

ChemBioChem

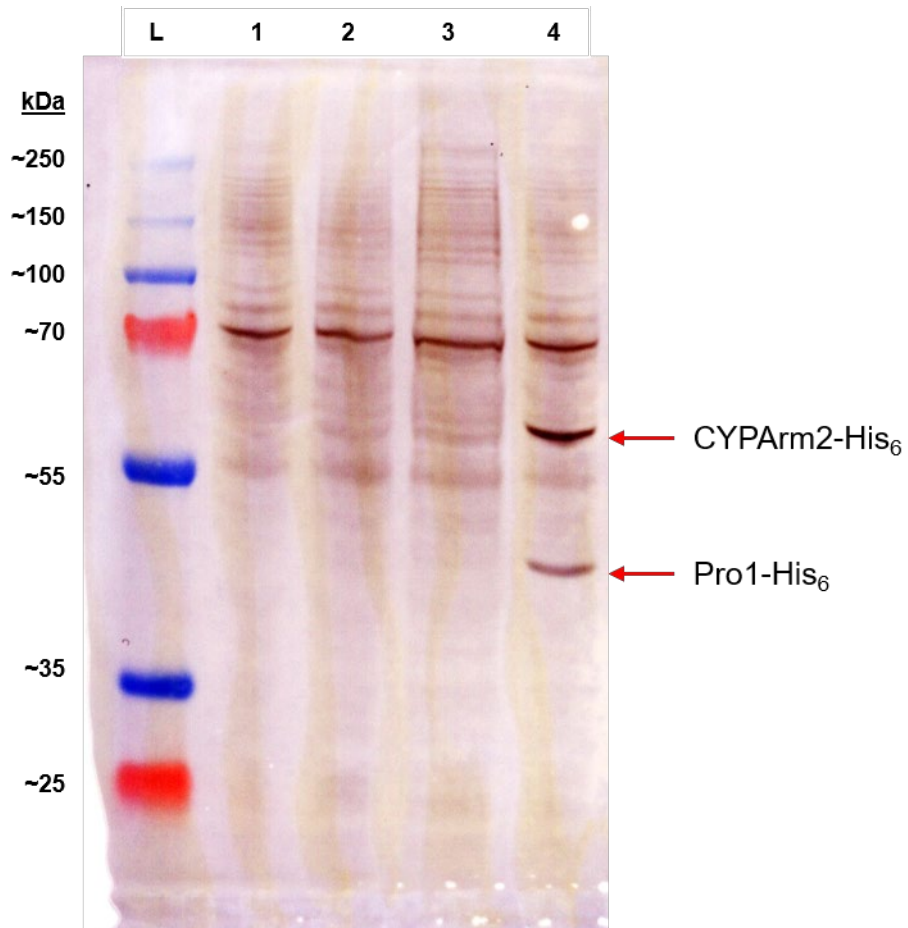
Supporting Information

CYPArm2 is a CYP450 Monooxygenase with Protoilludene 13-Hydroxylase Activity Involved in the Biosynthesis of Armillyl Orsellinate-Type Sesquiterpenoids

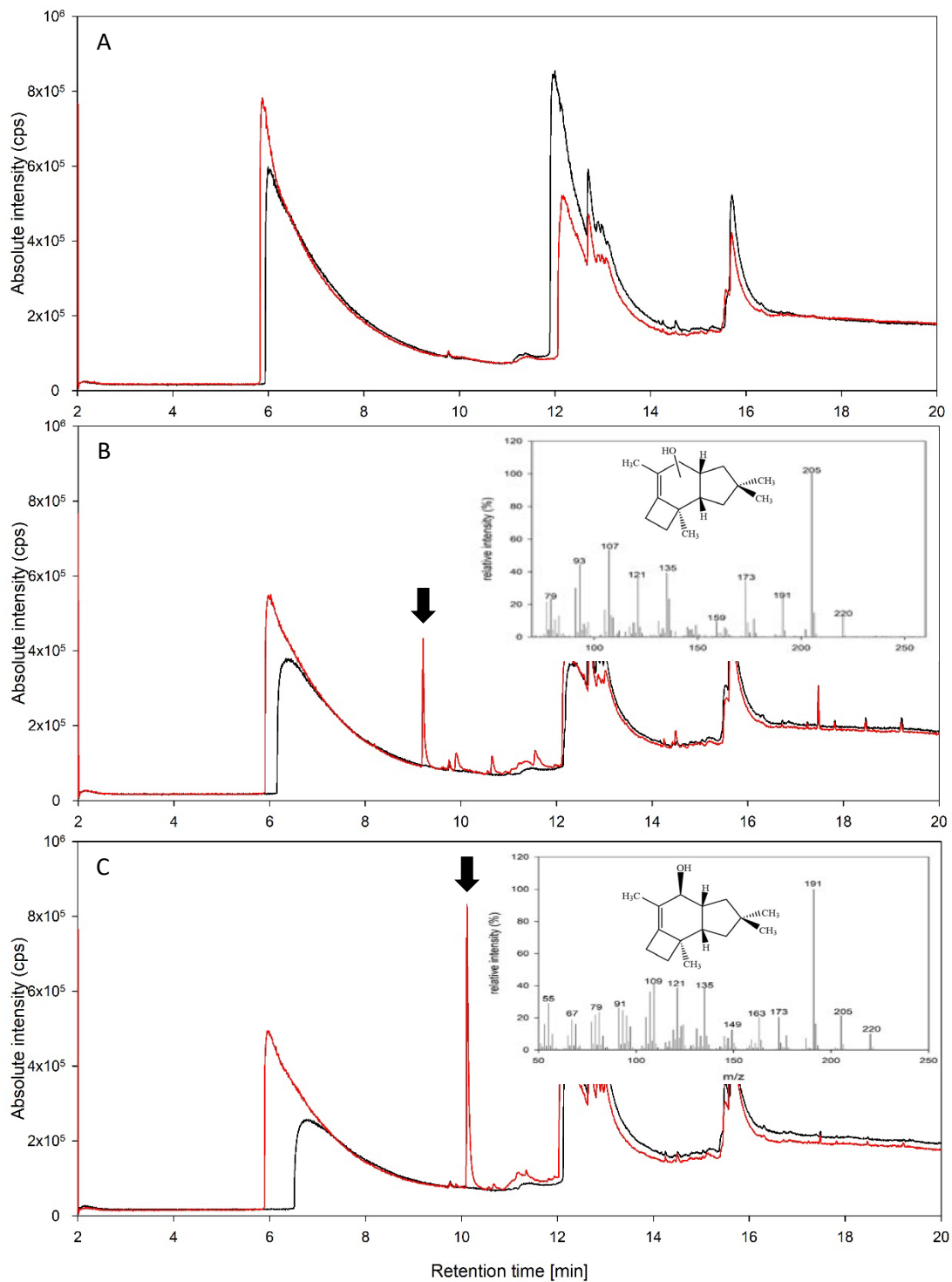
Christopher McElroy⁺, Benedikt Engels⁺, Reinhard Meusinger, and Stefan Jennewein*

Supplemental Table 1. Table of selected melleolides with activities and their publications

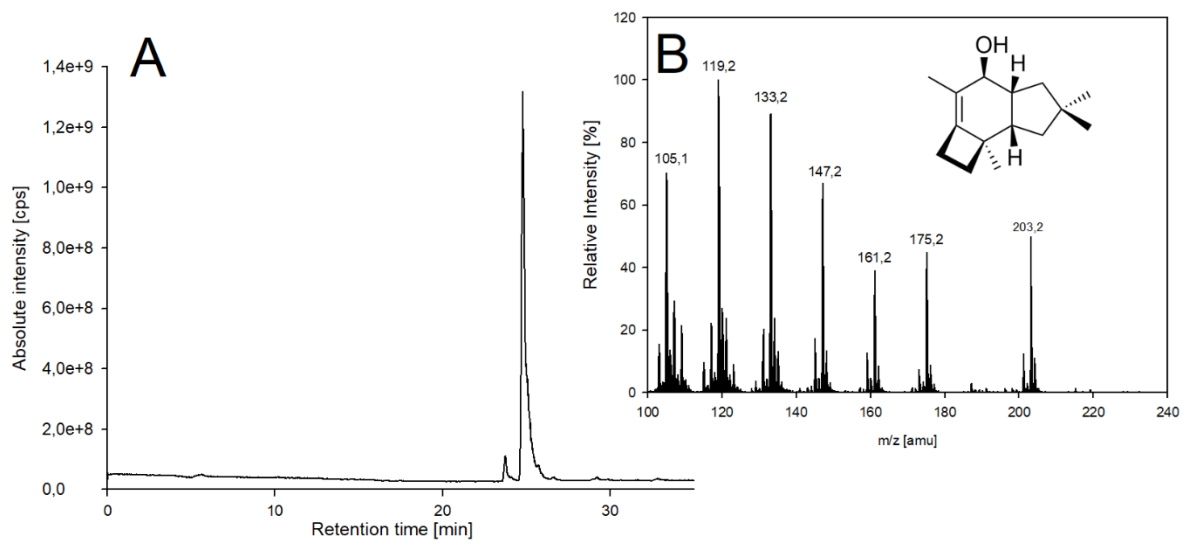
Fungal species	Compound	Activity	Tested organisms	Reference
<i>A. mellea</i> isolate FR-P 75	Arnamial	cytotoxic	Cancer and mammalian cell lines with respective IC ₅₀ from three in vitro assays, MCF7 15.4 μM, Jurkat 3.9 μM	[12]
<i>A. mellea</i> isolate FR-P 75	Arnamial	antifungal & cytotoxic	Inhibition of <i>P. notatum</i> , <i>A. flavus</i> and <i>A. nidulans</i> (14 μM). Inhibition of cancer cell growth GC ₅₀ , HUVEC 2 μM, K-562 2.3 μM, MCF-7 15.4 μM. Cytotoxicity assay CC ₅₀ , HeLa 4.9 μM	[13]
<i>A. mellea</i> isolate FR-P 75	Dehydroarmillyl-orsellinate	antifungal & cytotoxic	Inhibition of <i>P. notatum</i> , <i>A. flavus</i> and <i>A. nidulans</i> (31.3 μM). Inhibition of cancer cell growth GC ₅₀ , HUVEC 5.3 μM, K-562 5 μM, MCF-7 8 μM. Cytotoxicity assay CC ₅₀ , HeLa 15.2 μM	[13]
<i>A. mellea</i> isolate FR-P 75	Dehydroarmillyl-orsellinate	cytotoxic	Cancer and mammalian cell lines with respective IC ₅₀ from three in vitro assays, MCF7 8.0 μM, Jurkat 16.9 μM, HeLa 15.2 μM, K-562 5.0 μM	[12]
<i>A. mellea</i> (Vahl ex. Fr) Karst	Melleolide K	antifungal & antibacterial	Strong inhibition of gram positive bacteria (<i>S. aureus</i> , <i>B. subtilis</i> , yeast (<i>S. cerevisiae</i>) and fungi (<i>Aspergillus fumigatis</i>) but limited gram negative activity, LD ₅₀ in mice >100 mg/kg	[14]
<i>A. mellea</i> (# BCRC 36361)	Melleolide Q	cytotoxic	Inhibition of cancer cell lines with respective IC ₅₀ , MCF-7 1.5 μM, H460 80 μM, HT-29 54.2 μM, CEM 10.3 μM	[15]
<i>A. mellea</i> (# BCRC 36361)	Melleolide R	cytotoxic	Inhibition of cancer cell lines with respective IC ₅₀ , MCF-7 3.7 μM, H460 53.8 μM, HT-29 18.7 μM, CEM 3.4 μM	[15]
<i>A. mellea</i> isolate (50,063)	Armillasin	Cytotoxic	Inhibition of tested cell lines with respective IC ₅₀ (μg mL ⁻¹), HepG2 15.6, L02 14.4.	[16]
<i>A. mellea</i> isolate (50,063)	Armillarigin	Cytotoxic	Inhibition of tested cell lines with respective IC ₅₀ (μg mL ⁻¹), HepG2 37.7, L02 69.1.	[16]
<i>A. mellea</i> isolate (50,063)	Melleolide	Cytotoxic	Inhibition of tested cell lines with respective IC ₅₀ (μg mL ⁻¹), HepG2 5.0, L02 16.1.	[16]



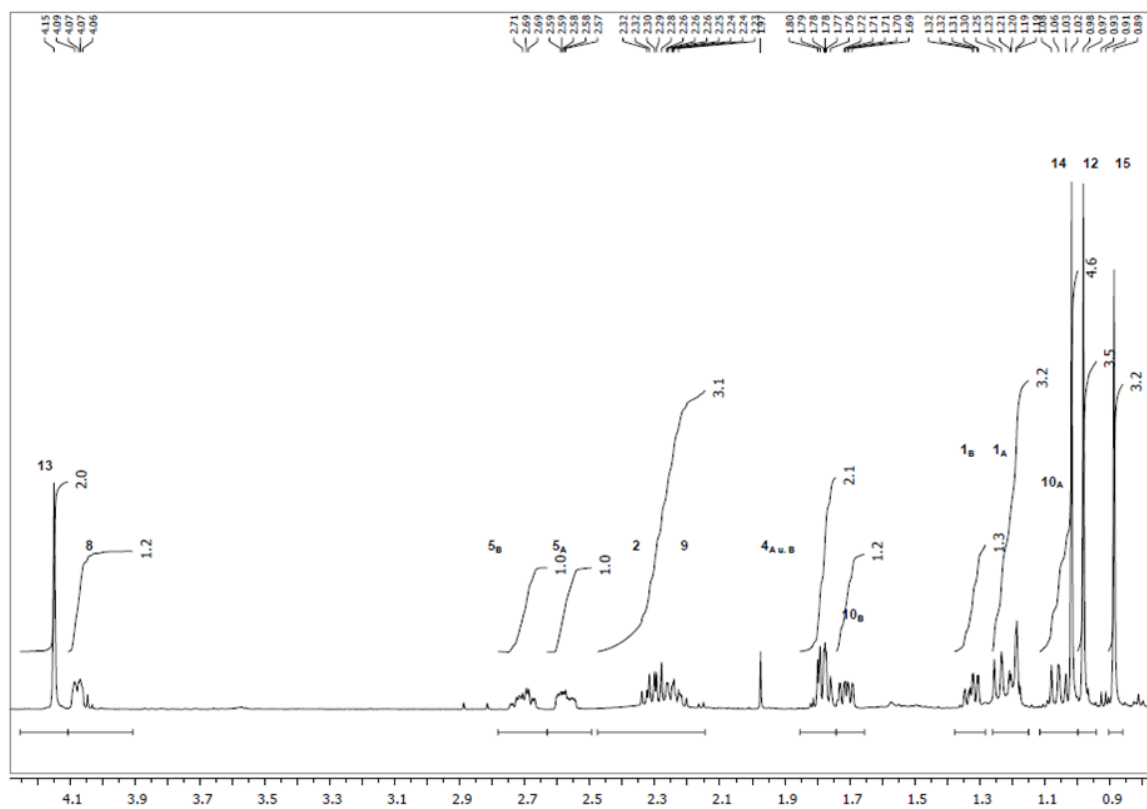
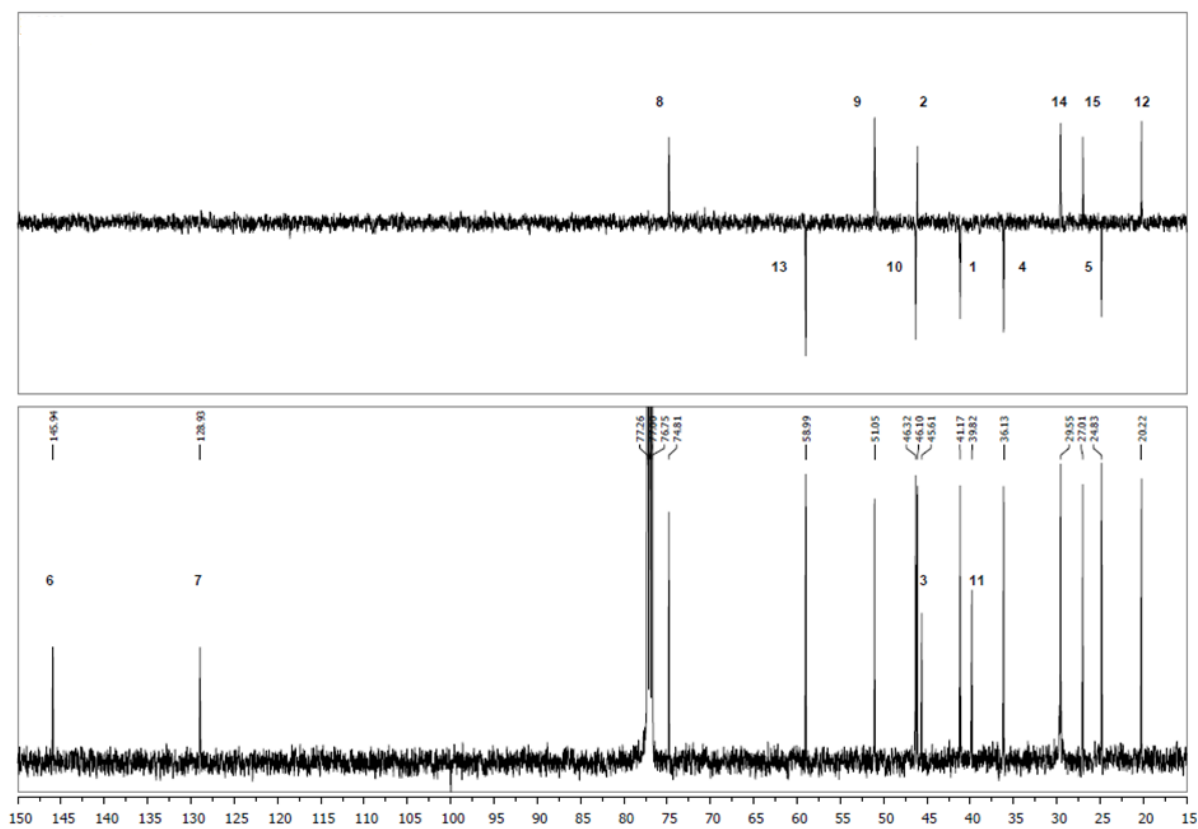
Supplemental Figure 1. Western blot of *A. gallica* proteins overexpressed in *S. cerevisiae*. A) L = 250 kDa ladder, 1 = Membrane and cytosolic protein from CEN-Empty strain (pCM183::*P450-Red-tc* and pYESDEST52::*Empty*) grown with glucose. 2 = Membrane and cytosolic protein from CEN-Empty strain fed on galactose to induce protein expression. 3 = Membrane and cytosolic protein from CEN-CYPArm2 strain (pRS315::*tHMGR* S.c., pRS423::*Pro1HIS6*, pCM183::*P450-Red-tc* and pYESDEST52::*CYPArm2HIS6*) fed on glucose. 4 = Membrane and cytosolic protein from CEN-CYPArm2 strain fed on galactose to induce protein expression. All lanes contain 60 μ g of protein. 10 % Bis-Tris SDS gel, MES buffer, 200 V 60 mins, transfer 30 V for 60 mins, 1:2000 dilution anti-his6 in PBST w. 1% BSA.



Supplemental Figure 2. Overlay of GC-MS chromatograms from induced (red) and non-induced cultures. (A) CEN-empty strain (CEN-PK2-1C strain containing plasmids pCM183::P450-Red-*tc* and pYESDEST52::Empty). (B) CEN-Arm2 (CEN.PK2-1C strain containing plasmids pCM183::P450-Red-*tc*, pRS315::tHMGR S.c., pRS423::Pro1HIS6 and pYESDEST52::CYPArm2HIS6). (C) CEN-22 (CEN.PK2-1C strain containing plasmids pCM183::P450-Red-T.c., pRS315::tHMGR S.c., pRS423::Pro1HIS6 and pYESDEST52::CYPArm3HIS6). Arrows indicate the hydroxylated protoilludene fermentation products. Inserts represent GC-MS spectra of the indicated products.

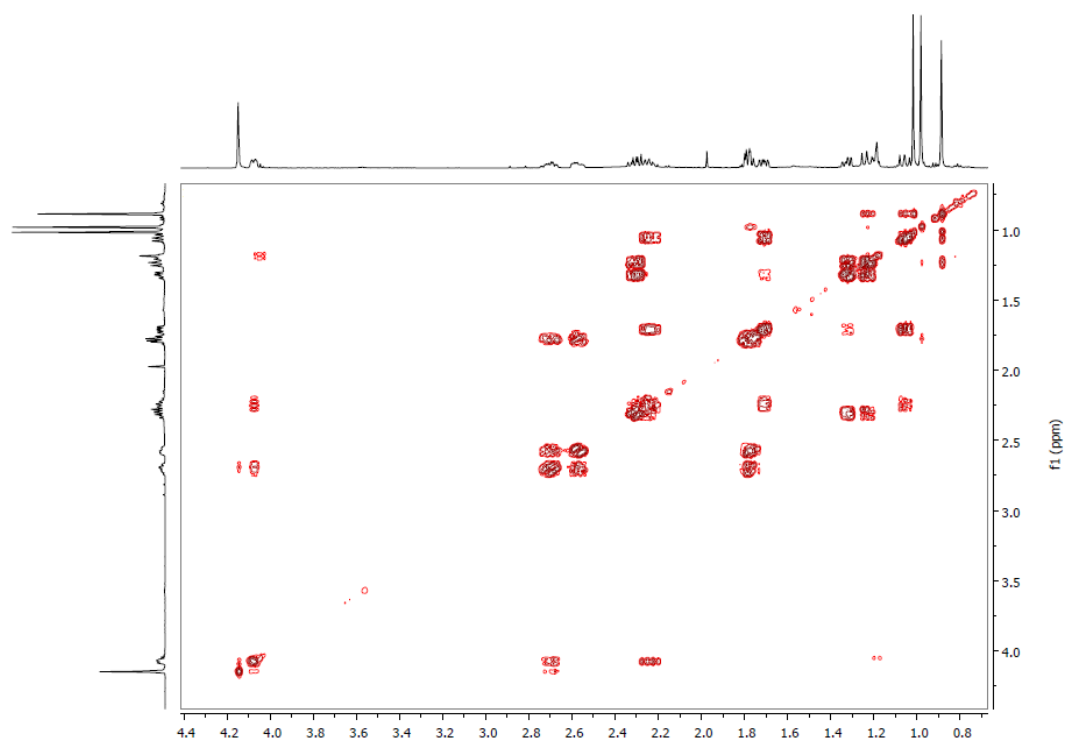


Supplemental Figure 3. LC-MS analysis of 8 α -hydroxy-6-protoilludene. (A) LC-diagram of the 8 α -hydroxy-6-protoilludene standard, (B) showing the corresponding APCI mass spectrum.

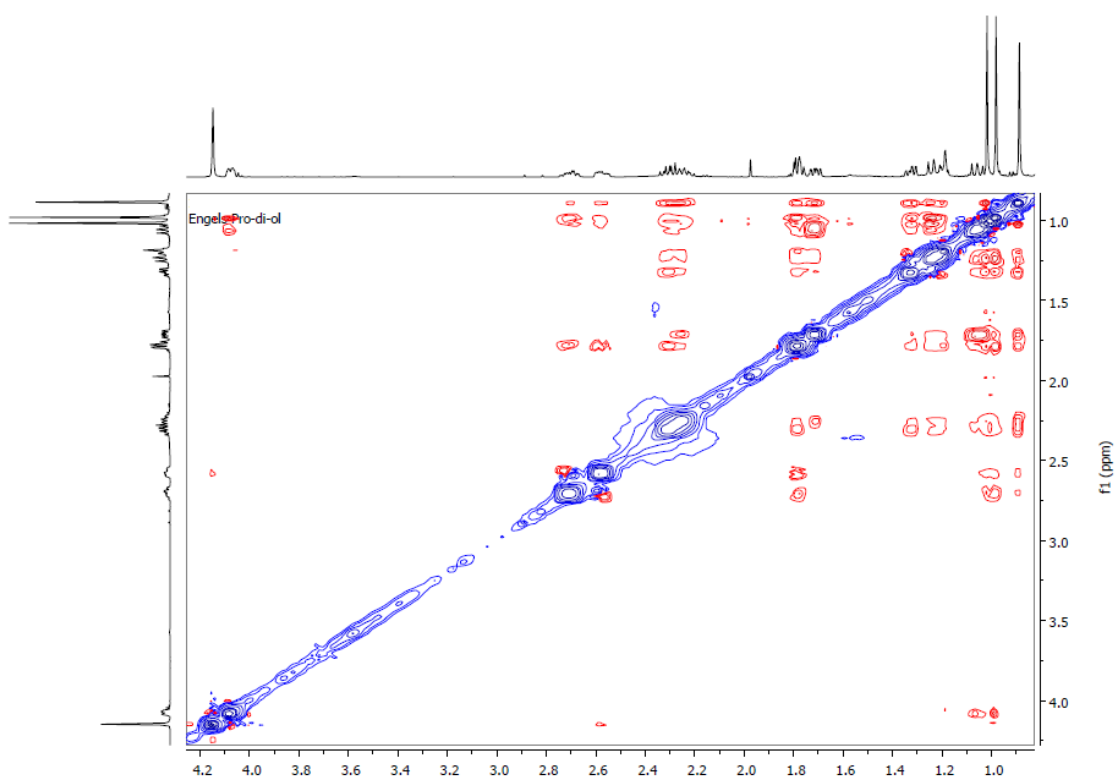
A**B**

Supplemental Figure 4. NMR spectra for the structural evaluation of the unknown dihydroxy-6-protoillidene product, identifying it as 8 α ,13-hydroxy-6-protoillidene. (A) ¹H NMR spectrum with integral identification. (B) ¹³C-DEPT NMR Spectrum

C

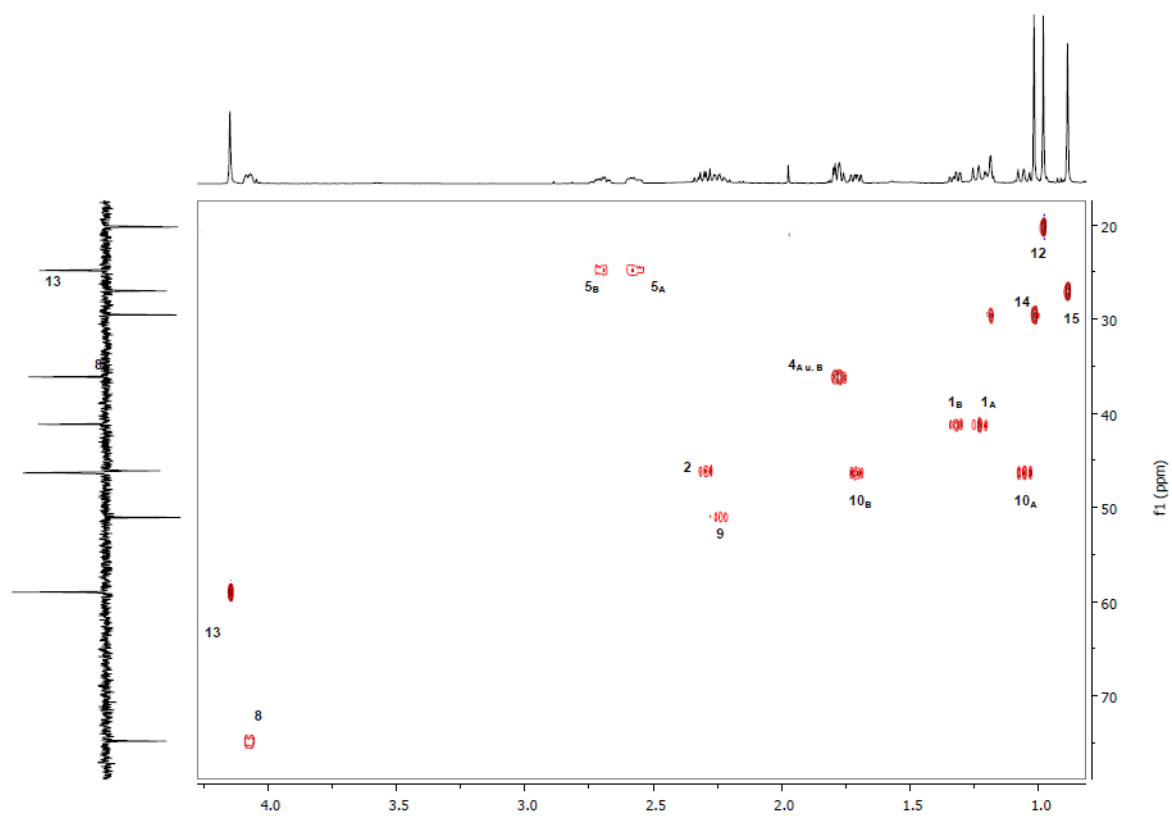


D

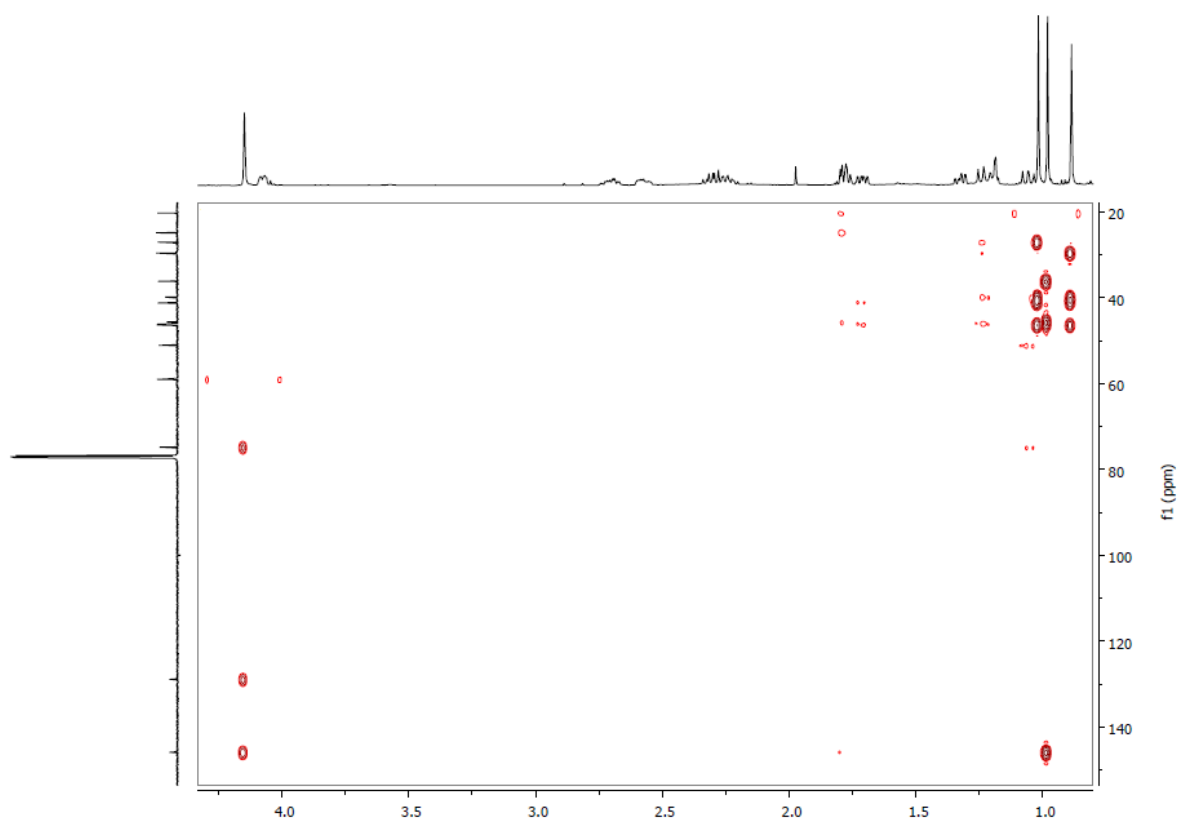


Continued Supplemental Figure 4. Two-dimensional NMR spectra for 8 α ,13-hydroxy-6-protoillidene. (C) gs-COSYDF spectrum. (D) gs-NOESY spectrum

E



F



Continued Supplemental Figure 4. Heteronuclear NMR spectra of $8\alpha,13$ -hydroxy-6-protoillidene. (E) gs-HSQC spectrum. (F) gs-HMBC spectrum.

Supplemental Table 2 – List of used expression plasmids.

Plasmid	Transgene	Function	Reference
pYESDEST52::Empty	-	Empty vector control	Invitrogen cat no.: 12286019
pCM183::P450-Red- <i>tc</i>	<i>Taxus chinensis</i> Cytochrome P450 reductase	NADPH: Cytochrome P450 reductase	[6]
pYESDEST52::CYP-Arm3His6	<i>Armillaria gallica</i> Cytochrome P450 monooxygenase CYP-Arm3 (GenBank Acc. No. MT277003)	Protoilludene-8 α -hydroxylase	[6]
pYESDEST52::CYP-Arm2His6	<i>Armillaria gallica</i> Cytochrome P450 monooxygenase CYP-Arm2 (GenBank Acc. No. MT277003)	Protoilludene-13-hydroxylase	[6], this study
pRS315 <i>tHMGR1</i> S.c.	truncated version of <i>S. cerevisiae</i> 3-hydroxyl-3-methylglutaryl-CoA reductase (HMG-CoA reductase) isoenzyme 1	3-hydroxyl-3-methylglutaryl-CoA reductase (HMG-CoA reductase) that is no more subject to feedback inhibition	[17]
pRS423::Pro1His6	<i>Armillaria gallica</i> Protoilludene synthase (<i>Pro1</i>) (GenBank Acc. No. KC852198)	<i>Armillaria gallica</i> Protoilludene synthase (<i>Pro1</i>) a terpene cyclase	[5], [6]
pRS423::Pro1His6::CYP-Arm3His6	<i>Armillaria gallica</i> Cytochrome P450 monooxygenase CYP-Arm3 and <i>Armillaria gallica</i> Protoilludene synthase (<i>Pro1</i>)	Protoilludene-8 α -hydroxylase and Protoilludene synthase	This study

Supplemental Table 3 – List of CEN-PK2-1C derived strains.

Strain name	Plasmids	reference
CEN-CYPArm2	pCM183::P450-Red- <i>tc</i> and pYESDEST52::CYP-Arm2His6	[6]
CEN-CYPArm3	pCM183::P450-Red- <i>tc</i> and pYESDEST52::CYP-Arm3His6	[6]
CEN-Empty	pCM183::P450-Red- <i>tc</i> and pYESDEST52::Empty	[6]
CEN-22	pCM183::P450-Red- <i>tc</i> , pRS315 <i>tHMGR1</i> S.c., pRS315 <i>tHMGR1</i> S.c., and pYESDEST52::CYP-Arm3His6	[6]
CEN-Pro1	pCM183::P450-Red- <i>tc</i> , pRS315 <i>tHMGR1</i> S.c., pRS423::Pro1His6	This study
CEN-Arm2	pCM183::P450-Red- <i>tc</i> , pRS315 <i>tHMGR1</i> S.c., pRS423::Pro1His6, and pYESDEST52::CYP-Arm2His6	This study
CEN-23	pCM183::P450-Red- <i>tc</i> , pRS315 <i>tHMGR1</i> S.c., pRS423::Pro1His6::CYP-Arm3His6, and pYESDEST52::CYP-Arm2His6	This study