SUPPLEMENTARY INFORMATION

Heterologous expression of cytotoxic sesquiterpenoids from the medicinal mushroom *Lignosus rhinocerotis* in yeast

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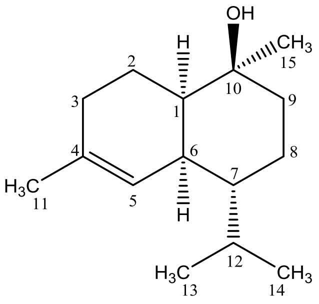
Table S1. Coding sequences of *GME3634*, *GME3638*, and *GME9210*

Gene ID	Coding sequence
GME3634	ATGGCTGTAACTCCTGCAAACGTCGCCTCCCCCGACTCGCAAGAGATCGTGCTCAAATTCCCCGATTTCATCTCTCCCATCCCCTACCCCCTTCGCTGCCATGTGCAGGAGCGAGAAGTGTCACGCCAATCCGAGGAATGGCTCCTCAGCATGGCCAACTTCTCCGAGAAACAGCGCAGCAAGTTCCTTACCCTCAACGGTGGTCTTCTTAGCGGCATGTGCTACATCGACTGCACGTTTGACGAACTGCGGGTCTGCACCGACTTCATGAACTTCCTGTTCACACTCGACGACTGGACGGACGAGTTCGACACGACCGGCACGCGGGGTCTCGCAGAGTGCGTCATGAACACGCTTTACTGGCCGCACAGCTACCAGGCAGACACCGCCGCGCACCGCTTGACAAAGTCCTTCTGGGTCCGCATGAAGCAGACGGCAGGCCCGGGCTGTCAGCAGCGCCTCATGTCGACGCTCGACACGTACTTCCAGGCCATCATGCAACAGGCCGCCGACCGTGGCTCACATAACATTCCGGAGTTGGAGGAGTACATCTTGCTCCGCCGAGACACGAGCGGGTGCAAGATCGGTTTCGCGTTCATCGAATACGCAGCCAACATTGATCTGCCCGACGACGTCATTGAGCACCCAATCATCAAGGCCATGGCCGATGCGACCAATGATCTCGTTTCGTGGGCGAATGATGTCCTTTCGTACAACGCTGAACAGTCACGCGGAGACACGCACAACCTCGTTTGTGTTCTCATGGCTCAGAACGGCCTCGACCGGCAGGGTGCGATCGAGCTGGCGGGCGAGCTATGGGAGAAGACCCTCCACTTGTTCTTCGAGTGCCGCAAGAACGTCCCTTCGTGGGGCTCCGAGATCGACCGTGCCGTCGCTCTGTACATCCAGGGGCTCGAAGACTGGATTATCGCGAACGCCGAGTGGAGCTTCGAGACGGAGCGCTACTTTGGCAAGGACGGGCATCTCGTGAAGAAGACGCGGCAGGTTACACTGCTTCCGGTCCGTACAGCTGCG
GME3638	ATGCGCGCTCGATCGTTCATTCTTCCCGACCTCGTGTCGGATTGCCCATATACGCTCAGGTGCAACTCCAATTGCGAAGCTGTTGCGCGCGCTTCAGAGGCCTGGATGCTCGAAGACGCGAATCTCTCACCGAAACGTCGTGACGCCTTCCTGCGTCTGCGGGGGGGAGAACTCACGGCAGCGTGCTACCCCGATACGGACGAGGCCTGCCTGCGAGTCGCCGCGGACTTCCTCAATTTCCTGTTTAGTTTGGACGACTGGTCGGACGAGTTCAGTATGGAGGACACTTGCGGGCTCGCGCAGTGCGTCATGTGTGTGCTCCATGATCCTGATGACTTCCAGACTGAAAAGGCTGCTGGCAAGCTCGCCAAAAGCTTTTTCAATCGGTTCCGGCAGACGGCGGGGCCGAGGTGTACTCGTCGATTCATCGATAGTATGGACCTCTTCTTTCATGCGATTGCACAGCAAGCCCAGGACCGCGCGTCCGGGTCTGCTCCCTCACTCGAAGAATATGTGGCCCTCCGCGAGGACACGAGCGGGTGCAAGCCCTGCTTCGCTCTCATCGAATACGCCGCGGGTATGGACCTTCCAGACCACGTCGCCCATCATCCAACAATCACCGCCCTCGAGCGGGAAGCCAACGCGTGTATATCATGGTCGAACGATCTCTTCTCGTACAACGTCGAGCAAGCGCGGGGTGACACACACAACATGATTGCGGTGATCATGCGCGAGGACGGGCGCAGCTTGCAAGAAGCCGTTGAATACTTGGGCGCTCTCTGCAAGCTCTGCATAGTGCACTTCGAGGAAAACCGAGCCATGCTGCCATCGTGGGGTCCGGAGATTGACGGGGAAGTCGACAGGTATGTGCTCGGCCTCCAGGACTGGATGGTCGGTGCCCTCCATTGGAGCTTCGACACCGCACGTTATTTCGGGGATGAAGGCCCTGCGATCAAGAAGCACGGTGTCGTGACACTACTACCGCGGAAGTCTTCATCG
GME9210	ATGCCTTTGTCTTCGTCTGTTGTCGCTTTCCGCCTTCCTGACACGCTTGGGTGCTGGCCATGGCGACGCTGCCTGAACACGCATTATGTGGAGGCCAAACAGGATTCGGCATCCTGGCTGGAGTCATTCCACCCGTTCGGCCCCAAGGCACAAAGGGCCTTCAACAAGTGCGACTTCAACCTTCTCGCGTCCCTAGCGTATCCGGTGGCCAGCAAAGACCAACTGCGTGCAGGGTGTGACCTGATGAACGTCTTCTTCGTCTTCGACGAGTACTCTGATGTCGAGAACGAGAAGACGGTCCAGCAGCTCGCGGACATCATCATGGACGCACTGCGAAACCCTCACAAGCCTCGTCCTGCAGGAGAATCTCTCGTGGGCGAAATCACTAGGCAGTTCTGGGCGCGCACGATCAAGGTAGCGAGCGAACCGTCGCAGCGCCGATTCATCGAGACCTTTGACGACTACTGCCAGTCCGTAGTCCAACAGGCTGCGGACCGGTCGCAGAACCACTTGCGTGACGTCGAGAGCTACTTGGAGAACCGGAGGGAGAACATCGGCGCCAAGCCGTCGTTTGCACTGCTCGAGTTGGACATGAACCTCCCCGACGAGGTGATCGAGCACCCGACCATCGTCAACCTGACGACATGGGCCATTGACATGATCATCCTGGGAAACGACATTGTCTCGTACAACGTGGAGCAGGCGCGTGGCGACGACGGACACAACGTCGTGACCATCGTCATGCATCACTACAACGTCGACGTGCAGGGTGCCATGGACCGCATCGCCGAGTGGCACCAGAGGCTGGCGGACCAGTTCCTCACCAACTACAACAAGCTGCCGTCGTGGGGACGCGAGATTGACGCGCAAGTTGAGCGGTACATCCAGGGCATCGGAAACTGGGTACGCGCGAACGACGCATGGAGTTTCGAGAGCGAGAGGTACTTTGGATTGAACGGACGCGAGATCGAGCAGAGTCGTTGGGTAACCCTACTCCCTCGGGTCTCCGCGGAGAAGCCTGCGGTTGTG

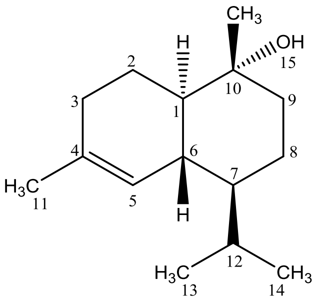
Table S2. PCR primers information

Gene ID	Primer name	Sequence	Product size (bp)
GME3634	XW-GME3634-F	ATCAACTATCAACTATTAACTATATCGTAATACCAATGGCTGTAACTCCTGCAAAC	410
	GME3634-fg1-r	GCTTCATGCGGACCCAGAAGGACTTTGTCAAGCGGTGCG	
	GME3634-fg2-f	CGCACCGCTTGACAAAGTCCTTCTGGGTCCGCATGAAGC	289
	GME3634-fg2-SOE-r	GTCTCCGCGTGACTGTTCAGCGTTGTACGAAAGGACATCATTCGCCCACGAAACGAGATC	
	GME3634-fg3-SOE-f	GATGTCCTTTCGTACAACGCTGAACAGTCACGCGGAGACACGCACAACCTCGTTTGTGT	297
	XW-GME3634-R	TGTCATTTAAATTAGTGATGGTGATGGTGATGCACCGCAGCTGTACGGACCG	
GME3638	XW-GME3638-F	ATCAACTATCAACTATTAACTATATCGTAATACCAATGCGCGCTCGATCGTT	374
	GME3638-fg1-r	GCCGGAACCGATTGAAAAAGCTTTTGGCGAGCTTGCCAGC	
	GME3638-fg2-f	GCTGGCAAGCTCGCCAAAAGCTTTTTCAATCGGTTCCGGC	289
	GME3638-fg2-r	ACGTTGTACGAGAAGAGATC-GTTCGACCATGATATACACGCG	
	GME3638-fg3-f	CGTGTATATCATGGTCGAACGATCTCTTCTCGTACAACGTCGA	336
	XW-GME3638-R	TGTCATTTAAATTAGTGATGGTGATGGTGATGCACCGATGAAGACTTCCGCGGTA	
GME9210	XW-GME9210-F	ATCAACTATCAACTATTAACTATATCGTAATACCAATGCCTTTGTCTTCGTCTGTTG	178
	GME9210-fg1-r	CTTTGCTGGCCACCGGATACGCTAGGGACGCGAGAAGGTTGAAGTCGCACTTGTTGAAGG	
	GME9210-fg2-f	ACCTTCTCGCGTCCCTAGCGTATCCGGTGGCCAGCAAAGACCAACTGCGTGCAGGGT	177
	GME9210-fg2-r	CTTGATCGTGCGCGCCCAGAACTGCCTAGTGATTTCGCCCA	
	GME9210-fg3-f	GGGCGAAATCACTAGGCAGTTCTGGGCGCGCACGATCAA	282
	GME9210-fg3-r	CACGTTGTACGAGACAATGTCGTTTCCCAGGATGATCATGTCA	
	GME9210-fg4-f	CATGATCATCCTGGGAAACGACATTGTCTCGTACAACGTGGAG	353
	XW-GME9210-R	TGTCATTTAAATTAGTGATGGTGATGGTGATGCACCACAACCGCAGGCTTCTCCG	

Table S3. 13C (150.903 MHz) and 1H (600.130 MHz) NMR spectral data for torreyol and α-cadinol (using $CDCl_3$ as internal standard, 77.16 ppm)



1



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Carbon*	**Torreyol (1)**		**α-Cadinol (2)**	
	δC (ppm)	**δH (ppm)**	**δC (ppm)**	**δH (ppm)**
1	45.55 d	1.60 m	50.15 d	1.21
2	18.52 t	1.89 m, 1.58 m	22.09 t	1.60, 1.14
3	31.14 t	1.99	31.07 t	1.98
4	134.37 s	---	135.12 s	---
5	124.61 d	5.51 m	122.46 d	5.50 s
6	36.79 d	2.02 d	40.00 d	1.72
7	44.10 d	1.3	46.83 d	1.04
8	21.54 t	1.51, 1.01	22.81 t	1.27, 2.00
9	35.34 t	1.56 t, 1.52 t	42.33 t	1.82, 1.80
10	72.58 s	---	72.62 s	---
11	23.64 q	1.66	23.99 q	1.67 s
12	26.42 d	1.97 m	26.12 d	2.15 m
13	21.69 q	0.89 d	21.67 q	0.92 d
14	15.33 q	0.81 d	15.26 q	0.77 d
15	27.97 q	1.29	20.91 q	1.10 s

*Carbon signal assignments are based on Van Eijk et al. [1].

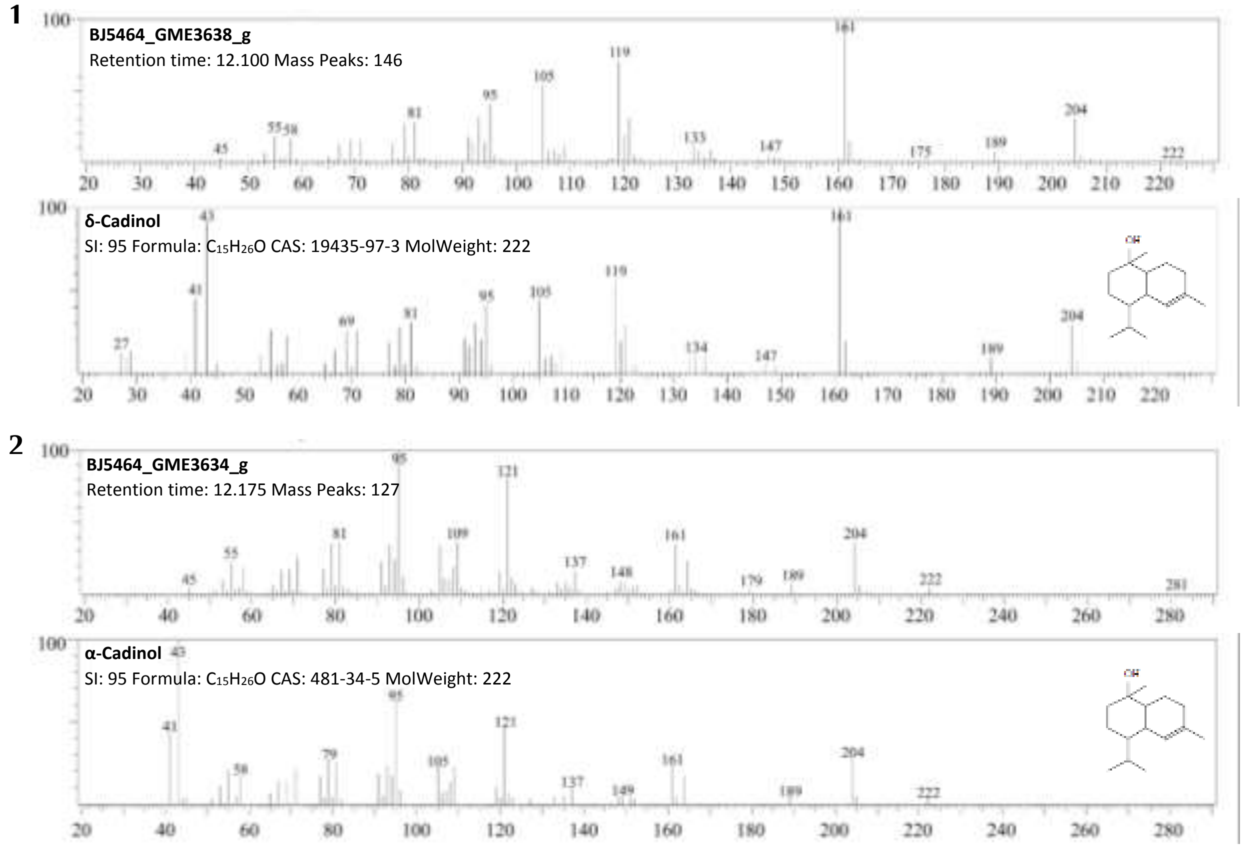

Figure S1. Mass spectrums of putative torreyol (1) and α-cadinol (2) to that of known compounds in NIST 05 library.

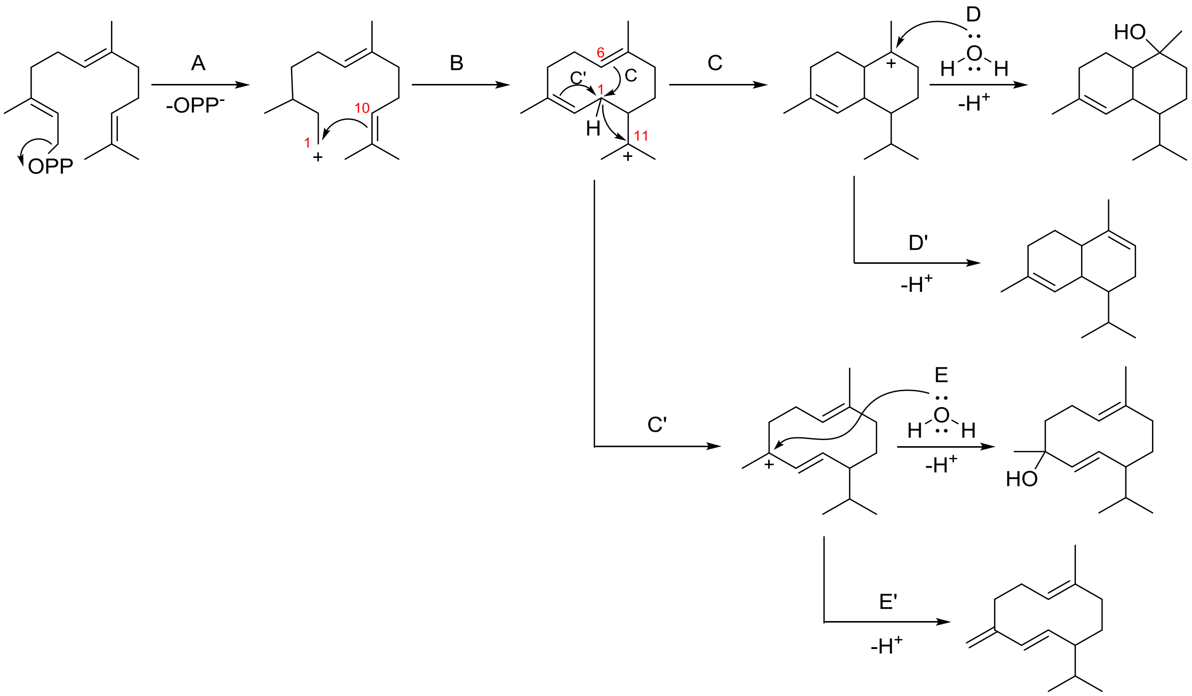

Figure S2. Proposed general mechanism for the biosynthesis of torreyol and α-cadinol from farnesyl pyrophosphate. The excision of the pyrophosphate group is catalysed by the enzyme producing the farnesyl cation (A: farnesyl carbocation formation). This is followed by the nucleophilic attack of carbon 10 (C-10) to the cation in C-1 that produces the first cyclisation reaction leading to germacranyl cation (B: cyclisation leading to germacranyl cation). At this point, the germacranyl cation can be quenched (C′: hydride shift) by deprotonation or hydrolysis to form germacrene (E: carbocation quenching by hydrolysis producing germacrene D-ol) and germacrene-4-ol [E’: hydride shift leading to a germacrene (germacrene D is presented here)] respectively, or continue the second cyclisation reactions via a Wagner-Meerwein rearrangement followed by the nucleophilic attack of C-6 over C-1. Depending on which face the hydride shift occurs, this leads to either the muurolenyl or cadinenyl cation (C: hydride shift). Finally, the resulted cation is quenched by either deprotonation [D’: hydride shift resulting in a cadinene (α-cadinene is shown here)] or hydrolysis (D: carbocation quenching by hydrolysis leading to torreyol and α-cadinol) to yield cadinene/muurolene or cadinols/muurolols, respectively. Note: The numbering in here follows the farnesyl numbering, not the final product numbering. In the case of GME3634 and GME3638, the deprotonation is a competing reaction that also yields sesquiterpene products that can be seen in Table 1; however, hydrolysis appears to be the preferred mechanism.

Reference

1. Van Eijk GW, Roeijmans HJ, Verwiel PEJ. Isolation and identification of the sesquiterpenoid (+)-torreyol from *Xylobolus frustulatus*. Exp Mycol. 1984;8:273-5.