Supplemental Material for:

$^{18}O_2$ labeling experiments illuminate the oxidation of *ent*-kaurene in bacterial gibberellin biosynthesis

Nagel, Raimund and Peters, Reuben J.*

Roy J. Carver Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, IA, 50011, USA. *E-mail: rjpeters@iastate.edu

Contents

Supplemental Table S1: Primers for cloning of synthetic *Et*CYP117 into pET100/pET101.

Primer name	Primer Sequence
forward	CACCATGGCGTTGCTGAACCCCTTTAAACG
reverse	TCATATGGACAGTGATCTACCTGCATCTTTTGAAAAAGC
reverse -stop	TATGGACAGTGATCTACCTGCATCTTTTGAAAAAGC
reverse +6xHistidine	TCAATGATGATGATGATGATGTATGGACAGTGATCTACCTGCATCTTTTGAAAAAGC

Underlined sequence in forward primer incorporated for dTOPO-based cloning method.

Supplemental Table S2: Potential labeling patterns from *ent*-kaurene, 1 (% isotopomer).

$[M^+]$	**(1) →**	**(2) →**	**(3) →**	**(6) →**	**(5)**
+0	100	0	0	0	0
+2	0	100	0	0	0
+4	0	0	100	0	100
+6	0	0	0	100	0
	(1) →	**(2) →**	**(3) →**	**(4) →**	**(5)**
+0	100	0	0	0	0
+2	0	100	0	100	0
+4	0	0	100	0	100
+6	0	0	0	0	0
	(1) →	**(2) →**	**(4) →**	**(3) →**	**(5)**
+0	100	0	0	0	0
+2	0	100	100	100	100
+4	0	0	0	0	0
+6	0	0	0	0	0
	(1) →	**(2) →**	**(4) →**	**(5)**	
+0	100	0	0	0	
+2	0	100	100	0	
+4	0	0	0	100	
+6	0	0	0	0	
	(1) →	**(2) →**	**(3) →**	**(5)**	
+0	100	0	0	0	
+2	0	100	0	0	
+4	0	0	100	100	
+6	0	0	0	0	

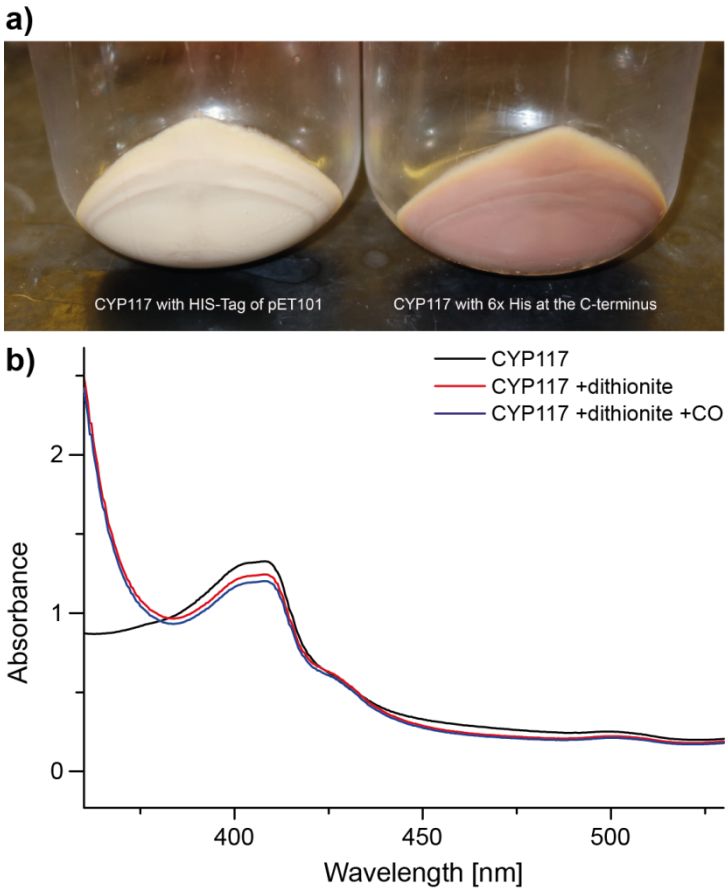
Supplemental Table S3: Potential labeling patterns from *ent*-kaurenol, 2 (% isotopomer).

$[M^+]$	**(2) →**	**(3) →**	**(6) →**	**(5)**
+0	100	0	0	0
+2	0	100	0	66
+4	0	0	100	33
	(2) →	**(3) →**	**(4) →**	**(5)**
+0	100	0	50	0
+2	0	100	50	50
+4	0	0	0	50
	(2) →	**(4) →**	**(5)**	
+0	100	100	0	
+2	0	0	100	
+4	0	0	0	
	(2) →	**(3) →**	**(5)**	
+0	100	0	0	
+2	0	100	100	
+4	0	0	0	

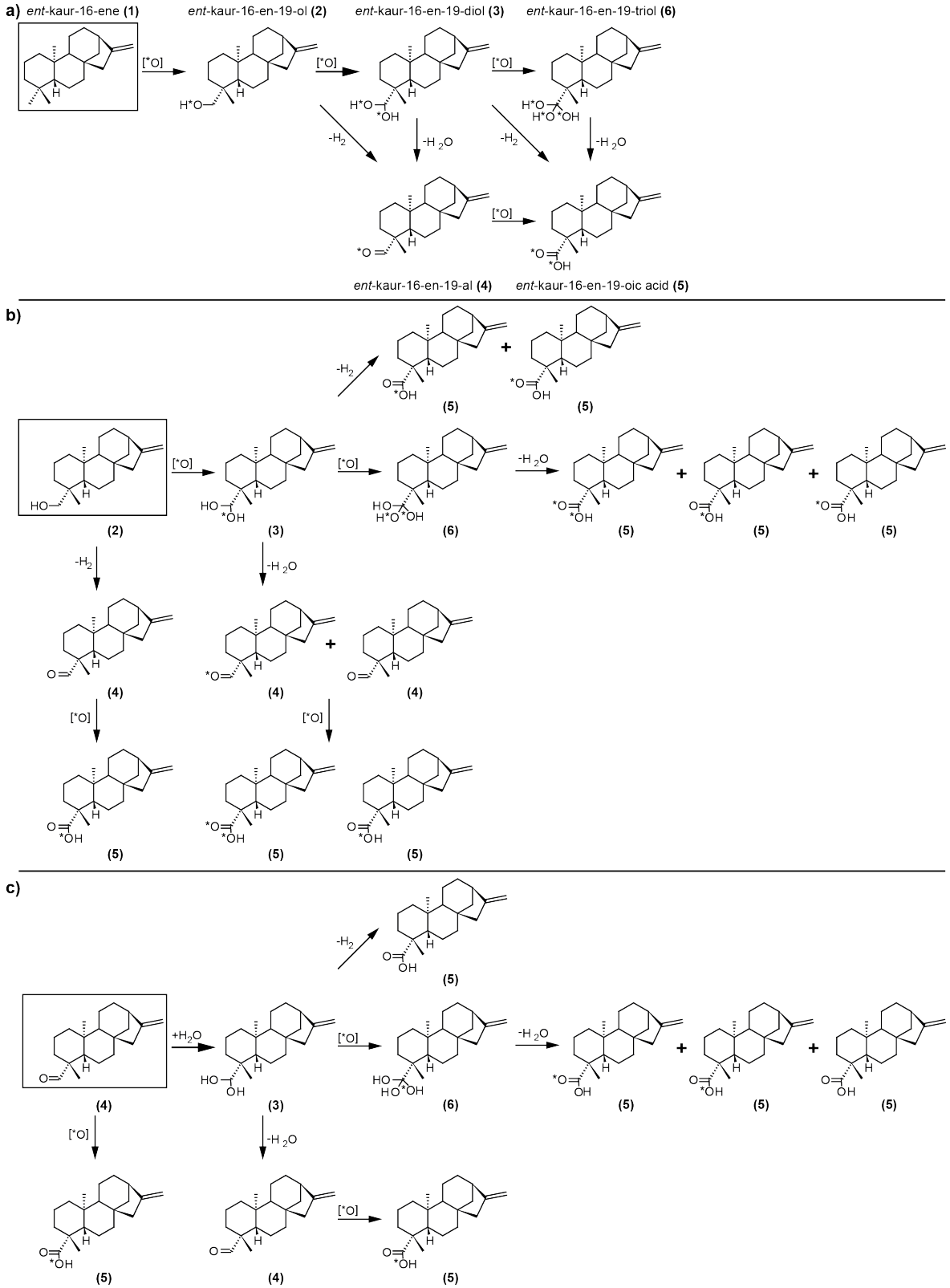
Supplemental Table S4: Potential labeling patterns from *ent*-kaurenal, 4 (% isotopomer).

$[M^+]$	**(4) →**	**(5)**		
+0	100	0		
+2	0	100		
+4	0	0		
	(4) →	**(3) →**	**(5)**	
+0	100	100	100	
+2	0	0	0	
+4	0	0	0	
	(4) →	**(3) →**	**(6) →**	**(5)**
+0	100	100	0	33
+2	0	0	100	66
+4	0	0	0	0

Supplemental Figure S1



Supplemental Figure S1: Expression and spectroscopic properties of *Et*CYP117. (a) Cell pellets from *E. coli* cultures expressing *Et*CYP117 with the C-terminal His-Tag encoded by pET101, or only 6 histidines directly incorporated at the C-terminus (i.e., without any linker sequence). **(b)** UV-VIS absorbance measurement of E. coli cell lysate expressing *Et*CYP117 with the short 6xHis-tag, before and after reduction by dithionite, as well as subsequent addition of carbon monoxide (CO).



Supplemental Figure S2. Theoretical position of ^{18}O labels. Schemes depicting the position of the ^{18}O label, marked by an asterisk (*), for all pathways from *ent*-kaurene **(a)**, *ent*-kaurenol **(b)** or *ent*-kaurenal **(c)**. For **(a)** and **(b)** reverse reactions were omitted.