

## Supplementary Information

Table S1. Nucleotide sequences of primers used in this study

Primer	Name	Sequence <sup>a</sup>
1	<i>AaADS</i> <sub>forward</sub>	<u>CACCATGTC</u> ACTTACAGAAGAAAAACCTA
2	<i>AaADS</i> <sub>reverse</sub>	TCATATACTCATAGGATAAACGAGT
3	Specific <i>AmaOS</i> <sub>forward</sub>	AAACGGAGCGTTCAAGCAATCT
4	Specific <i>AmaOS</i> <sub>reverse</sub>	TAGCACCGCCAGTAATGAATAA
5	Specific <i>AmbOS</i> <sub>forward</sub>	AAGTGGAGCGTTCAAGCAATTG
6	Specific <i>AmbOS</i> <sub>reverse</sub>	TAGCACCGCCAAGATTGACTGC
7	<i>Actin</i> <sub>forward</sub>	CCAGGCTGTTCAAGTCTCTGTAT
8	<i>Actin</i> <sub>reverse</sub>	CGCTCGGTAAGGATCTTCATCA

<sup>a</sup>Sequence necessary for directional cloning into pENTR/D-TOPO is underlined.

Table S2. Percent amino acid sequence identities of AmaOS as compared to other characterized sesquiterpene synthases

Species <sup>a</sup>	Name	Abbreviation	% Identity
<i>Artemisia maritima</i>	(+)- $\alpha$ -Bisabolol synthase	AmbOS	86
<i>Artemisia annua</i>	Amorpha-4,11-diene synthase	AaADS	84
	$\alpha$ -Bisabolol synthase	AaBOS	85
	8- <i>epi</i> -Cedrol synthase	AaECS	54
	( <i>E</i> )- $\beta$ -Farnesene synthase	AaFS	49
	Germacrene A synthase	AaGAS	33
	$\beta$ -Caryophyllene synthase	AaQHS	60
<i>Artemisia absinthium</i>	Koidzumiol synthase	AabKOS	87
<i>Artemisia kurramensis</i>	(+)- $\alpha$ -Bisabolol synthase	AkBOS	86
<i>Mikania micrantha</i>	$\beta$ -Caryophyllene synthase	MmQHS	52
<i>Solidago canadensis</i>	Germacrene A synthase	ScGAS	53
	Germacrene D synthase	ScGDS	55
<i>Tanacetum parthenium</i>	$\beta$ -Caryophyllene synthase	TpQHS	59

<sup>a</sup>Only characterized sesquiterpene synthases from *A. annua* or from other plants with higher than 50% amino acid sequence identity to AmaOS were selected in this comparison.

Table S3. NMR Spectroscopic Data (600 MHz, CDCl<sub>3</sub>) for 4-amorphen-11-ol

Position	$\delta_C$ , type	$\delta_H$ , mult. ( <i>J</i> in Hz)	HMBC <sup>a</sup>	COSY <sup>b</sup>	NOESY <sup>c</sup>
1	39.9, CH	1.51, m	6, 9, 10	2	6, 9a, 14
2 a b	25.3, CH <sub>2</sub>	1.45, m 1.92, m	4, 6	1, 3	3
3 a b	29.4, CH <sub>2</sub>	1.95, m 1.95, m	4, 5	2, 5, 15	2a, 15
4	135.7, C				
5	126.3, CH	5.51, dt (3.02, 1.51)		3, 6, 15	6, 12, 15
6	34.6, CH	2.33, m		5, 7, 15	1, 5, 12
7	51.0, CH	1.45, m	5, 6, 9, 11	6, 8	
8 a b	23.4, CH <sub>2</sub>	1.35, m 1.49, m	10	7, 9	10 <sup>d</sup> , 12 13
9 a b	29.0, CH <sub>2</sub>	1.32, m 1.60, m	1, 8	8, 10	1, 14
10	31.5, CH	1.62, m		1, 9, 14	8a <sup>d</sup> , 14
11	74.4, C				
12	26.2, CH <sub>3</sub>	1.24, s	7, 11, 13		5, 6, 8a, 13
13	30.0, CH <sub>3</sub>	1.20, s	7, 11, 12		8b, 12
14	19.8, CH <sub>3</sub>	0.97, d (7.02)	1, 9, 10	10	1, 9a, 10
15	23.7, CH <sub>3</sub>	1.65, s	3, 4, 5	3, 5, 6	3, 5

<sup>a</sup>HMBC correlations, optimized for 6 Hz, are from proton(s) stated to the indicated carbon.

<sup>b</sup>COSY correlations are from proton(s) stated to the indicated proton.

<sup>c</sup>NOESY correlations, optimized mixing time for 1.2 ms, are from proton(s) stated to the indicated proton.

<sup>d</sup>Signal partially obscured.

AmAOS	1	ATGTCACCTTACAGAAGAAAAACAAATTCGCCCCATTGCCAACTTTCTCCAAGCATTTGGGAGATCAGTTTCTTATCTATGCCAAGCAA	90
AmBOS	1	ATGTCACCTTACAGAAGAAAAACCTAATTCGCCCCATTGCCAACTTTCTCCAAGCATTTGGGAGATCAGTTTCTTATCTATGCCAAGCAA	90
AmAOS	91	GTAGAGCAAGGGGTGGAACAGATAGTGGAAAGATTTAAAAAAGAAGTGGCGCACTACTAAAAGAACTTTGGATAATCCTACGAAACAT	180
AmBOS	91	GTAGAGCAATGGGGTGGAAACAGAGAGTGAAGATTTAAAAAAGAAGTGGCGCACTACTAAAAGAACTTTGGATAATCCTATGAAACAT	180
AmAOS	181	GCCAATTTGTTGAAGATGATTGATGAAATACAACGCCTTGGAAATGCCGTATCTCTTTGAACAGGAGATTGATCAAGCATTGCAACATAT	270
AmBOS	181	GCCAATTTGTTAAAGCTGATTGATGAAATACAACGCCTTGGAAATATCGTATCTCTTTGAACAGGAGATTGATCATGCATTGCAACATATC	270
AmAOS	271	TATGAAACATATGGTGATAAGTGGAGTGGTGACCGCTCTTCCTTGGTTCCTGCTTATGCGAAAAAAGGATATTTTGTACCTGTGAT	360
AmBOS	271	TATGAAACATATGGTGATAACTGGAGTGGTGACCGCTCTTCCTTATGGTTCCTGCTTATGCGTAAACAAGGATATTTTGTACATGTGAT	360
AmAOS	361	GTTTTCAATAACTATAAAGACGAAACGGAGCGTTCAAGCAATCTTAGCTAATGATGTTGAAGTTTGGCTTAGAGTTGACGAAGCAACG	450
AmBOS	361	GTTTTCAATAACATAAAGACGAAAGTGGAGCGTTCAAGCAATCTTAGCTAATGATGTTGAAGTTTGGCTTAGAGTTGACGAAGCAACG	450
AmAOS	451	TCTATGAGGGTACCAGGGGAGATTATATTAGAGATGCTCTGTTTTTACACGATCTTATCTTAGCGTTATAGCAAAAAGATACTCTTTCCG	540
AmBOS	451	TCTATGAGGGTACCAGGGGAGATTATATTAGATGATGCTCTGTTTTTACACGATCTAATCTTAGCATATAGCAAAAAGATACTCTTTCCG	540
AmAOS	541	ACCAACCCTGCTCTTTCTACCAGAAATACAACGGGCACTAAAGCAACCCCTTTGGAAAAGATTGCCAAGAATAGAGCGGCACAATACATT	630
AmBOS	541	ACCAACCCTGCTCTTTCTACCAGAAATACAACGGGCACTAAAGCAACCCCTTTGGAAAAGTTGCCAAGAATAGAGCGGCACAATACATT	630
AmAOS	631	CCTTCTATCTACAACAAGATTCTCATAACAAGACTTTACTTAAACTTGCTAAATTAGAGTTCAATTTGCTTCAGTCATTGCACAAGGAA	720
AmBOS	631	CCTTCTATGAAACAACAAGATTCTCATAACAAGACTTTACTTAAACTTGCTAAATTAGAGTTCAATTTGCTTCAGTCATTGCACAAGGAA	720
AmAOS	721	GAGCTCAGCCAAAGTGTCCAAATGGTGGAAAGCTTTCGATGTCAAGAAACAACGCACCTTATTCAGAGATAGAATTGTTGAATGCTACTTT	810
AmBOS	721	GAGCTCAGCCAAAGTGTCCAAATGGTGGAAAGCTTTCGATGTCAAGAAACAACGCACCTTATTCAGAGATAGAATTGTTGAATGCTACTTT	810
AmAOS	811	TGGGCACTAGGTTCAAGCTATGAGCGGCACTAATTTCTCAGGTAGAAATTTCTTGGCAAAAGTTATTGCACTTATAACTCTTATAGATGAG	900
AmBOS	811	TGGGCACTAGGTTCAAGCTTGGAGCCACAATTTCTCGGGTAGAAATTTCTTGGCAAAAGTTATTGCACTTGTGACTCTTATAGATGAG	900
AmAOS	901	ACTTATGACCGGTATGGTACTTATGAAGAACTTAAGATCTTACTGAAGCAATTCAAAGGTGGTCAATTACATGCTTAGACATGATTCCA	990
AmBOS	901	ACTTATGATCGGTATGGTACTTATGAAGAACTTAAGATCTTACTGAAGCAATTCAAAGGTGGTCAATTACATGCTTAGACATGATTCCG	990
AmAOS	991	GAATACATGAACCCGATATACAAATTTATTCATGGATACGTACGCCGAAATGGAAGAAATTCCTTGGAAAGGAGGGCAAAAACAGATATATTT	1080
AmBOS	991	GAATACATGAACCCGATATACAAATTTATTCATGGATACGTACGCCGAAATGGAAGAAATTCCTTGGAAAGGAGGGCAAAAACAGATATATTT	1080
AmAOS	1081	AACTGCGCAAGAATCTGTGAAGAAGTTTGTAGAGCCCTGATGGTTGAAGCAACATGGTTAAATGAGGGACACATACCAACCCCTGAA	1170
AmBOS	1081	AACTGTGCAAGAATTTGTGAAGAGTTTGTAGAGTCTGATGGTTGAAGCAACATGGTTAAATGAGGGACACATACCAACCCCTGAA	1170
AmAOS	1171	GAGCATGATTCAATTTATTCATTAAGTGGCGGTGCTAACCTGCTTACAACAACCTAGTTATCTTGGCATGAGTGATATAGTCACAAAAGAG	1260
AmBOS	1171	GAGCTTGATTCAATTTGAGTCAATTTTGGCGGTGCTAACCTGCTTACAACAACCTTGTATCTTGGCATGAGTGATATAGTCACAAAAGAG	1260
AmAOS	1261	GCTGTGCAATGGTGTCTCTGTACCTCTCTTTTAGATACTCAGGTATACTTGGTCGACGCCCTAAATGATATCAGGCCACAGGTG	1350
AmBOS	1261	GCTGTGCAATGGCTGTCTCTGAACCTCTCTTTTAGATACAAAGGTATACTTGGTCGACGCCCTAAATGATCTTGGGCCACAAAGGAG	1350
AmAOS	1351	GAGCAAGAAAGAAAGCATGTTTCATCGAGCGTTGAAAGTTACATGAATGAATAAATAATCAATGAGGAGTATGCCAAAACCTGTTGTAC	1440
AmBOS	1351	GAGCAAGAAAGAAAGCATGTTTCATCGAGCGTTGAAAGTTACATGAAGGAATAAATGTCAGTGGAGGATATGCCAAAACCTGTTGTAC	1440
AmAOS	1441	AAGCATGTAGAAGATCTGTGGAAGAATATAAACCGAGAGTACCTCATAACTAAAAACATTCAAAGGCCACTATTGATGGCGGTGATCAAT	1530
AmBOS	1441	AAGCAATGTAGAAGATCTGTGGAAGAATATAAACCGAGAGTACCTCATAACTAAAAACATTCAAAGGCCACTATTGATGGCGGTGATCAAT	1530
AmAOS	1531	TTGGCACAGTTTGTGGAAGTTCAATATGCAGGAAAGGATAACTTCACCTCATAATGGGAGACGATTACAAAATCTCTGTAAGTCTTTACTC	1620
AmBOS	1531	TTGGTACATTTTCTGGAAGTTCTATATGCAGAAAGGATAACTTCACACGTACGGGAGACGAATACAAAATCTCTGTAAGTCTTTACTC	1620
AmAOS	1621	GTTTATCCTATGAGTATATGA	1641
AmBOS	1621	GTTTATCCTATGAGTATATGA	1641

Figure S1. Alignment of the full-length ORF of *AmAOS* and *AmBOS*. Target sequences of each specific primer are shown in red boxes.

AmAOS	1	-----MSLTEEKPIRPIANF--PPSIWGDQFLIY-AKQVEQ-GVEQIVEDLKKEVRQLKE--	52
AmBOS	1	-----MSLTEEKPIRPIANF--SPSIWEDQFLIY-AKQVEH-GVEQRVKDLKKEVRQLKE--	52
AaADS	1	-----MSLTEEKPIRPIANF--PPSIWGDQFLIY-EKQVEQ-GVEQIVNDLKKEVRQLKE--	52
AaBOS	1	-----MSLTEEKPIRPIANF--SPSIWGDQFLIY-DNQVEQ-GVEQIVKDLKKEVRQLKE--	52
AaECS	1	-----MSLIVEDVIRPNANF--PSEIWGDQFLAYDQDE--QEGVEQVIKDLKKEVKSSELLT--	52
AaFS	1	MSTLPISSVSFSSTSPLVVDDKYSTKPDVIRHTMNF--NASIWGDQFLTY-DEPEDLVMKKQLVEELKEEVKKEELITIK	77
AaGAS	1	-----MAAVQANVTGIKANTKTSAPVVRPLANF--PPSVWGDQFLSFLDRSELERYAIAMEKPKEDLKLIVD--	67
AaQHS	1	-----MSVKEEKVIRPIVHF--PPSVWADQFLIFDDKQAEQANVEQVNVNELREDVRKDLVS--	54
AabKOS	1	-----MSLTEEKPIRPIANF--PPSIWGDQFLIN-AKQVEQ-GVEQIVEDLKKEVRQLKE--	52
AkBOS	1	-----MSLTEEKPIRPIANF--SPSIWEDQFLIY-AKQVEH-GVEQRVKDLTKEVRQLKE--	52
MmQHS	1	-----MASRENIIRPKANY--HPSVWGDQFLIYNEQEV-QAEVDQIIGSLKEEVRKQLLI--	53
ScGAS	1	-----MAAQQGEVVRPDADYSYHPSLWGDQFLHYDEQEDDQVEVDQIEILKEETRREILS--	56
ScGDS	1	-----MGCKQESFRPFRSTSPSVWGDQFLNY-EKKAQCDELTVEDLKEKVEAITG--	52
TpQHS	1	-----MSAKEEKVIRPIVHF--PPSVWADQFLIFHEEQAEQANLEQVFNESREDVRKDIVS--	54
AmAOS	53	TLDNPTKHANLLKLMIDEIQRLGMPYLFEEQEIHQALQHIYETYGDKWSGDRS----SLWFRMLMRKQGYFVTCDFVNNY-KD	127
AmBOS	53	ALDIPMKHANLLKLIDEIQRLGISYLFEEQEIHDALQHIYETYGDNWSGDRS----SLWFRMLMRKQGYFVTCDFVNNH-KD	127
AaADS	53	ALDIPMKHANLLKLIDEIQRLGIPYHFEREIDHALQCIYETYGDNWNGDRS----SLWFRMLMRKQGYVTCDFVNNY-KD	127
AaBOS	53	ALDIPMKHANLLKLVDIEIQRLGISYLFEEQEIHDALQHIYETYGDNWSGDRS----SLWFRMLMRKQGYFVTCDFVNNH-KD	127
AaECS	53	ALNSPTQHTLLEKFDIAIERLGIAYYFEEINQVFOHMYTAYGDKWTGGNT----SLWFRMLRQHGFVSSDIFSTY-KD	127
AaFS	78	GSNEPMQHVKLIELIDAVQRLGIAYYHFEIEEALQHIHTVYGEQWVDKENLQSIWFRMLRQGGFNYSVSGVFKDF-MD	156
AaGAS	68	---PTMDSNEKGLIYSVHRLGLTYMFLQEIESQLDKLFNKFSLDQYEEVDLYTISINWQVFRHVGYKLPDVFNFKFDV	144
AaQHS	55	SLDVQTEHTNLLKLIDAIQRLGIAHYHFEIEEQALQHIYDTYGDWKGKRSPP----SLWFRMLRQGGFYVSCDIFKNY-KK	129
AabKOS	53	ALDIPMKHANLLKLIDEIQRLGIPYLFEEQEIHDALQHIYETYGDNWSGDRS----SLWFRMLMRKQGYVTCDFVNNY-KD	127
AkBOS	53	ALDIPMKHANLLKLIDEIQRLGISYLFEEQEIHDALQHIYETYGDNWSGDRS----SLWFRMLMRKQGYFVTCDFVNNH-KD	127
MmQHS	54	GLDDPTKHTDLLKLVVVIQRGLGIAYYFEMEIDQALQHIHTVYGDHWKGGST----FIWFRMLRSHGFYVSCDIFNDA-KD	128
ScGAS	57	SLDDPAKHTNLLKLIDVIQRLGIAHYHFEIEITQALDHIYVYGDWNGGST----SLWFRMLRQGGFYVSCDIFNIY-KL	131
ScGDS	53	ALGNPKHEVNLKLLIDAIQRLGIPYFEEITNALQHLYEAYGDEWVGGSP----SIWFRMLRQGGFYVSCDIFNKYKQD	128
TpQHS	55	SLDVQAEHTNLLKLIDAIQRLGIAHYHFEIEINQALQHIYDTYGDWKGKSP----SLWFRMLRQGGFYVSCDIFKNY-KE	129
AmAOS	128	ENGAFKQSLANDVEGLLELYEATSMRVPGEITLEDALVFTRSYLSVIAKDTLSTNPALSTEIQRALKQPLWKRLPRIEAA	207
AmBOS	128	ESGAFKQLLANDVEGLLELYEATSMRVPGEITLDDALVFTRSNLSIAKDTLSTNPALSTEIQRALKQPLWKRLPRIEAA	207
AaADS	128	KNGAFKQSLANDVEGLLELYEATSMRVPGEITLEDALVFTRSRLSINTKDAFSTNPALFTEIQRALKQPLWKRLPRIEAA	207
AaBOS	128	ESGVFKQSLKNHVEGLLELYEATSMRVPGEITLEDALVFTQSHLSIAKDTLSINPALSTEIQRALKQPLWKRLPRIEAV	207
AaECS	128	KEGRFKESLEKDVHGLLELYEAAYMFVPGEGITLDDALVFTRTCLDETAKNPSLSNAVSSQITREALTQPLHKRLPRLEAL	207
AaFS	157	EKGKFKESLCNDAQGILALYEAAMRVEDEITLDNALEFTKVLHDIIAKDP-SCDSSLRTQIHOALKQPLRRRLARIEAL	235
AaGAS	145	SSGTFKASITSDV-GVVGLYESAQLRIRGEKILDEASVFTFAKLKSVVNT---LEGDLAQVQTSLRRPFHQGMPGLGIRQ	220
AaQHS	130	EDGSFKESLTDNVEGLLELYEATYLRVQGEGLVDDALVFTRTCLEIAKDLVHTNPTLSTYIQEALKQPLHKRLTRLEAL	209
AabKOS	128	DNGAFKQLLANDVEGLLELYEATSMRVPGEITLEDALVFTRSRLSIMAKDALSTNPALFTEIKRALKQPLWKRLPRIEAA	207
AkBOS	128	ESGAFKQSLANDVEGLLELYEATSMRVPGEITLDDALVFTRSNLSIAKDTLSTNPALSTEIQRALKQPLWKRLPRIEAA	207
MmQHS	129	NTGSFKESLTDNVEGLLELYEATYSMVAGEAVLDDAHAFAKAHLEKIANDPLQRNSFLSRRILEALEQPIHRRLPRIINAL	208
ScGAS	132	DNGSFKDSLTKDIECMLLELYEAAYMRVQGEITLDEALEFTKTHLEQIAKDLPRCNNTLSRHIEYALKRPIRKLPRVDAL	211
ScGDS	129	KHGDFKESLINDDEEMVELYEATSLRVRGEGVLDFAFEFTRNFHANIAKEPRCSNATLSTHIQVALETPLHKRIPRLDAL	208
TpQHS	130	EDGSFKESLTDNVEGLLELYEATYLVQGEGLVDDALVFTRTCLDKIAKDLVQSNPTLSTQIQEALQQSVHKRLTRLEAL	209
AmAOS	208	QYIPFYLQQDShnKTL-LKLAKLEFNLLQSLHKEELSQVSKWKKAFDVKNNAPYSRDRIVECYFWALGSSYEPQYSHGRI	286
AmBOS	208	QYIPFYEQQDShnMTL-LKLAKLEFNLLQSLHREELSOLSKWKKAFDVKNNAPYSRDRIVECYFWGLASRFEPQFSRARI	286
AaADS	208	QYIPFYQQDShnKTL-LKLAKLEFNLLQSLHKEELSHVCKWKKAFDIKKNAPCLRDRIVECYFWGLGSGYEPQYSRARI	286
AaBOS	208	QYIPFYEQQDShnKTL-IKLAKLEFNLLQSLHREELSOLSKWKKAFDVKNNAPYSRDRIVECYFWALASRFEPQYSRARI	286
AaECS	208	RYIPFYQQQASHSETL-LKLAKLGFNLLQSLHKEELSIISKWKKSFVANNLPYARNRPVECYFWALAVYFEPQYSERSRV	286
AaFS	236	HMPYIYQGETSHDEVL-LKLAKLDFSVLQSMHKKELSHICKWKKDLDLQNKLPYVRDRVVEGYFWILSLIYEPQHARTRM	314
AaGAS	221	GSISLTMKKNVPLMTHCKLAKLHFKYLELQQKEELRIVSKWKKDMRFHETTPYIRDRVPEIYLWILGLYFEPYSLARI	300
AaQHS	210	RYIPMYEQQASHNESL-LKLAKLGFNLLQSLHREELSEVSRWKKGLDVPNNLPYARDRMVECYFWALGVYFEPKYSQARI	288
AabKOS	208	QYIPFYLQQDShnKTL-LKLAKLEFNLLQSLHKEELSHVCKWKKAFDVKNNAPYSRDRIVECYFWGLGSRFEPQYSRARI	286
AkBOS	208	QYIPFYEQQDShnMAL-LKLAKLEFNLLQSLHREELSOLSKWKKAFDVKNNAPYSRDRIVECYFWGLASRFEPQFSRARI	286
MmQHS	209	RFIPIYEQQVSHNDSL-LRLAKLDFNRVQSVHKKELSQLSKWKKDFDAPKNAPYMRDLVEGYFWGTGVYFEPQYSDSRI	287
ScGAS	212	QYMPFYEQQDShnKSL-LRLAKLGFNRLQSLHKEELSQLSKWKKFEDAPKNLRYVRDLVELYFWLGVYFEPKYSRSRI	290
ScGDS	209	RYIRFYEKQASHNESL-LKLAKLGFNLLQSLHREELSQVSKWKKDQVQKNLPYARDRMVSEYFAIGVYSEPKYSLGRV	287
TpQHS	210	RYIPIYEQQLASHNESL-LKLAKLGFNLLQSLHREELSQVSRWKKGLDVPNNLPYARDRMVECYFWALGVYFEPKYSRARI	288

Figure S2 (cont.)

AmAOS	287	FFAKVIAVITLIDDDTYDAYGTYEELKIFTEAQRWSITCLDMIP	PEYMKPIYNLFMDTYTEM	EEILAKEGKTDIFNCAKE	365	
AmBOS	287	FLAKVIALVTLIDDDTYDAYGTYEELKIFTEAERWSITCLDMIP	PEYMKPIYKLLMDTYAEM	EEVLAKEGKTDIFNCGKE	365	
AaADS	287	FFTKAVAVITLIDDDTYDAYGTYEELKIFTEAVERWSITCLDTL	PEYMKPIYKLFMDTYTEM	EEFLAKEGRDLDLFCNGKE	365	
AaBOS	287	FLAKVIALVTLIDDDTYDAYGTYEELKIFTEAERWSITCLDMIP	PEYMKPIYKLFMDTYTEM	EEILAKEGKTNIFNCGKE	365	
AaECS	287	FLSRFFSIQTFLLDDTYDAYGTYEELQFTEAQRWSITCLDGL	PESMKLIFQMLVKIFEETI	EEILSKDGKQHHVNYIKE	365	
AaFS	315	FLMKTCMWLVLDDTFDNYGTYEELIIFTQAVERWSISCLDMIP	PEYMKLIYQELVNLHVEM	EEISLEKEGKTYQIHVYKE	393	
AaGAS	301	IATKITLFLVVLDDTYDAYATIEEIRLLTDAINKWDISAMEQIPE	YIRPFYKILLDEYAGNWRKWLK	KGQIILLLLQKK	380	
AaQHS	289	FLAKVISLATVLDLDDTYDAYGTYEELKIFTEAQRWSITCIDML	PEYLKLLYQGVLDIYIEM	EEIMGKEGKAHHSYAKE	367	
AabKOS	287	FFAKVIAIITLIDDDSYDAYGTYEELKIFTEAQRWSITCLDTL	PEYMKPIFKLFMDTYTEM	EENLAKGRTDLFCNGKE	365	
AkBOS	287	FLAKVIALVTLIDDDTYDAYGTYEELKIFTEAERWSITCLDMIP	PEYMKPIYKLLMDTYTEM	EEVLAKEGKTDIFDCGKE	365	
MmQHS	288	FVVKSFVMAALLDDTYDYSYGTFFEELEIFTKAVERWSMTCMDT	LPDYMKMYKTLLDIYDEL	EETMEKKGKTYHLNYAKD	366	
ScGAS	291	FLTTKVIMATILDDTYDIHGTYEELIIFTKAVQRWSITCMDTL	PDYMKMIYKSLLDVYEM	EEIIEKDGKAYQVHYAKD	369	
ScGDS	288	FLAKVIQLATAIDDDTFDAYGTYEELQFTTRAVERWSITCLDEL	PEYMKLLYQVLLDLYGEM	EPIMEKEQLTFLNCSKE	366	
TpQHS	289	FLAKVISLATVLDLDDTYDAYGTYEELKIFTEAQRWSITCIDTL	PEYMKLLYAGILNIYKEM	EEIMGKEGKAHHSYAKE	367	
AmAOS	366	SVKFEVRALMVEATMLNEGHIPTPEEHDLSILFITGGANLTTT	SYLGM-SDIVTKEAVEVWVSP	PPLFRYSGILGRRLND	444	
AmBOS	366	FVTDFVRVLMVEAQMLNEGHIPTTEELDSIAVNLGGANLTTTC	YLG-SDIVTKEAVEWAVSE	PPLFRYSGILGRRLND	444	
AaADS	366	FVKFVRVLMVEAKWANEAGHIPTTEEHDPVVIITGGANLTTTC	YLG-SDIFTKESVEWAVSAP	PPLFRYSGILGRRLND	444	
AaBOS	366	FVKDFVRVLMVEAQMLNEGHIPTTEELDSIAVNLGGANLTTTC	YLG-SDIVTKEAFWAVSE	PPLFRYSGILGRRLND	444	
AaECS	366	TLKEAVQSYMTEARMAKEYIPTIEEHTKVSYSISYKALVAG	FACM-GDVIADDSFEWFTN	PPLVNAACCLCRIMDD	444	
AaFS	394	MAKELVRNYLVEARNLKEGYMPTLEEYMSVSMVTGTGLMI	ARSYVGR-GDIVTEDTFKVVSS	YPIIKASCIVRLMDD	472	
AaGAS	381	RSKTLARGYLEEAENTNSGYVASTPEYMKVNLITSAYNVISK	SALVGM-GEIVSEDALAWY	SHPTLQASELISRLQDD	459	
AaQHS	368	SMKEFIRSYMMEAKWANEGYVPTAEHMSVAFVSSGYSML	ATTFCVGM-GDIVTDEAFK	WALTKPPIIKASCAIARL	MDD 446	
AabKOS	366	FMKEIVRALMVEAKWVNEAGHIPTTEEHDSVAFITGGANL	STTCYLG-SDIFTKESVEWAV	SEPPPLFRYSGILGRRLND	444	
AkBOS	366	FVKDFVRVLMVEAQMLNEGHIPTTEELDSIAVNLGGANLTTTC	YLG-SDIVTKEAVEWAVSE	PPLFRYSGILGRRLND	444	
MmQHS	367	TMKEFVRVLMVEAKWRNEGYIPTVEEHKSVAFLSCGYKML	ITAAVGM-GDII TEDSF	KVWSTFPPPLVKASSAVCR	IMDD 445	
ScGAS	370	SMIDLVTSYMTAKWLEHGHPVTFEEYNSITNLTGGYKML	TSSFDVMPGDIVTQESFR	WALNPNPLIKASADVSR	IMDD 449	
ScGDS	367	FMIETVKAYMVEAKWVYEGHIPTKDEHTSIAFATGGG	PLLMSSCYLG-GDIITNDS	INWVISEPPLKATNMIGR	LLND 445	
TpQHS	368	SMKEFIRSYMMEAKWANEAGHIPTPEEHMSVAFVSSGYSML	ATTFCVGM-SDIVTDEAF	EWALTDPPPIIKASCAIAR	LMD 446	
AmAOS	445	ITGHQVEQERKRVSSVSESYMNEYNI-EEYAQNLLYKQV	EDLWKDINREYLI-TKNI	QRPLLMVAVINLAQFVE	VQYAGK 522	
AmBOS	445	LAGHKEEQERKRVSSVSESYMKEYNVS-EEYAQNLLYKQV	EDLWKDINREYLI-TKT	IPRPLLVAVINLVHFL	LEVLYAEK 522	
AaADS	445	LMTHKAEEQERKHSSSLESYMKEYNVN-EEYAQTLIYKE	VEDVWKDINREYLI-TKNI	IPRPLLMVAVIYL	CQFLEVOYAGK 522	
AaBOS	445	LAGHKEEQERKRVSSVSESYMKEYNVS-EEYAKNLLYKQV	EDLWKDINREYLI-TKT	IPRPLLVAVINLVHFL	LDVLYAEK 522	
AaECS	445	LGSHKGEQDRKRVASTTECYMKQFDAS-EQQAYESLNKK	VEDAWKEINREFMITCK	DVNIHVAMRVLNF	SRSVDVLYKNK 523	
AaFS	473	IVSHKEEQERGHVASSIECYSKSGAS-EEEAACEYISR	KVEDAWKVINRESLR-PT	AVPPLLMPAINLARM	CEVLYSVN 550	
AaGAS	460	VMTYQFERERQGSATGVDAYIKTYGVSE-KEAIDALKIMI	ENAWKDNREGCLK-PR	QVSMDLLAPILN	LARMIDVVRYD 537	
AaQHS	447	IHSQKEEQERIHVASSVSESYMKQYDVT-EEHVLKVF	NKKI EDAWKDIR	TRESLV-RKDI	PMPLMMRVINLAQVMD	VLYKHK 524
AabKOS	445	LVSHKDEQERKHISVSESYMKEYNVN-EEYAQNLLYKQV	EDVWKDINREYLI-TKNI	IPRPLLMVAVINL	CQFLEVOYARK 522	
AkBOS	445	LAGHKEEQERKRVSSVSESYMKEYNVS-EEYAQNLLYKQV	EDLWKDINREYLI-TKT	IPRPLLVAVINLVH	FLLEVLYAEK 522	
MmQHS	446	IVGHKEEQEREHVASSVSESYMKQHDVTN-ENAYEFL	YQQVEDRWKEMNWEL	LT-CKDV	PMPVMTWVINQVR	MDLKYKD 524
ScGAS	450	IVGHKEEQERKHLPSRVEYMKKYHLA-EDVYD	LKQVVEDAWKDLNRE	TLT-CKD	IHMALKMRPINLAR	VIDMLYKND 527
ScGDS	446	IVSSKKEQERVHFQSIYQCYKKQYDVS-EEHAINL	VREEIEDVWKDINKD	SLT-AKV	PRPLILVVVNYART	LYLYKYN 523
TpQHS	447	IHSQKEEQERIHVASSVSESYMKQYDVT-EEHVHKV	FLKKI EDAWKDIR	TRESLV-CKD	IPMLTRVINLAR	VMDVLYKHK 524
AmAOS	523	DNFTHMGDDYKHLVKSLLVYPMSI-			546	
AmBOS	523	DNFTRTGDEYKDLVKSLLVYPMSI-			546	
AaADS	523	DNFTRMGDEYKHLIKSLLVYPMSI-			546	
AaBOS	523	DNFTRMGEEYKHLVKSLLVYPMSI-			546	
AaECS	524	DHFTHVGEVINHIKSLFVDAIIT-			547	
AaFS	551	DGFTHAEGDMKSYMKSFFVHPMVV-			574	
AaGAS	538	DGFTFPRKDSERVYQSFCLWVLPV			562	
AaQHS	525	DGFTNVGEELKDHKSLLVHPIPT-			548	
AabKOS	523	DNFTRMGDEYKHLVKSLLVYPMSI-			546	
AkBOS	523	DNFTRMGDEYKDLVKSLLVYPMSI-			546	
MmQHS	525	DTYTRVGEELINNIKQCFVHSMTV-			548	
ScGAS	528	DNLKNVGQEIQDYIKSCFINAISV-			551	
ScGDS	524	DNFFVEEDIKEDIKSLLIHPMSI-			547	
TpQHS	525	DGFTNVGKELKDHKSLLVHPIPT-			548	

Figure S2. Alignment of deduced amino acid sequences of AmAOS and selected plant sesquiterpene synthases. Full name of the enzymes and % amino acid sequence identity of the aligned enzymes compared with AmAOS are shown in Table S2. Only characterized sesquiterpene synthases from *A. annua* or other plants with greater than 50% amino acid sequence identity to

AmAOS were selected in this alignment. Typical motifs of sesquiterpene synthases are underlined: R(R,P)(X)<sub>8</sub>W, pink; RXR, blue; DDXXD, red; and NSE/DTE, green. Putative catalytic residues located within 12Å from the active site (Salmon et al. 2015) are indicated by brown boxes. Black shading indicates conserved amino acid residues. Gray shading indicates amino acid residues with >51% conservation. Black triangle indicates the magnesium ligands of the NSE/DTE motif (residue 447) mentioned in the main text. GenBank accession numbers of aligned enzymes are as follow: AmAOS, LC153278; AmBOS, LC106015; AaADS, LC106014; AaBOS, JQ717161; AaECS, AF157059.1; AaFS, AY835398.1; AaGAS, DQ447636.1; AaQHS, AF472361.1; AabKOS, LC106017; AkBOS, LC106016; MmQHS, ACN67535.1; ScGAS, CAC36896.1; ScGDS, CAE47440.1; TpQHS, AEH41845.1.

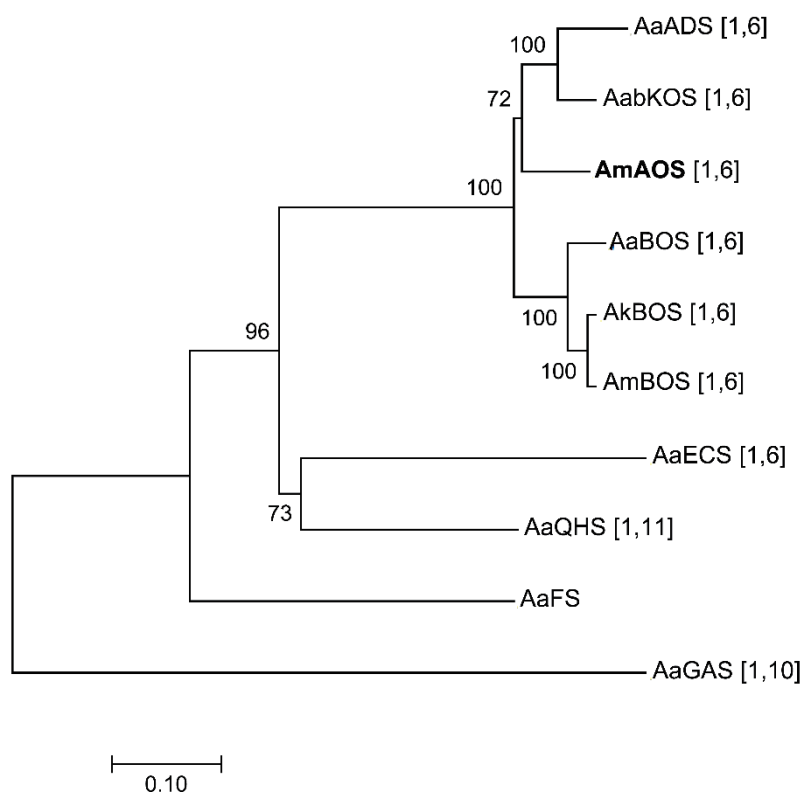


Figure S3. Phylogenetic relationships among *Artemisia* spp. sesquiterpene synthases. The rooted neighbor-joining tree was generated based on amino acid sequences of sesquiterpene synthases identified in *Artemisia* species using CLUSTAL W and MEGA7 software. The numbers indicate the bootstrap value (%) from 1,000 replications. AaGAS was used as the out-group. The enzyme cloned in this study is indicated in bold. The first cyclization mechanisms are indicated in square brackets.

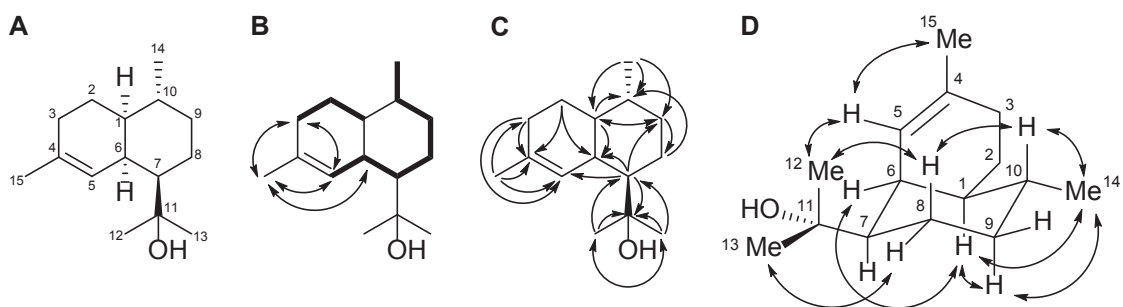


Figure S4. (A) Numbering and observed correlations from (B) COSY, (C) HMBC, and (D) NOESY spectra of 4-amorphen-11-ol. Double-headed arrows indicate (B–D) reciprocal correlations and (B) long range couplings detected in COSY spectrum. Only NOESY correlations necessary for determining the relative configurations are shown.



4-Amorphen-11-ol <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 600 MHz)

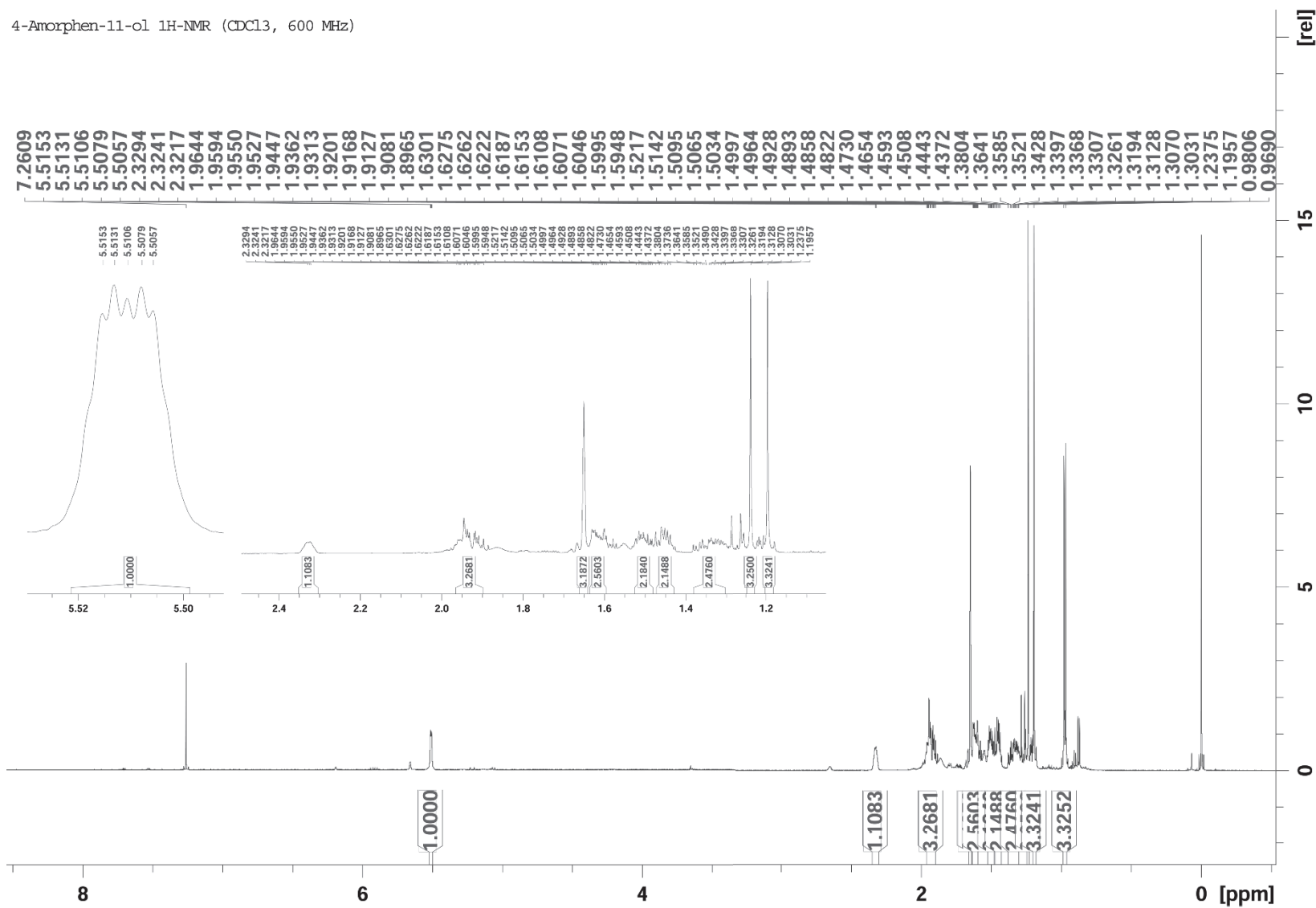


Figure S5. <sup>1</sup>H NMR spectrum for 4-amorphen-11-ol (CDCl<sub>3</sub>, 600 MHz).

4-Amorphen-11-ol  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ , 600 MHz)

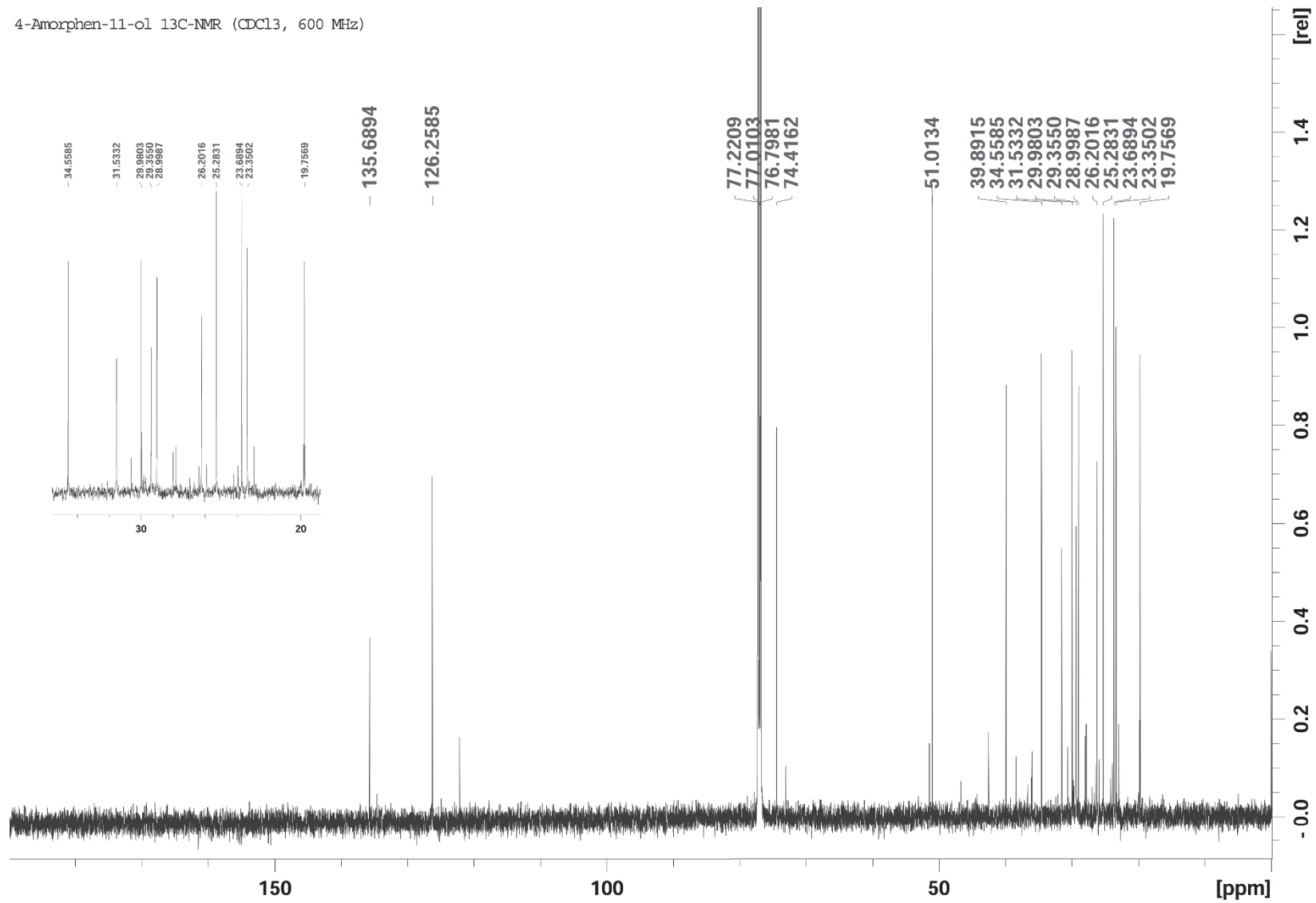


Figure S6.  $^{13}\text{C}$  NMR spectrum for 4-amorphen-11-ol ( $\text{CDCl}_3$ , 600 MHz).

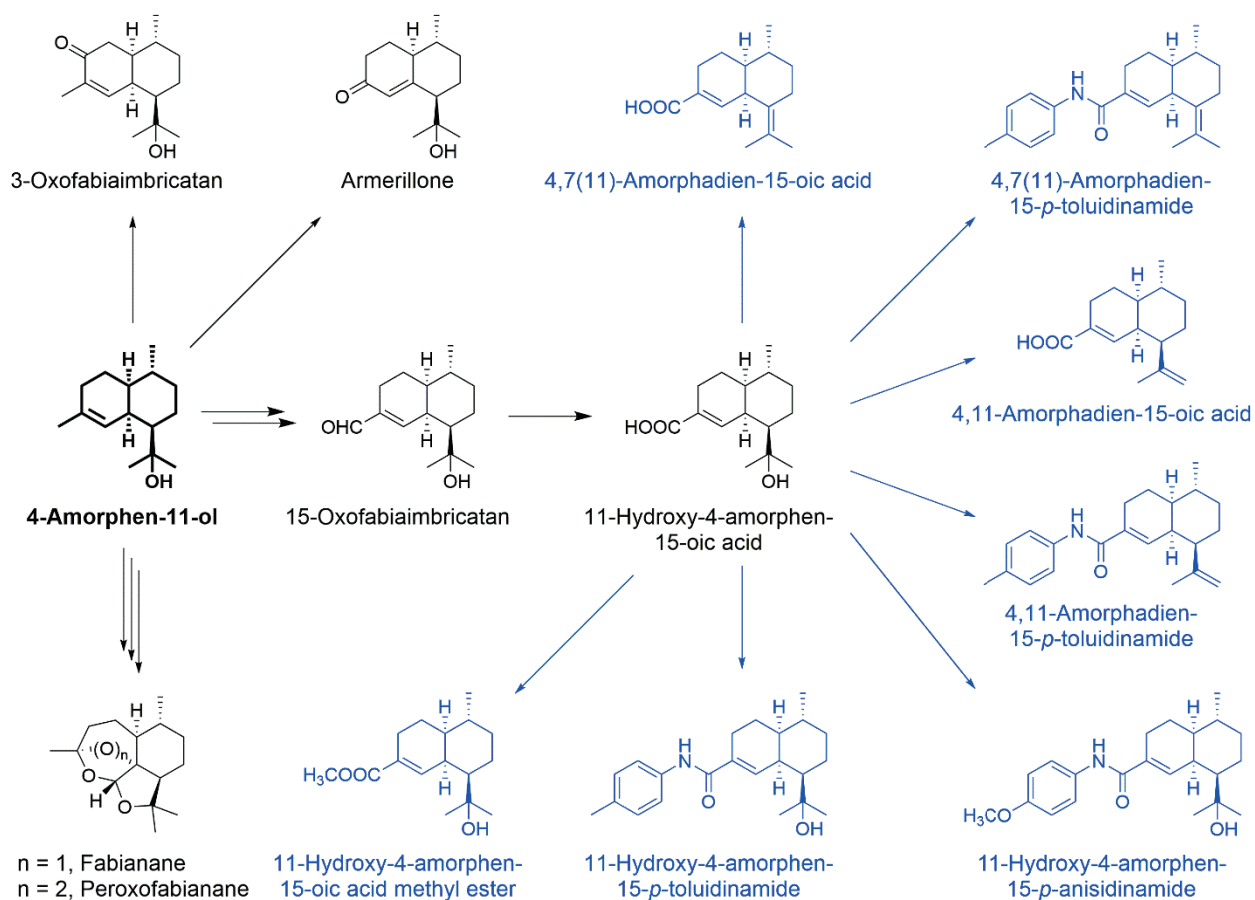


Figure S7. Natural and chemically synthesized sesquiterpenoids derived from 4-amorphen-11-ol. Compounds shown in black were previously isolated from *Fabiana imbricata* (Brown 1994a; Schmeda-Hirschmann and Papastergiou 1994). Fabianane can be either chemically synthesized or natural autoxidation/rearrangement from 4-amorphen-11-ol inside *F. imbricata* via the generation of peroxofabianane (Brown 1994b; Jun and Youn 1996; Ngo and Brown 1999). Compounds shown in blue were chemically synthesized from 11-hydroxy-4-amorphen-15-oic acid and tested their bioactivities against gastric lesions as well as cytotoxicity towards human gastric adenocarcinoma cell line (AGS) (ATCC CRL-1739) and normal human lung fibroblast (MRC-5) (ATCC CCL-171) (Reyes et al. 2005).

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