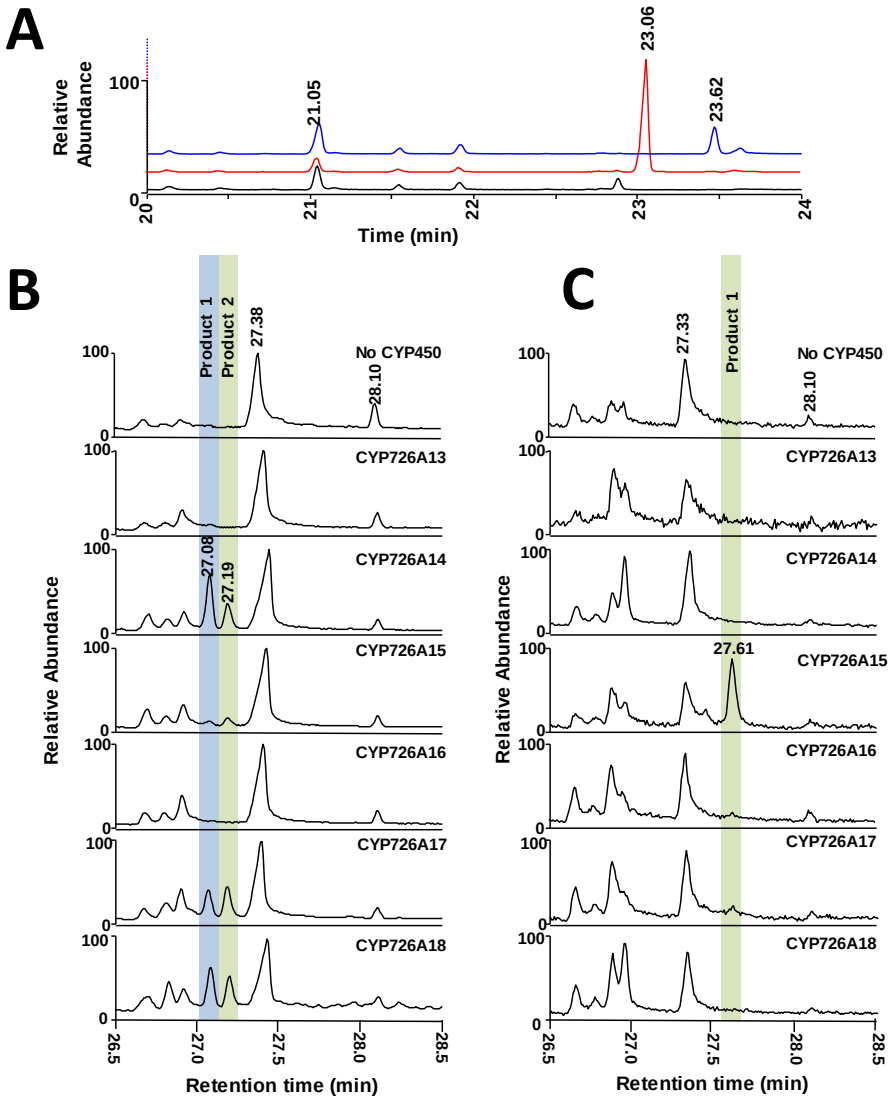
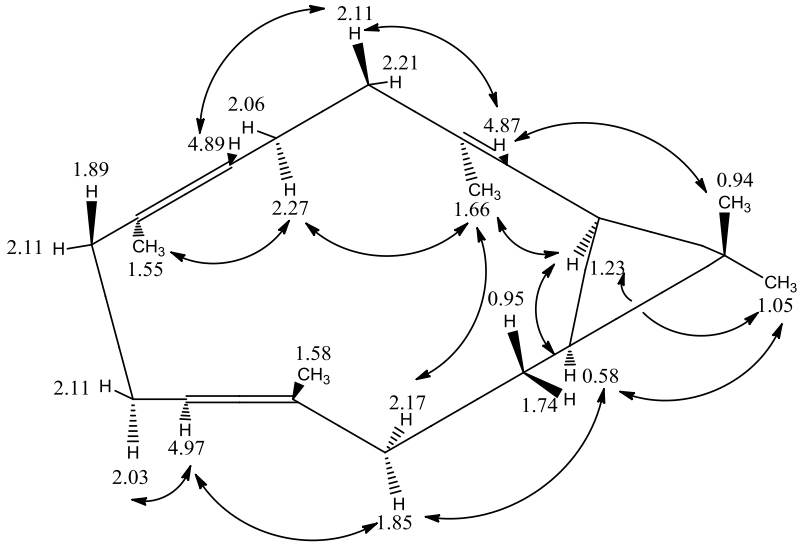
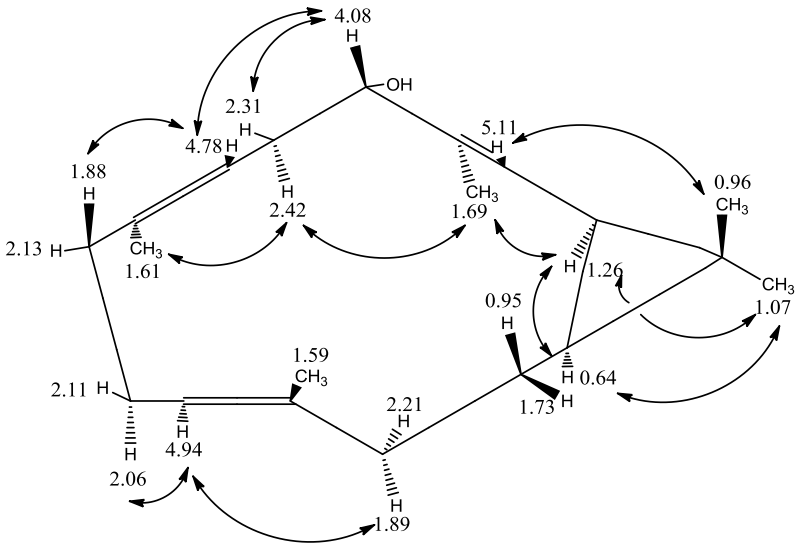


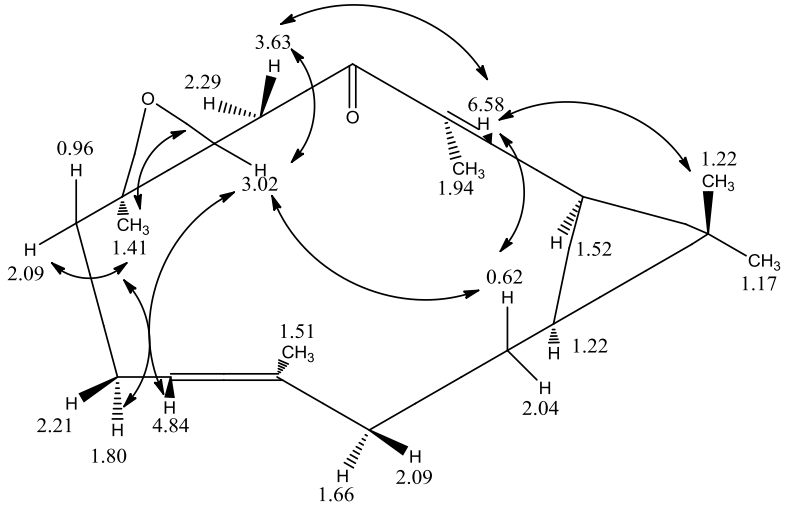
Supplemental Figure 1. *A* - GC analysis of a chloroform extract of *N. benthamiana* leaves infiltrated with a p19 vector only (black line) or the p19 vector plus a pFGC5951 vector containing a casbene synthase ORF from *J. curcas* (red line) or the neocembrene synthase ORF from *R. communis.* The elution times of casbene (23.06 min) and neocembrene (23.62) are indicated. *B* – GC analysis of a chloroform extract of *N. benthamiana* leaves infiltrated with a p19 vector, a pFGC vector containing a casbene synthase ORF for *J. curcas* and pFGC vectors contain the ORF for a cytochrome P450 gene as indicated in the top right of each graph. *C* - GC analysis of a chloroform extract of *N. benthamiana* leaves infiltrated with a p19 vector, a pFGC vector containing the neocembrene synthase ORF for *R. communis* and pFGC vectors contain the ORF for a cytochrome P450 gene as indicated in the top right of each graph.



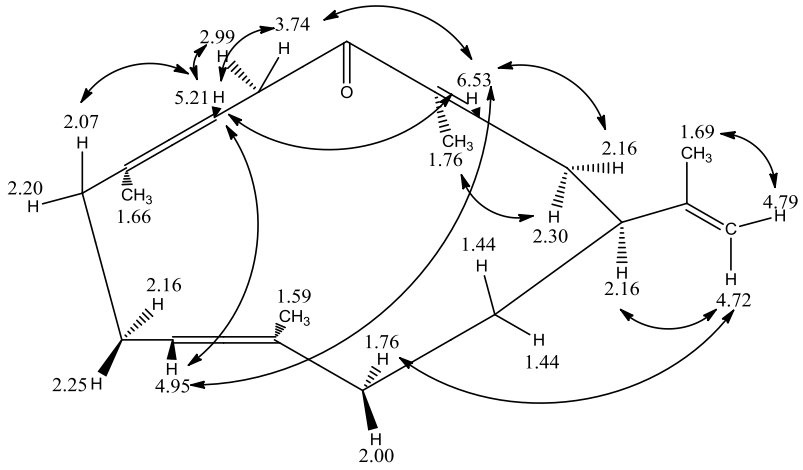
Supplemental Figure 2. Critical NOESY correlations observed for casbene, indicated by double-headed arrows between protons.



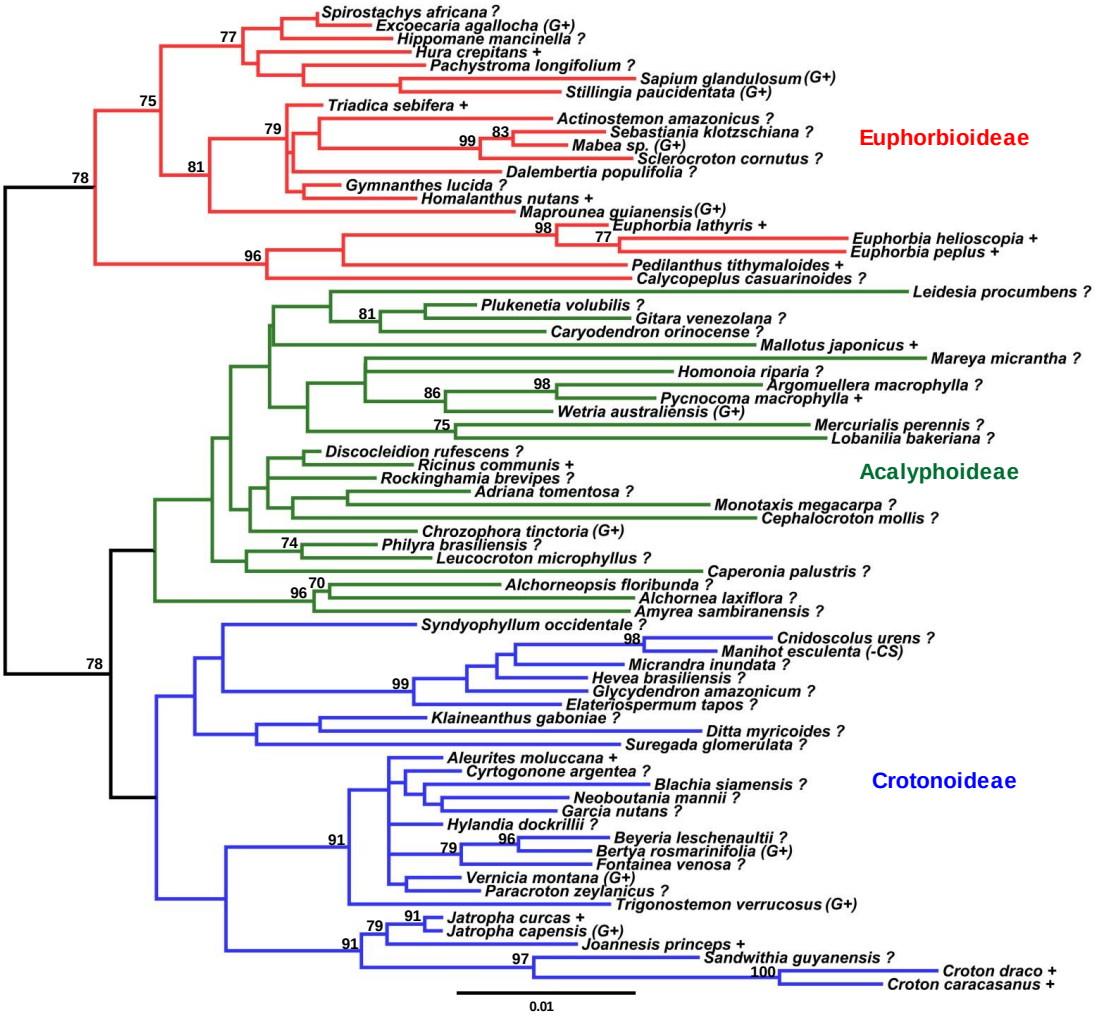
Supplemental Figure 3. Critical NOESY correlations used in establishing the relative configuration at the 5-position of 5α-hydroxy-casbene, indicated by double-headed arrows between protons (the conformation adopted by the 14-membered ring is similar to that of casbene itself – see Supplemental Figure 2).



Supplemental Figure 4. Critical NOESY correlations observed for 5-keto-7,8-epoxy-casbene, indicated by double-headed arrows between protons.

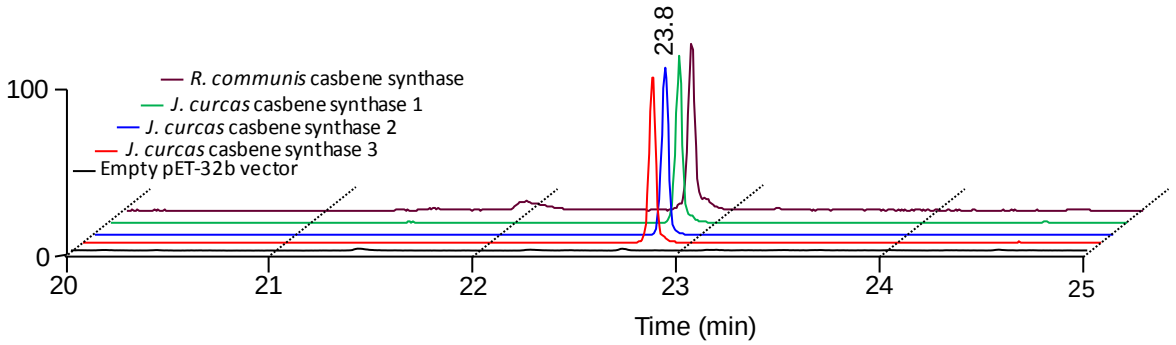


Supplemental Figure 5. Critical NOESY correlations observed for 5-keto-neocembrene, indicated by double-headed arrows between protons.

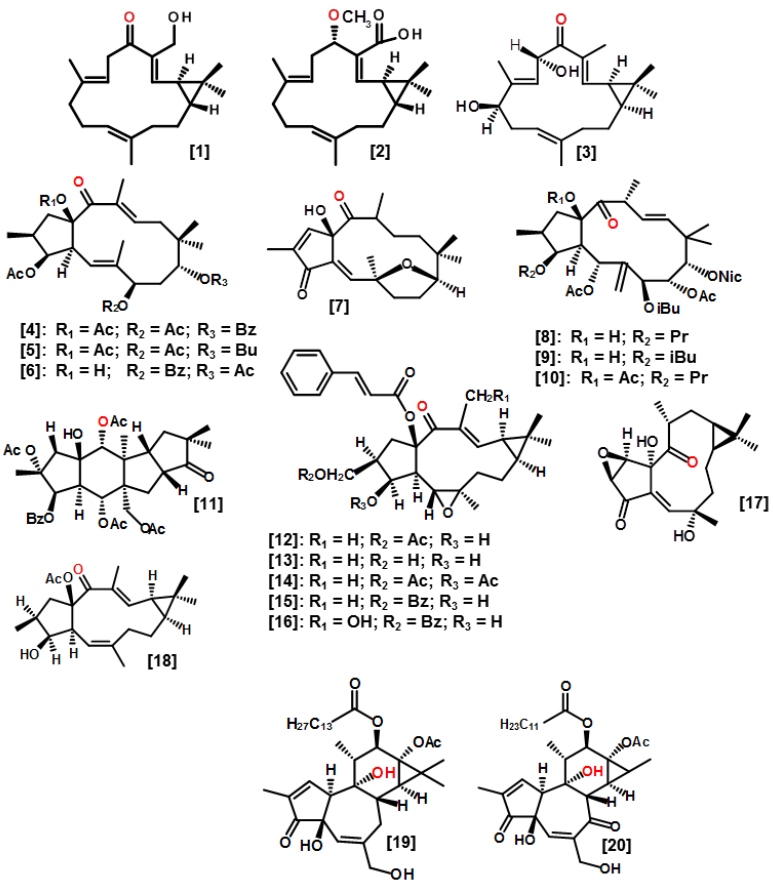


Supplemental Figure 6. Midpoint rooted phylogenetic tree of the Euphorbiaceae based on the plastidial ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) DNA sequence. The taxa included are: ACALYPHOIDEAE subfamily highlighted in green; *Leidesia procumbens* [AY794932], *Plukenetia volubilis* [AY794929], *Gitara venezolana* [AY794924], *Caryodendron orinocense* [AY794931], *Mallotus japonicas* [AY794934], *Mareya micrantha* [AY794941], *Homonoia riparia* [AY794978], *Argomuellera macrophylla* [AY794937], *Pycnocoma macrophylla* [AY794939], *Wetria australiensis* [AY794940], *Mercurialis perennis* [AY794944], *Lobanilia bakeriana* [AY794947], *Discocleidion rufescens* [AY794952], *Ricinus communis* [AY794915], *Rockinghamia brevipes* [AY794983], *Adriana tomentosa* [AY794917], *Monotaxis megacarpa* [AY947535], *Cephalocroton mollis* [AY794950], *Chrozophora tinctoria* [AY794951], *Philyra brasiliensis* [AY794920], *Leucocroton microphyllus* [AY794980], Caperonia palustris [AY794923], *Alchorneopsis floribunda* [AY794962], *Alchornea laxiflora* [AY794957] and *Amyrea sambiranensis* [AY794964]. EUPHORBIODEAE subfamily highlighted in red; *Spirostachys africana* [AY794838], *Excoecaria agallocha* [AY794839],

Hippomane mancinella [AY794835], *Hura crepitans* [AY788177], *Pachystroma longifolium* [AY794847], *Sapium glandulosum* [AY794841], *Stillingia paucidentata* [AY794845], *Triadica sebifera* [AY794859], *Actinostemon amazonicus* [AY794861], *Sebastiania klotzschiana* [AY794850], *Mabea* sp. [AY794852], *Sclerocroton cornutus* [AY794850], *Dalembertia populifolia* [AY794857], *Gymnanthes lucida* [AY794858], *Homalanthus nutans* [AB267957], *Maprounea guianensis* [AJ418810], *Euphorbia lathyris* [HM849989], *Euphorbia helioscopia* [HM849988], *Euphorbia peplus* (from transcriptome assembly), *Pedilanthus tithymaloides* [AY794825] and *Calycopeplus casuarinoides* [AY794829]. CROTONOIDEAE subfamily highlighted in blue; *Syndyophyllum occidentale* [AY794967], *Cnidoscolus urens* [AY794874], *Manihot esculenta* [AB233880], *Micrandra inundata* [AY794877], *Hevea brasiliensis* [HQ285842], *Glycydendron amazonicum* [AY794876], *Elateriospermum tapos* [AY794873], *Klaineanthus gaboniae* [AY794869], *Ditta myricoides* [AY794871], *Suregada glomerulata* [AY794866], *Aleurites moluccana* [AY794883], *Cyrtogonone argentea* [AY794974], *Blachia siamensis* [AY794888], *Neoboutonia mannii* [AY794896], *Garcia nutans* [AY794890], *Hylandia dockrillii* [AY794882], *Beyeria leschenaultia* [AY794879], *Bertya rosmarinifolia* [AY794878], *Fontainea venosa* [AY794881], *Vernicia montana* [AY794899], *Paracroton zeylanicus* [AY794894], *Trigonostemon verrucosus* [AY788192], *Jatropha curcas* [HQ153096], *Jatropha capensis* [AM234978], *Joannesia princeps* [AJ418808], *Sandwithia guyanensis* [AY794904], *Croton draco* [EF405840] and *Croton caracasanus* [EF405834]. The majority of the sequences were obtained in a study by Wurdack *et al* (Wurdack et al., 2005). The + symbols after the taxon names indicates species known to produce diterpenoids with structural relationships to casbene or neocembrene, whereas a (G+) denotes a member of the same genus produces casbene or neocembrene derived diterpenoids (Sakata et al., 1971; Seip et al., 1983; Ghisalberti et al., 1985; Beutler et al., 1989; Yamamura et al., 1989; Brooks et al., 1990; Kashman et al., 1994; Achenbach and Benirschke, 1997; Murillo et al., 2001; Satyanarayana et al., 2001; Mongkolvisut and Sutthivaiyakit, 2007; Busch et al., 2008; Johnson et al., 2008; Xie et al., 2010; Yamaguchi et al., 2012; Chavez et al., 2013; Li et al., 2013; Lu et al., 2014)]. The ? symbol indicates no reported production of casbene of neocembrene derived diterpenoids whereas the (-CS) denotes genome does not contain a casbene/neocembrene synthase ortholog. The bootstrap values for 1,000 replicates are shown as percentages. Bootstrap values below 70% are not shown. The edited sequence alignment used for construction of this tree is available in Supplemental Dataset 2.



Supplemental Figure 7. Conversion of GGPP into casbene by a crude lysate obtained from the expression of thioredoxin fusion proteins of casbene synthase from *R. communis* and three casbene synthase homologs from *J. curcas*. Cleared lysates were incubated with GGPP and then extracted with hexane and analyzed by GC-MS. An extract from reaction using the cleared lysate of the empty pET32b vector was also analyzed as a negative control.



No.	Compound	Source species	Biologicial activity	Ref.
Casbanes				
[1]	Pekinenin A	*Euphorbia pekinesis*	Cytotoxic	(Shao et al., 2011)
[2]	Pekinenin B	*Euphorbia pekinesis*	Cytotoxic	(Shao et al., 2011)
[3]	1,4-dihydroxy-2*E*,6*E*,12*E*-trien-5-one-casbane	*Croton nepetaefolius*	Antibacterial	(Sá et al., 2012)
Jatrophanes				
[4]-[6]	Pubescenes A-C	*Euphorbia pubescens*	MDR reversal	(Valente et al., 2004)
[7]	Japodagrone	*Jatropha podogrica*	Antibacterial	(Aiyelaagbe et al., 2007)
[8]-[10]	Euphodendrophanes A-C	*Euphorbia dendroides*	MDR reversal	(Aljančić et al., 2011)
Rearranged jatrophane				
[11]	Portlandicine	*Euphorbia portlandica*	MDR reversal	(Madureira et al., 2004)
Lathyranes				
[12]-[16]	Latilagascenes A-E	*Euphorbia lagascae*	Cytotoxic	(Pusztai et al., 2007)
[17]	Japodagrin	*Jatropha podogrica*	Antibacterial	(Aiyelaagbe et al., 2007)
[18]	Jolkinol D	*Euphorbia piscatoria*	MDR reversal	(Reis et al., 2013)
Tiglianes				
[19]	Phorbol-12-myristate-13-acetate	*Croton tiglium*	Co-carcinogenic	(Van Duuren and Orris, 1965)
[20]	Trigowiin A	*Trigonostemon howii*	Antiviral	(Bourjot et al., 2012)

Supplemental Figure 8. Structures of biologically active diterpenoids that have been isolated from a number of Euphorbiaceae. The oxygen atom labeled in red on each of the structures corresponds to the 5-position of casbene which was oxidized by castor enzymes CYP726A14, CYP726A17 and CYP726A18 (this study). The abbreviated functional groups are as follows; Ac = acetate, Bu = butyl, Bz = benzoyl, iBu = isobutyl, Nic nicotinoyl and Pr = propanoyl.

Supplemental Table 1: Primers used for qPCR analysis of *R. communis* genes

Transcript ID	Gene Annotation	Forward primer	Reverse primer	Amplicon size (bp)	Ta (ºC)
Housekeeping genes					
Rc29836.m000559	Actin 1	5'-ACCGTATGAGCAAGGAGATCACTG-3'	5'-CCCTTGGAAATCCACATCTGCTG-3'	148	60
Rc29983.m003140	Nuclear cap-binding protein	5'-AGCTGGGTCATTGGGCTCTTTC-3'	5'-GGAACCGCTTCCGATGGTAATCTC-3'	119	63
Rc30169.m006323	Ubiquitin 1	5'-ATTCCTCCTGTCCAGCAAAGGC-3'	5'-CAAGCACAAGATGAAGCACAGAAC-3'	109	58
Target genes					
Rc30169.m006273	CYP726A13	5'-CCAGTGCTCCTGCTGAGCTTCC-3'	5'-CCGGAGGTGAGTTGAAGCTGCC-3'	88	60
Rc30169.m006274	Short-chain ADH	5'-CCAACAGGCTTGAAGGAAAGGTAG-3'	5'-TGTTTGCAAAGATTGTGGCCAAGC-3'	145	60
Rc30169.m006275	CYP726A14	5'-AACACAGAGCCACCAGGAATCG-3'	5'-AGCTCCAAACATATCCAGAATGGC-'3	146	65
Rc30169.m006276	Neocembrene synthase	5'-ACATAATGTCTCATGTGAGCGAGCAA-3'	5'-GTCGTGCAAGGTTGACCACTCT-3'	210	63
Rc30169.m006277	CYP726A15	5'-GCTAGACCACCGCAACCGCA-3'	5'-AGGCGGTGATGGGGAAGAGC-3'	149	65
Rc30169.m006279	CYP726A16	5'-TCCAAGAATGGTGGTGGTGACG-3'	5'-ACCGCCAAGCATTTCCAGAATGG-3'	146	65
Rc30169.m006281	BAHD acyltransferases	5'-ACGCATGTCGAGGTGGTGTC-3'	5'-CATGCGACGATGCAGTTGGC-3'	97	65
Rc30169.m006282	CYP726A17	5'-CGCTTGAGCTTGTTGCTAAGACC-3'	5'-GCTCCCAGCACCAAACATTTCCAG-3'	144	65
Rc30169.m006283	Casbene synthase	5'-TGGTGGTTCCTCTCCTTGTGAAAC-3'	5'-TCCAAGCTAGTGGTGGGTAAGC-3'	113	63
Rc30169.m006285	CYP726A18	5'-ACAGTGTTGTTGCGGCAGGA-3'	5'-TCCTGCCGGCTTTGTGTTCA-3'	162	65
Rc30169.m006287	Short-chain ADH	5'-TGAGATTGGAAGCTCGCTCTGC-3'	5'-ACGGCTAAATCCACAGCGTTCC-3'	111	65

Supplemental Table 2: Primers used for cloning of *R. communis* genes

Gene ID & annotation	Primer name	Purpose	Sequence
Rc30169.m006273 (CYP726A13)	Rc6273F	Cloning	5'-ATGGACAAGCAAATCCTATCATATCC-3'
	Rc6275R	Cloning	5'-TCAGTCCGTTGTTGGTGAAGGG-3'
	Rc6273_AscI_F	Subcloning (pFGC-5941)	5'-AAAAGGCGCGCCAAAAATGGACAAGCAAATCCTATC-3'
	Rc6273_PacI_R	Subcloning (pFGC-5941)	5'-AAAATTAATTAATCAGTCCGTTGTTGGTGAAG-3'
Rc30169.m006275 (CYP726A14)	Rc6275F	Cloning	5'-ATGGAGCAGCAATTGCTATCG-3'
	Rc6275R	Cloning	5'-CTATGGCAAAGTAGTGAATGGAATGG
	Rc6275_AscI_F	Subcloning (pFGC-5941)	5'-AAAAGGCGCGCCAAAAATGGAGCAGCAATTGCTATCG-3'
	Rc6275_PacI_R	Subcloning (pFGC-5941)	5'-AAAATTAATTAACTATGGCAAAGTAGTGAATG-3'
	Rc6275_AgeI_F	Subcloning (pEAQ-HT)	5'-AAAACCGGTAAAAATGGAGCAGCAATTGCTATCG-3'
	Rc6275_XhoI_R	Subcloning (pEAQ-HT)	5'-AAAACTCGAGCTATGGCAAAGTAGTGAATG-3'
Rc30169.m006276 (Neocembrene synthase)	Rc6276F	Cloning	5'-ATGGCACTGCAATCACTACTATTC-3'
	Rc6276R	Cloning	5'-TTACACATGTTTTGTTTTGGTTTCTCC-3'
	Rc6276_AscI_F	Subcloning (pFGC-5941)	5'-AAAAGGCGCGCCAAAAATGGCACTGCAATCACTACTATTC-3'
	Rc6276_PacI_R	Subcloning (pFGC-5941)	5'-AAAATTAATTAATTACACATGTTTTGTTTTGGTTTCTC-3'
	Rc6276_AgeI_F	Subcloning (pEAQ-HT)	5'-AAAACCGGTAAAAATGGCACTGCAATCACTACTATTC-3'
	Rc6276_XhoI_R	Subcloning (pEAQ-HT)	5'-AAAACTCGAGTTACACATGTTTTGTTTTGGTTTCTC-3'
Rc30169.m006277 (CYP726A15)	Rc6277F	Cloning	5'-ATGTCATTGCAACCTGCACCTG-3'
	Rc6277R	Cloning	5'-TTAAGGATGAAATAGAACAGGAATC-3'
	Rc6277_AscI_F	Subcloning (pFGC-5941)	5'-AAAAGGCGCGCCAAAAATGTCATTGCAACCTGCACCTG-3'
	Rc6277_PacI_R	Subcloning (pFGC-5941)	5'-AAAATTAATTAAGGATGAAATAGAACAGGAATC-3'
	Rc6277_AgeI_F	Subcloning (pEAQ-HT)	5'-AAAAACCGGTAAAAATGTCATTGCAACCTGCACCTG-3'
	Rc6277_XhoI_R	Subcloning (pEAQ-HT)	5'-AAAACTCGAGTTAAGGATGAAATAGAACAGGAATC-3'
Rc30169.m006279 (CYP726A16)	Rc6279F	Cloning	5'-ATGGAAAGTGCTGCTCACCAATC-3'
	Rc6279R	Cloning	5'-TTATGGTAAAGGACTGACGGGAATGG-3
	Rc6279_AscI_F	Subcloning (pFGC-5941)	5'-AAAAGGCGCGCCAAAAATGGAAAGTGCTGCTCACCAATC-3'
	Rc6279_PacI_R	Subcloning (pFGC-5941)	5'-AAAATTAATTAATTATGGTAAAGGACTGACG-3'
	Rc6279_AgeI_F	Subcloning (pEAQ-HT)	5'-AAAACCCGGTAAAAATGGAAAGTGCTGCTCACCAATC-3'
	Rc6279_XhoI_R	Subcloning (pEAQ-HT)	5'- AAAACTCGAGTTATGGTAAAGGACTGACGGGAATG-3'
Rc30169.m006282 (CYP726A17)	Rc6282F	Cloning	5'-ATGGAGAAACAAATCCTATCATTTCCAG-3'
	Rc6282R	Cloning	5'-CTAAGGAGTAAATGGAATGGGAATC-3'
	Rc6282_AscI_F	Subcloning (pFGC-5941)	5'-AAAAGGCGCGCCAAAAATGGAGAAACAAATCCTATCATTTC-3'
	Rc6282_PacI_R	Subcloning (pFGC-5941)	5'-AAAATTAATTAACTAAGGAGTAAATGGAATG-3'
Rc30169.m006283 (Casbene synthase)	Rc6282F	Cloning	5'-ATGGCATTGCCATCAGCTG-3'
	Rc6282R	Cloning	5'-TCAAATAGAAATTGGATCAACGGAC-3'
	Rc6282_NcoI_F	Subcloning (pET32b)	5'-AGCCATGGCCTCCTCAACAACCCACCAAGAA-3'
	Rc6282_NotI_R	Subcloning (pET32b)	5'-AAGCGGCCGCTCAAATAGAAATTGGATCAACG-3'
Rc30169.m006285 (CYP726A18)	Rc6285F	Cloning	5'-ATGTCATCACAACCAGCAGTTTTAC-3'
	Rc6285R	Cloning	5'-TCAATGTGTAGGATATAGAACAGG-3'
	Rc6285_AscI_F	Subcloning (pFGC-5941)	5'-AAAAGGCGCGCCAAAAATGTCATCACAACCAGCAGTTTTAC-3'
	Rc6285_PacI_R	Subcloning (pFGC-5941)	5'-AAAATTAATTAATCAATGTGTAGGATATAGAAC-3'
	Rc6285_AgeI_F	Subcloning (pEAQ-HT)	5'-AAAAACCGGTAAAAATGTCATCACAACCAGCAGTTTTAC-3'
	Rc6285_XhoI_R	Subcloning (pEAQ-HT)	5'-AAAACTCGAGTCAATGTGTAGGATATAGAACAGG-3'

Supplemental Table 3: Primers used for cloning and subcloning of *J. curcas* genes

Gene ID/ function	Primer name	Purpose	Sequence
Casbene synthase	CAS10	Degenerate PCR, forward	5'-CTYGAGTTTTCGAAGYTGGA-3'
	CAS21	Degenerate PCR, forward	5'-ATTCCWTATGCAAGAGAYAGAATG-3'
	CAS9	Degenerate PCR, reverse	5'-ACTARTTCTTGAWGCGCTTC-3'
JcCAS1	JcCAS1_F	Cloning	5'-TTCATATTTGTTGCTAATCCTC-3'
	JcCAS1_R	Cloning	5'-CAAGGTACAGGATTTATGCAAATCC-3'
	JcCAS1_NcoI_F	Subcloning (pET32b)	5'-AGCCATGGCATCAACAAAATCTGAGACAGAAG-3'
	JcCAS1_NotI_R	Subcloning (pET32b)	5'-AAGCGGCCGCTCAAGTGGCAATAGGTTCAATG-3'
	JcCAS1_AscI_F	Subcloning (pFGC-5941)	5'-AAAAGGCGCGCCAAAAATGGCAATGCAACCTGCAATTG-3'
	JcCAS1_PacI_R	Subcloning (pFGC-5941)	5'-AAAATTAATTATCAAGTGGCAATAGGTTCAATGAAC-3'
	JcCAS1_AgeI_F	Subcloning (pEAQ-HT)	5'-AAAACCGGTAAAAATGGCAATGCAACCTGCAATTG-3'
	JcCAS1_XhoI_R	Subcloning (pEAQ-HT)	5'-AAAACTCGAGTCAAGTGGCAATAGGTTCAATGAAC-3'
JcCAS2	JcCAS2_F	Cloning	5'-CCTCTTTAGTTCTCAAAGATG-3'
	JcCAS2_R	Cloning	5'-CCTCCAACCACAAAACAAATAGG-3'
	JcCAS2_NcoI_F	Subcloning (pET32b)	5'-AGCCATGGCCTTATCAACAAAATCTGAGAC-3
	JcCAS2_NotI_R	Subcloning (pET32b)	5'-AAGCGGCCGCTCAAATGGCAATAGGTTCAATG-3
JcCAS3	JcCAS3_F	Cloning	5'-CTTACATTCTACACTTTCCAA-3'
	JcCAS3_R	Cloning	5'-CGTTAATTTCTTTAAATTGCAAGG-3'
	JcCAS3_NcoI_F	Subcloning (pET32b)	5'-AGCCATGGCCTTGTCAACCAAACCTAGTG-3
	JcCAS3_NotI_R	Subcloning (pET32b)	5'-AAGCGGCCGCTCAAATTGGAATAGGTTCAA-3
CYP726A20	CYP726A20_F	Cloning	5'-ATGGAACACCAAATCCTCTCATTTCC-3'
	CYP726A20_R	Cloning	5'-TTAGGGACGGAATGGAATGGGGA-3'
CYP726A21	CYP726A21_F	Cloning	5'-ATGGAACAACAAATCCTCTCTTTTC-3'
	CYP726A21_R	Cloning	5'- TTAAGGACGGAATGGTGCGGGAATCA-3'
CYP726A22	CYP726A22_F	Cloning	5'-ATGGAACAGCAAATCCTCTCAGTTTC-3'
	CYP726A22_R	Cloning	5'-TTAAGGATGGTATGGAATGGGAATCAGTTC-3'
CYP726A23	CYP726A23_F	Cloning	5'- ATGGAACATCAAATCCTCTCATTTCCAGC-3'
	CYP726A23_R	Cloning	5'- GTACGGAATGGAATAGGAATGAGTTC-3'
CYP726A24	CYP726A24_F	Cloning	5'-ATGCTCTCATTTCCAGTTATTTTCAGTTTCC-3'
	CYP726A24_R	Cloning	5'-TTAAGGACGGAATGCAACAGGGATCAGT-3'
CYP726A25	CYP726A25_F	Cloning	5'-ATGGACCACCGAATTCTCTCATTCC-3'
	CYP726A25_R	Cloning	5'-CTATGGGATTGGAATCAAATTGAGATC-3'
CYP726A26	CYP726A26_F	Cloning	5'-ATGGAGTATCAAATCCTCTCATCTC-3'
	CYP726A26_R	Cloning	5'-CTAAGGATTAAACGCGGTCGGAA-3'

Supplemental Table 4: Primers used for cloning and subcloning of *E. peplus* genes

Gene ID/ function	Primer name	Purpose	Sequence
CYP726A3	CYP726A3_F	Cloning	5'-ACTCAGTAATAGTTATCAGAAAAATGG-3'
	CYP726A3_R	Cloning	5'-CTAAATAATAGAAAACTTATTGACAAGGC-3'
CYP726A4	CYP726A4_F	Cloning	5'-ATGGAGCTTCAATTTCAAATCCC-3'
	CYP726A4_R	Cloning	5'-CACTTACAGCAGCAACTAATTAACT-3'
CYP726A5	CYP726A5_F	Cloning	5'-AAAATATATATGCAAGTATGGAGTTCAC-3'
	CYP726A5_R	Cloning	5'-TATGCACTAGATAGATTGGTTCAAAT-3'
CYP726A6	CYP726A6_F	Cloning	5'-CACAGAAAAATGAAAATGCTTGAG-3'
	CYP726A6_R	Cloning	5'-CTACATGAATAAGTACCATTTATTCTCC-3'
CYP726A19	CYP726A19_F	Cloning	5'-CACTTTCACGGAACTCTCCCAA-3'
	CYP726A19_R	Cloning	5'-ACAGTCTCAAAAATCAGTTTGCAG-3'
	CYP726A19_AgeI_F	Subcloning (pEAQ-HT)	5'-AAAACCGGTAAAAATGGCAACACTTCAACATTCAATGC-3'
	CYP726A19_XhoI_R	Subcloning (pEAQ-HT)	5'-AAAACTCGAGTCAGTTTGCAGGTGAAGTATGGAATGG-3'
Casbene synthase	EpCAS_INV	Long distance PCR	5'-GACGAGGCTTTCGATTTCTCGAAGG-3'
CYP726A19	CYP726A19_INV	Long distance PCR	5'-GAATGACTCTGCCTAAGCGCTC-3'

Supplemental Table 5: Primers used for linkage mapping in *J. curcas*

Marker	GenBank	*J. curcas* genome build 4.5	Forward primer(s)	Reverse primer
Simple Sequence Repeat (SSR) markers				
CYP726A20	KF986815	Jcr4S15541 & Jcr4S12007*	5'-[M13†]-GCTTTCTCCGTCGCAAACGTGG-3'	5'-AGTGGTTTAAAGATCTTACATGGCGAA-3'
CYP726 (partial)	N/A	Jcr4S16860.10	5'-[M13]-ACGTTTGAAGAAGCGCAGTCA-3'	5'-TGCTATCCCTAGCTAACACTCCCT-3'
BAC JHL23C09	AP011970	N/A	5'-[M13]-GCGGATTTGAATTTATTGTTTTGGC-3'	5'-GGGATAGTCCATTGAGGCTTAGTTAGAA-3'
Single Nucleotide Polymorphism (SNP) marker – KASP assay				
3 x casbene synthase	KJ026361	N/A	5'-[FAM‡]-TGAGCAACTCATCGTGCGGTCT-3' 5'-[VIC§]-TGAGCAACTCATCGTGCGGTCC-3'	5'-CGGTCGTCGTCGCGCTTGTA-3'

Supplemental Table 5 footnotes:

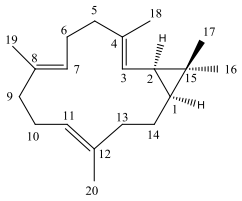
*Pseudomolecules Jcr4S15541 & Jcr4S12007 assembled were assembled into a single contig using CAP3. The presence of a single CYP726A gene within this contig was confirmed by cloning and sequencing of the corresponding cDNA sequence.

†The sequence of the M13 tail incorporated to permit labeling of PCR products as detailed in King *et al*. (2013) was 5'-TGTAAAACGACGGCCAGT-3.

‡The sequence of the FAM tail added for the KASP assay was 5'-GAAGGTGACCAAGTTCATGCT-3'

§The sequence of the VIC tail added for the KASP assay was 5'-GAAGGTCGGAGTCAACGGATT-3'

Supplemental Table 6: NMR analysis of casbene

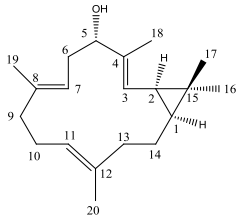


Position†	δ_C (ppm)	Multiplicity in edited-HSQC	δ_H (ppm)	Multiplicity in ^{1}H NMR	Correlations from ^{13}C to ^{1}H observed in HMBC (*via* $^2J_{CH}$ or $^3J_{CH}$)	Correlations from ^{1}H to ^{1}H observed in COSY (principally $^3J_{HH}$)
1	30.6	CH	0.58	*ddd*, *J* = 9, 9, 2 Hz	2.17 (H-13β), 1.85 (H-13α), 1.74 (H-14α), 1.23 (H-2), 1.05 (H-16), 0.94 (H-17)	1.74 (H-14α), 1.23 (H-2), 0.95 (H-14β)
2	25.7	CH	1.23	*dd*, *J* = 9, 9 Hz	1.74 (H-14α), 1.05 (H-16), 0.95 (H-14β), 0.94 (H-17)	4.87 (H-3), 0.58 (H-1)
3	121.2	CH	4.87	*d*, *J* = 9 Hz	2.21 (H-5α), 1.66 (H-18)	1.66 (H-18), 1.23 (H-2)
4	136.1	C	-	-	2.27 (H-6α), 2.11 (H-5β), 1.66 (H-18), 1.23 (H-2)	-
5	39.3	CH_2	2.21	*dd*, *J* =14, 5 Hz	4.87 (H-3), 2.27 (H-6α), 1.66 (H-18)	2.27 (H-6α), 2.11 (H-5β)
			2.11	*m*		2.27 (H-6α), 2.21 (H-5α)
6	25.0	CH_2	2.27	*dddd*, *J* =14, 10, 6, 4 Hz	4.89 (H-7), 2.21 (H-5α)	4.89 (H-7), 2.21 (H-5α), 2.11 (H-5β), 2.06 (H-6β)
			2.06	*m*		4.89 (H-7), 2.27 (H-6α)
7	125.6	CH	4.89	*dd*, *J* = 6, 6 Hz	2.27 (H-6α), 1.89 (H-9β), 1.55 (H-19)	2.27 (H-6α), 2.06 (H-6β), 1.55 (H-19)
8	133.3	C	-	-	2.27 (H-6α), 2.11 (H-9α/10β), 1.89 (H-9β), 1.55 (H-19)	-
9	39.5	CH_2	2.11	*M*	4.89 (H-7), 2.11 (H-10β), 2.03 (H-10α), 1.55 (H-19)	1.89 (H-9β)
			1.89	*dd*, *J* = 12, 12 Hz		2.11 (H-9α/10β), 2.03 (H-10α)
10	24.0	CH_2	2.11	*M*	1.89 (H-9β)	4.97 (H-11), 2.11 (H-10β), 1.89 (H-9β)
			2.03	*M*		4.97 (H-11), 2.03 (H-10α)
11	123.4	CH	4.97	*dd*, *J* = 6, 6 Hz	2.17 (H-13β), 2.03 (H-10α), 1.85 (H-13α), 1.58 (H-20)	2.11 (H-10β), 2.03 (H-10α), 1.58 (H-20)
12	135.5	C	-		2.17 (H-13β), 2.03 (H-10α), 1.85 (H-13α), 1.74 (H-14α), 1.58 (H-20), 0.95 (H-14β)	-
13	40.4	CH_2	2.17	*ddd*, *J* = 14, 7, 7 Hz	1.74 (H-14α), 1.58 (H-20), 0.95 (H-14β), 0.58 (H-1)	1.85 (H-13α), 1.74 (H-14α), 0.95 (H-14β)
			1.85	*ddd*, *J* = 14, 8, 6 Hz		2.17 (H-13β), 1.74 (H-14α), 0.95 (H-14β)
14	23.9	CH_2	1.74	*dddd*, *J* = 14, 7, 6, 2 Hz *m*	1.85 (H-13α), 1.23 (H-2)	2.17 (H-13β), 1.85 (H-13α), 0.95 (H-14β), 0.58 (H-1)
			0.95			2.17 (H-13β), 1.85 (H-13α), 1.74 (H-14α), 0.58 (H-1)
15	19.8	C	-		1.74 (H-14α), 1.23 (H-2), 1.05 (H-16), 0.94 (H-17), 0.58 (H-1)	-
16	28.9	CH_3	1.05	[3H] *s*	1.23 (H-2), 0.94 (H-17), 0.58 (H-1)	-
17	15.8	CH_3	0.94	[3H] *s*	1.23 (H-2), 1.05 (H-16)	
18	16.2	CH_3	1.66	[3H] *s*	4.87 (H-3), 2.11 (H-5β)	4.87 (H-3)
19	15.7	CH_3	1.55	[3H] *s**	4.89 (H-6), 1.89 (H-9β)	4.89 (H-7)
20	16.5	CH_3	1.58	[3H] *s*	4.97 (H-11), 1.85 (H-13α)	4.97 (H-11)

Supplemental Table 6 footnotes:

† Numbering is based on Tan *et al.* (2009), which is shown directly above the table.
*Obscured by water peak

Supplemental Table 7: NMR analysis of 5α-hydroxy-casbene

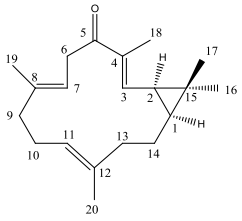


Position†	δ_C (ppm)	Multiplicity in edited-HSQC	δ_H (ppm)	Multiplicity in ^{1}H NMR	Correlations from ^{13}C to ^{1}H observed in HMBC (*via* $^2J_{CH}$ or $^3J_{CH}$)	Correlations from ^{1}H to ^{1}H observed in COSY (principally $^3J_{HH}$)
1	31.5	CH	0.64	*dd*, *J* = 10, 10 Hz	1.07 (H-16), 0.96 (H-17)	1.26 (H-2), 0.95 (H-14β)
2	25.5	CH	1.26	*M*	1.07 (H-16), 0.96 (H-17)	5.11 (H-3), 0.64 (H-1)
3	125.9*	CH	5.11	*d*, *J* = 9 Hz	1.69 (H-18)	1.69 (H-18), 1.26 (H-2)
4	137.4*	C	-	-	1.69 (H-18)	-
5	79.4	CH	4.08	*br d*, *J* =12 Hz	1.69 (H-18)	2.42 (H-6α), 2.31 (H-6β), 1.42 (5-OH)
6	33.1	CH_2	2.42	*ddd*, *J* =14, 12, 7 Hz	-	4.78 (H-7), 4.08 (H-5), 2.31 (H-6β)
			2.31	*ddd*, *J* = 14, 6, 4 Hz		4.78 (H-7), 4.08 (H-5), 2.42 (H-6α)
7	120.5	CH	4.78	*dd*, *J* = 7, 6 Hz	1.61 (H-19)	2.42 (H-6α), 2.31 (H-6β), 1.61 (H-19)
8	135.3*	C	-	-	1.61 (H-19)	-
9	39.3	CH_2	2.13	*m*	1.61 (H-19)	1.88 (H-9β)
			1.88	*m*		2.13 (H-9α)
10	24.0	CH_2	2.11	*m*	-	4.94 (H-11), 2.11 (H-10β)
			2.06	*m*		4.94 (H-11), 2.06 (H-10α)
11	123.4	CH	4.94	*dd*, *J* = 7, 6 Hz	1.59 (H-20)	2.11 (H-10β), 2.06 (H-10α), 1.59 (H-20)
12	135.5*	C	-	-	1.59 (H-20)	-
13	40.4	CH_2	2.21	*ddd*, *J* = 14, 7, 7 Hz	1.59 (H-20)	1.89 (H-13α), 1.73 (H-14α), 0.95 (H-14β)
			1.89	*m*		2.21 (H-13β), 1.73 (H-14α), 0.95 (H-14β)
14	23.6	CH_2	1.73	*ddd*, *J* = 14, 7, 7 Hz	-	2.21 (H-13α), 1.89 (H-13β), 0.95 (H-14β)
			0.95	*m*		2.21 (H-13α), 1.89 (H-13β), 1.73 (H-14α), 0.64 (H-1)
15	20.7*	C	-	-	1.07 (H-16), 0.96 (H-17)	-
16	28.8	CH_3	1.07	[3H] *s*	0.96 (H-17)	-
17	15.8	CH_3	0.96	[3H] *s*	1.07 (H-16)	-
18	10.4	CH_3	1.69	[3H] *s*	-	5.11 (H-3)
19	16.2*	CH_3	1.61	[3H] *s*	-	4.78 (H-7)
20	16.8	CH_3	1.59	[3H] *s*	-	4.94 (H-11)

Supplemental Table 7 footnotes:

† Numbering is based on Tan *et al.* (2009).
*Signal to noise ratio too low for detection in 1D- ^{13}C NMR, but observed indirectly in HSQC and/or HMBC

Supplemental Table 8: NMR analysis of 5-keto-casbene

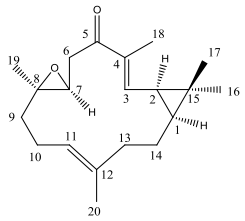


Position†	δ_C (ppm)	Multiplicity in edited-HSQC	δ_H (ppm)	Multiplicity in ^{1}H NMR	Correlations from ^{13}C to ^{1}H observed in HMBC (*via* $^2J_{CH}$ or $^3J_{CH}$)	Correlations from ^{1}H to ^{1}H observed in COSY (principally $^3J_{HH}$)
1	27.6	CH	0.83	*M*	1.16 (H-16), 1.10 (H-17)	-
2	35.4	**	1.48	*M*	1.16 (H-16), 1.10 (H-17)	6.39 (H-3)
3	143.3	CH	6.39	*d*, *J* = 10 Hz	1.87 (H-18)	1.87 (H-18), 1.48 (H-2)
4	136.8	C	-	-	1.87 (H-18)	-
5	200.1	C	-	-	1.87 (H-18)	-
6	39.5	CH_2	3.55	*dd*, *J* =14, 9 Hz	-	5.08 (H-7), 2.99 (H-6)
			2.99	*dd*, *J* = 14, 6 Hz		5.08 (H-7), 3.55 (H-6)
7	119.4	CH	5.08	*dd*, *J* = 9, 6 Hz	1.57 (H-19)	3.55 (H-6), 2.99 (H-6), 1.57 (H-19)
8	137.2	C	-	-	1.57 (H-19)	-
9	39.3	CH_2	2.10	*m*	1.57 (H-19)	2.00 (H-9)
			2.00	*m*		2.10 (H-9)
10	24.1	CH_2	2.16	*m*	-	4.84 (H-11), 1.97 (H-10)
			1.97	*m*		4.84 (H-11), 2.16 (H-10)
11	124.4	CH	4.84	*dd*, *J* = 7, 6 Hz	1.56 (H-20)	2.16 (H-10), 1.97 (H-10), 1.56 (H-20)
12	136.3	C	-		1.56 (H-20)	-
13	40.1	CH_2	2.19	*m*	1.56 (H-20)	1.75 (H-13), 0.87 (H-14)
			1.75	*m*		2.19 (H-13)
14	**	**	2.07	*m*	-	1.75 (H-13), 0.87 (H-14)
			0.87	*m*		2.07 (H-14)
15	25.6	C	-		1.16 (H-17), 1.10 (H-16)	-
16	29.1	CH_3	1.16	[3H] *s*	1.10 (H-17)	-
17	16.0	CH_3	1.10	[3H] *s*	1.16 (H-16)	-
18	11.8	CH_3	1.87	[3H] *s*	-	6.39 (H-3)
19	15.6	CH_3	1.57	[3H] *s*+	-	5.08 (H-6)
20	15.4	CH_3	1.56	[3H] *s*+	-	4.84 (H-11)

Supplemental Table 8 footnotes:

† Numbering is based on Tan *et al.* (2009).
*Signal to noise ratio too low for detection in 1D- ^{13}C NMR, but observed indirectly in HSQC and/or HMBC
** Not detected in either ^{13}C NMR or HSQC
+ Obscured by water peak

Supplemental Table 9: NMR analysis of 5-keto-7,8-epoxy-casbene

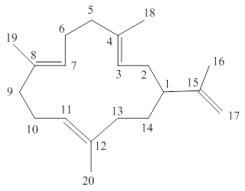


Position†	δ_C (ppm)	Multiplicity in edited-HSQC	δ_H (ppm)	Multiplicity in ^{1}H NMR	Correlations from ^{13}C to ^{1}H observed in HMBC (*via* $^2J_{CH}$ or $^3J_{CH}$)	Correlations from ^{1}H to ^{1}H observed in COSY (principally $^3J_{HH}$)
1	35.4	CH	1.22	*M*	1.66 (H-13α), 1.22 (H-17), 1.17 (H-16), 0.62 (H-14β)	1.52 (H-2), 0.62 (H-14β)
2	27.1	CH	1.52	*M*	1.22 (H-17), 1.17 (H-16)	6.58 (H-3), 1.22 (H-1)
3	144.5	CH	6.58	*d*, *J* = 9 Hz	1.94 (H-18)	1.94 (H-18), 1.52 (H-2)
4	139.3	C	-	-	2.28 (H-6α), 1.94 (H-18)	-
5	196.9	C	-	-	6.58 (H-3), 3.63 (H-6β), 2.28 (H-6α), 1.94 (H-18)	-
6	40.8	CH_2	3.63 (Hβ) 2.28 (Hα)	*dd*, *J* =12, 5 Hz *dd*, *J* = 12, 9 Hz	3.02 (H-7)	3.02 (H-7), 2.28 (H-6α) 3.63 (Hβ), 3.02 (H-7)
7	60.6	CH	3.02	*dd*, *J* = 9, 5 Hz	2.28 (H-6α), 2.09 (H-9α), 0.96 (H-9β), 1.41 (H-19)	3.63 (H-6β), 2.28 (H-6α)
8	61.0	C	-	-	3.63 (H-6β), 2.21 (H-10β), 1.41 (H-19)	-
9	40.2	CH_2	2.09 (Hα) 0.96 (Hβ)	*m* *ddd*, *J* = 13, 13, 4 Hz	2.21 (H-10β), 1.80 (H-10α), 1.41 (H-19)	2.21 (H-10β), 0.96 (H-9β) 2.21 (H-10β), 2.09 (H-9α), 1.80 (H-10α)
10	23.1	CH_2	2.21 (Hβ) 1.80 (Hα)	*dddd*, *J* = 14, 10, 4, 4 Hz *ddd*, *J* = 14, 13, 6 Hz	4.84 (H-11), 2.09 (H-9α), 0.96 (H-9β)	4.84 (H-11), 2.09 (H-9α), 1.80 (H-10α), 0.96 (H-9β) 4.84 (H-11), 2.21 (H-10β), 0.96 (H-9β)
11	123.3	CH	4.84	*dd*, *J* = 10, 6 Hz	2.21 (H-10β), 2.09 (H-13β), 1.66 (H-13α), 1.51 (H-20)	2.21 (H-10β), 1.80 (H-10α), 1.51 (H-20)
12	137.4	C	-		2.21 (H-10), 2.09 (H-13β), 1.66 (H-13α), 1.51 (H-20), 0.62 (H-14β)	-
13	39.7	CH_2	2.09 (Hβ) 1.66 (Hα)	*m* *dd*, J = 12, 12 Hz	4.84 (H-11), 2.04 (H-14α), 1.51 (H-20), 0.62 (H-14β)	1.66 (H-13α), 0.62 (H-14β) 2.09 (H-13β), 2.04 (H-14α), 0.62 (H-14β)
14	26.9	CH_2	2.04 (Hα) 0.62 (Hβ)	*m* *ddd*, J =12, 12, 12 Hz	2.09 (H-13β), 1.66 (H-13α)	1.66 (H-13α), 0.62 (H-14β) 2.09 (H-13β), 2.04 (H-14α), 1.66 (H-13α), 1.22 (H-1)
15	24.1	C	-		6.58 (H-3), 1.22 (H-17), 1.17 (H-16)	-
16	28.5	CH_3	1.17	[3H] *s*	1.52 (H-2), 1.22 (H-17)	-
17	15.5	CH_3	1.22	[3H] *s*	1.17 (H-16)	-
18	11.8	CH_3	1.94	[3H] *s*	6.58 (H-3)	6.58 (H-3)
19	16.4	CH_3	1.41	[3H] *s*	2.09 (H-9α), 0.96 (H-9β)	-
20	14.4	CH_3	1.51	[3H] *s*	4.84 (H-11), 1.66 (H-13α)	4.84 (H-11)

Supplemental Table 9 footnotes:

† Numbering is based on Tan *et al.* (2009).

Supplemental Table 10: NMR analysis of neocembrene

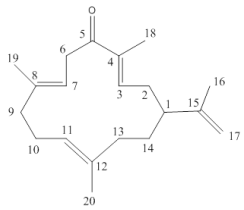


Position†	δ_C (ppm)	Multiplicity in edited-HSQC	δ_H (ppm)	Multiplicity in ^{1}H NMR	Correlations from ^{13}C to ^{1}H observed in HMBC (*via* $^2J_{CH}$ or $^3J_{CH}$)	Correlations from ^{1}H to ^{1}H observed in COSY (principally $^3J_{HH}$)
1	46.0	CH	2.03	*M*	4.71 (H-17), 4.65 (H-17), 1.99 (H-2), 1.95 (H-2), 1.75 (H-13α), 1.67 (H-14β), 1.37 (H-14α)	4.65 (H-17), 1.37 (H-14α)
2	32.4	CH_2	1.99 1.95	*m* *m*	5.19 (H-3), 2.03 (H-1), 1.67 (H-14β), 1.37 (H-14α)	5.19 (H-3) 5.19 (H-3)
3	124.0	CH	5.19	*dd*, *J* = 7, 7 Hz	2.17 (H-5), 1.99 (H-2), 1.95 (H-5), 1.57 (H-18)	1.99 (H-2), 1.95 (H-2), 1.57 (H-18)
4	134.8	C	-	-	1.99 (H-2), 1.95 (H-2), 1.57 (H-18)	-
5	39.0	CH_2	2.17 2.12	*m* *m*	5.19 (H-3), 4.98 (H-7), 1.57 (H-18)	
6	24.9	CH_2	2.27 (Hβ) 2.17 (Hα)	*m* *m*	4.98 (H-7), 2.17 (H-5), 2.12 (H-5)	4.98 (H-7) 4.98 (H-7)
7	125.9	CH	4.98	*dd*, *J* = 6, 6 Hz	2.07 (H-9), 2.03 (H-9), 1.59 (H-19)	2.27 (H-6α), 2.17 (H-6β), 1.59 (H-19)
8	133.4	C	-	-	2.07 (H-9), 2.03 (H-9), 1.59 (H-19)	-
9	39.4	CH_2	2.07 2.03	*m* *m*	5.06 (H-11), 4.98 (H-7), 2.12 (H-10), 1.59 (H-19)	
10	23.7	CH_2	2.12 2.12	*m* *m*	5.06 (H-11), 2.07 (H-9), 2.03 (H-9)	5.06 (H-11) 5.06 (H-11)
11	121.8	CH	5.06	*dd*, *J* = 6, 6 Hz	2.12 (H-10), 2.07 (H-9), 2.03 (H-9), 1.56 (H-20)	2.12 (H-10), 1.56 (H-20)
12	133.9	C	-	-	2.12 (H-10), 1.67 (H-14β), 1.56 (H-20), 1.37 (H-14α)	-
13	34.0	CH_2	1.95 (Hβ) 1.75 (Hα)	*m* *ddd*, *J* = 13, 10, 3 Hz	5.06 (H-11), 2.03 (H-1), 1.67 (H-14β), 1.56 (H-20), 1.37 (H-14α)	1.75 (H-13α), 1.37 (H-14α) 1.95 (H-13β), 1.67 (H-14β), 1.37 (H-14α)
14	28.1	CH_2	1.67 (Hβ) 1.37 (Hα)	*dddd*, *J* = 14, 10, 4, 4 Hz *dddd*, *J* = 14, 10, 7, 4 Hz	2.03 (H-1), 1.95 (H-13β), 1.75 (H-13α)	1.75 (H-13α), 1.37 (H-14α) 2.03 (H-1), 1.95 (H-13β), 1.75 (H-13α), 1.67 (H-14β)
15	149.3	C	-	-	4.71 (H-17), 4.65 (H-17), 2.03 (H-1), 1.95 (H-2), 1.66 (H-16), 1.37 (H-14)	-
16	19.3	CH_3	1.66	[3H] *s*	4.71 (H-17), 4.65 (H-17), 2.03 (H-1)	4.71 (H-17), 4.65 (H-17)
17	110.1	CH_2	4.71 4.65	*s* *s*	2.03 (H-1), 1.66 (H-16)	1.66 (H-16) 2.03 (H-1), 1.66 (H-16)
18	15.5	CH_3	1.57	[3H] *s*	5.19 (H-3), 2.17 (H-5), 2.12 (H-5)	5.19 (H-3)
19	15.3	CH_3	1.59	[3H] *s*	4.98 (H-7), 2.07 (H-9), 2.03 (H-9)	4.98 (H-7)
20	18.0	CH_3	1.56	[3H] *s*	5.06 (H-11), 1.75 (H-13α)	5.06 (H-11)

Supplemental Table 10 footnotes:

† Numbering is based on Tan *et al.* (2009).

Supplemental Table 11: NMR analysis of 5-keto-neocembrene



Position†	δ_C (ppm)	Multiplicity in edited-HSQC	δ_H (ppm)	Multiplicity in ^{1}H NMR	Correlations from ^{13}C to ^{1}H observed in HMBC (*via* $^2J_{CH}$ or $^3J_{CH}$)	Correlations from ^{1}H to ^{1}H observed in COSY (principally $^3J_{HH}$)
1	44.8	CH	2.16	*M*	6.53 (H-3), 4.79 (H-17), 4.72 (H-17), 2.30 (H-2β), 2,16 (H-2α), 1.76 (H-13α), 1.69 (H-16), 1.44 (H-14)	1.44 (H-14)
2	34.7	CH_2	2.30 (Hβ) 2.16 (Hα)	*ddd*, *J* = 15, 11, 11 Hz *m*	6.53 (H-3), 1.44 (H-14)	6.53 (H-3) 6.53 (H-3)
3	144.8	CH	6.53	*dd*, *J* = 10, 5 Hz	2.30 (H-2β), 2.16 (H-2α), 1.76 (H-18)	2.30 (H-2β), 2.16 (H-2α), 1.76 (H-18)
4	136.5	C	-	-	3.74 (H-6β), 2.30 (H-2β), 2.16 (H-2α), 1.76 (H-18)	-
5	201.7	C	-	-	6.53 (H-3), 3.74 (H-6β),1.76 (H-18)	-
6	40.4	CH_2	2.99 (Hα) 3.74 (Hβ)	*br d*, *J* = 14 Hz *dd*, *J* = 14, 10 Hz	5.21 (H-7)	5.21 (H-7), 3.74 (H-6β), 2.20 (H-9α), 1.66 (H-19) 5.21 (H-7), 2.99 (H-6α)
7	120.5	CH	5.21	*br d*, *J* = 10 Hz	3.74 (H-6β), 2.07 (H-9β), 1.66 (H-19)	3.74 (H-6β), 2.99 (H-6α), 1.66 (H-19)
8	136.2	C	-	-	2.07 (H-9β), 1.66 (H-19)	-
9	38.7	CH_2	2.20 (Hα) 2.07 (Hβ)	*m* *ddd*, *J* = 13, 11, 3 Hz	4.95 (H-11), 1.66 (H-19)	2.99 (H-6α)
10	23.9	CH_2	2.16 (Hα) 2.25 (Hβ)	*m* *m*	2.07 (H-9β)	4.95 (H-11) 4.95 (H-11)
11	121.7	CH	4.95	*dd*, *J* = 6, 6 Hz	1.59 (H-20)	2.25 (H-10β), 2.16 (H-10α), 1.59 (H-20)
12	134.3	C	-	-	1.59 (H-20), 1.44 (H-14)	-
13	33.3	CH_2	2.00 (Hβ) 1.76 (Hα)	*d*, *J* = 16 Hz *m*	4.95 (H-11), 1.59 (H-20), 1.44 (H-14)	1.76 (H-13α), 1.44 (H-14) 2.00 (H-13β), 1.44 (H-14)
14	28.8	CH_2	1.44 1.44	*m* *m*	2.30 (H-2β), 2.16 (H-2α), 1.76 (H-13α)	2.16 (H-1), 2.00 (H-13β), 1.76 (H-13α)
15	147.9	C	-		4.79 (H-17), 4.72 (H-17), 1.69 (H-16), 1.44 (H-14)	-
16	19.0	CH_3	1.69	[3H] *s*	4.79 (H-17), 4.72 (H-17), 2.16 (H-1)	4.79 (H-17), 4.72 (H-17)
17	111.5	CH_2	4.79 4.72	*s* *s*	1.69 (H-16)	1.69 (H-16) 1.69 (H-16)
18	11.5	CH_3	1.76	[3H] *s*	6.53 (H-3)	6.53 (H-3)
19	15.2	CH_3	1.66	[3H] *s*	2.07 (H-9β)	5.21 (H-7), 5.21 (H-7)
20	18.8	CH_3	1.59	[3H] *s*	4.95 (H-11)	4.95 (H-11)

Supplemental Table 11 footnotes:

† Numbering is based on Tan *et al.* (2009).

Supplemental Methods

Structural analysis of casbene and the two casbene oxidation products via ^{1}H and ^{13}C NMR.

All NMR spectra were acquired using a Bruker AVIII 700 MHz instrument, equipped with a TCI cryogenically-cooled probe, operating at ambient temperature (298 K). Samples were dissolved in $CDCl_3$ (0.5 ml), incorporating 0.03% (*v/v*) tetramethylsilane (TMS) as the spectral reference, then transferred to a 5 mm NMR tube.

The ^{1}H NMR spectra of all three samples were recorded using 32 repetitions of the pulse sequence "zg30" (2 dummy scans) digitised over 131072 points, covering a sweep width of 15 ppm, resulting in an FID resolution of 0.08 Hz (total acquisition time: 6.2 s). The time data was weighted using a negative exponential function (line broadening of 0.2 Hz), followed by one-dimensional Fourier transformation to produce a spectrum containing 131072 points.

The ^{13}C NMR spectra were recorded using 10240 repetitions for neocembrene and 5-keto-neocembrene, 14336 repetitions for 5-keto-7,8-epoxy-casbene, 15360 repetitions for casbene and 5-hydroxy-casbene and 22528 repetitions in the case of 5-keto-casbene, of the pulse sequence "zgpg60" (2 dummy scans) digitised over 131072 points, covering a sweep width of 270 ppm, resulting in an FID resolution of 0.36 Hz (total acquisition time: 1.4 s). The time data was weighted using a negative exponential function (line broadening of 1 Hz), followed by one-dimensional Fourier transformation to produce a spectrum containing 131072 points.

The edited-HSQC spectra were recorded using between 32-128 repetitions (32 for casbene (*ca.* 300 μg, neocembrene (*ca.* 1 mg) 5-keto-neocembreme (*ca.* 1 mg) and 5-keto-7,8-epoxy-casbene (*ca.* 400 μg); 64 for 5α-hydroxy-casbene (*ca.* 30 μg); and 128 for 5-keto-casbene (*ca.* 15 μg)) of the pulse sequence "hsqcedetgpsisp2.2" (32 dummy scans) with 2048 points in F2 and 256 points in F1. The sweep width in F2 was 10 ppm and the sweep width in F1 was 180 ppm, resulting in a FID resolution of 3.4 Hz in F2 and 123.8 Hz in F1 (total acquisition time: 0.15 s in F2). The time data was weighted using a Qsine function (SSB=2) in both dimensions, followed by two-dimensional Fourier transformation producing a spectrum with 2048 points in F2 and 1024 points in F1.

The HMBC spectra were recorded using between 32 and 196 repetitions (32 of neocembrene, 5-keto-neocembrene and 5-keto-7,8-epoxy-casbene; 48 for casbene; 64 for 5α-hydroxy-casbene; and 196 for 5-keto-casbene) of the pulse sequence "hmbcetgpl3nd" (32 dummy scans) with 4096 points in F2 and 256 points in F1. The sweep width in F2 was 10 ppm and the sweep width in F1 was 240 ppm, resulting in a FID resolution of 1.7 Hz in F2 and 165 Hz in F1 (total acquisition time: 0.29 s in F2). The time data was weighted using a Qsine function (SSB=2) in both dimensions, followed by two-

dimensional Fourier transformation and processing in magnitude mode in F2, thereby producing a spectrum with 4096 points in F2 and 1024 points in F1.

The ^{1}H-^{1}H COSY spectra were recorded using 4 repetitions for neocembrene and 5-keto-neocembrene, and 5-keto-7,8-epoxy-casbene, 8 repetitions for casbene and 5-hydroxy-casbene and 16 in the case of 5-keto-casbene of the pulse sequence "cosygpmfqf" (8 dummy scans) with 2048 points in F2 and 256 points in F1.The sweep width was 10 ppm in both F2 and F1, resulting in a FID resolution of 3.4 Hz in F2 and 27.4 Hz in F1 (total acquisition time: 0.15 s in F2). The time data was weighted using a sine function in both dimensions prior to Fourier transformation, producing a spectrum with 2048 points in both F2 and F1.

The NOESY spectra were recorded using 16 repetitions (32 in the case of 5-keto-casbene) of the pulse sequence "noesygpph" (16 dummy scans) with 2048 points in F2 and 256 points in F1.The sweep width was 10 ppm in both F2 and F1, resulting in a FID resolution of 3.4 Hz in F2 and 27.4 Hz in F1 (total acquisition time: 0.15 s in F2). The mixing time for nOe build up was set at 800 ms. The time data was weighted using a Qsine function (SSB=2) in both dimensions prior to Fourier transformation, producing a spectrum with 2048 points in F2 and 1024 points in F1.

The cyclopropyl group of casbene has been depicted as 1*S*,2*R*-*cis* in Figure 4, Supplemental Figure 2 and Supplemental Table 6, in accordance with the absolute stereochemistry assigned to (-)-casbene from *Ricinus communis* by Crombie *et al* (Crombie et al., 1980). The same absolute stereochemistry has been assigned to the 5α-hydroxy derivative in Figure 4, Supplemental Figure 3 and Supplemental Table 7 and the 5-keto derivative in Figure 4 and Supplemental Figure 4, on the basis that this same (-)-casbene is the biosynthetic precursor to both compounds. The configuration has been observed for the majority of casbene-derived diterpenoids (Crombie et al., 1980), including those extracted from *J. curcas* (Haas et al., 2002; Zhang et al., 2012) and *E. peplus* (Hohmann et al., 2000).

Cloning of casbene synthase genes from *J. curcas* and heterologous expression in *E. coli*.

Prior to the release of the draft of the *J. curcas* genome sequence (Hirakawa et al., 2012) we had cloned partial fragments of three casbene synthase genes from cDNA prepared from total RNA extracted from *J. curcas* roots via degenerate PCR, using primers detailed in Supplemental Table 3. A combination of 5'- and 3'-RACE was used to obtain full length cDNA and then genomic clones (Frohman, 1993). Long-distance PCR was used to clone the regions between the casbene synthase genes. To assay each of the casbene synthase homologues, the translated peptide sequences were first analysed using ChloroP to obtain predicted chloroplast transit peptide sequences (Emanuelsson et al., 1999). The coding region without the transit peptide was then cloned into pET32b and transformed

into *E. coli* Rosetta 2 (Merck Millipore, Darmstadt, Germany). For each construct and an empty vector control, cultures were grown in 50 ml of LB medium containing 125 µg ml-1 of carbenicillin inside a 250 ml flask at 37 ºC with gyrotary shaking at 250 r.p.m. After reaching an OD600 of 2.0 to 2.5, IPTG was added to a final concentration of 250 µM. The cultures were then incubated at 20 ºC, 180 r.p.m. After an additional 18 hours of cultivation, the cultures were harvested by centrifugation at 4,000 g for 10 mins. The cell pellets were washed once by resuspending in 20 ml of phosphate buffered saline (PBS, pH 7.4) and centrifugation at 4,000 g for 10 mins. The cell pellets were then resuspended in 1 ml of PBS containing 1 mM dithiothreoitol (DTT) and transferred to a 1.5 ml Eppendorf tube and placed in a salt-ice-water bath at approximately -10 ºC. The cell pellets were then lysed with 6 cycles of 10 seconds using an ultrasonic probe, with 10 seconds pauses between cycles to allow cooling. The cell extracts were then centrifuged for 15 mins at 20,000 g to obtain a "cleared" lysate containing soluble proteins. Assays were then performed in 1.5 ml Eppendorf tubes with 340 µl of PBS containing 1 mM DTT, 100 µl of cleared lysate, 50 µl of 50 mM $MgCl_2$ and 10 µl of 1 mg ml^{-1} geranylgeranylpyrophosphate (GGPP) in 7:3 methanol:NH4OH (Sigma-Aldrich). The reactions were incubated for 3 hours at 30 ºC, after which 500 µl of hexane as added and the tubes were vortexed. After centrifugation at 12,000 g for 2 minutes, the hexane layer was recovered and 5 µl of the extract was analysed by GC-MS.

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