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Supporting Information

Regioselective Hydroxylation of Oleanolic Acid Catalyzed by Human CYP3A4 to Produce Hederagenenin, a Chiral Metabolite

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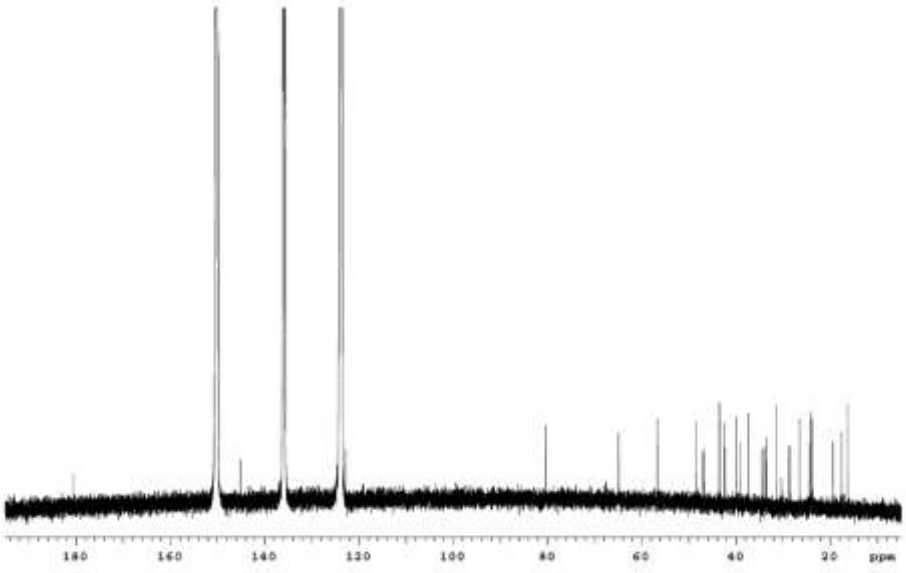
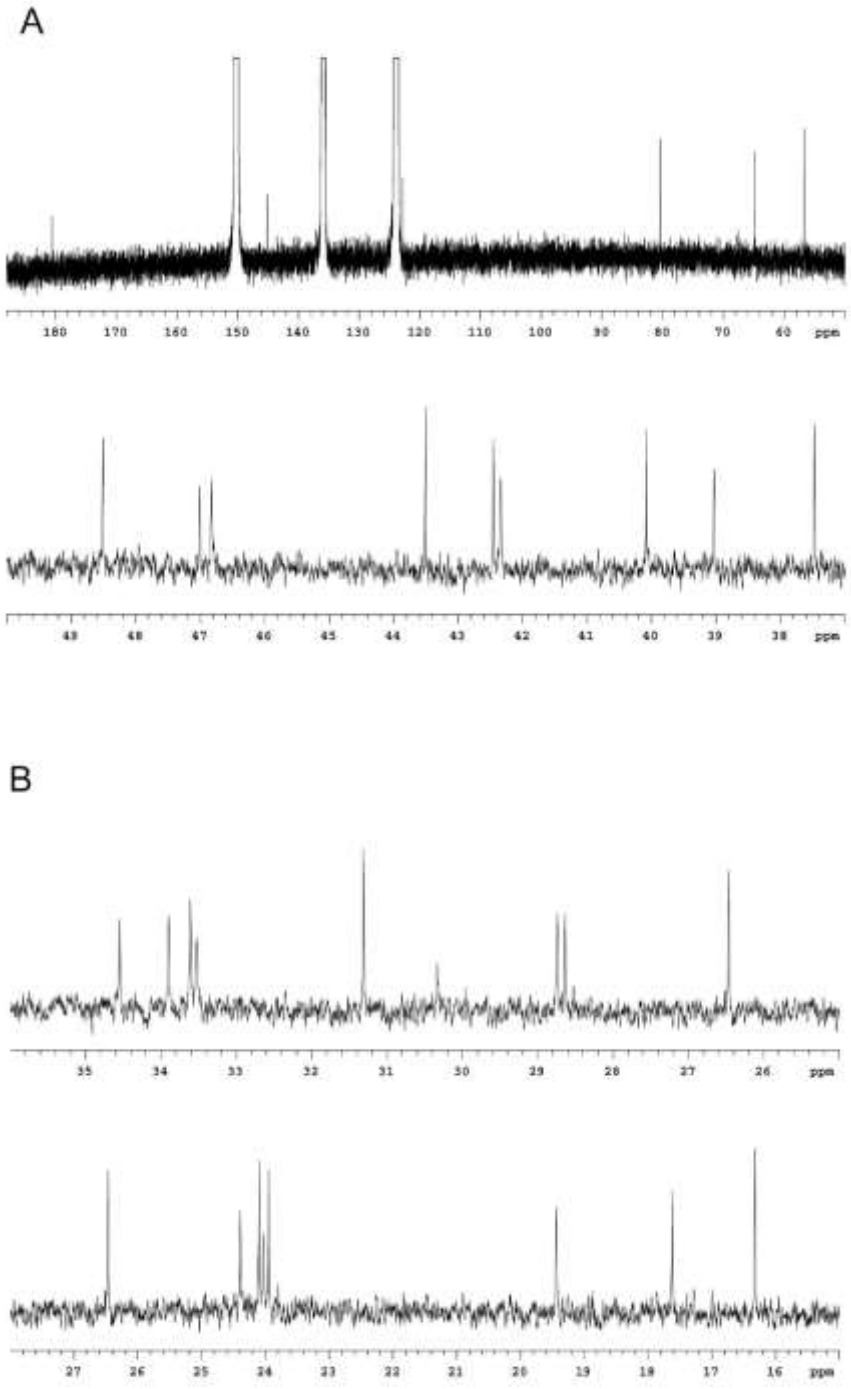
Supplementary Table S1. ^{13}C NMR chemical shifts of metabolite, M1

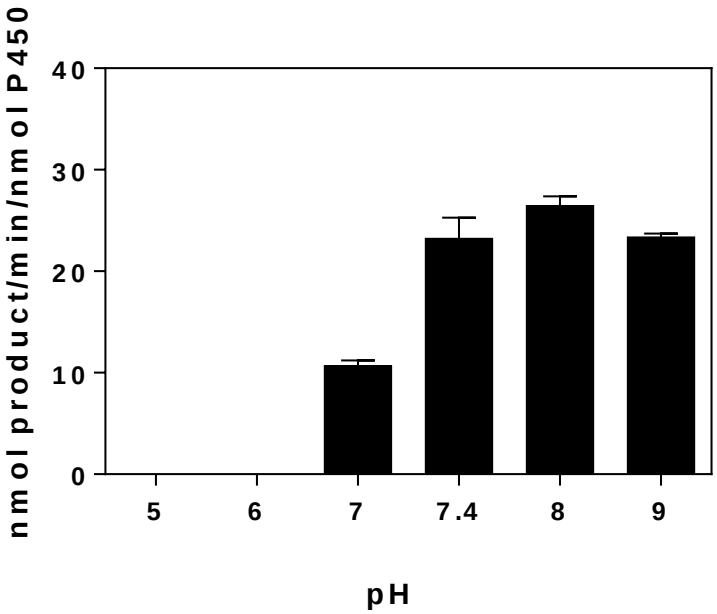
Position	δ_C $^{13}C(\delta)$, ppm
1	39.03
2	28.74
3	80.46
4	43.51
5	56.71
6	19.44
7	33.90
8	40.08
9	48.51
10	37.47
11	24.40
12	122.81
13	145.16
14	42.46
15	28.64
16	24.03
17	47.01
18	42.34
19	46.82
20	30.34
21	34.55
22	33.53
23	64.91
24	23.95
25	16.32
26	17.62
27	26.47
28	180.54
29	24.10
30	33.61



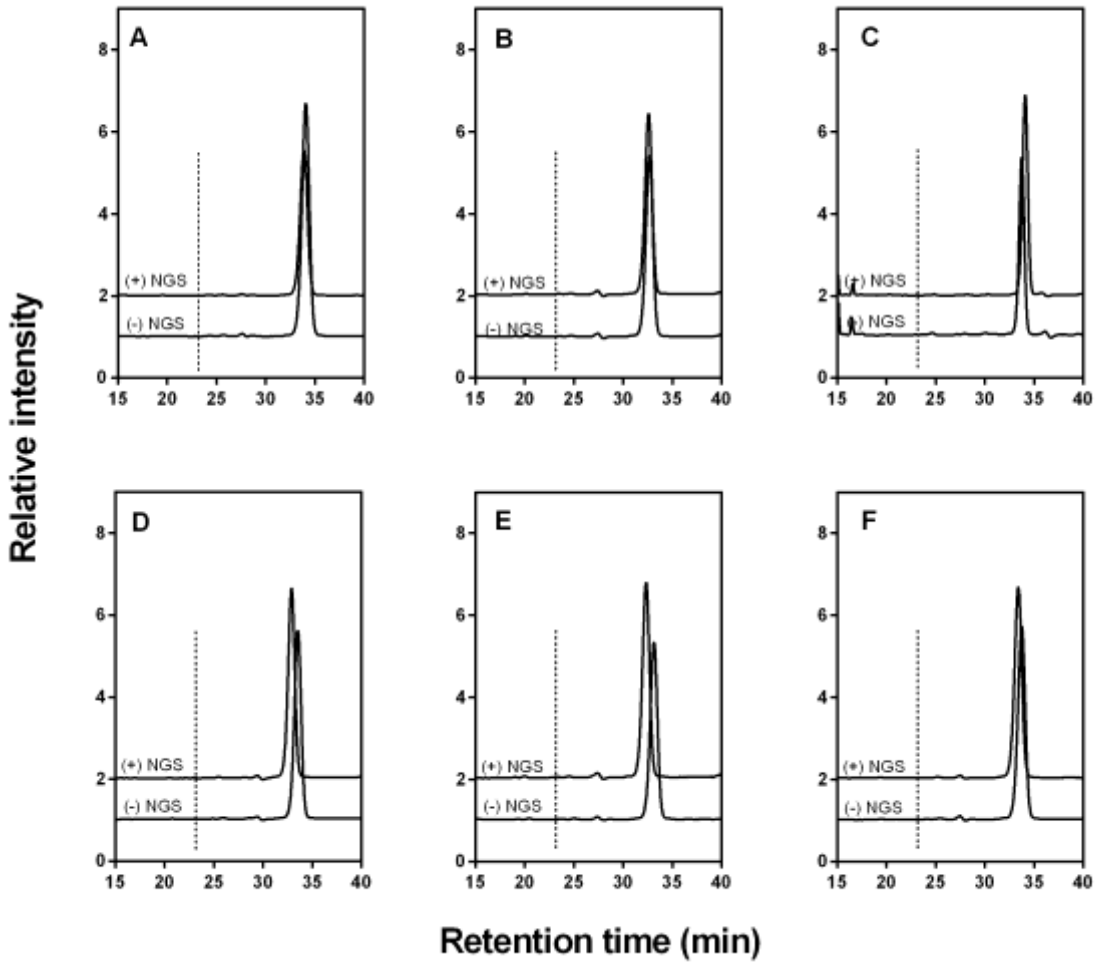
Supplementary Figure S1. ^{13}C NMR spectra of metabolite, M1.



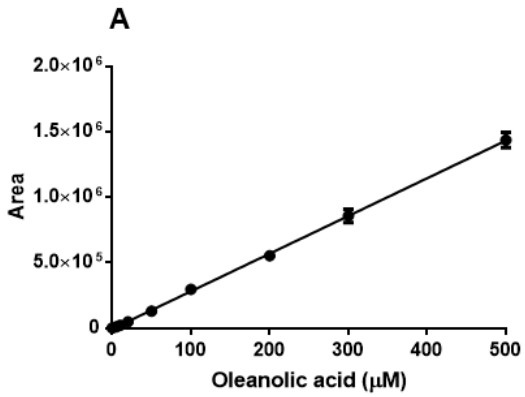
Supplementary Figure S2. Expanded regions of ^{13}C NMR spectra of metabolite, M1. (A) 37-190 ppm. (B) 15-37 ppm.

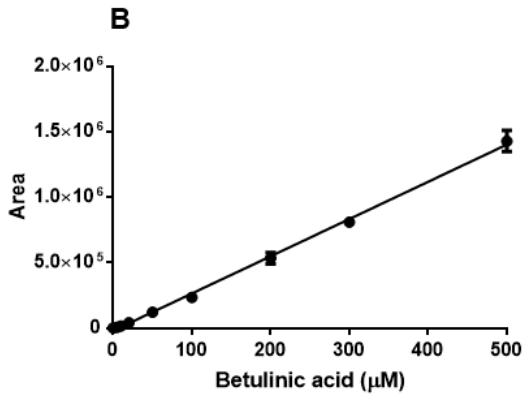


Supplementary Figure S3. pH-dependence of 4-epi-hederagenin formation by CYP3A4. Values represent the mean ± SD of the three determinations.



Supplementary Figure S4. HPLC chromatograms of OA and its metabolite produced by human P450s with (+NGS) and without NADPH (-NGS). The reaction mixture containing 0.20 μM P450 and 100 μM OA was incubated at 37 °C for 60 min. Retention times: 4-epi-hederagenin (M1), t_R = 22.4 min; OA, t_R = 32.3 min. (A) CYP2E1, (B) CYP2D6, (C) CYP2C19, (D) CYP1B1, (E) CYP2C9, and (F) CYP1A2. The position of 4-epi-hederagenin is indicated with a vertical dot line.

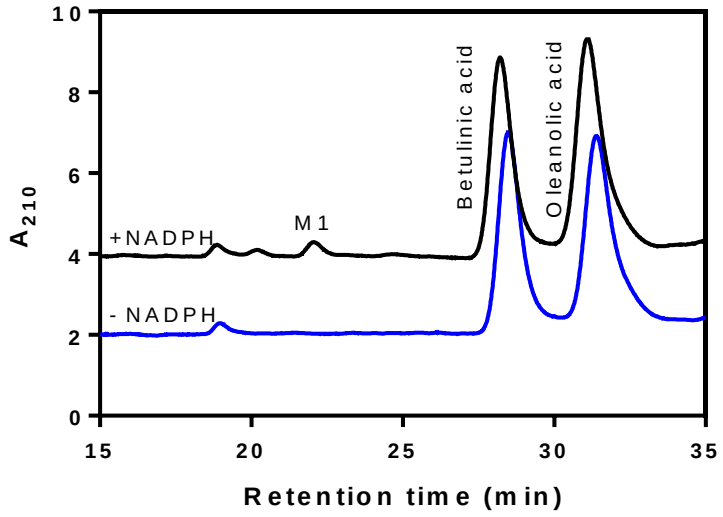




OA-slope	2878 ± 31.60
Y-intercept when X=0.0	-6996 ± 6605
R square	0.9981
Equation	Y = 2878*X - 6996

BA-slope	2844 ± 44.51
Y-intercept when X=0.0	-20420 ± 9301
R square	0.9961
Equation	Y = 2844*X - 20420

Supplementary Figure S5. Standard curves of internal standard (BA), and OA. Different concentration of OA and BA were used at 0.2, 0.5, 1, 2, 5, 10, 20, 30, 50, 100, 200, 300, and 500 μM.



Supplementary Figure S6. HPLC chromatograms of OA and its metabolite produced by CYP3A4 with and without NADPH. The reaction mixture containing 0.20 μM CYP3A4 enzyme and 100 μM OA was incubated at 37 °C for 60 min. Internal standard BA (100 μM) was added after the reaction: BA; t_R =27.2 min, 4-epi-hederagenin (M1); t_R = 22.4 min, and OA: t_R =32.3 min.