissoschizine. C. CrAS with tetrahydroalstonine. Data represent mean of three technical replicates.



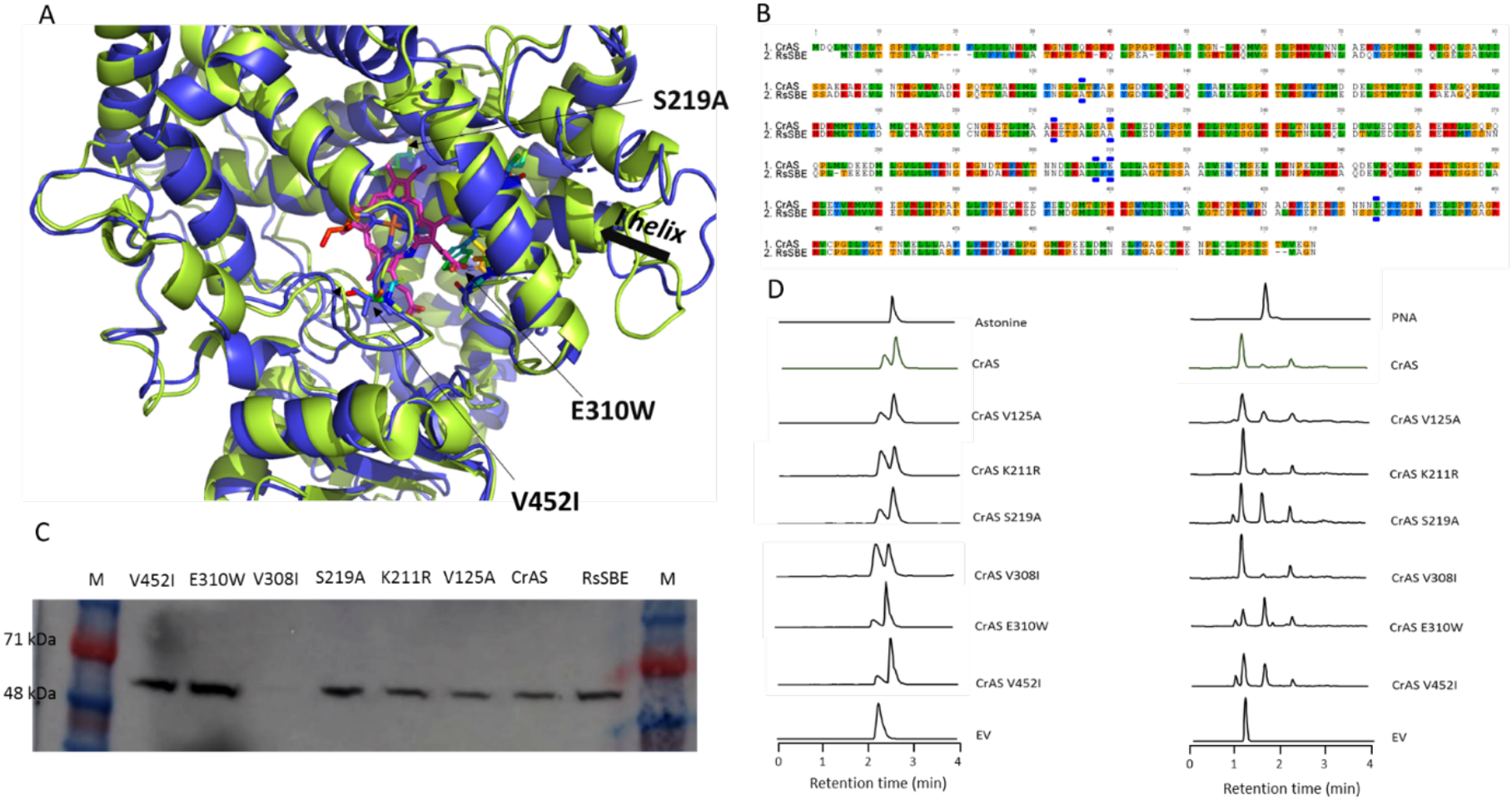
Supplementary Figure 9. Relative expression of *RsSBE*, *GsSBE* and *CrAS* in different organs. A. FPKM values of *CrAS* and *RsSBE* obtained from RNA-seq data (previously made available at http://medicinalplantgenomics.msu.edu/ as reported in reference 9). B. RT-PCR of *GsSBE* in comparison to 7DLH positive control. This experiment was not repeated, but is consistent with recently released RNA-seq data for *Gelsimium sempervirens* (reported in reference 11).



Supplementary Figure 10. Substrate specificity of RsSBE, GsSBE and CrAS. Substrates originate from different subgroups of MIA, including heteroyohimbanes (ajmalicine, tetrahydroalstonine, mayumbine, geissoschizine), yohimbanes (corynantheine, yohimbine, rauwolscine, isositssirikine), methyl ether alkaloids (geissoschizine methyl ether, hirsuteine, hirsutine and rhynchophylline) and their unmethylated semisynthetic derivatives (desmethylhirsuteine, desmethylhirsutine and desmethylrhynchophylline). Colors refer to the enzyme: red, RsSBE; blue, GsSBE; green, CrAS. Numbers indicate relative conversion of the substrate compared to the conversion of geissoschizine in case of RsSBE/GsSBE, or compared to tetrahydroalstonine in case of CrAS. "-" means no conversion.



Supplementary Figure 11. Catalytic activity of recombinant RsSBE, GsSBE and CrAS with additional substrates. Extracted ion chromatograms showing the catalytic activity of RsSBE (Rs_CYP12057), GsSBE (GsSBE) and CrAS (CrCYP71AY1). The negative control is empty vector (EV). RsSBE, GsSBE and CrAS oxidized ajmalicine to form serpentine and geissoschizine to form a compound with a mass and UV spectrum consistent with oxidized geissoschizine methyl ether. Whole cell yeast cultures expressing the corresposnding proteins were fed with the indicated substrate (10 µM) for 24 hours. **A.** Ajmalicine as substrate. **B.** Geissoschizine methyl ether (GME) as substrate. **C.** Unifying mechanism for the action of SBE and its *C. roseus* homologue AS with substrate with (pathway I) or without (pathway II) internal nucleophile. All experiments were repeated three times independently with similar results.


A
S219A
αhelix
E310W
V452I
B
1. CrAS
2. RsSBE
C
M
V452I
E310W
V308I
S219A
K211R
V125A
CrAS
RsSBE
M
71 kDa
48 kDa
D
Astonine
CrAS
CrAS V125A
CrAS K211R
CrAS S219A
CrAS V308I
CrAS E310W
CrAS V452I
EV
0
1
2
3
4
Retention time (min)
PNA
CrAS
CrAS V125A
CrAS K211R
CrAS S219A
CrAS V308I
CrAS E310W
CrAS V452I
EV
0
1
2
3
4
Retention time (min)

Supplementary Figure 12. Homology modeling and mutagenesis of CrAS. **A.** RsSBE and CrAS protein structure homology modeling using Phyre2, heme is highlighted in pink. **B.** Sequence alignment of CrAS and Rs SBE. Blue squares indicate mutations made in this study. **C.** Western blot showing expression of mutants. Protein expression was induced by adding galactose. Recombinant P450 proteins were detected using α-FLAG antibodies. This experiment was repeated two times independently with similar results. **D.** LC-MS chromatograms of assays with CrAS mutants with tetrahydroalstonine (left) and geissoschizine (right). This experiment was repeated three times independently with similar results.

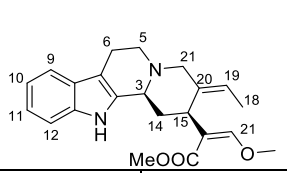
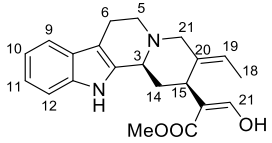
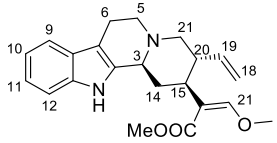
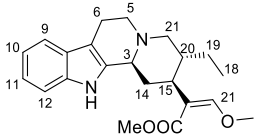
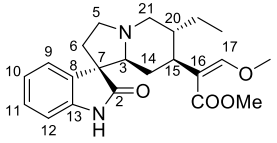
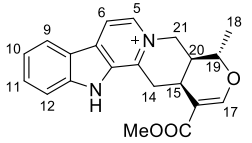
Supplementary Tables

Supplementary Table 1. Primers used for the assembly of expression vectors, creating mutants and for RT-PCR

Vector name	Forward primer (5'-3')	Reverse primer (5'-3')	Insert size (bp)
pESC-Leu2d-5437	ACC CTC ACT AAA GGG CGG CCG CAA CCA TGG ACT TAT TAC AAA TCT TA TT	GTC ATC CTT GTA ATC CAT CGA TAC AGG TTC ATA CAA GCA CTT GG CA	1575
pESC-Leu2d-379	ACC CTC ACT AAA GGG CGG CCG CAA CCA TGA TGG AGC TCA TCG TTC TCC TTT TTG	GTC ATC CTT GTA ATC CAT CGA TAC TTC CCC TTT GTA ATC AAC ATG AG	1527
pESC-Leu2d-3375	ACC CTC ACT AAA GGG CGG CCG CAA CCA TGA TGG AGT TCT CTT TCT CCT TTC C	GTC ATC CTT GTA ATC CAT CGA TAC TTT TGC AAG ATG AGG AAC CAG TTT	1503
pESC-Leu2d-12057	ACC CTC ACT AAA GGG CGG CCG CAA CCA TGG AGA TAA TGA ATT TCT CTC TCA AC	GTC ATC CTT GTA ATC CAT CGA TAC ATT TCC TGC AAC GGA GAT GCT G	1580
pESC-Leu2d-2384	ACC CTC ACT AAA GGG CGG CCG CAA CCA TGG CGT CTA TCT GTC TCT ACT TTC	GTC ATC CTT GTA ATC CAT CGA TAC AAT CTG AGC AAG TAG ATT GAG C	1536
pESC-Leu2d-1138	ACC CTC ACT AAA GGG CGG CCG CAA CCA CCA TGG AGC TCA TCT TTC TCT TCT	GTC ATC CTT GTA ATC CAT CGA TAC GCG ATC TCT TTT TTC AGA GCA GAA	1503
pESC-Leu2d-3859	ACC CTC ACT AAA GGG CGG CCG CAA CCA CCA TGG CAA TCA AGA TTG C	GTC ATC CTT GTA ATC CAT CGA TAC GCC CAT TTT GCA GAA GAA CAA GCA	1458
pESC-Leu2d-32520	ACC CTC ACT AAA GGG CGG CCG CAA CCA CCT TGT TCA TGA AGT CCA CCC TTG A	GTC ATC CTT GTA ATC CAT CGA TAC GCG CCT GAA GAA TTT CTC CT	1566
pESC-Leu2d-Gs_207-0.13	ACC CTC ACT AAA GGG CGG CCG CAA CCA TGC AGC TCT CTT TCT CCT ATC CTG	GTC ATC CTT GTA ATC CAT CGA TAC TGG AAC AAC AGT CTT GGG AA	1509
pESC-Leu2d-Cr71AY1	ACC CTC ACT AAA GGG CGG CCG CAA CCA TGG ATC AGC TGA TGA ACT TCT C	GTC ATC CTT GTA ATC CAT CGA TAC GTT TCC TTC AAC TAC AGT T	1464
7DLH-RT	ATG GGA GTA AAC TTC AAG TGG T	GTT TGT GCA AAA TTA AGT GAG CAC	1555
GsSBE-RT	CGA ACT GCC CCA TCA CAC	AAG ATC AAT TGC AAA ACG ATG GC	753
CrAS_V125A_	ACC TAA ACT ATT ATA CAA CAT GAT TTT AGC CAC AGT AGT	ATG TTG TAT AAT AGT TTA GGT GCC ACT TTT GCT CCC TAT GGT GA	
CrAS_K211R	AGC TGC CAT AAT TAG AGT CTC TCG TCC ATT A	AGA CTC TAA TTA TGG CAG CTA GAG AAA CGT CAG CTC TTT CT	

CrAS_S219A	AGC AGA AAG AGC TGA CGT TTC TTT AGC TGC CA	AAA GAA ACG TCA GCT CTT TCT GCT GCC ATT AGG ATT GAA GAT
CrAS_V308I	GGC CTT GAT GTC ATT GTT GGT GAC TCT AAA TTT	AAC AAT GAC ATC AAG GCC ATC ATT TTT GAA CTG ATT TTG GCT GGA
CrAS_E310W	AAA AAC GAT GGC CTT GAT GTC ATT GTT GGT G	ATC AAG GCC ATC GTT TTT TGG CTG ATT TTG GCT GGA ACT
CrAS_V452I	CCT TCT TCC AGC ACC AAA TGG TAT CAA CTC	TTT GGT GCT GGA AGA AGG ATT TGT CCT GGA ATA TTA TTT GGA AC

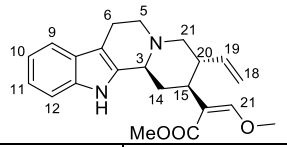
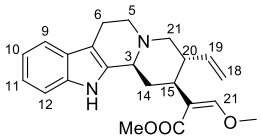
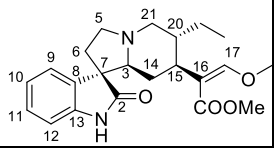
Supplementary Table 2. ^{1}H NMR data of isolated and semisynthetic alkaloids used in this study.

	Geissoschizine methyl ether 12 		Geissoschizine 2 		Hirsuteine 		Hirsutine 		Rhynchophylline 		Alstonine 7 	
No	**Reference**[1]	**^{1}H ($CDCl_3$, 298 K, 400 MHz)**	**Reference** [1]	**^{1}H ($CDCl_3$, 298 K, 400 MHz)**	**Reference** [2]	**^{1}H ($CDCl_3$, 298 K, 400 MHz)**	**Reference** [2]	**^{1}H ($CDCl_3$, 298 K, 400 MHz)**	**Reference**[3]	**^{1}H ($CDCl_3$, 298 K, 400 MHz)**	**Reference**[4]	**^{1}H (CD_3OD, 298 K, 400 MHz)**
3	3.52 (dd, 11.3, 2.0)	3.55 (br d, 11.9)	3.85 (dd, 11.6, 6.2)	3.85 (dd, 11.3, 6.5)	4.49 (br s)	4.50 (br s)	4.45 (d, 2.4)	4.46 (m)	2.27 (br d, 11.4)	2.28 (m)	-	-
5	3.07 (ddd, 11.1, 5.4, 3.2) 2.65 (ddd, 11.1, 9.6, 4.6)	3.08 (ddd, 11.1, 5.3, 3.0) 2.66 (ddd, 10.9, 9.8, 4.8)	3.21 (dd, 11.7, 5.4) 2.72 (ddd, 11.7, 11.7, 4.1)	3.22 (dd, 11.5, 5.3) 2.72 (ddd, 11.9, 11.9, 4.0)	3.32 (m)	3.33 (m)	3.32 (m)	3.32 (m)	3.40 (dd, 10.3, 8.2) 2.44 (dt, 10.3, 8.2)	3.40 (m) 2.46 (m)	8.53 (d, 6.5)	8.52 (d, 6.6)
6	2.98 (dddd, 15.0, 9.6, 5.4, 2.0) 2.73 (br d, 15.0)	2.99 (m) 2.74 (m)	3.07 (dddd, 15.6, 11.7, 5.4, 2.2) 2.82 (dd, 15.6, 4.1)	3.07 (m) 2.82 (br d, 15.7)	3.04 (dd, 12.0, 3.6) 2.59 (m)	3.05 (m) 2.60 (m)	3.02 (m) 2.59 (d, 11.4)	3.04 (m) 2.58 (dddd, 15.9, 4.7, 1.6, 1.6)	2.52 (ddd, 12.8, 10.3, 8.2) 2.02 (dt, 12.8, 8.2)	2.54 (m) 2.03 (m)	8.47 (d, 6.5)	8.46 (d, 6.4)
9	7.46 (dd, 7.8, 1.2)	7.47 (br d, 7.8)	7.48 (d, 8.0)	7.48 (d, 7.8)	7.52 (d, 8.4)	7.50 (d, 7.8)	7.52 (d, 7.8)	7.50 (d, 7.7)	7.20 (d, 7.3)	7.22 (d, 7.5)	8.39 (dt, 8.1, 1.1)	8.38 (d, 8.4)
10	7.07 (td, 7.8, 1.2)	7.08 (ddd, 7.4, 7.4, 1.2)	7.11 (td, 8.0, 1.1)	7.11 (ddd, 8.0, 7.0, 1.1)	7.14 (t, 7.2)	7.12 (ddd, 8.3, 7.2, 1.1)	7.13 (t, 7.8)	7.11 (ddd, 8.0, 7.2, 1.2)	7.03 (td, 7.7, 0.9)	7.04 (dd, 7.5, 7.5)	7.47 (ddd, 8.1, 6.8, 1.2)	7.46 (dd, 7.4, 7.4)
11	7.11 (td, 7.8, 1.2)	7.12 (7.5, 7.5, 1.3)	7.16 (td, 8.0, 1.1)	7.18 (ddd, 8.1, 7.0, 1.3)	7.21 (t, 7.2)	7.18 (ddd, 8.3, 7.1, 1.3)	7.18 (t, 7.8)	7.16 (ddd, 8.1, 7.1, 1.3)	7.17 (td, 7.7, 1.0)	7.18 (dd, 7.6, 7.6)	7.83-7.75, m, 2H	7.79 (dd, 7.6, 7.6)
12	7.26 (dd, 7.8, 1.2)	7.27 (ddd, 8.1, 0.9, 0.9)	7.31 (d, 8.0)	7.32 (d, 8.0)	7.43 (d, 7.2)	7.39 (d, 7.9)	7.42 (d, 7.8)	7.39 (d, 7.9)	6.59 (d, 7.6)	6.80 (d, 7.7)	7.83-7.75, m, 2H	7.75 (m)
14	2.33 (ddd, 12.5, 12.5, 11.3) 1.89 (ddd, 12.5, 5.0, 2.0)	2.35 (ddd, 12.5, 12.5, 11.9) 1.90 (ddd, 12.5, 5.0, 2.8)	2.65 (ddd, 13.7, 11.3, 6.2) 2.10 (ddd, 13.7, 11.6, 1.5)	2.61 (ddd, 13.5, 11.3, 6.2) 2.11 (ddd, 13.6, 11.9, 1.9)	2.48 (m) 2.02 (m)	2.50 (ddd, 13.1, 13.1, 5.2) 2.04 (ddd, 13.2, 2.8, 1.6)	2.47 (m) 2.04 (m)	2.46 (ddd, 13.0, 13.0, 5.1) 1.99 (ddd, 13.7, 2.3, 2.3)	2.00 (br) 1.20 (br)	1.95 (br) 1.20 (br)	4.18 (dd, 17.7, 6.3) 3.38 (dd, 17.7, 9.3)	4.17 (dd, 17.8, 6.0) 3.38 (dd, 18.1, 9.6)
15	3.70 (d, 12.5)	3.72 (m)	4.51 (dd, 11.3, 1.5)	4.49 (dd, 11.2, 1.3)	n.r. [a]	2.38 (ddd, 13.9, 11.0, 2.9)	2.22 (m)	2.21 (m)	2.23 (br)	2.23 (br)	3.22 (dtd, 9.2, 6.3, 1.1)	3.22 (ddd, 8.5, 6.5, 6.5)
17	7.35 (s)	7.36 (s)	7.85 (s)	7.83 (s)	7.29 (s)	7.28 (s)	7.33 (s)	7.32 (s)	7.25 (s)	7.26 (s)	7.75 (s)	7.74 (s)
18	1.55 (dt, 7.2, 1.4)	1.56 (ddd, 7.2, 1.3, 1.3)	1.82 (dd, 6.9, 1.7)	1.83 (ddd, 6.8, 2.4, 0.7)	5.00 (d, 16.8) 4.95 (d, 10.2)	4.93 (ddd, 17.2, 2.1, 0.8) 4.85 (dd, 10.3, 2.1)	0.77 (t, 7.2)	0.77 (m)	0.82 (t, 7.4)	0.81 (t, 7.4)	1.49 (d, 6.2)	1.49 (d, 6.3)
19	5.42 (br q, 7.2)	5.43 (br q, 7.2)	5.41 (br q, 6.9)	5.41 (br q, 6.8)	5.33 (dd, 10.8, 7.8)	5.36 (ddd, 17.2, 10.2, 8.5)	1.34 (m) 0.83 (m)	1.31 (m) 0.77 (m)	1.37 (br) 0.96 (br)	1.37 (br) 0.96 (br)	2.57 (dq, 9.6, 5.8)	2.56 (m)

20	-	-	-	-	2.99 (m)	2.99 (m)	n.r. [a]	2.19 (m)	2.28 (br dd, 11.4, 3.3)	2.27 (m)	4.03 (dq, 9.7, 6.2)	4.03 (dddd, 9.5, 6.3, 6.3, 6.3)
21	3.44 (d, 12.5) 3.16 (dd, 12.5, 1.0)	3.44 (d, 12.3) 3.17 (br d, 12.1)	3.96 (dt, 13.4, 2.4) 3.18 (d, 13.4)	3.95 (ddd, 13.7, 2.3, 2.3) 3.18 (d, 13.7)	2.67 (m)	2.68 (dd, 11.2, 4.4) 2.59 (m)	2.82 (m)	2.79 (dd, 11.0, 3.4) 2.37 (dd, 10.7, 10.7)	3.42 (dd, 10.6, 3.3) 1.66 (t, 10.6)	3.45 (m) 1.67 (m)	5.04 (dd, 14.4, 5.9) 4.70 (dd, 14.5, 5.2)	5.04 (dd, 14.3, 6.0) 4.69 (dd, 14.5, 5.2)
O**Me**	3.82 (s)	3.83 (s)	-	-	3.79 (s)	3.76 (s)	3.78 (s)	3.76 (s)	3.71 (s)	3.74 (s)	-	-
COO**Me**	3.72 (s)	3.72 (s)	3.69 (s)	3.67 (s)	3.65 (s)	3.68 (s)	3.69 (s)	3.69 (s)	3.61 (s)	3.62 (br s)	3.80 (s)	3.79 (s)
NH	7.79 (br s)	7.69 (br s)	7.97 (br s)	7.68 (br s)	8.32 (s)	7.98 (br s)	n.r. [a]	7.97 (br s)	8.52 (br s)	7.42 (br s)	-	

[a] not reported

Supplementary Table 3. ^{13}C NMR data of major isolated alkaloids used in this study.

	Hirsuteine 		Hirsutine 		Rhynchophylline 	
No	**Reference** [5]	**^{13}C ($CDCl_3$, 298 K, 100 MHz)**	**Reference** [6]	**^{13}C ($CDCl_3$, 298 K, 100 MHz)**	**Reference** [3]	**^{13}C ($CDCl_3$, 298 K, 100 MHz)**
2	132.7	133.0	133.1	133.5	181.6	n.d.[a]
3	54.1	54.2	54.2	54.3	75.3	75.3
5	51.2	51.3	51.4	51.6	55.0	55.0
6	17.0	17.1	17.0	17.2	34.8	34.7
7	107..8	108.1	107.8	108.1	55.1	n.d.[a]
8	127.8	128.1	127.9	128.1	133.9	133.8
9	117.9	118.2	118.0	118.1	123.1	123.3
10	119.3	119.6	119.3	119.4	122.4	122.5
11	121.3	121.5	121.3	121.4	127.7	127.7
12	111.2	111.3	111.2	111.2	109.4	109.0
13	136.0	136.1	135.9	136.0	141.1	140.9
14	31.1	31.3	31.7	32.0	29.0	29.0
15	34.0	34.2	34.9	35.2	37.9	37.9
16	111.6	111.9	111.7	112.0	111.9	112.0
17	159.6	159.7	159.8	159.9	159.7	159.4
18	115.4	115.5	11.4	11.5	11.3	11.2
19	139.3	139.5	24.3	24.4	24.2	24.2

20	42.9	43.2	39.0	39.2	39.7	39.6
21	51.2	51.3	50.7	50.9	58.2	58.2
O**Me**	61.4	61.6	61.5	61.6	61.4	61.5
COOMe	168.7	168.9	169.0	169.1	169.0	169.0
COO**Me**	51.2	51.4	51.3	51.4	51.1	51.1

[a] not detected by HMBC

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Supplementary Table 4. Chromatographic and spectral data used for the identification and relative quantification of enzymatic products by LC-MS/MS.

Compound	found $[M]^+$ or $[M+H]^+$	expected $[M]^+$ or $[M+H]^+$	Retention time (min)	Cone voltage (eV)	Collison energy (eV)	ESI-CID, *m/z*	Source of reference compound
Alstonine	349.1612	349.1547	2.84	36	50	206.02	Semi-synthetic
					31	234.96	
					30	263.06	
					26	317.10	
Polyneuridine aldehyde	351.1707	351.1703	2.23	26	21	158.2	Gift
					14	166.0	
					19	319.0	
					16	323.0	
Ajmalicine	353.1942	353.1860	2.76	50	46	117.2	Commercial
					26	144.0	
					20	210.1	
					20	222.0	
Tetrahydroalstonine	353.1942	353.1860	2.76	50	40	114.16	Commercial
					40	117.19	

Serpentine	349.1612	349.1547	2.86	54	54	205.88	Commercial
					24	234.95	
					30	262.98	
					24	317.12	
Geissoschizine	353.1860	353.1860	1.77	50	40	114.16	Semi-synthetic
					40	117.19	
Geissochizine methyl ether (GME)	367.2035	367.2016	4.56				Isolated