

Identification of enzymes responsible for the reduction of geraniol to citronellol

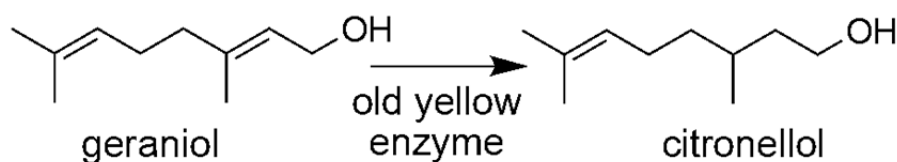
Tian-Tian YUAN,^{a,b} Qian-Qian CHEN,^a Pei-Ji ZHAO,^a Ying ZENG,^{a,*} Xiao-Zhu LIU,^b and Shan LU^c

^aState Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

^bLife Science College, Southwest Forestry University, Kunming 650224, China

^cState Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, China

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Scheme. The enzyme-catalyzed reduction of geraniol to citronellol.

*To whom correspondence should be addressed. E-mail: biochem@mail.kib.ac.cn.

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Figure S2. GC-MS analyses of the product 3-methyl-2-cyclohexanone (RT=4.02 min) formed by the crude recombinant OYE2 enzyme with 3-methyl-2-cyclohexenone (RT=5.71 min) as substrate (11082702.D), using the cell lysate of BL21 (DE3) containing original pET32a+ as the control (11082700.D).

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Figure S4. GC-MS analyses of whole-cell transformation vs crude enzyme activity including *S. cerevisiae* yeast cells (11092806.D), the induced OYE2/pET32a+/BL21 (DE3) intact cells (11092802.D), and the crude recombinant OYE2 extracts (11092804.D).

Figure S5. GC-MS analyses of the crude enzyme products formed by the recombinant HbOPR as a fusion protein (11090201.D) and a 'net' protein (11090202.D), respectively. The retention time of the product citronellol peaks at 9.33 min, and the internal standard at 13.25 min.

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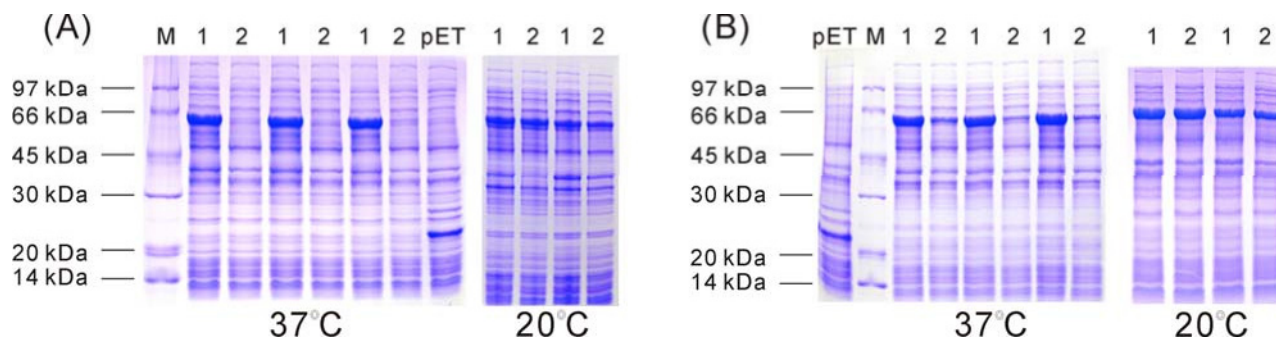
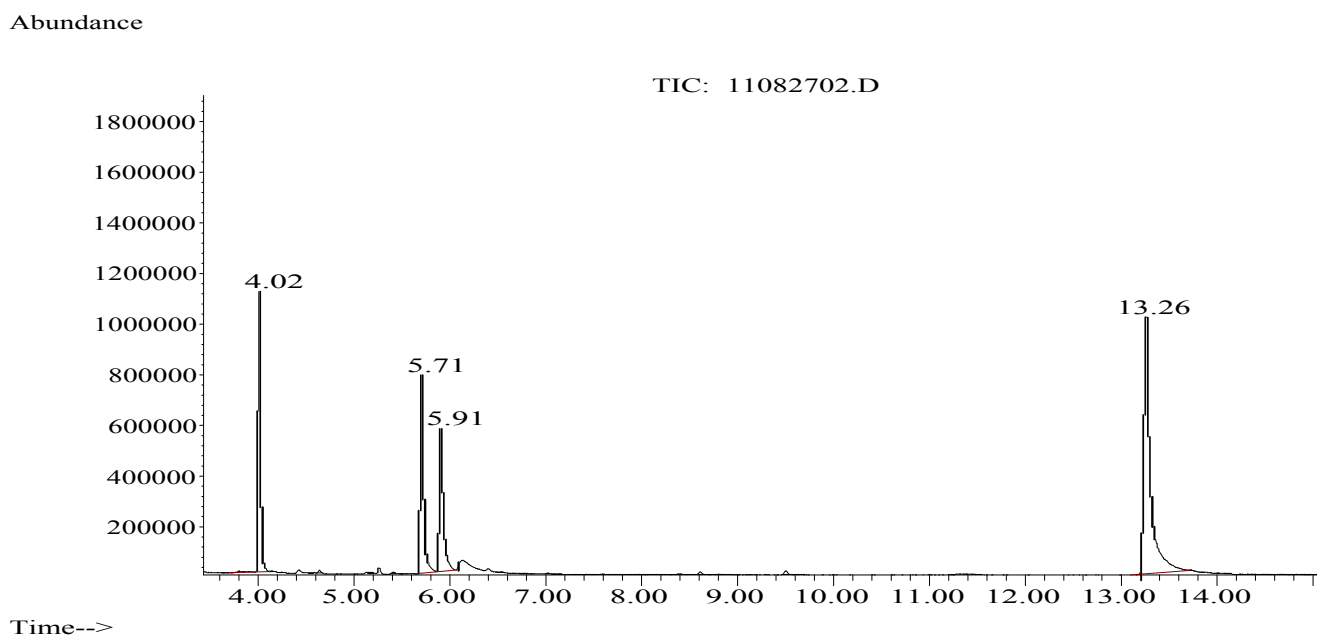


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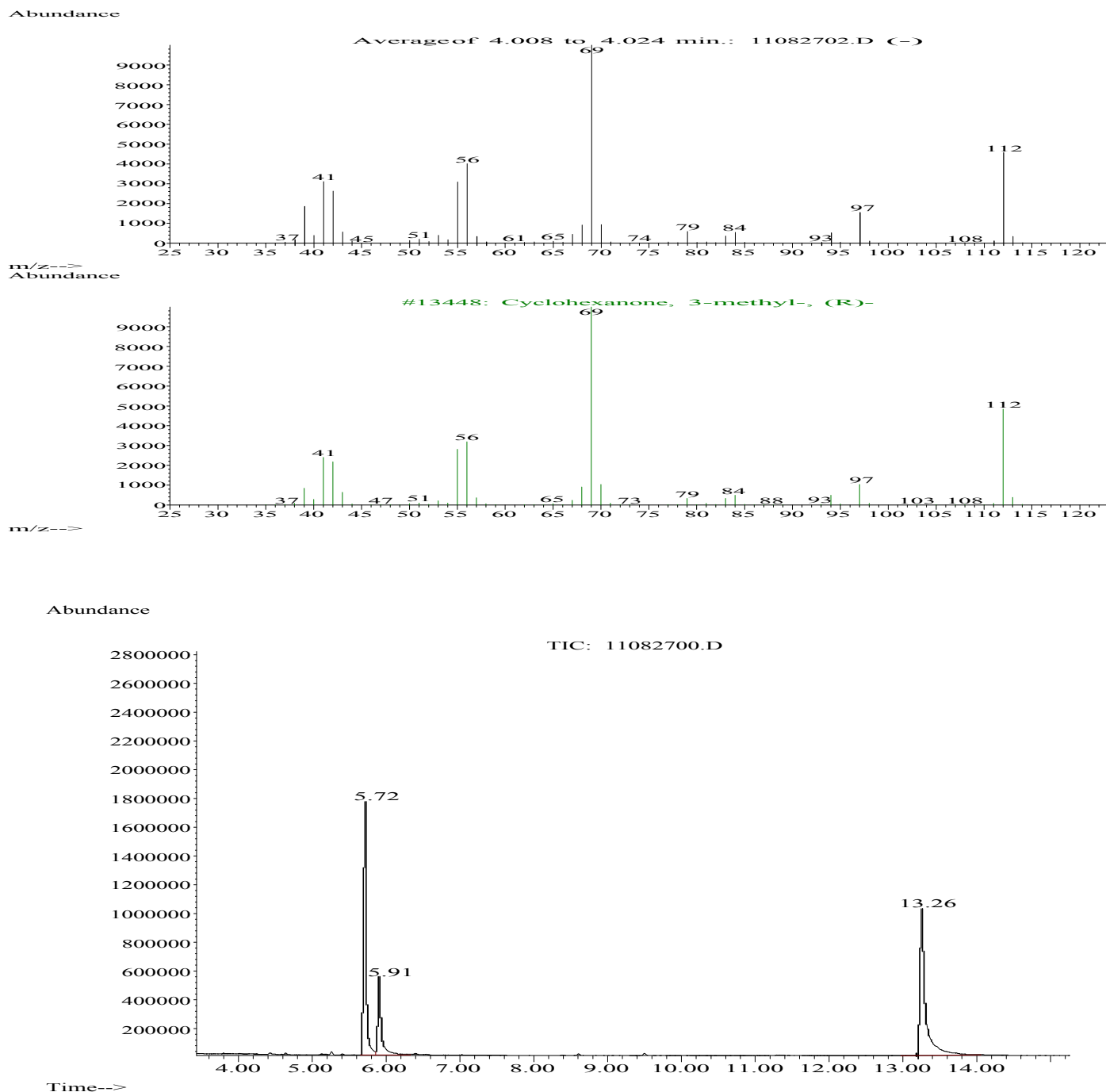
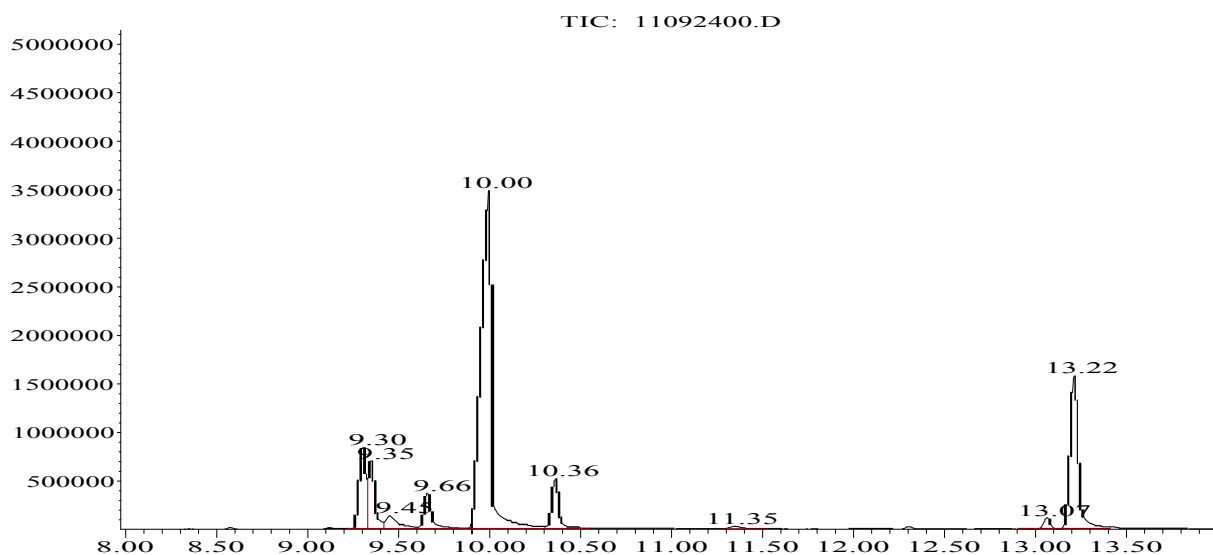


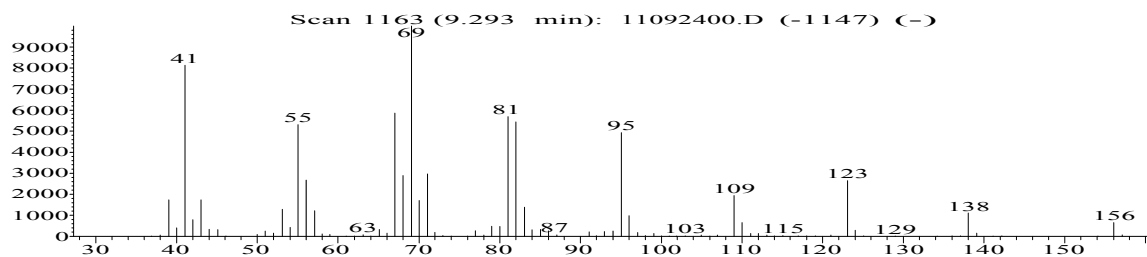
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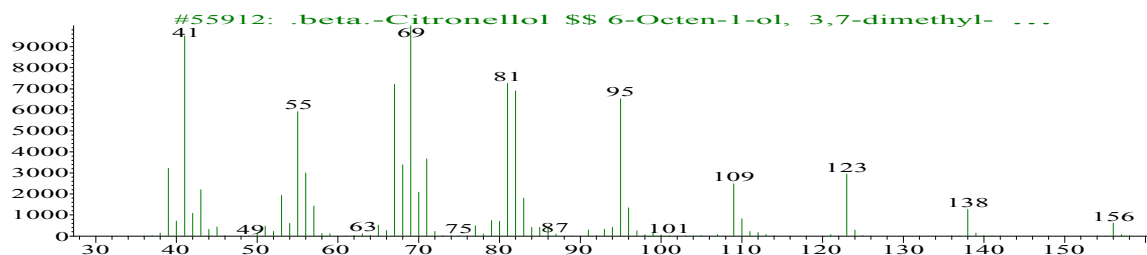
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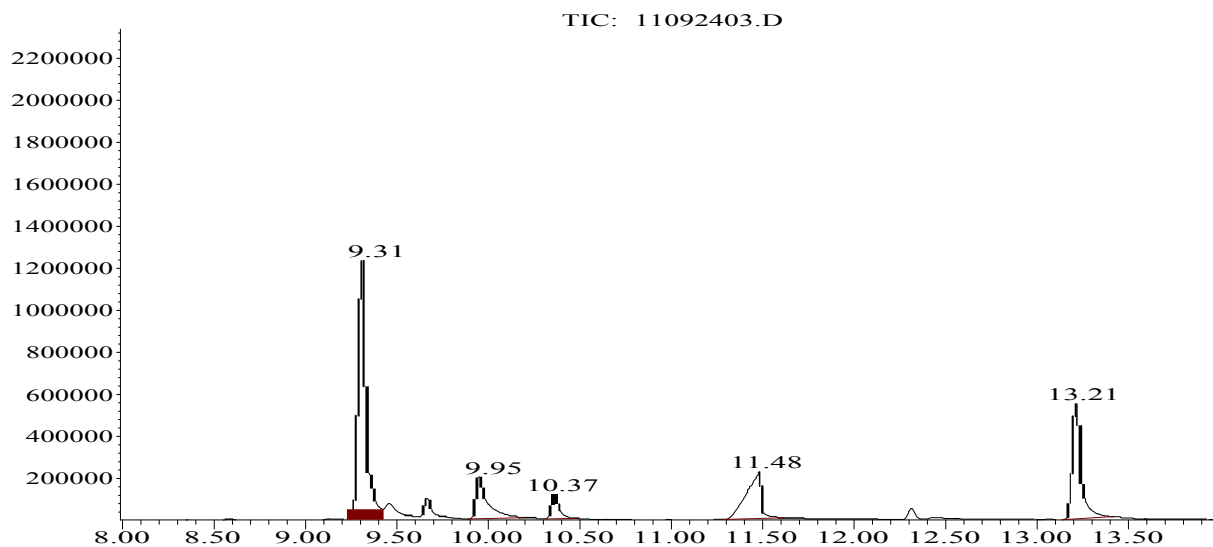
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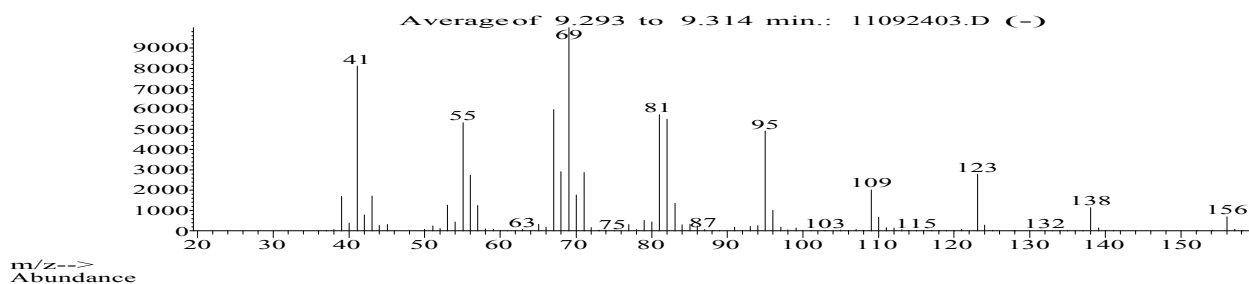
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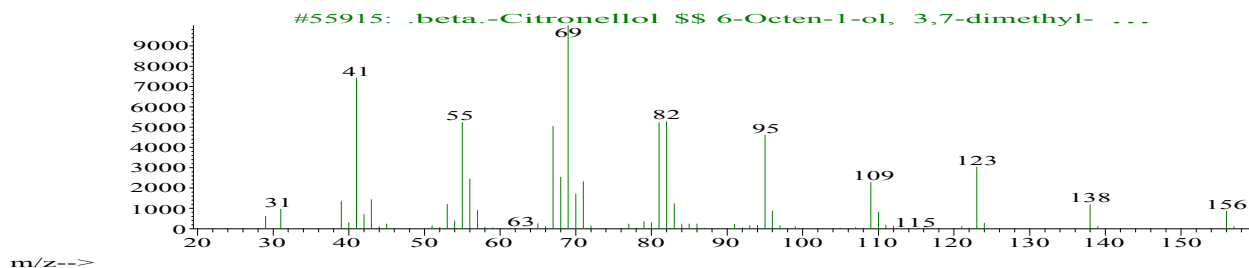


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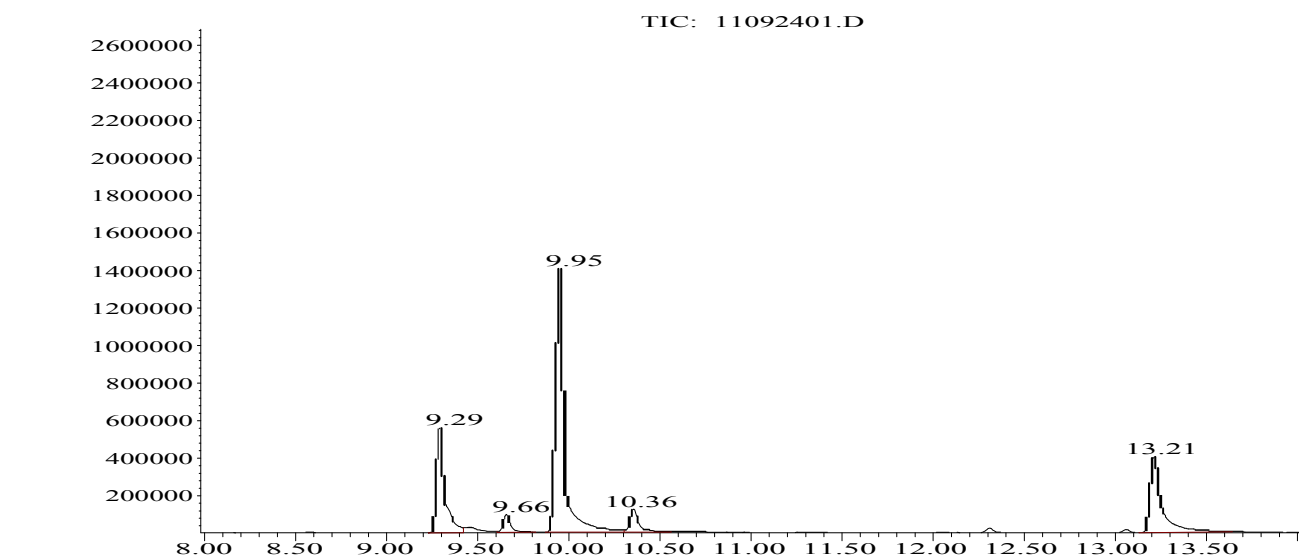
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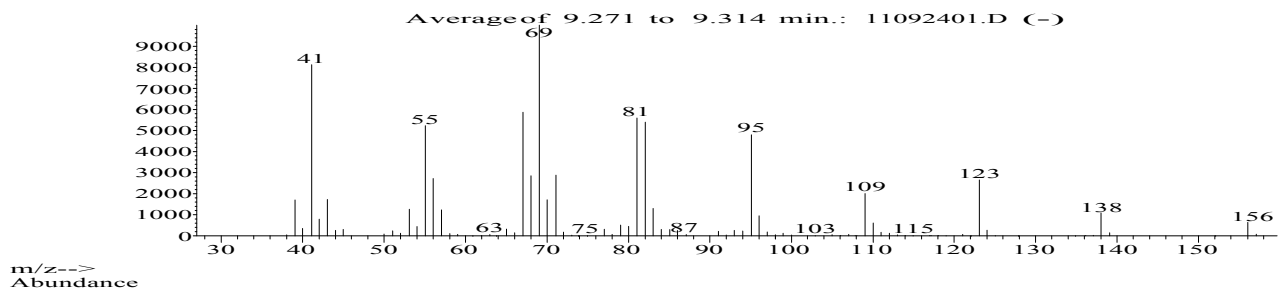
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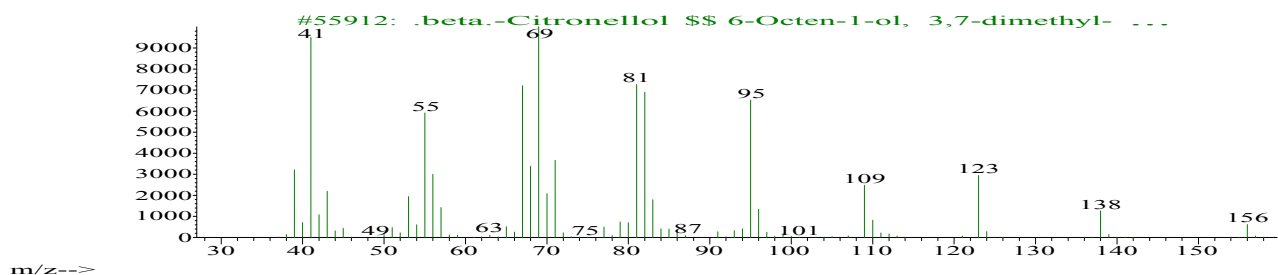
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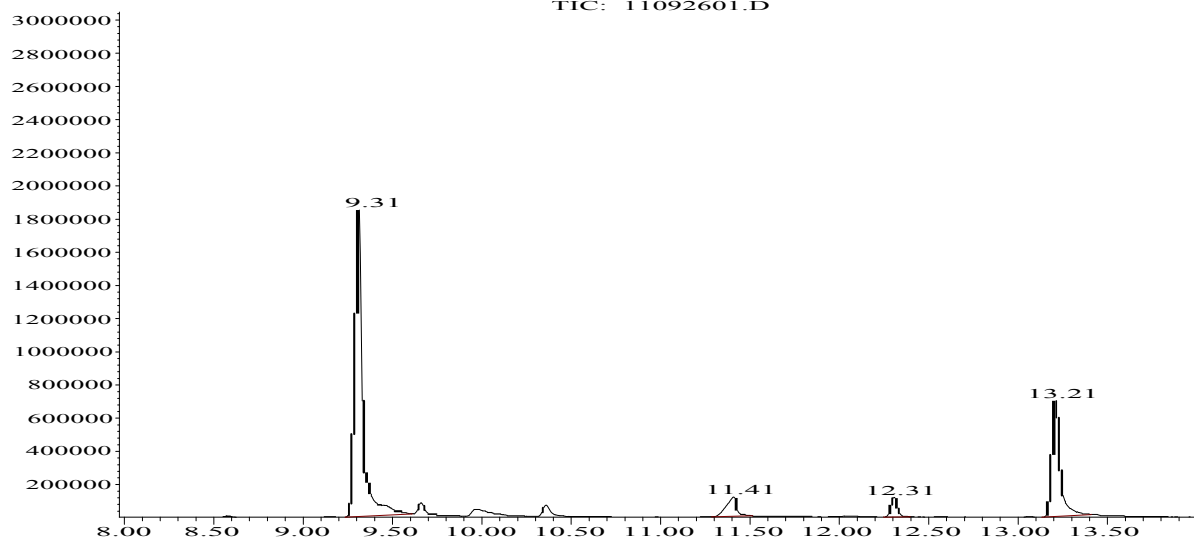


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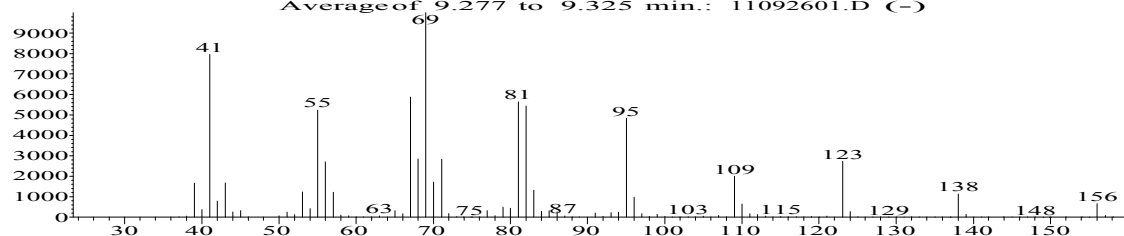
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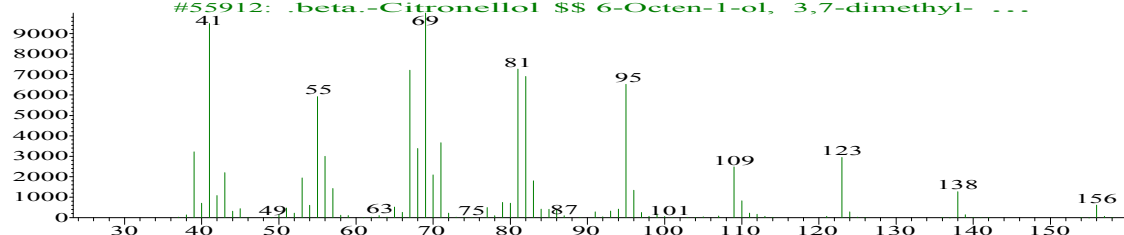
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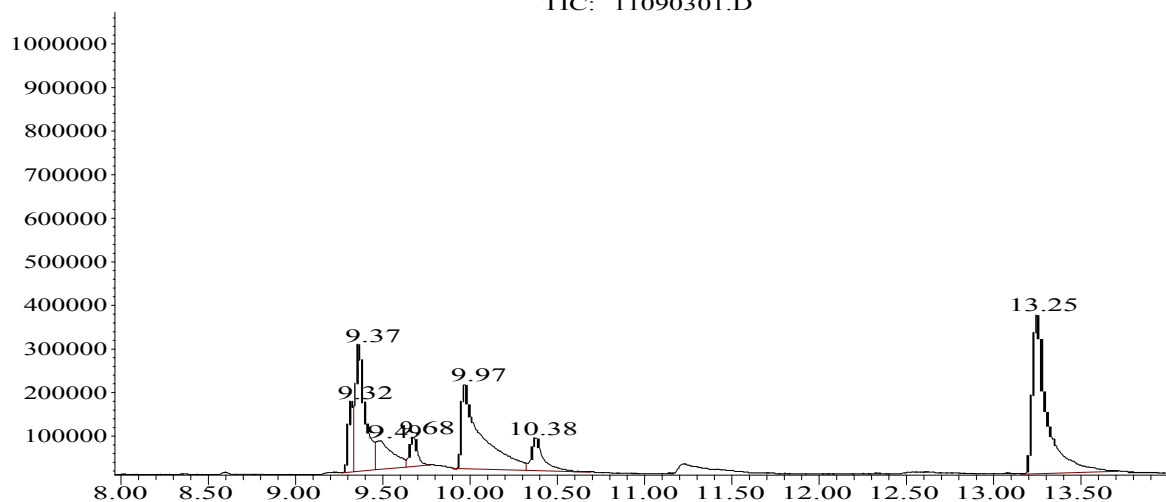
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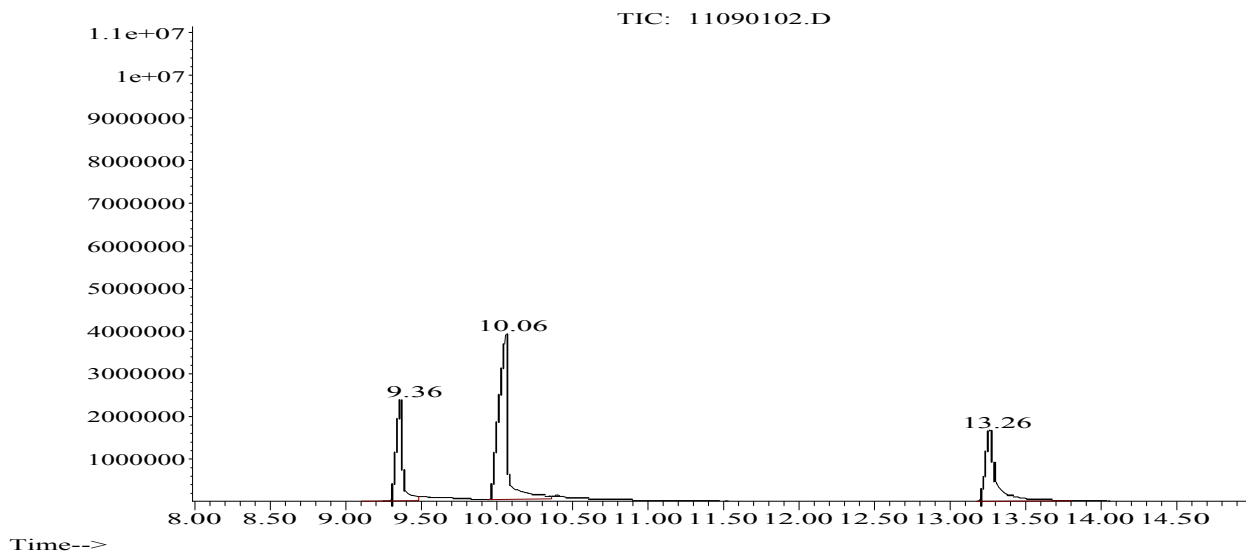
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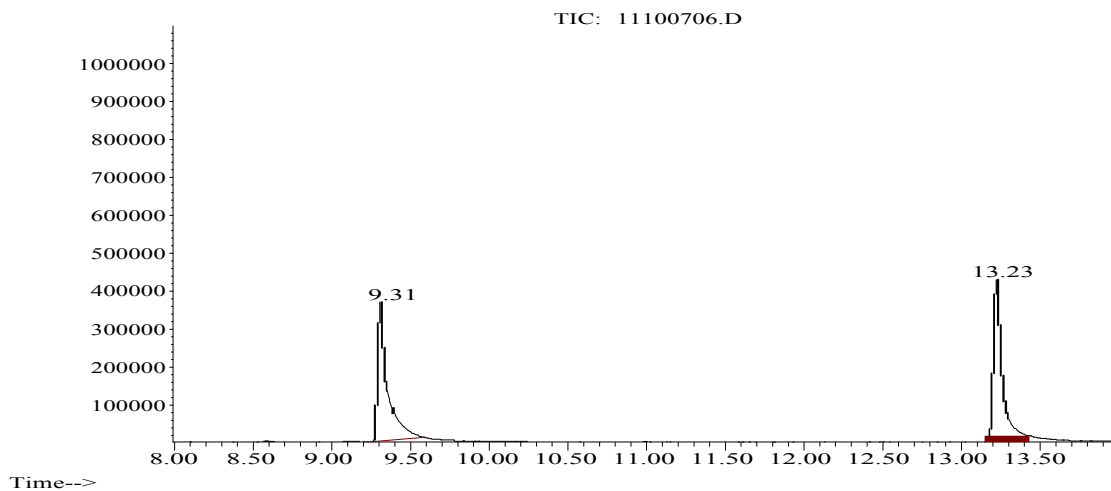


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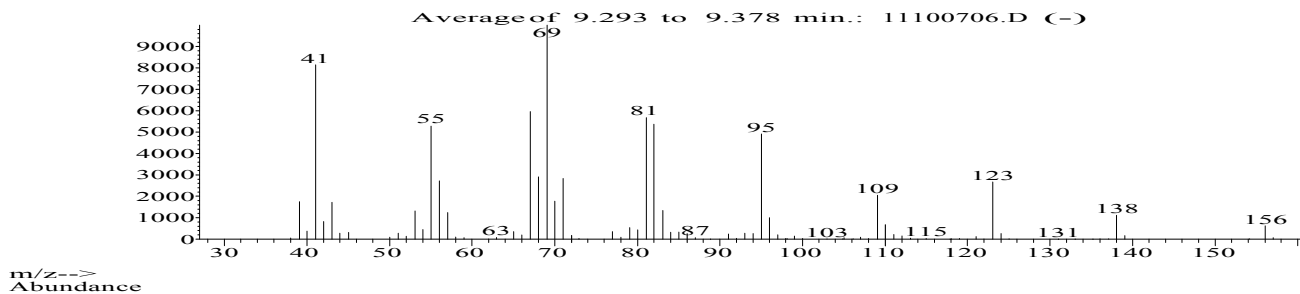
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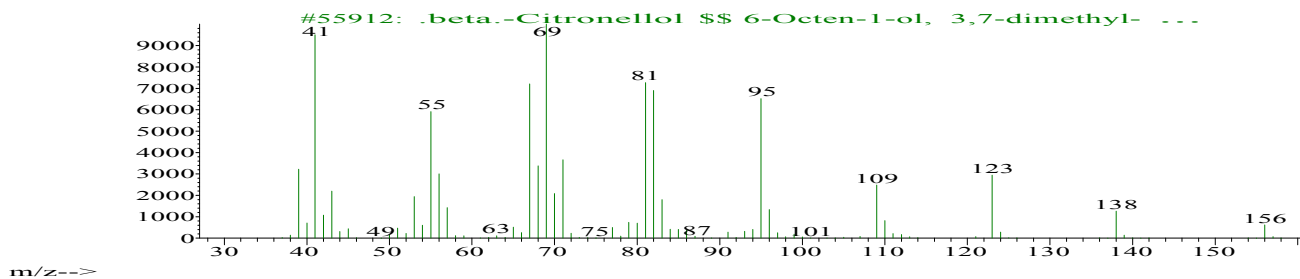
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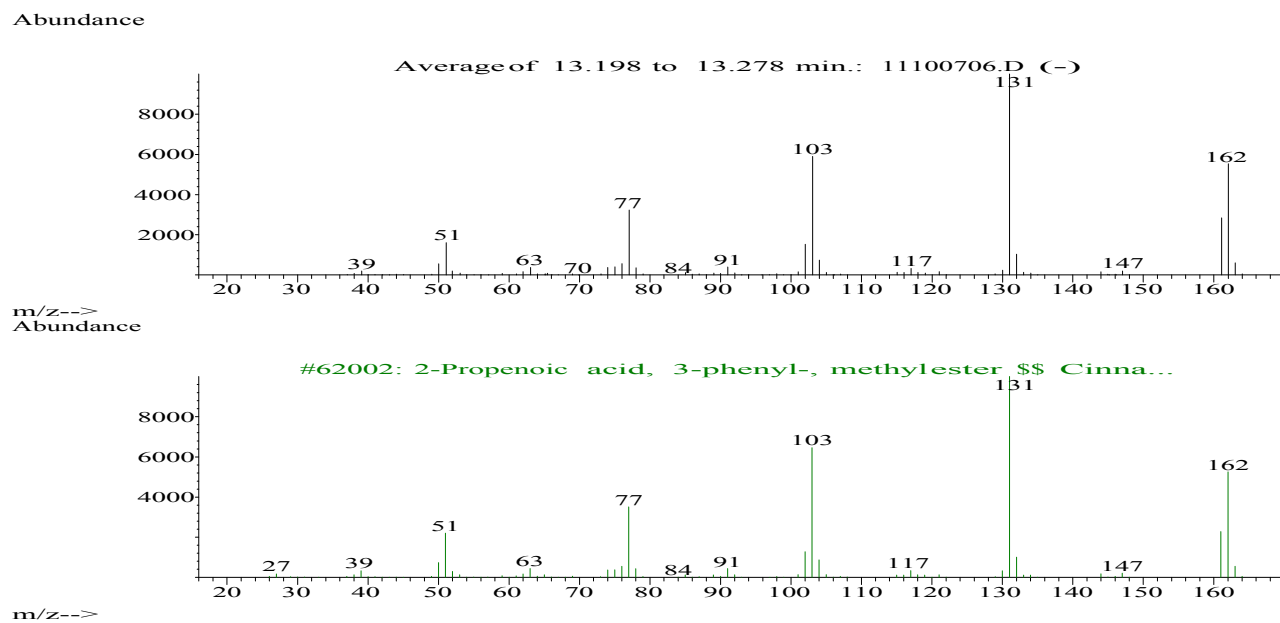
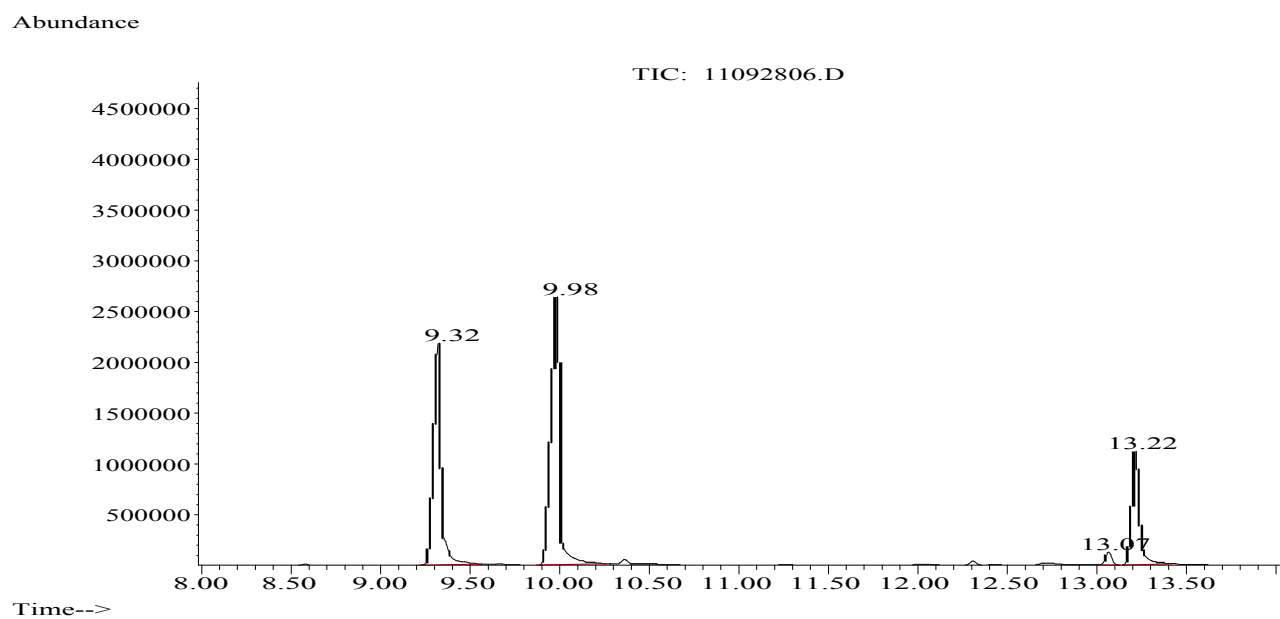


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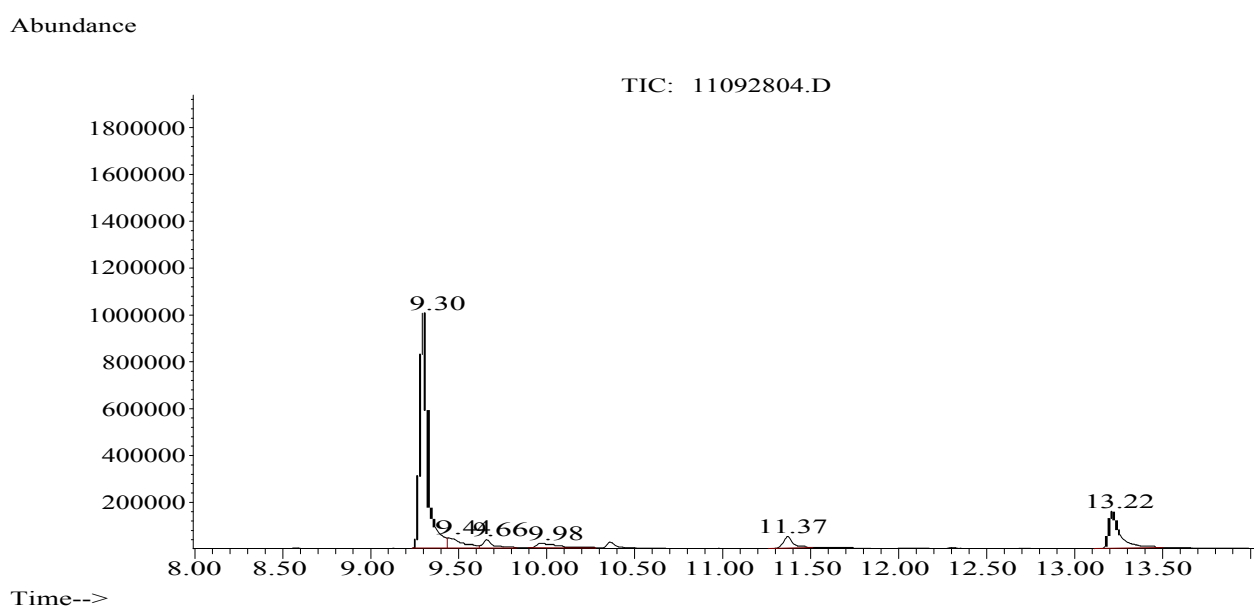
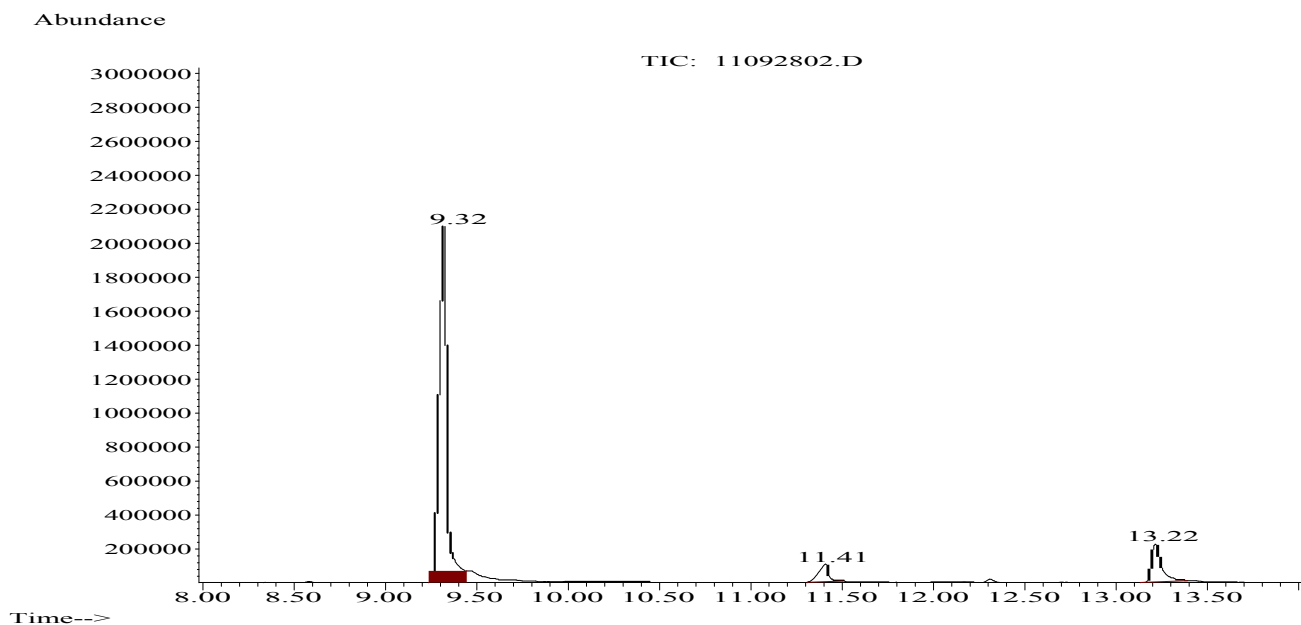
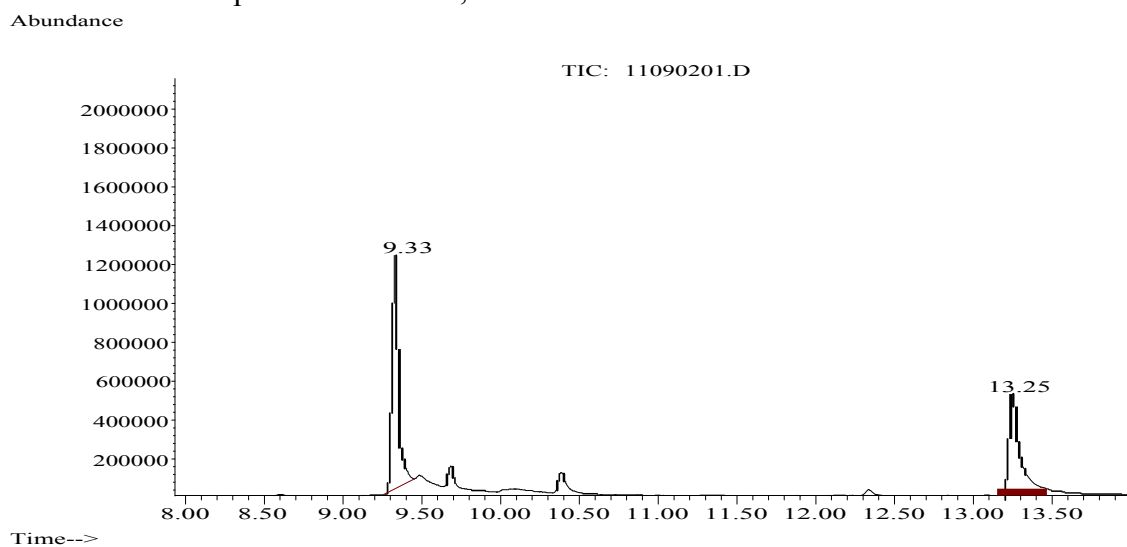


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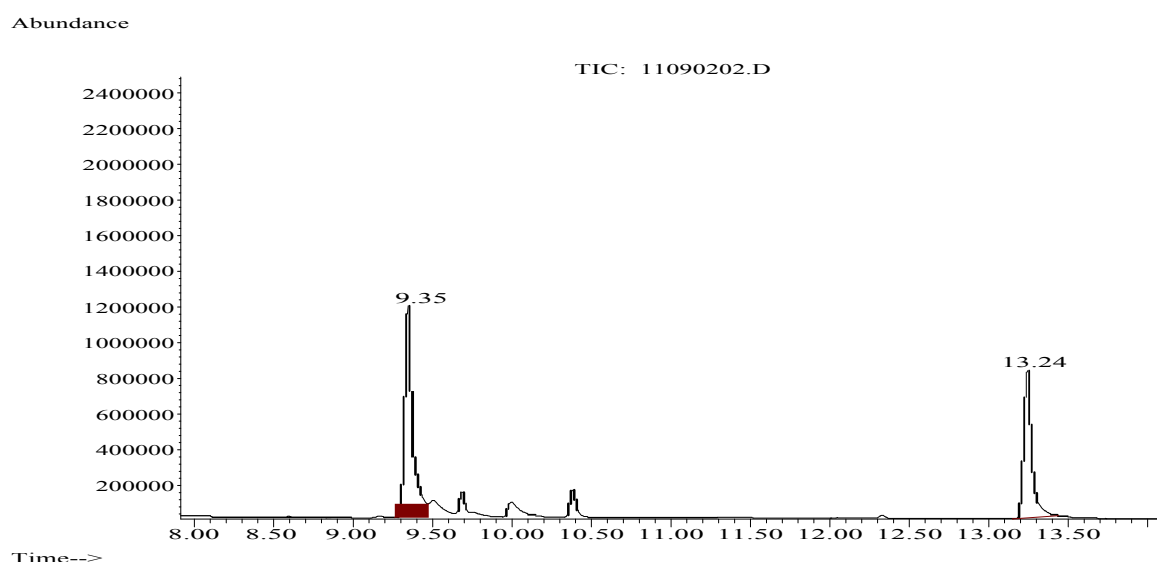


Table S1. Degenerate primers for cloning *EaOPR* and *IeOPR* and primer pairs for cloning *EaOPR*, *HbOPR*, *IeOPR* and *OYE2* into pET32a+

	Sequence (5'-3')
Degenerate	Forward: CCYTACAARATGGGCAASTTC
Primers	Reverse: CCACCATAYTCRTCYGTYCKGTC
Ea	Forward: <u>GGAATTC</u> ATGGGATCCTATACTGAATCG
	Reverse: CG <u>CTCGAGT</u> CAAGTCGAAATCTCAGAAG
Ie	Forward: T <u>GAATTC</u> ATGGCGGAAACGACTCCCG
	Reverse: GAC <u>CTCGAGT</u> TTAAAATCGCGATGCAGC
Hb	Forward: C <u>GGATCC</u> ATGGCTGAAACTGGAACAG
	Reverse: GAC <u>CTCGAGT</u> TCAAAGGCGTGATCGTGGCT
Sc	Forward: GG <u>GAAATTC</u> ATGCCATTTGTTAAGG
	Reverse: GGC <u>CTCGAGT</u> TTAATTTTGTCCC

Table S2. Primary data for enzyme activity comparison, estimation of whole-cell transformation vs crude enzyme activity, and fusion tag cleavage

Comparison of Enzyme Activity Level						
	pET32a+ / BL21 (DE3)	BL21 (DE3)	EaOPR	HbOPR	IeOPR	ScOYE2
C / IS	0.2256	0.2266	0.3777	2.3185	0.9142	2.3677
(GC integral)	0.2697	0.2320	0.4455	2.5584	1.0281	2.4010
Whole-Cell Transformation vs Crude Enzyme Activity						
	<i>Saccharomyces cerevisiae</i>		OYE2/pET32a+/ BL21 (DE3)		OYE2/ pET32a+/ BL21 (DE3)	
	intact cells		intact cells		cell lysates	
	28°C, 12h	37°C, 4h	28°C, 12h	37°C, 4h	28°C, 12h	37°C, 4h
C / IS	1.9131	1.1372	6.9931	4.4072	4.600	2.3677
(GC integral)	2.1113	1.2916	7.6456	4.5827	4.7840	2.4010
Fusion Tag Cleavage						
	fusion protein	EK-cleaved protein	pET32a+ / BL21 (DE3)	BL21 (DE3)	EK + BL21 (DE3)	EK + pET32a+/ BL21 (DE3)
C / IS	1.21	1.35	0.13	0.11	0.17	0.15
(GC integral)	1.43	1.14	0.19	0.15	0.18	0.18

C, Citronellol; IS, internal standard (methyl *trans*-cinnamate)