Supplementary Material
on

Crystal structure and biochemical analysis of a cytochrome P450 CYP101D5 from *Sphingomonas echinoides*

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Keywords: α/β-ionone; crystal structure; cytochrome P450; X-ray crystallography

Figure S1. (A) SDS-PAGE analysis of purified CYP101D5 (lane 1, 45.97 kDa) and the redox partners PxR (lane 2, 58 kDa) and Pdx (lane 4, 10 kDa), where M is the standard protein marker with the size of the protein marker presented in kilodaltons (kDa). (B) Absorbance spectra of the carbon-monoxide-bound and dithionite reduced forms of CYP101D5.

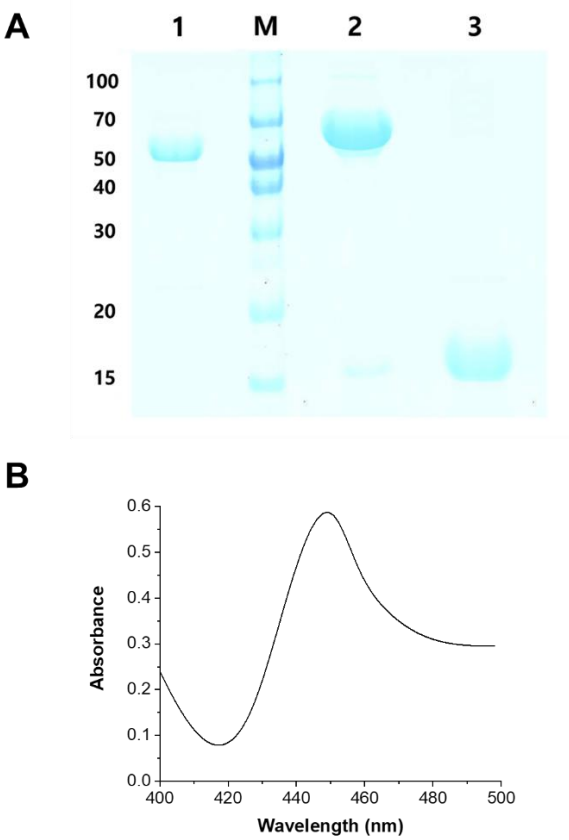


Figure S2. Gas chromatogram (GC) spectra of β-ionone conversion by CYP101D5. GC of (A) the control reaction and (B) reaction mixture. The peak (t_{RS} = 13.028 min) is of β-ionone, and the product peak (t_{RP} = 14.134 min) was identified as 4-hydroxy-β-ionone. The structures of both the substrate and product are shown. (C) (D) The mass spectra of the peak and library hits for both the substrate and product are presented.

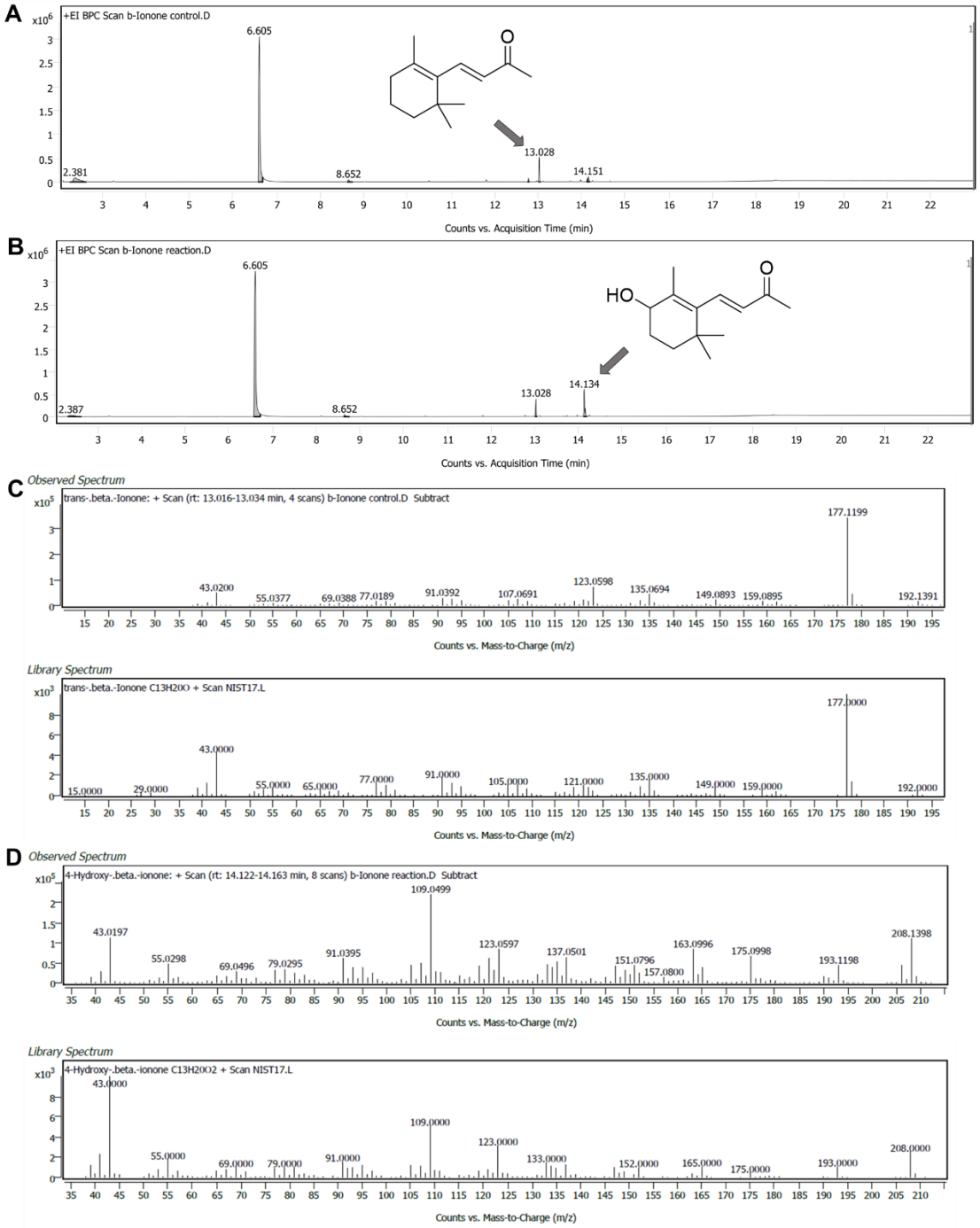
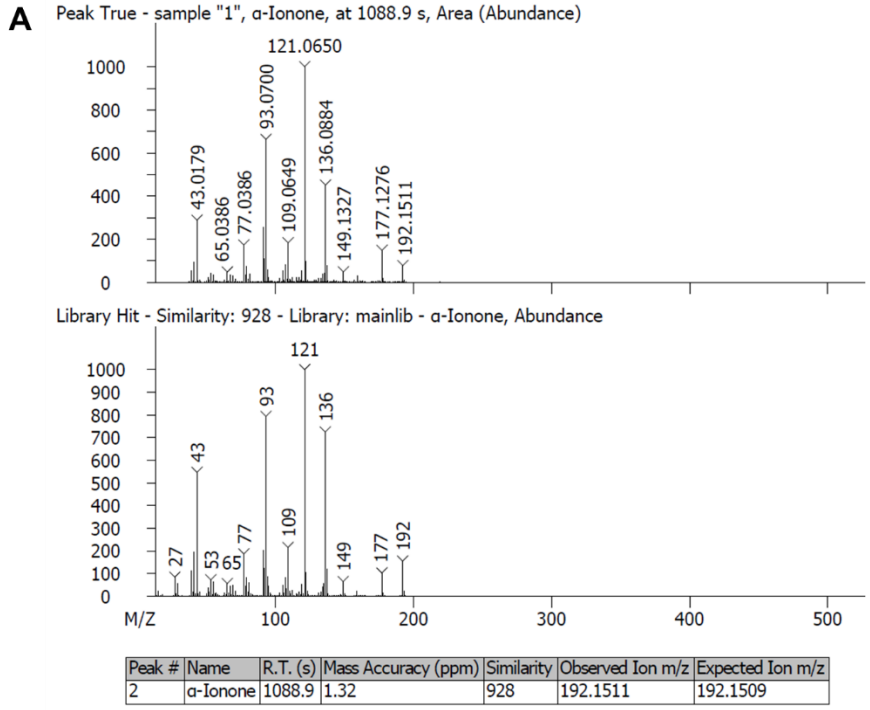
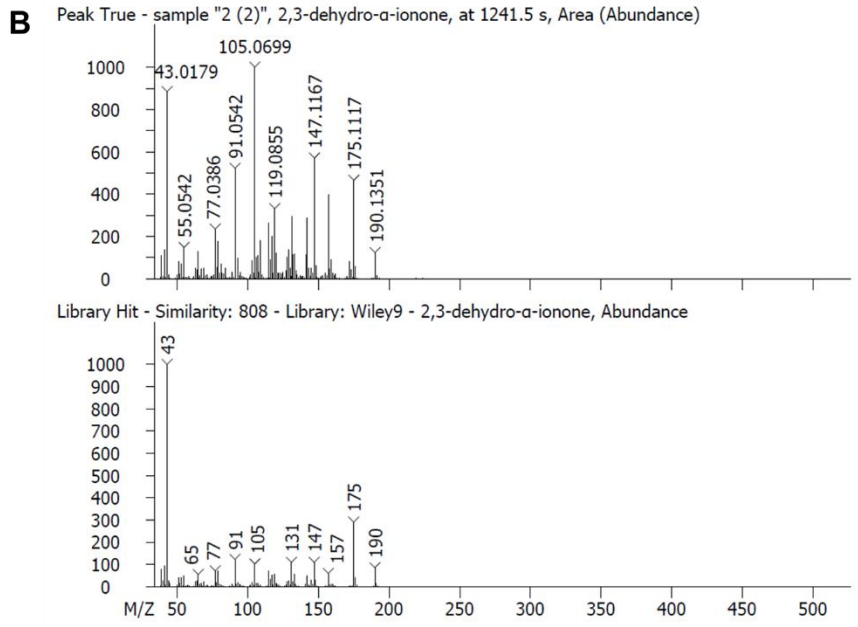


Figure S3. MS spectra of both the substrate (α-ionone) (A) and product (2,3-dehydro-α-ionone) (B) peaks. The observed mass spectrum of the peak and library spectrum for both the substrate and product are presented.

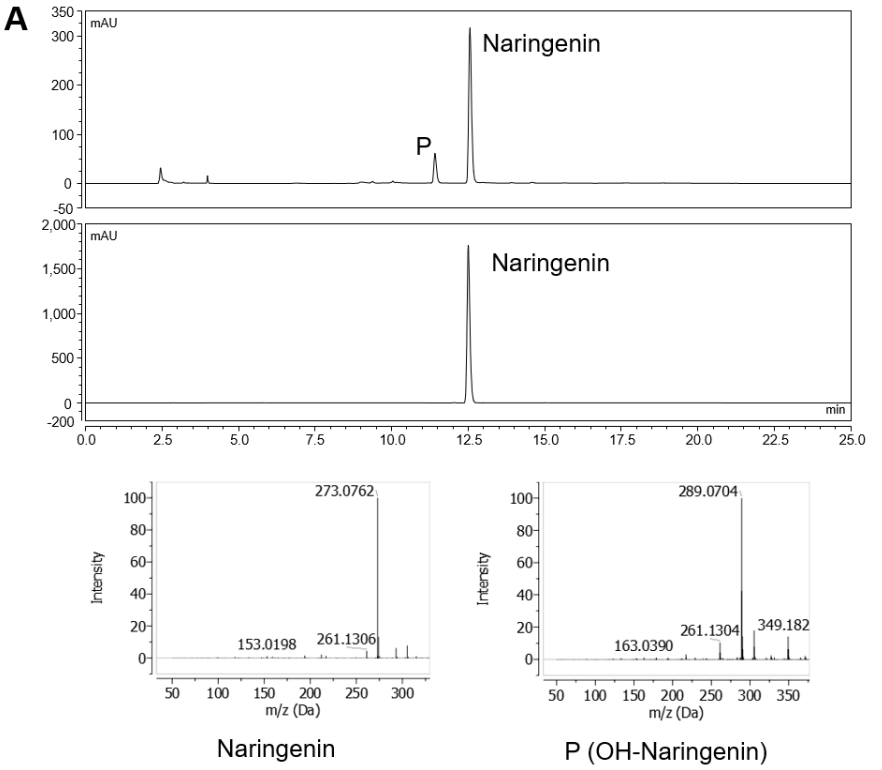


Peak #	Name	R.T. (s)	Mass Accuracy (ppm)	Similarity	Observed Ion m/z	Expected Ion m/z
2	α-Ionone	1088.9	1.32	928	192.1511	192.1509



Peak #	Name	R.T. (s)	Mass Accuracy (ppm)	Similarity	Observed Ion m/z	Expected Ion m/z
2	2,3-dehydro-α-ionone	1241.5	-0.71	808	190.1351	190.1352

Figure S4. HPLC-PDA and HR-QTOF ESI/MS analyses of the reaction products. The product of naringenin (A) and apigenin (B) is denoted by P in their respective HPLC chromatograms. The mass spectra of the substrate and their corresponding hydroxylated product are also shown.



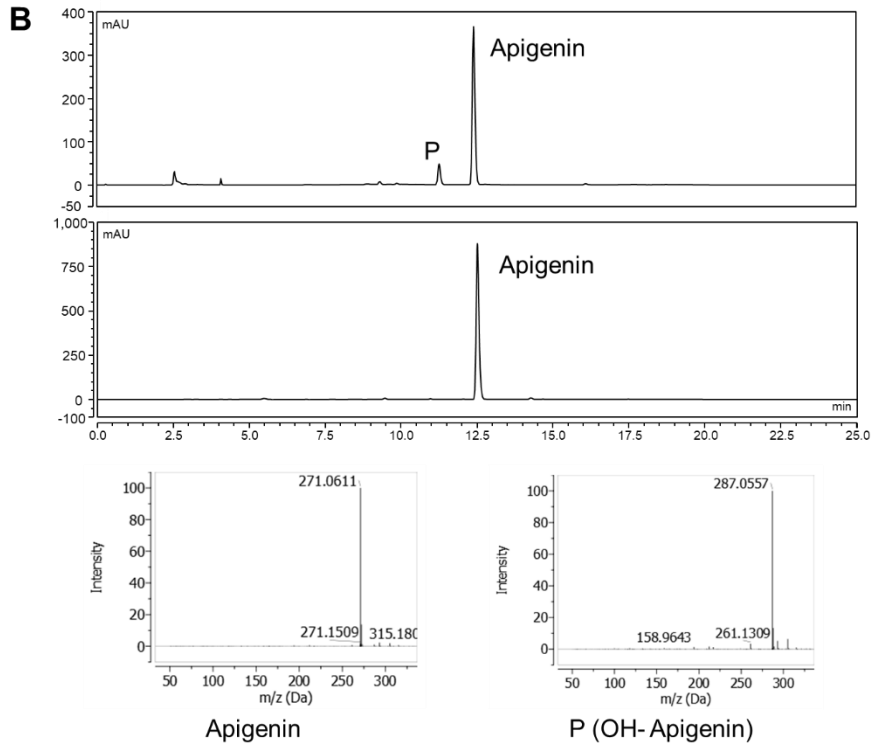
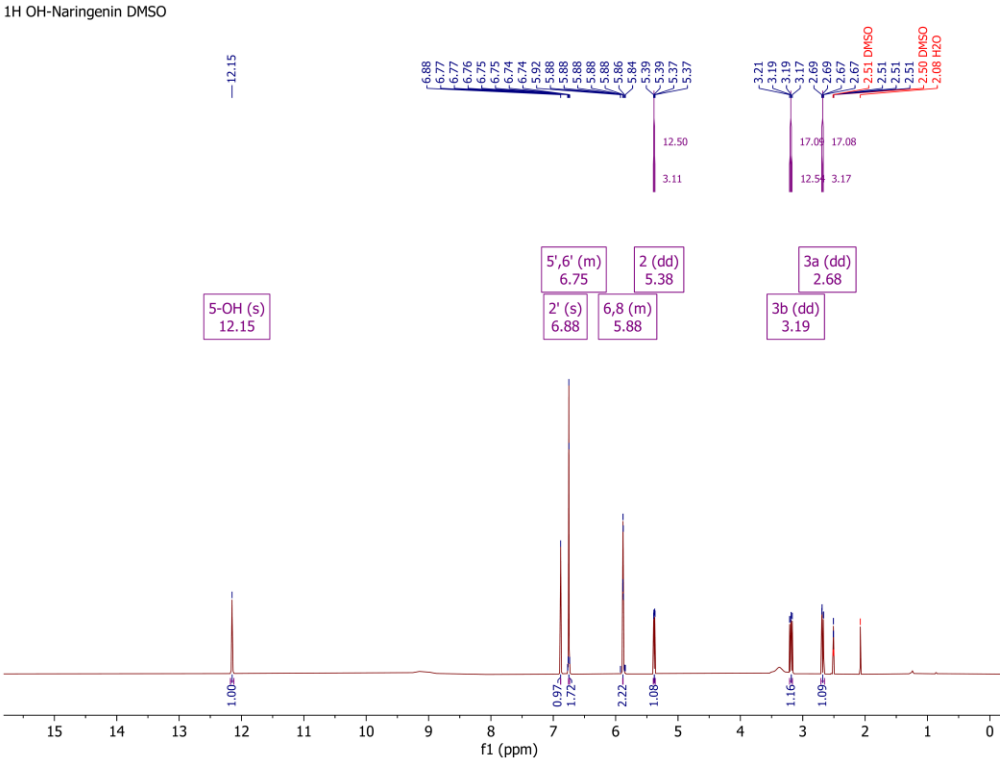
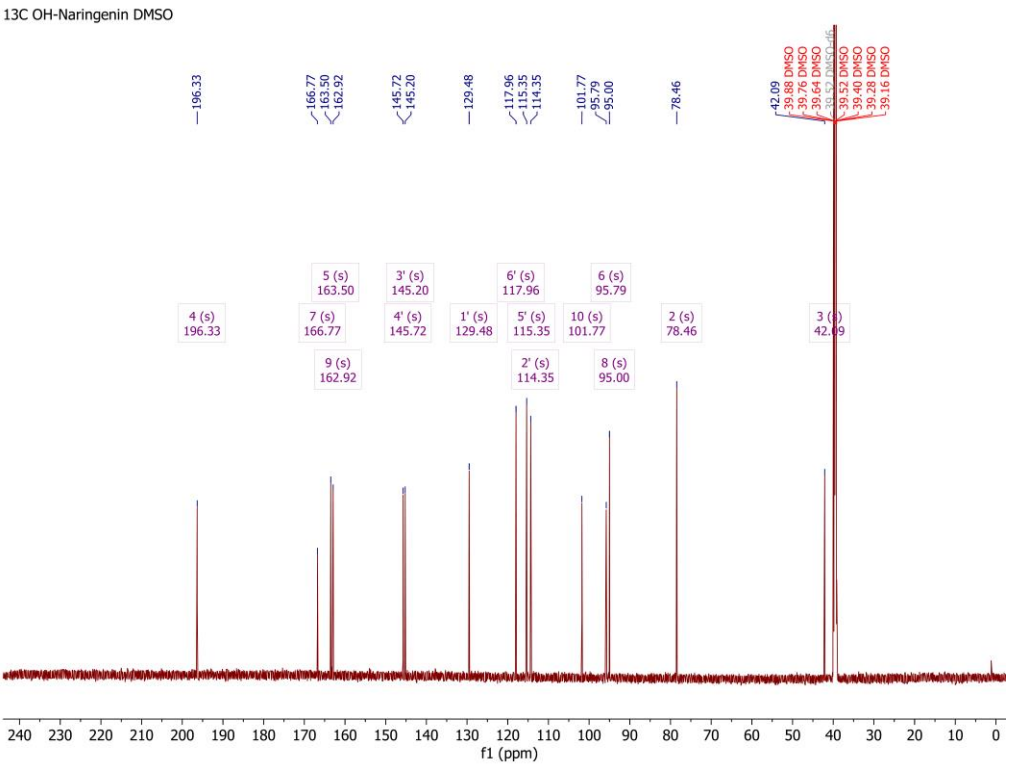


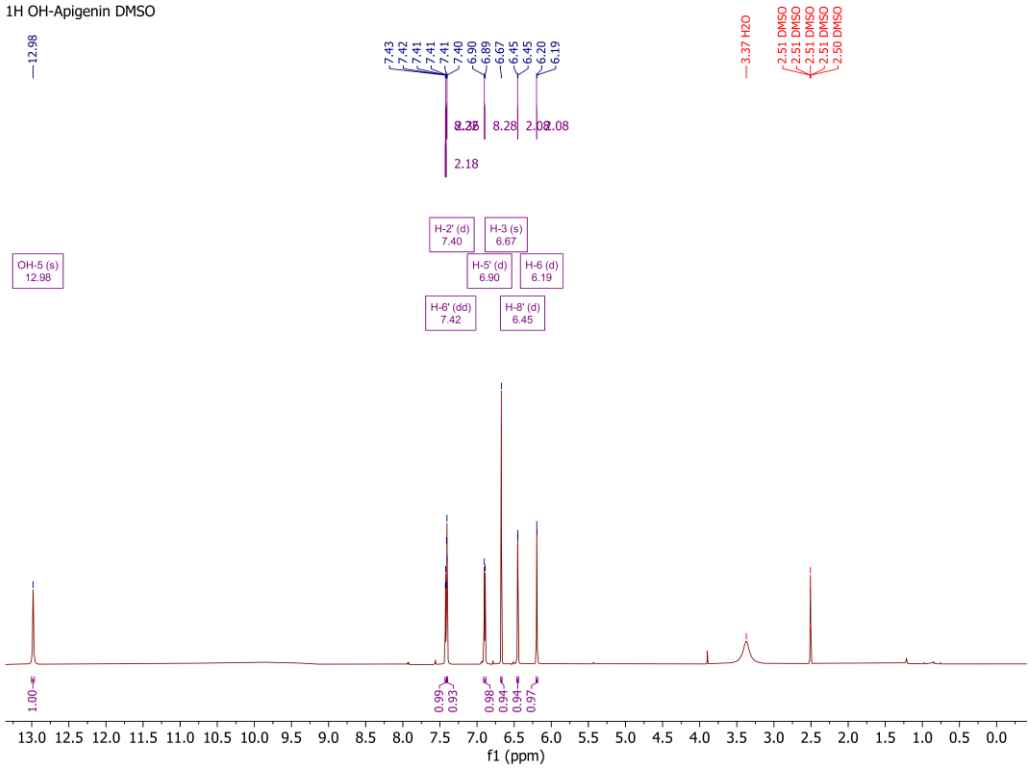
Figure S5. ^{1}H and ^{13}C NMR analyses of the hydroxylated product of naringenin (A) and apigenin (B).

A





B


1H OH-Apigenin DMSO
12.98
7.43
7.42
7.41
7.41
7.41
7.40
6.90
6.89
6.67
6.45
6.45
6.20
6.19
3.37 H2O
2.51 DMSO
2.51 DMSO
2.51 DMSO
2.51 DMSO
2.50 DMSO
8.28
8.28
2.08
2.08
2.18
H-2' (d) 7.40
H-3 (s) 6.67
OH-5 (s) 12.98
H-5' (d) 6.90
H-6 (d) 6.19
H-6' (dd) 7.42
H-8' (d) 6.45
1.00
0.99
0.93
0.98
0.94
0.94
0.97
13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

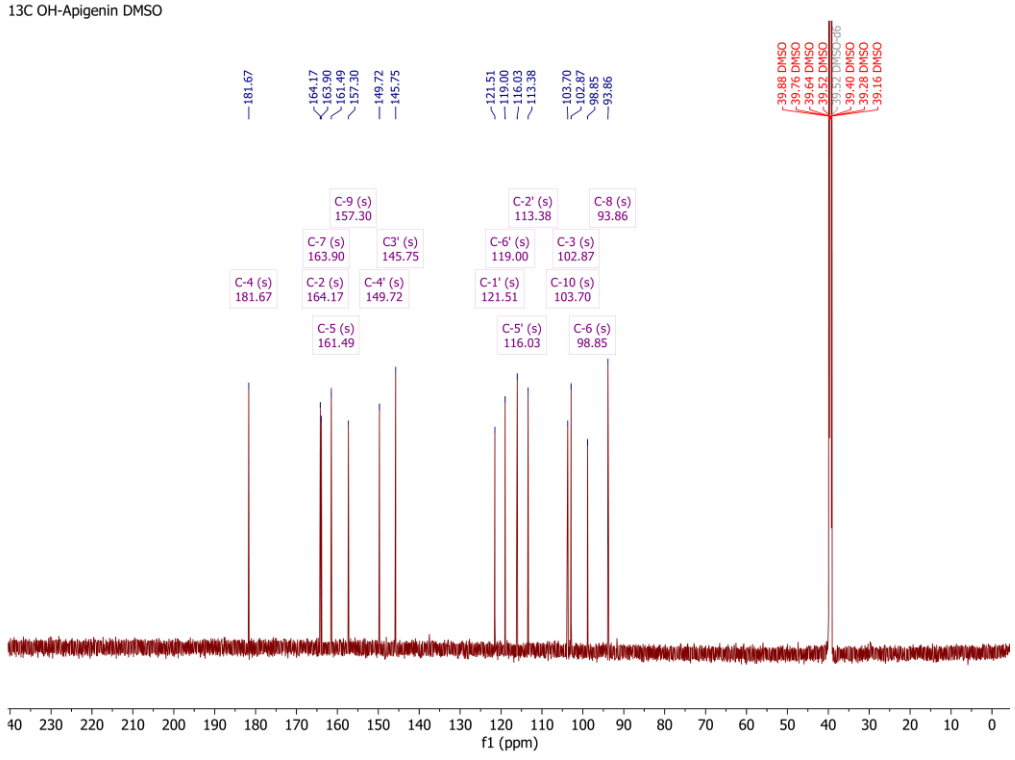

13C OH-Apigenin DMSO
181.67
164.17
163.90
161.49
157.30
149.72
145.75
121.51
119.00
116.03
113.38
103.70
102.87
98.85
93.86
39.88 DMSO
39.76 DMSO
39.64 DMSO
39.52 DMSO
39.40 DMSO
39.28 DMSO
39.16 DMSO
C-9 (s) 157.30
C-2' (s) 113.38
C-8 (s) 93.86
C-7 (s) 163.90
C3' (s) 145.75
C-6' (s) 119.00
C-3 (s) 102.87
C-4 (s) 181.67
C-2 (s) 164.17
C-4' (s) 149.72
C-1' (s) 121.51
C-10 (s) 103.70
C-5 (s) 161.49
C-5' (s) 116.03
C-6 (s) 98.85
40 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

Figure S6. The αK′–αL loops are mainly involved in the interaction with the heme molecule through a combination of hydrophobic and hydrogen bonds and salt bridges.

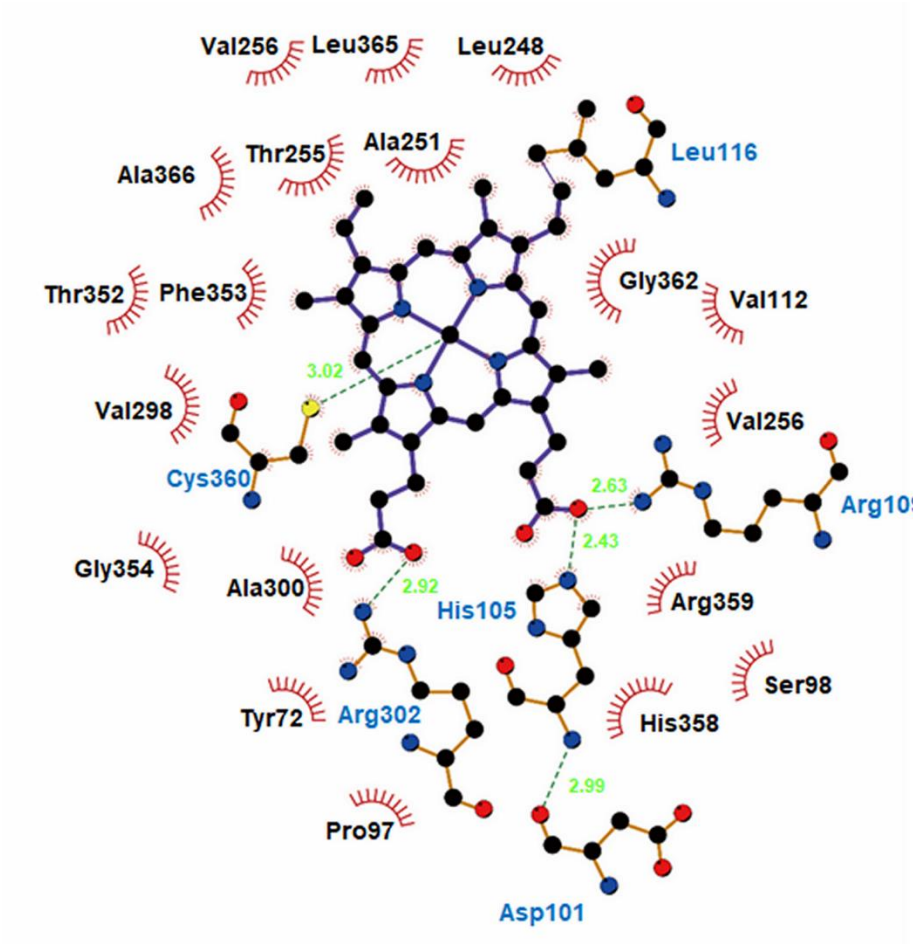


Figure S7. Conservation analysis of the protein sequence of CYP101D5 using ConSurf [35]. The conservation of the amino acids among the 400 CYP homologues are indicated using nine-grade color coding. The average scores of the edge B/C and F/G loops were 4.3 and 3.6, respectively.

Figure S8. Substrate-dependent conformation of the B/C loop in the CYP101 family. Ionone-binding CYPs (A) and camphor-binding CYPs (B) were grouped and presented with different colors. The residues from the B/C loop comprising substrate binding sites are shown with sticks.