

Supplemental Data

Giddings et al. - “A stereoselective hydroxylation step of alkaloid biosynthesis in *Catharanthus roseus*”

```

Locus_88716      AAACAAC TAGATTTCTCTAGCAAGAAAAATATGTTGTCTTCATTGAAAGATTTCTTCGTT
Locus_88716      CTACTTCTGCCTTTCTTCATTGGCATTGCATTTATATATAAATATGGAATTTTACATCC
Locus_88716      AAGAAAAATCTTCCGCCATCGCCAAGAAGGCTACCGATTATCGGAAACCTTCATCAGCTC
Locus_88716      AGTAAATTTCTCAACGTTCACTGAGAACATTATCAGAAAAATATGGGCCTGTCATGTTA
Locus_88716      CTCATTTTGGTAGCAAGCCAGTTTTGGTTATATCCTCAGCAGAAGCAGCTAAAGAAGTA
Locus_88716      ATGAAAATCAATGATGTTTCTTCGCTGATAGGCCTAAATGGTATGCTGCTGGTAGGGTT
Locus_88716      CTTTATGAATTTAAGGATATGACATTTTCACCCATGGTGAGTATTGGAGACAAGCTAGA
Locus_87898      GGTGAGTATTGGAGACAAGCTAGA
Locus_87898      AGTATATGTGTTCTTCAGCTTCTAAGTAACAAAAGGGTTCAATCTTTTAAAGGTATTAGA
Locus_87898      GAAGAAGAGATAAAGGCAATGTTGGAATAAATAAATCAAGCTAGTAATAATTCAGTATC
Locus_87898      ATTAATGGTGATGAGATTTTCTCGACACTTACAAATGACATAATTGGAAGATCTCCTTTT
Locus_87898      GGGAGGAAATTTTCTGAAGAAGAAAGTGAAGTAAATTAAGAAAAGTCTTCAAGATTTG
Locus_87898      CCACCTTGTAGGTTCTTTCAATGTTGGTGATTTTATTCCATGGCTTCTTGGGTTAAT
Locus_87898      TATTTAAATGGTTTTGAAAAAAATGAACCAAGTTTCAAAAGATTGTGATCAATATCTT
Locus_87898      GAGCAAGTTATTGATGATACGAGGAAGAGAGATGAAGAAAATGGAGCTAATAACAATGGC
Locus_87898      GGAAATCATGGAAATTTTGTAGTGTCTACTTCATCTCCAGAAAGAAGATGTCAAGGGT
Locus_87898      TTTCCTTCAGAAAAGATTTTCTTAAAGCTATCATTTTGGACATGATGTTGGGGGAACA
Locus_87898      GACACAACACATTTGCTTTTGCATTGGGTAATCACAGAGCTTCTCAAAAACAAAGATGTA
Locus_87898      ATGACTAAATTACAAAAGAGGTGAGAGAAATAGTCGGAAGAAAATGGAGATAACTGAT
Locus_87898      GAAGACAAAGAAAAATGAAATATTTACACGCAGTTATAAAAGAGGCACTCCGGTTACAT
Locus_87898      CCTTCTCTCCTTTACTCGTTCACAGAGTAGCAAGAGAGGATATAAACCTAATGGGTTAT
Locus_87898      CGCGTAGCGAAAGGCACAGAAGTGATTATTAATGCTTGGGCAATAGCGAGAGATCCATCG
Locus_87898      TACTGGGATGAAGCCGAGGAGTTAAGCCGGAGAGATTCTTGAGTAATAATTTGATTTT
Locus_87898      AAAGGACTTAATTTGAGTATATCCATTTGGTCTGGAAGAAGAAGTTGCCCTGGATCT
Locus_87898      TCATTTGCAATCCCATTGTAGAACACACAGTGGCACATTTGATGCATAAATTCATATT
Locus_87898      GAGTTGCCAAATGGAGTCAGTCTGAAGATTTTGTCCACAGATGCCGTTGGACTCGTA
Locus_87898      TCTCATGACCAAAACCTCTCTCGTTTTGTGGCAACTCCGGTTACCATTTTTACTTTT
Locus_87898      CACTCGGTGCTAAGTAAAAGGTTCAAATAGTTTA

```

Figure S1. Assembly of Locus_88716 and Locus_87898 to form SOC-001 contig. Overlapping region is in bold. Start codon is highlighted in green. Stop codon is highlighted in red.

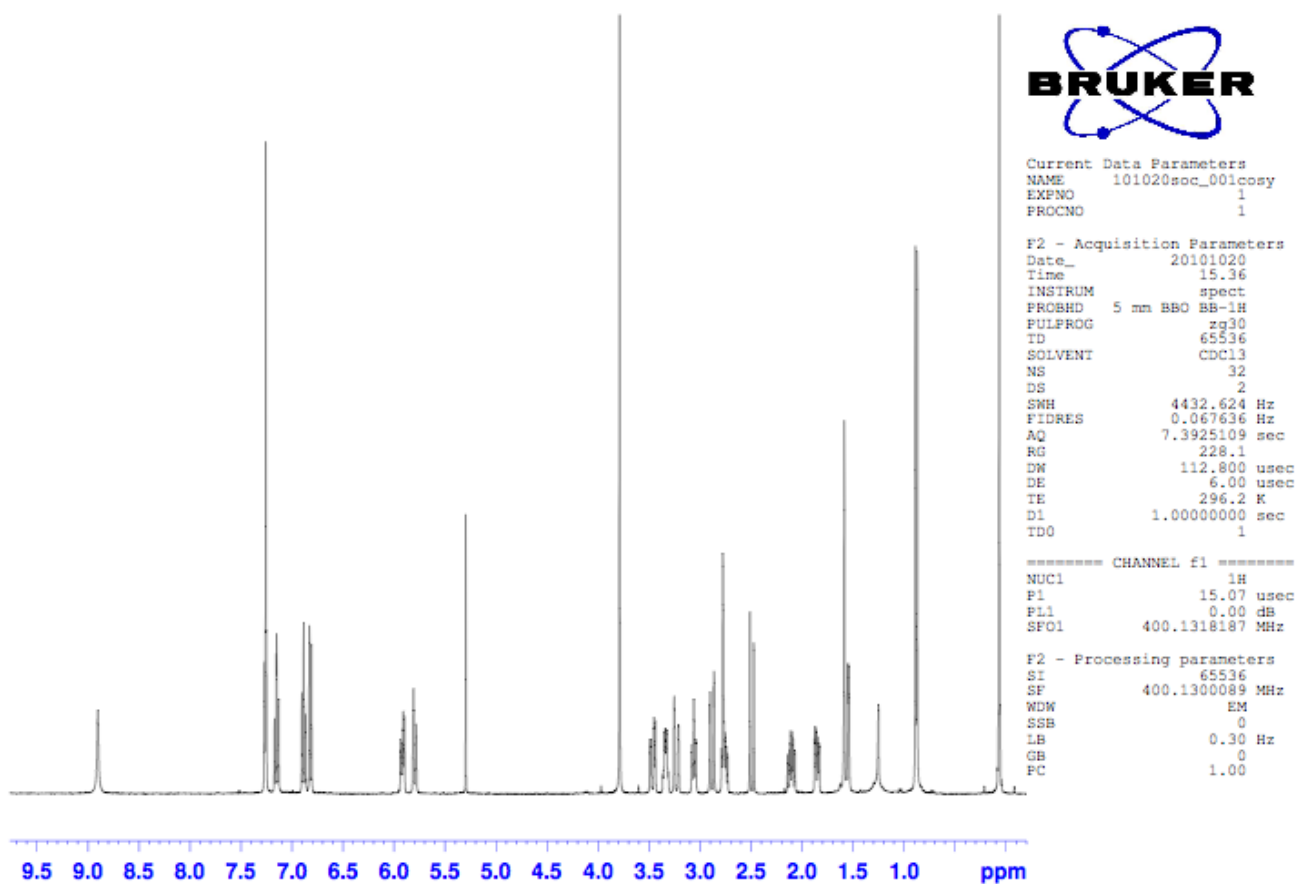


Figure S2. ¹H NMR spectrum of 19-hydroxytabersonine.

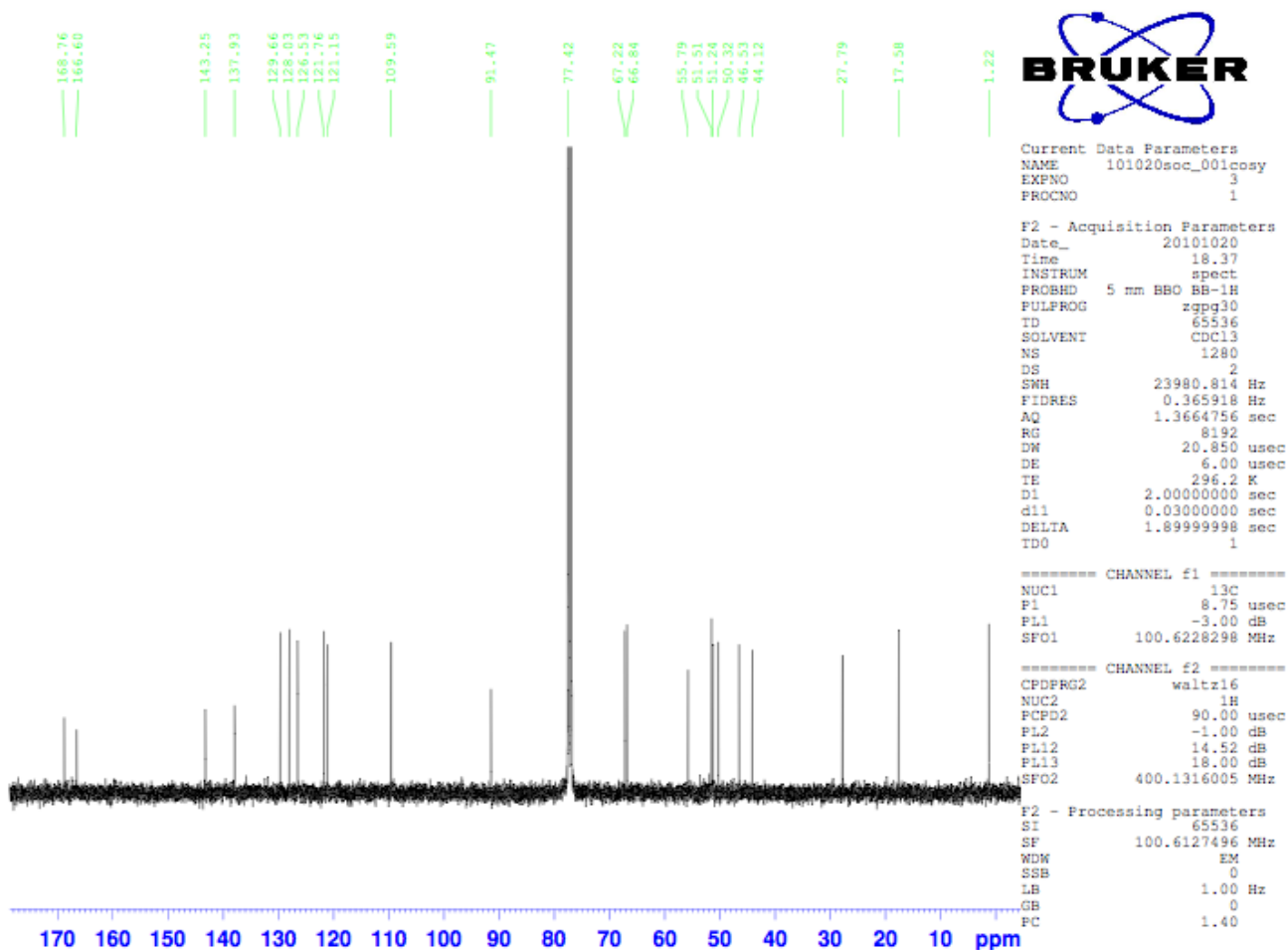


Figure S3. ¹³C NMR spectrum of 19-hydroxytabersonine.

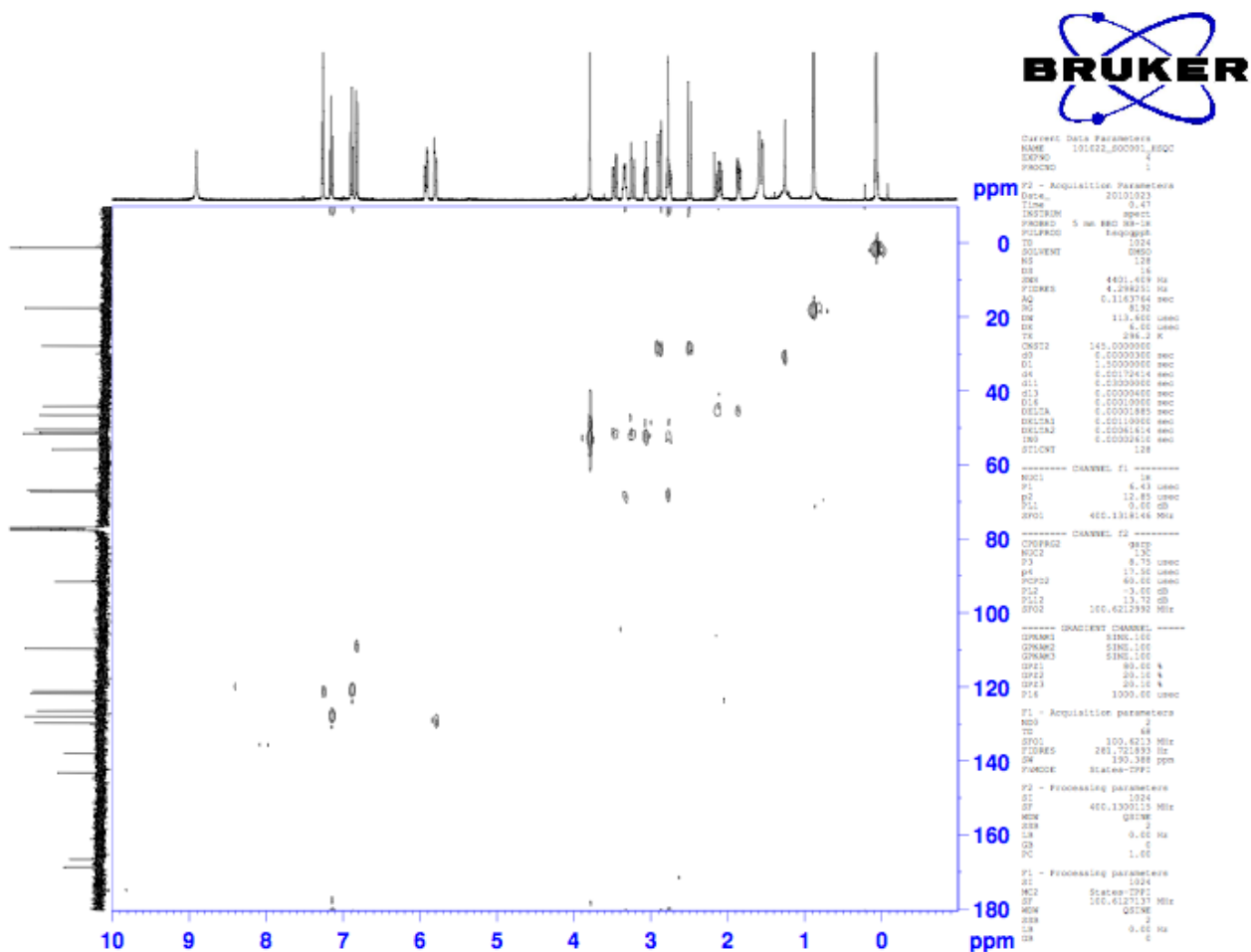


Figure S4. ^1H , ^{13}C HSQC spectrum of 19-hydroxytabersonine.

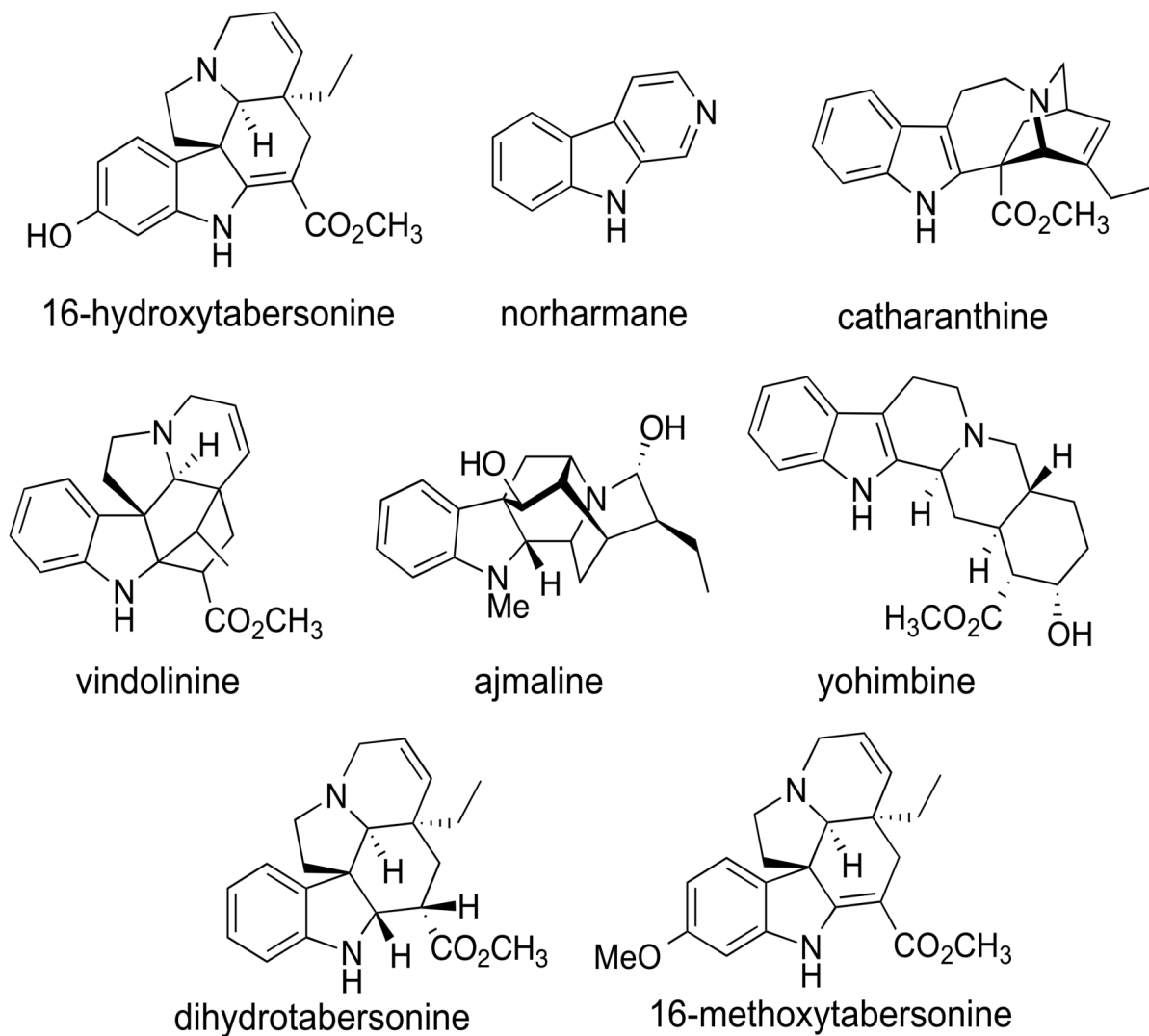


Figure S5. Substrates that were not turned over by enriched CYP71BJ1 microsomes

Supplemental Data - Giddings et al.

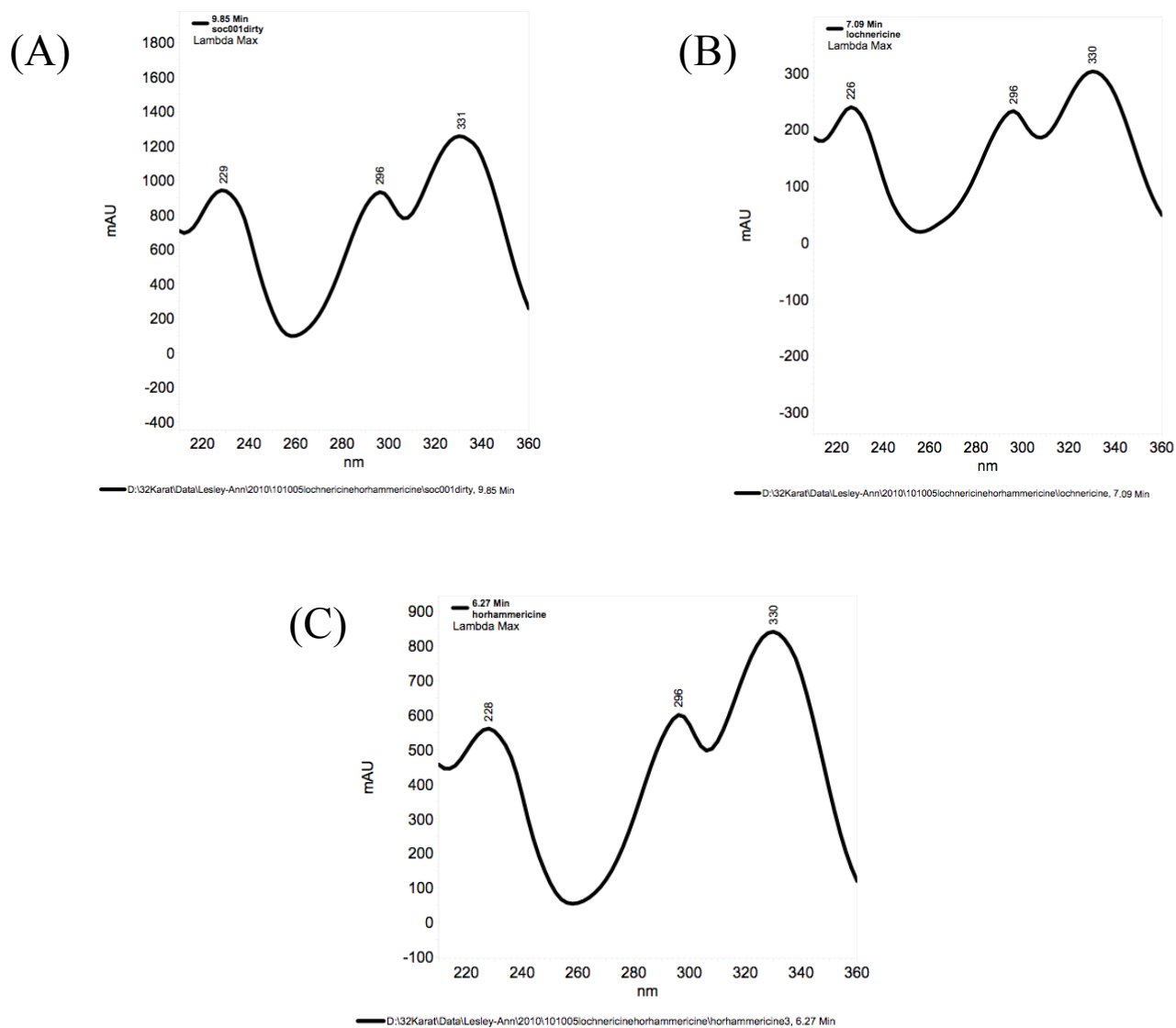


Figure S6. UV spectra of 19-hydroxytabersonine (A), lochnericine (B), and horhammericine (C) isolated from *C. roseus* hairy root cultures.

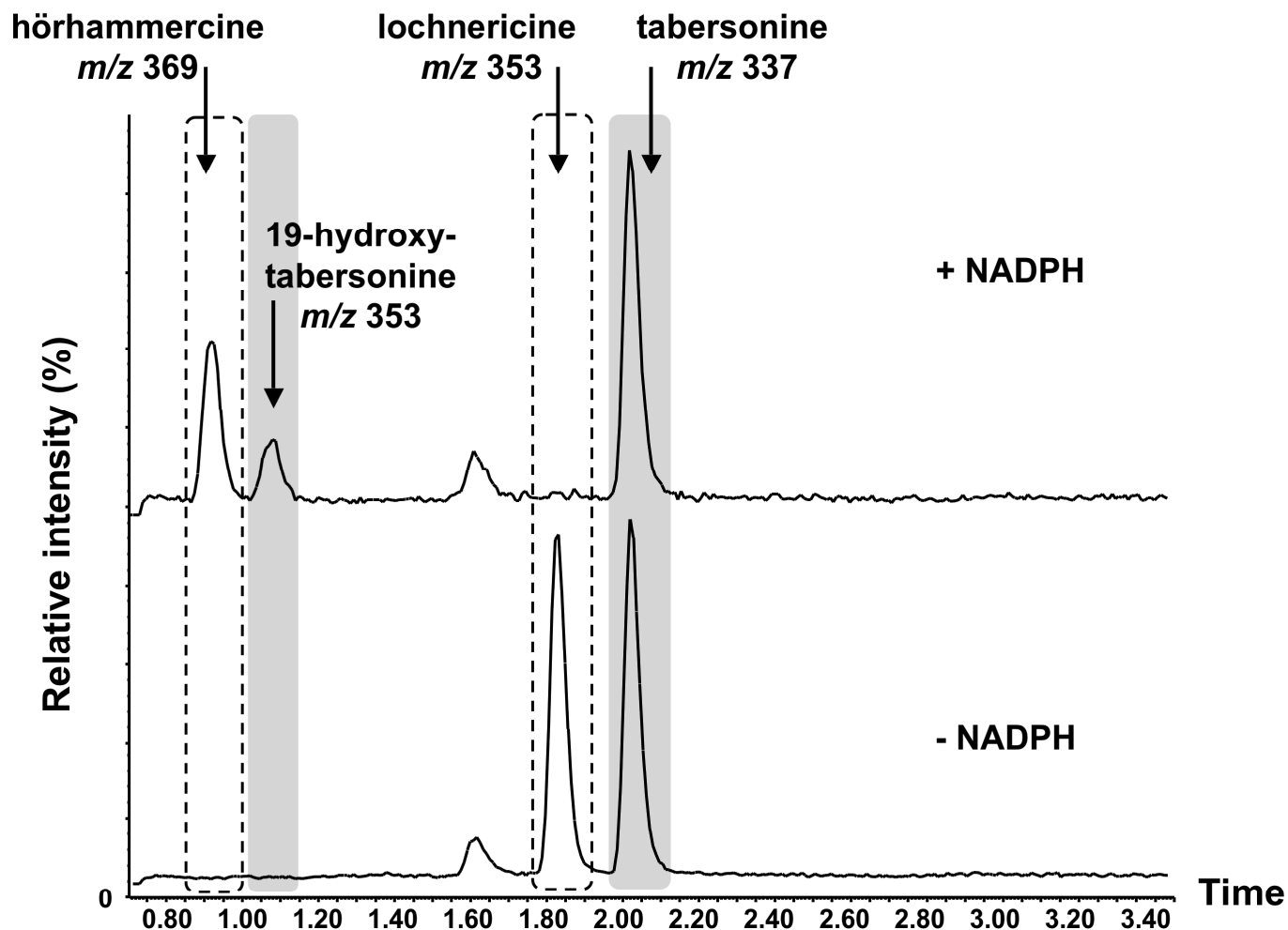


Figure S7. Incubation of microsomes containing CYP71BJ1 with a 1-1 mixture of tabersonine and lochnericine (55 μ M concentration) in the presence and absence of NADPH. Samples were incubated for 15 hours at 30°C; conditions were as reported in Materials and Methods. Assays were analyzed by LC-MS.

Table S1. Exact mass data for compounds assayed with CYP71BJ1.

Compound	Calculated Mass	Observed Mass	Error
19-hydroxytabersonine	353.1860	353.1848	0.0012
lochnericine	353.1860	353.1866	0.0006
hörhammericine	369.1809	369.1804	0.0005
16-hydroxytabersonine	353.1860	353.1857	0.0003
16-methoxytabersonine	367.2016	367.2006	0.0010