

Electronic Supporting Information

β -Amyrin synthase from *Euphorbia tirucalli* L. Functional analyses of the highly conserved aromatic residues Phe413, Tyr259 and Trp257 disclose the importance of the appropriate steric bulk, and cation- π and CH- π interactions for the efficient catalysis of the polyolefin cyclization cascade

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* 220 * 240 * 260 * 280 * 300

EtAS : CMRLGEGPNG-GQDNACSRARKWIIDHGGATYI PSWGKTWLSILGVYEWISGNSNMPPEFWILPFLMHPAKM WY CRMVYMPMSYLYGKRFVGPITPL : 282

PNY1 : CMRILGEGPDG-GVNNACARGRKWILDHGSVTAI PSWGKTWLSILGVYEWIGNSNMPPEFWILPFLMHPAKM WY CRMVYMPMSYLYGKRFVGPITPL : 284

BPY : CMRILGEGPDG-GQDNACARARKWILDHGGVTHMP SWGKTWLSILGIFEWIGNSNMPPEFWILPFLMHPAKM WY CRMVYMPMSYLYGKRFVGPITPL : 282

PSY : CMRILGEGPDG-GEDNACVRARNWIRQHGGVTHI PSWGKTWLSILGVFDWLGNSNMPPEFWILPFLMHPAKM WY CRLVYMPMSYLYGKRFVGPITPL : 282

AtLUP1 : CLRMLGENP----EQDACKRARQWILDRGGVIFI PSWGFVWLSILGVYDWSGTNETPPELLMLPFLPIHPGKIL YSRMVSI PMSYLYGKRFVGPITPL : 280

TRW : TLRLGEEADSV AEDMALVGRK WILDHGGAVGI PSWGFVWLSILGVYEWGGCNMPPEFWLMPKFFPIHPGKML YCRLVYMPMSYLYGKRFVGPITGL : 283

OEW : TLRLGEGQED-GEDKAVARGRK WILDHGGAVGI PSWGFVWLSILGVYEWGGCNMPPEFWLMPKFFPIHPGKML YCRLVYMPMSYLYGKRFVGPITGL : 281

BPW : ALRILGEGLED-GEDGAMAKSRK WILDHGGGLVAI PSWGFVWLSILGVYEWGGCNPLPPEFWLPEDFPIHPGKML YCRLVYMPMSYLYGKRFVGPITGL : 280

AtCAS1 : TLRLGEGPNDG--DGDMEKGRDWILNHGGATNIT SWGKMWLSVLGAFEWISGNNPLPPEIWLLEBYFLPIHPGRM WY CRMVYLPMSYLYGKRFVGPITST : 280

PsCAS : TLRLGEGPNDG--EGDMEGRDWILEHGGATYIT SWGKMWLSVLGVFEWISGNNMPPEIWLLEBYALPVHPGRM WY CRMVYLPMSYLYGKRFVGPITPT : 280

PgCAS : TLRLGEGANDG--QGAMKGRQWILDHGSATAIT SWGKMWLSVLGVFEWISGNNPLPPEIWLLEBYILPIHPGRM WY CRMVYLPMSYLYGKRFVGPITPT : 280

BpCAS1 : TLRLGEG-PEDG--MGAVEKARK WILDHGGATAIT SWGKMWLSVLGVYEWISGNNPLPPEIWLLEBYILPIHPGRM WY CRMVYLPMSYLYGKRFVGPITST : 290

AtLAS1 : ALRLMGEELDGG--DGAMESARSWIHHHGGATFI PSWGFVWLSVLGAYEWISGNNPLPPEIWLLEBYSLPFHPGRM WY CRMVYLPMSYLYGRRFVCRVTNGT : 280

HsLAS : SLRILGVGPD----DPDLVRARNILHKKGGAVAI PSWGFVWLSVLAFLNVYSWEGLNTEFPMMWLEPDWAPAHHPSTI WY CRMVYLPMSYLYGRRFVCRVTNGT : 255

MmLAS : ALRILGIGPD----DPDLVRARNVILHKKGGAVAI PSWGFVWLSVLAFLNVYSWEGLNTEFPMMWLEPDWAPAHHPSTI WY CRMVYLPMSYLYGRRFVCRVTNGT : 256

ScLAS : ILRLGLPKD----HPVCAKARSTLLRLGGAIGSPHWGKIWLSALNLYKWEQVNBAPPETWLLBYSLPMPHGRW WY CRMVYLPMSYLYGRRFVCRVTNGT : 257

SHC : ALKYIGMSRD----EEPQKALRFIQSQGGEISSRVE TRMMLALVGEY PWEKVPMPPEIMFLGKRMP LNIYE GSWARATVVALSIVMSRQ---PVFPPL : 189

64 6G r 6 Gg s5g4 W6 6g 5 W g np pPE w p P PNY1 Y261 5p6Sy y f

* 320 * 340 * 360 * 380 * 400

EtAS : ILQLR-QEIHTEPYHHINWTKTRHLCAHE DVYYPHPLIQDLMDSDLYIFTEPLLTRWPFNKIIRKKALEVTMKHIHYEDENSRYITIGCVER--VLCMLA : 379

PNY1 : ILQLR-EELYGOPYNEINWRKTRRVCAKE DIYYPHPLIQDLLWDSLYVLTEPLLTRWPFNKL-REKALQTTMKHIHYEDENSRYITIGCVER--VLCMLV : 380

BPY : ILQLR-EELYTQPYHQVNWKKVRHLCAKE DIYYPHPLIQDLLWDSLYIFTEPLLTRWPFNKLVRKRALEVTMKHIHYEDENSRYITIGCVER--VLCMLA : 379

PSY : ILQLR-EELHTEPYEKINWTKTRHLCAKE DIYYPHPLIQDLIWDSDLYIFTEPLLTRWPFNKLVRKRALEVTMKHIHYEDENSRYLTIGCVER--VLCMLA : 379

AtLUP1 : ILLLR-EELYLEPYEEINWKKSRRLYAKE DMYAHPLVQDLLSDTLQNFVEPLLTRWPLNKLVRKALQTMKHIHYEDENSHYITIGCVER--VLCMLA : 377

TRW : VRDLR-QELYTDPYDEINWNKARNTCAKE DLYYPHPFVQDMVWGVLHNVEPVLTSRPISTL-REKALKVAMDHVHYEDKSSRYLCIGCVER--VLCMLA : 379

OEW : VLSLR-QEITYTEPYHGINWNRARNTCAKE DLYYPHPLAQDMLWGLDHHFAEPVLTWRPF SKL-REKALKVAMHVHYEDMNSRYLCIGCVER--VLCMLA : 377

BPW : IQSLR-QELYNEPYHQINWNRARNTCAKE DLYYPHPLIQDLLWGLDHHVAEPVLTWRPF SML-REKALKAAIGHVHYEDENSKYLCIGSVER--VLCMLA : 376

AtCAS1 : VLSLR-KELETPYHEVNWNEARNLCAKE DLYYPHPLVQDILWASLDHKIVEPVLMRWPGANL-REKAIKTAIEHIHYEDENTRYICIGPVNK--VLNMLC : 376

PsCAS : VLSLR-KELETPYHDIWQARNLCAKE DLYYPHPLVQDILWATLDHKIVEPVFMNWPGKKL-REKAIKTAIEHIHYEDENTRYICIGPVNK--VLNMLC : 376

PgCAS : VLSLR-KEVFSVPYHEIDWQARNLCAKE DLYYPHPLIQDILWASLDKVVWEPFMHWPAKKL-REKALRTVMEHIHYEDENTRYICIGPVNK--VLNMLC : 376

BpCAS1 : IQSLR-KELYTPYHEIDWNRARNTCAKE DLYYPHPLVQDILWASLDLYYAYEPIFMYWPAKRL-REKALDTVMQHIHYEDENTRYICIGPVNK--VLNMLC : 386

AtLAS1 : ILSLR-RELYTIPYHHIDWDTARNQCAKE DLYYPHPLIQDVLWVSLNKFGEPLLRWPLNLL-RNHALQTVMQHIHYEDQNSHYICIGPVNK--VLNMLC : 376

HsLAS : VQSLR-QELYVEDFASIDWLAQRNNVAPDELYTPHSMWLRVYVALLN----LYEHHHSAHL-RQRAVQKLYEHTVADDRFTKSI SIGPISK--TINMLV : 346

MmLAS : VQSLR-QELYVQDYASIDWPAQRNNVSPDEMYTPHSMWLRVYVALLN----LYERFHSTSL-RKWAVQMLYEHIAADDCFTKCSI SIGPISK--TINMLV : 347

ScLAS : LEELR-NEIYTKPFDKINE SKNRNTVCGVDLYYPHSTTLNIA-NSLVVYFYEKYLRRNRFIYLSLKKVYDL----IKTELQNTDSLCTAPVWQ--AFCALV : 349

SHC : PERARVPELYETDV-----PPRRRGA KGG----GGWIFDALDRALHGYQK--LSVHPFRRAAEI RALDWLLEERQAGDG-----SWGGLQPPWFYALIA : 271

1R E6 p R a d yyph L e p a h e d i g 6 k 6

EtAS F413

EtAS F474

EtAS : CM-AEDPNGVPEKKHLARIIPDYMWVAEDGMKMQSF-GSQQWDTGFAIQALLASNDTE--EIGQVLKKGHDFIKKSQVKENP-SGDFKSMHRHISKGSWQEF : 474
 PNY1 : CM-VEDPNGDYERKHLARIIPDYIWWVAEDGMKMQSF-GSQEWDGTGFSIQALLSDSLTH--EIGPTLMKKGHDFIKKSQVKDNP-SGDFKSMYRHISKGSWQEF : 475
 BPY : CM-VEDPNGDYERKHLARIIPDYIWWVAEDGIKMQSF-GSQEWDGTGFAIQALLASNDTD--EIGPTLARGHDFIKKSQVKDNP-SGDFESMHRHISKGSWQEF : 474
 PSY : CM-VEDPNGDAEKKHLARVDPDYLWVISEDGMTMQSF-GSQEWDAGFAVQALLATNLE--EIKPALAKGHDFIKKSQVTENP-SGDFKSMHRHISKGSWQEF : 474
 AtLUP1 : CM-VENPNGDYERKHLARIIPDYMWVAEDGMKMQSF-GCQLWDTGFAIQALLASNDLP--ETDDALKRGHNYIKASQVRENK-SGDFRSMYRHISKGSWQEF : 472
 TRW : TM-VEDPNGDAYKRHLARIIPDYFVVAEDGMKMQSF-GCOMWDAAFAIQAIFFSSNDTE--EYGPTLKKAHAEFVKASQVRDNP-PGDFSKMYRHISKGSWQEF : 474
 OEW : CM-VEDPNSEAYKRHLARIIPDYFVVAEDGLKMQSF-GCOMWDAAFAIQAILLSNDLAE--EYGPTLMKAHAEFVKASQVQENP-SGDFNEMYRHISKGSWQEF : 472
 BPW : CM-AEDPNGEAYKLHLGRIPDNYVVAEDGLKIQSF-GCOMWDAGFAIQAILSCNDNE--EYWPTLRKAHAEFVKASQVPENP-SGDFKAMYRHINKGSWQEF : 471
 AtCAS1 : CM-VEDPNSEAEKLHLPRIHDFLWLAEDGMKMQGYNGSOLWDTGFAIQAILATNLE--EYGPVLEKAHAEFVKASQVLEDC-PGDLNRYWRHISKGSWQEF : 472
 PsCAS : CM-VEDPNSEAEKLHLPRIDYLDLWVAEDGMKMQGYNGSOLWDTAFAAQAIISTNLEID--EFGPTLKKAHAEFVKASQVSEDC-PGDLNRYWRHISKGSWQEF : 472
 PgCAS : CM-VEDPNSEAEKLHLPRIDYLDLWVAEDGMKMQGYNGSOLWDTAFVQAIISTNLEAE--EYGPVLEKAHAEFVKASQVLEDC-PGDLNRYWRHISKGSWQEF : 472
 BpCAS1 : CM-AEDPNSEAEKLHLPRIDYLDLWVAEDGMKMQGYNGSOLWDTTFAVQAIISTNLEAE--EYGPVLEKAHAEFVKASQVLEDC-PGDLNRYWRHISKGSWQEF : 482
 AtLAS1 : CM-VSSNSEAEKSHLSRIKDYLDLWVAEDGMKMQGYNGSOLWDTTFAVQAIISTNLEAE--EYGPVLEKAHAEFVKASQVLEDC-PGDLNRYWRHISKGSWQEF : 472
 HsLAS : RMYVDGPASTAEQEHVSRIIPDYLDLWVGLDGMKMQGTNGSOLWDTAFAIQALLEAGGHRRPEFSSCLQKAHEFLRLSQVDPNP-P-DYQKYYRQMRKGGGESE : 444
 MmLAS : RMYVDGPSSPAEQEHVSRIKDYLDLWVGLDGMKMQGTNGSOLWDTTFAVQAIISTNLEAE--EYGPVLEKAHAEFVKASQVLEDC-P-DYQKYYRQMRKGGGESE : 445
 ScLAS : TLIEEGVDSEAEQRLQYRFKDALFHGPQGMITMGTVGVQWDCAFAIQYFFVAGLAERPEFYNTIIVSAYKFLCHAQFDTECVPGS----YRDKRKGSWQEF : 445
 SHC : LKILDMTQHPAEIKGWEGLELYGVELDYGGWVFOASISPVWDTGLAVLALRAAGLPADHD---RLVKGAEWLLDRQIT---VPGDWAVKRPNLKPGGGEAE : 365
 w e p 5 h r d d g k 6 q g q W D f a q a e 6 h 5 6 Q g d r h k G 5 E

D⁴⁸⁶CTAE

EtAS C564

EtAS : SDQDHGMQVSDCTAEGLKCCLLFSMMPPEIVGKEMDAQHLYNAVNIILISLQS----KNGGLAAWE PAGAQQWLEMLNPTEFEADIVIEHEHYVBCIASAI : 569
 PNY1 : SDQDHGMQVSDCTAEGLKCCLLFSTMPPEIVGKKIKPERLYDSVNVLLSLQS----KNGGLSAWE PAGAQEWLELLNPTEFEADIVIEHEHYVBCISSAI : 570
 BPY : SDQDHGMQVSDCTAEGLKCCLLFSIMPPPEIVGKEMPEQLYDSVNVLLSLQS----KNGGLAAWE PAGAQEWLELLNPTEFEADIVIEHEHYVBCIASAM : 569
 PSY : SDQDHGMQVSDCTAEGLKCCLLLSLLPPPEIVGKEMPERLFDVSNVLLSLQS----KNGGLAAWE PAGAQEWLELLNPTEFEADIVIEHEHYVBCITGSAI : 569
 AtLUP1 : SDRDHGMQVSDCTAEALKCCLLLSMMSA-DIVGQKIDDEQLYDSVNVLLSLQS----GNGGVNAWE PSRAYKWLELLNPTEFEADIVIEHEHYVBCITSSVI : 567
 TRW : SIQDHGMQVSDCTAEGLKVSLLYSQMNP-KLVGKQVETEHLYDAVNVILSLQS----ENGGFPAWE PQRAYAWLEKFNPTFEEDVLIERYVBCITSSAI : 569
 OEW : SMQDHGMQVSDCTAEGLKAALLFSQMPE-ELVGAETETGHLYDAVNVILTQS----ASGGFPAWE PQRAYRWLEKFNPTFEEDVLIERYVBCITSSAV : 567
 BPW : SMQDHGMQVSDCTAEGLKVAALLFSQMPP-DLVGKQIEKERLYDAVNVILSLQS----SNGGFPAWE PQRAYGWLEKFNPTFEEDVLIERYVBCITSSAV : 566
 AtCAS1 : STADHGMPI SDCTAEGLKAALLLSKVPK-AIVGEPIDAKRLYEAVNVIIISLQN----ADGGLATYELTRSYPWLELINPAETE GDIVIDYYPVBCITSAI : 567
 PsCAS : STADHGMPI SDCTAEGLKAVLLLSKIAP-EIVGEPIDSKRLYDAVNVILSLQN----ENGGLATYELTRSYTWLEIINPAETE GDIVIDCPYVBCITSAI : 567
 PgCAS : STADHGMPI SDCTAEGLKAVLQLSKLPS-ELVGEPLDAKRLYDAVNVILSLQN----SDGGYATYELTRSYSWLELVNPAETE GDIVIDYYPVBCITSAI : 567
 BpCAS1 : STADHGMPI SDCTAEGLKAVILLSQFPS-ETVGSVDVKRLYDAVNVILSLQN----TDGGFATYELTRSYHWLELINPAETE GDIVIDYYPVBCITSAI : 577
 AtLAS1 : STGDNPMVSDCTAEALKAALLSQMPV-NLVGEPMPPEHLVDAVNVILSLQN----KNGGFASYELTRSYPELVINPSETEGDIIIDYQYVBCITSAI : 567
 HsLAS : STLDCGMIVSDCTAEALKAALLLQEKCP-HVT-EHIPRERLCAVAVLLNMRN----PDGGFATYETKRGHLLLELLNPSEVEGDIMIDYTYVBCITSAI : 538
 MmLAS : STLDCGMIVADCTAEGLKAVLLLQNCQP-SIT-EHIPRERLCAVDVLLSLRN----ADGGFATYETKRGHLLLELLNPSEVEGDIMIDYTYVBCITSAI : 539
 ScLAS : STKTQGYTVADCTAEAIKAILMVKNSPVSEVHHMISSERLFEIGIDVLLNQNIGSFYEGSFATYETKIKAPLAME TLNPAEVEGDIMVEYYPVBCITSSV : 545
 SHC : QFDNYYYPDVDDTA-----VWVWALNTLRLPDERRRRDAMTKGFRWIVGMQS----SNGGWGAYDVDNTSDLPNHI-PFCDEGEVT-DPPSEDVTAHVL : 453
 s g 5 s DcTAE k 6 v 6 66 6 q Gg 5e e n p e f e c t 6

DXDD

SHC H451

D485C486TAE involved in EtAS β-amylin synthase

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EtAS      : HALIMFKKLYPGHRKKEIENFITNAVRYLEDVQTADGSMYGNWGVCFITYGTWFAVGGAAAGKNYNN---CAAMRKAVDFLLRTOQKQDGGWGESYLSCPH : 666
PNY1     : QALVLFKKLYPGHRKKEIDNFITNAVRYLEDTQMPDGSWYGNWGVCFITYGSWFALGGLAAAGKTYYN---CAAVRKAVEFLLKSOQDDGGWGESYLSCPK : 667
BPY      : QTLVLFKKLYPGHRKKEIENFIKNAAQEIQVIQMPDGSWYGNWGVCFITYGTWFAVGGAAAGKTYNN---CLAVRRRAVDFFLRAQRDNGGWGESYLSCPK : 666
PSY      : QALVLFKKLYPGHRKKEIENFIFNAVRELEDTQTEDGSWYGNWGVCFITYGSWFALGGLAAAGKTYTN---CAAIRKGVKFFLLTTOREDGGWGESYLSSPK : 666
AtLUP1   : CALDLFRKLYBDHRKKEINRSIEKAVQEIQDNQTQPDGSWYGNWGVCFITYATWFAVGGAAAGETYN---CLAMRNGVHFLLTTOREDGGWGESYLSQSE : 664
TRW      : OGLTLFKKLHPEGHRKKEIEHCIISRAVKYVEDTQESDGSWYGCWGIQYTYGTWFAVDALVACGKNYHN---CPALQKACKFLLSKQLPDGGWGESYLSQSN : 666
OEW      : QALKLFKQLHPEGHRKKEIASCISKAIQYIEATQNPDGSWYDGSWGIQYTYGTWFAVEGLVACGKNYHN---SPTLRRACEFLLSKQLPDGGWGESYLSQSN : 664
BPW      : HGLALFRKLYPRHRGTEIDSSIYRGIQYIEADVQEPDGSWYGHWGIQYTYGTWFAVGGAAAGKTYNN---CPALRKSCEFLLSKQLPNGGWGESYLSQSN : 663
AtCAS1   : QALISFRKLYPGHRKKEVDECIKAVKFEIESIQAADGSWYGSWAVCFITYGTWFGVKGLVAVGKTLKN---SPHVAKACEFLLSKQPPSGGWGESYLSQCD : 664
PsCAS    : QALATFGLKLYPGHRREEIQCCIEKAVAFIEKIQASDGSWYGSWGVCFITYGTWFGIKGLIAAGKNEFSN---CLSRKACEFLLSKQLPSGGWGESYLSQCN : 664
PgCAS    : QALATFGLKLYPGHRREEIQHSIEKAAFEIEKIQSSDGSWYGSWGVCFITYGTWFGIKGLVTAAGRTFSS---CASIRKACDFLLSKQVSGGWGESYLSQCN : 664
BpCAS1   : CALTLFKKLHPEGHRREEIENCIKAAAEIENIQASDGSWYGSWGVCFITYAGWFGIKGLVAAGRTYKN---CSSIHKACDYLLSKELASGGWGESYLSQCD : 674
AtLAS1   : OGLVLFITLNSYKRKEIVGSINKAVBEIEKTOLEDGSWYGSWGVCFITYATWFGIKGLVLAAGKTYES---SLCIRKACGELLSKQLCCGGWGESYLSQCN : 664
HsLAS    : CALKYFHKRFPEHRAAEIRETLTQGLEECRRQQRADGSWEGSWGVCFITYGTWFGLEAFACMGQTYRDGTACABVSRACDFLLSRQADGGWGEDFESCEE : 638
MmLAS    : QALKHFHEHFPEDYRAAEVRETLNQLGLDECRKQRADGSWEGSWGVCFITYGTWFGLEAFACMGHTYQDGAACABVAQACNELLSSQADGGWGEDFESCEQ : 639
ScLAS    : LGLTYFHKYF-DYRKEEIRTRIRIAIEFIKKSQLPDGSWYGSWGIQYTYAGMFALEALHTVGETYEN---SSTVRKGCDFLVSKQMKDGGWGESYLSSEL : 641
SHC      : ECFGSE-----GY--DDAWKVIIRRAVEYLLKREKQKPDGSWYGRWGVNYLYGTGAVVSAKAVGIDTRE----PYIQKALDWEHQHONPDGGWGEDCRSYED : 542
1 F p e 6 a 5 Q DGSw G Wg6c5tY wf 6 G 6 56 2 GGWgEs s

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EtAS F728

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EtAS      : KKYVPLEDNRSNLVHTSMALMGLISAGQMDRDPDPTPLHRAAKLLINSQLEDGDFPQOEITGV-EMKNCMLHYAAYRNIYPLWALAEYRNRVPLPSTTL--- : 762
PNY1     : KKYVPLEGNRSNLVHTSMALMGLIHSEQAERDPTPLHRAAKLLINSQMEDGDFPQOEITSGV-EMKNCMLHYAAYRNIYPLWALAEYRRRVPLPSLGT--- : 763
BPY      : KKYVPLEGNKSNLVHTAMAMMGLIHAGQAERDPTPLHRAAKLLINSQLEDGDFPQOEITGV-EMKNCMLHYAAYKNIYPLWALAEYRKHVPLP-LGKNLN : 764
PSY      : KKYVPLEGNRSNLVHTAMALMGLIHAGQSERDPTPLHRAAKLLINSQLEQGDWVPPQOEITGV-EMKNCMLHYPMYRDIYPLWALAEYRRRVPLP----- : 758
AtLUP1   : QRYIPSEGERSNLVQTSWAMMALIHTGQAERDLIPLHRAAKLLINSQLENGDFPQOEITVGA-EMNTCMLHYATYRNTFPLWALAEYRKHVPLP----- : 755
TRW      : KKYTNLEGNRSNLVHTSMALLSLIKAGQAEIDPTPLHRAAKLLINSQMEEGDFPQOEITGV-EMKNCMLHYAAYRNIYPLWALAEYRRRVPLP----- : 755
OEW      : KKYTNLEGNRSNLVQTSWALLSLIKAGQVEIDPGPIHRRGIRKLLVNSQMEDGDFPQOEITGA-EMKNCMLHYAAYRNIYPLWALAEYRRRVPLP----- : 753
BPW      : KKYTNLEGNRSNLVQTSWALLSLIKAGQAEIDPTPLHRAAKLLINSQMEDGDFPQOEITGV-EMKNCMLHYAAYRNIYPLWALAEYRRRVPLP----- : 752
AtCAS1   : KKYSNLDGNRSNLVHTAMAMMALIAGQAERDPTPLHRAAKLLINSQMEDGDFPQOEITGV-EMKNCMLHYAAYRNIYPLWALAEYRRRVPLP----- : 753
PsCAS    : KKYSNLEGNRSNLVHTAMAMMALIAGQAERDPTPLHRAAKLLINSQMEDGDFPQOEITGV-EMKNCMLHYAAYRNIYPLWALAEYRRRVPLP----- : 752
PgCAS    : KKYTNLEGNRSNLVHTAMAMMALIAGQAERDPTPLHRAAKLLINSQMEDGDFPQOEITGV-EMKNCMLHYAAYRNIYPLWALAEYRRRVPLP----- : 753
BpCAS1   : KKYTNLKDNRPHIVNTAMAMMALIAGQAERDPTPLHRAAKLLINSQMEDGDFPQOEITGV-EMKNCMLHYAAYRNIYPLWALAEYRRRVPLP----- : 763
AtLAS1   : KKYTNLPGNKSHIVNTAMAMMALIAGQAERDPTPLHRAAKLLINSQMEDGDFPQOEITGV-EMKNCMLHYAAYRNIYPLWALAEYRRRVPLP----- : 753
HsLAS    : RRY--LQSAQSQIHNTCWMAMMGLMAVRHP--DIEAQERGVRCLEKQLPNGDWPOENIAGV-ENKSCAISYTSYRNIYPLWALAEYRRRVPLP----- : 732
MmLAS    : RRY--VQSARSQVHSTCWMAMMGLMAVRHP--DITAQERGVRCLEKQLPNGDWPOENISGV-ENKSCAISYTSYRNIYPLWALAEYRRRVPLP----- : 733
ScLAS    : HSY--VDSEKSLVQTSWALLIAGQAERDPTPLHRAAKLLINSQMEDGDFPQOEITGV-EMKNCMLHYAAYRNIYPLWALAEYRRRVPLP----- : 731
SHC      : PAVAGKGASTPS--QTAMAMMALIAGGRAESEA--RRGVQYLVETQRPDGGWDEPPYTTGTSEPGDFYLGYTMYRHVFPPLALGRYKQAT----- : 628
5 s v T WA66 L6 d r 6 n Q e Gd5p Gv E c 6 Y 54 5P wAL 5

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EtAS      : ----- : -
PNY1     : ----- : -
BPY      : QVVNCIGQSLYKKYK : 779
PSY      : ----- : -
AtLUP1   : -----VN : 757
TRW      : -----QNI-- : 758
OEW      : -----LHAQT : 758
BPW      : -----LEA-- : 755
AtCAS1   : -----LLQQGE : 759
PsCAS    : -----L--QAC : 756
PgCAS    : -----L-QGPS : 758
BpCAS1   : -----L--KAL : 767
AtLAS1   : -----L---SL : 756
HsLAS    : ----- : -
MmLAS    : ----- : -
ScLAS    : ----- : -
SHC      : -----ERR : 631

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The triterpene cyclases amino acids sequences were aligned using Clustal W, as implemented in the (CLC Sequence Viewer (CLC bio), and the figure was made by GenDoc (<http://www.nrbcs.org/gfx/genedoc/>). EtAS: *Euphorbia tirucalli* β -amyirin synthase (AB206469), PNY1: *Panax ginseng* β -amyirin synthase (AB009030), BPY: *Betula platyphylla* β -amyirin synthase (AB055512), PSY: *Pisum sativum* β -amyirin synthase (AB034802), AtLUP1: *Arabidopsis thaliana* multifunctional triterpene cyclase (At1g78970), TRW: *Taraxacum officinale* lupeol synthase (AB025345), OEW: *Olea europaea* lupeol synthase (AB025343), BPW: *Betula platyphylla* lupeol synthase (AB055511), AtCAS1: *Arabidopsis thaliana* cycloartenol synthase (At2g07050), PsCAS: *Pisum sativum* cycloartenol synthase (D89619), PgCAS: *Panax ginseng* cycloartenol synthase (AB009029), BpCAS1: *Betula platyphylla* cycloartenol synthase (AB055509), AtLAS1: *Arabidopsis thaliana* lanosterol synthase (At3g45130), HsLAS: *Homo sapiens* lanosterol synthase (P48449), MmLAS: *Mus musculus* lanosterol synthase (AK044016), ScLAS: *Saccharomyces cerevisiae* lanosterol synthase (P38604), SHC: *Alicyclobacillus acidocaldarius* squalene-hopene cyclase

Fig. S2. EIMS and NMR spectra of Product 10-acetate

Fig. S2-1. EIMS spectrum of Product 10 acetate

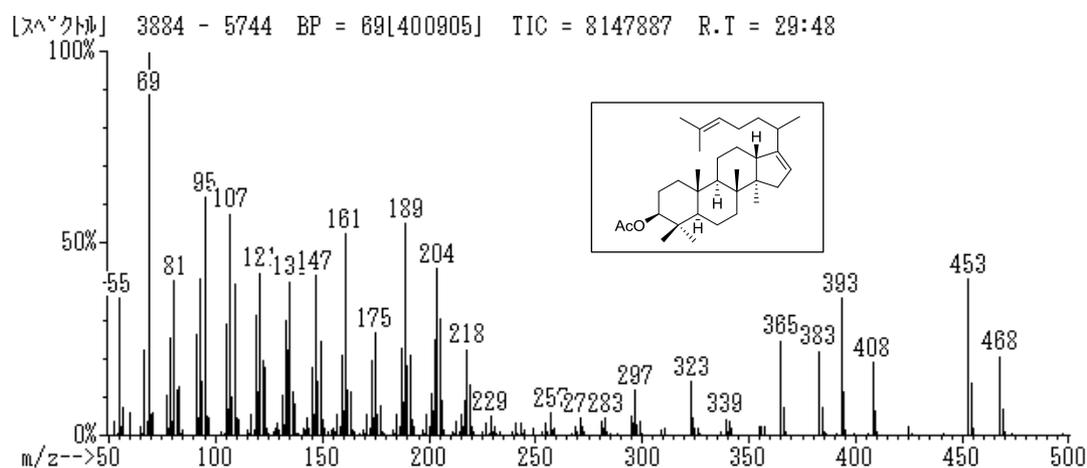


Fig. S2-2. ¹H-NMR spectrum of product 10-Ac in CDCl₃ (600 MHz).

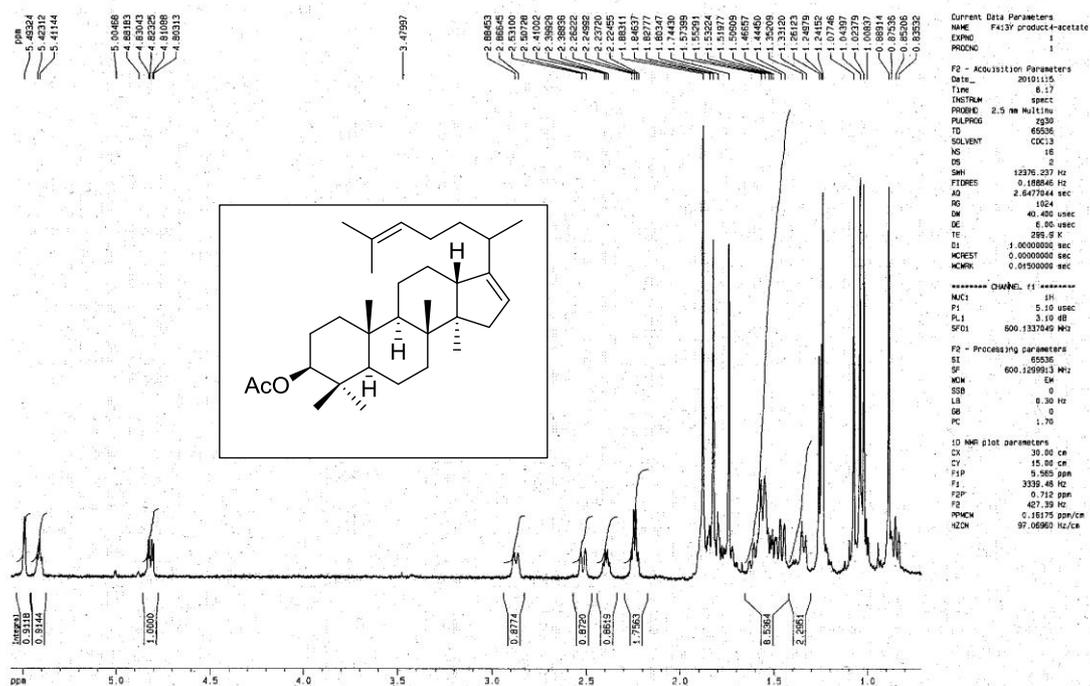


Fig. S2-3. ^{13}C -NMR spectrum of product 10 Ac (150 MHz)

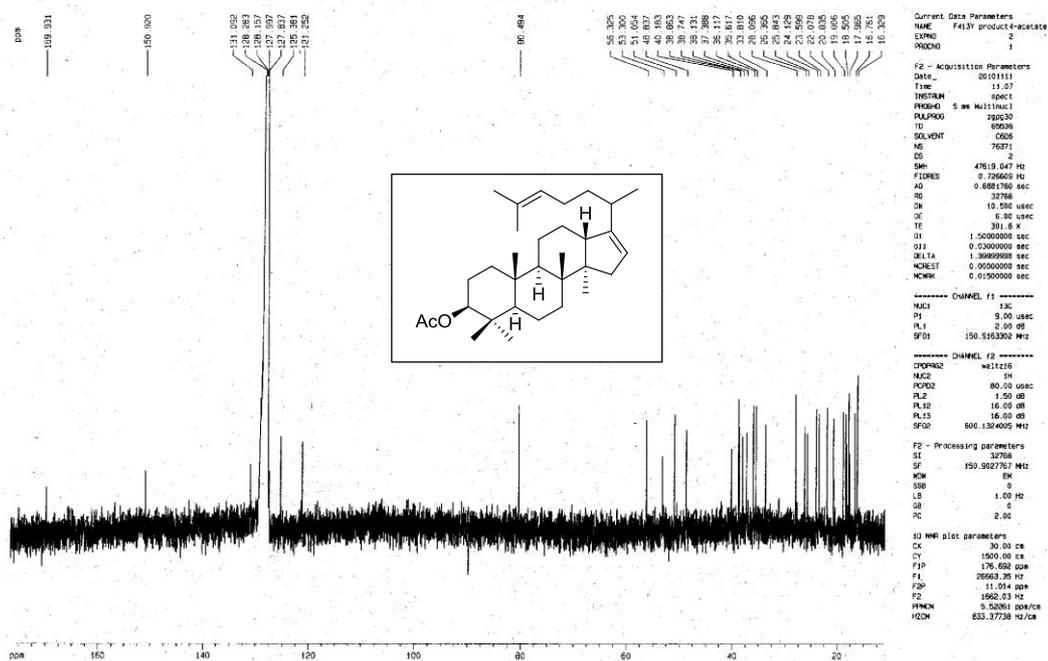


Fig. S2-4. ^1H - ^1H COSY 90 of product 10 Ac

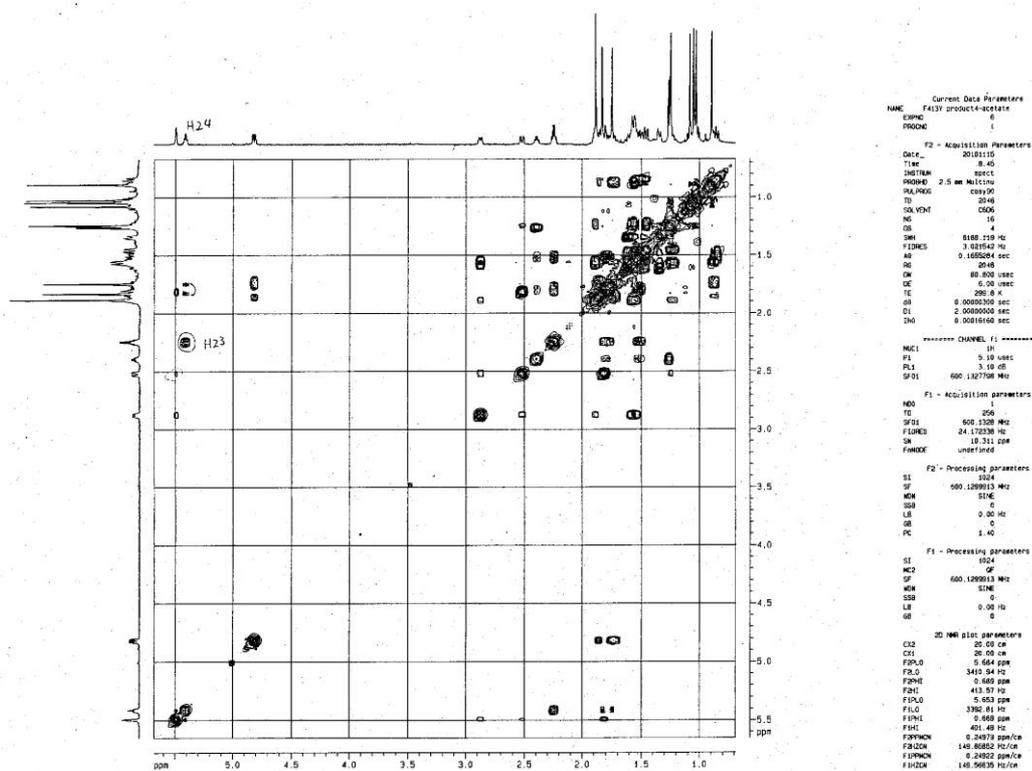


Fig. S2-5. HOHAHA spectrum of product 10 Ac

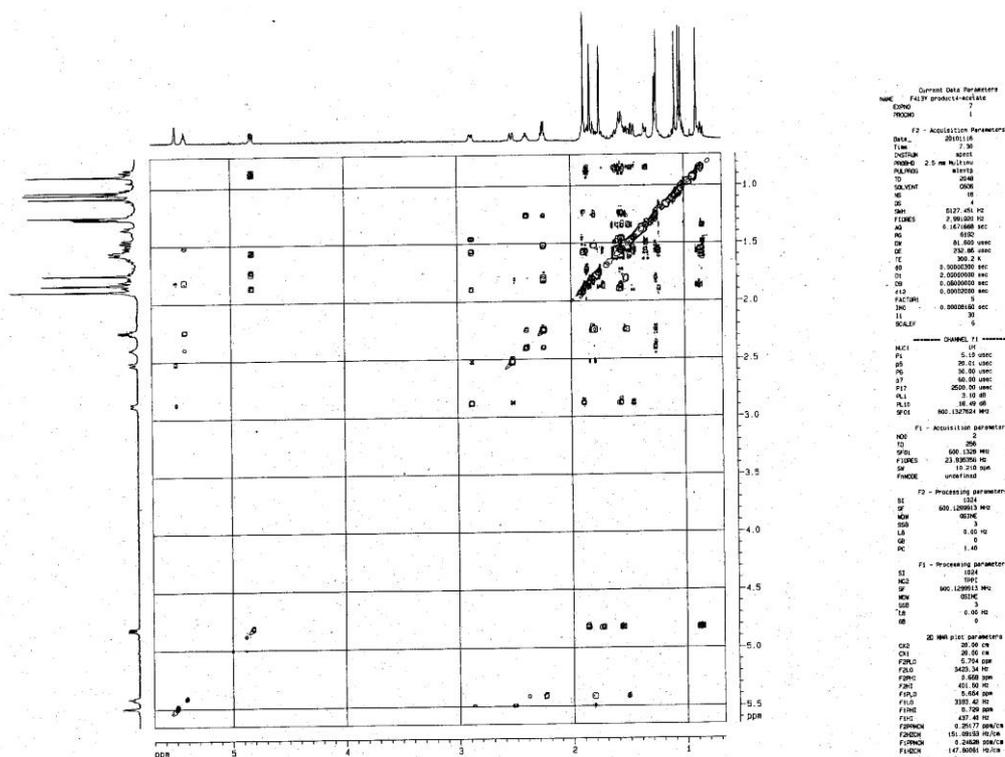


Fig. S2-6. NOESY spectrum of product 10 Ac

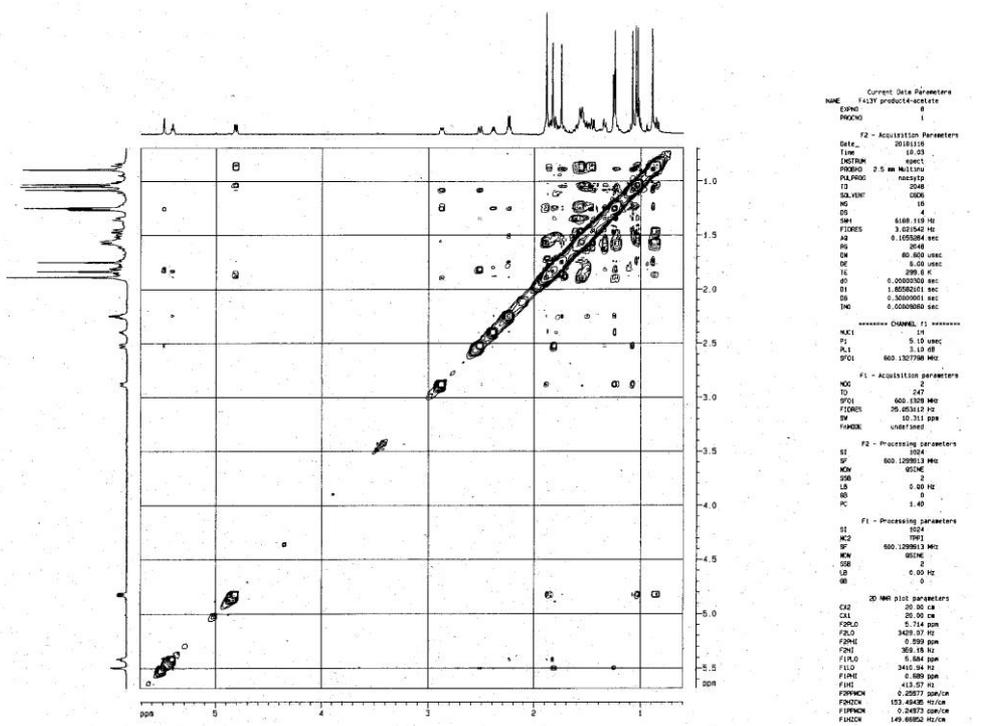


Fig. S2-7. HSQC spectrum of product 10 Ac

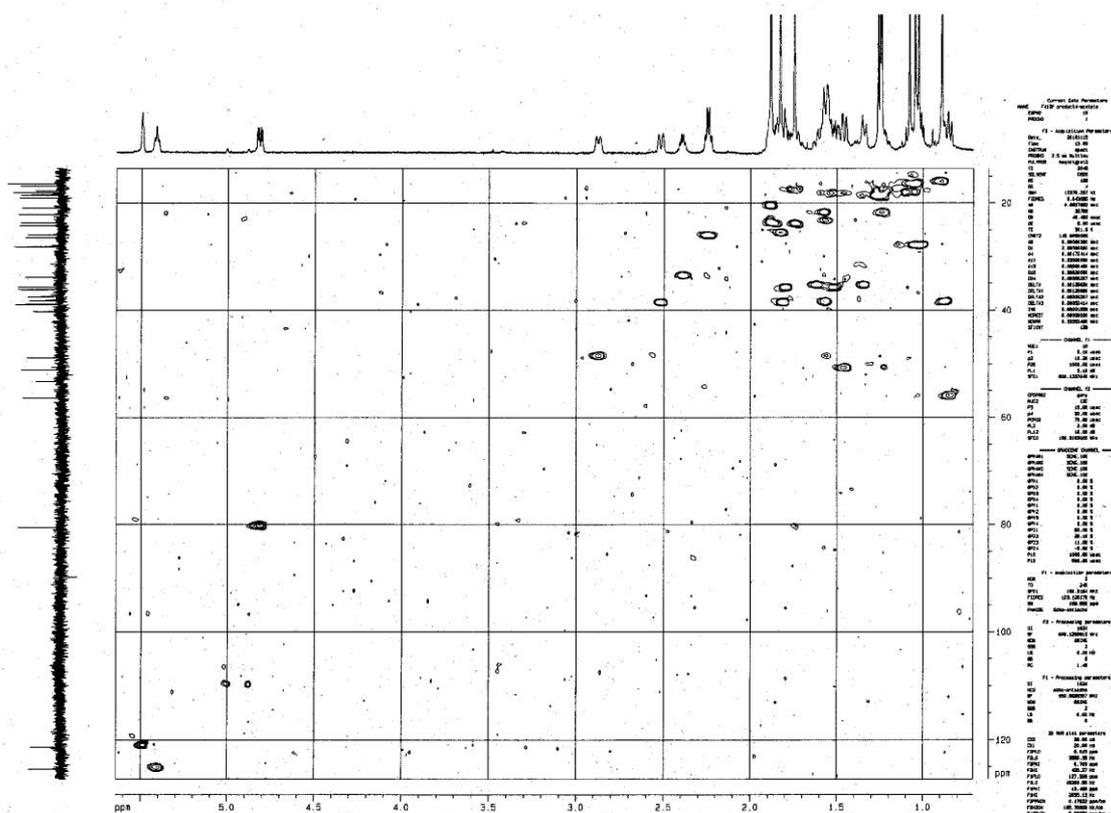


Fig. S2-8. HMBC spectrum of product 10 Ac

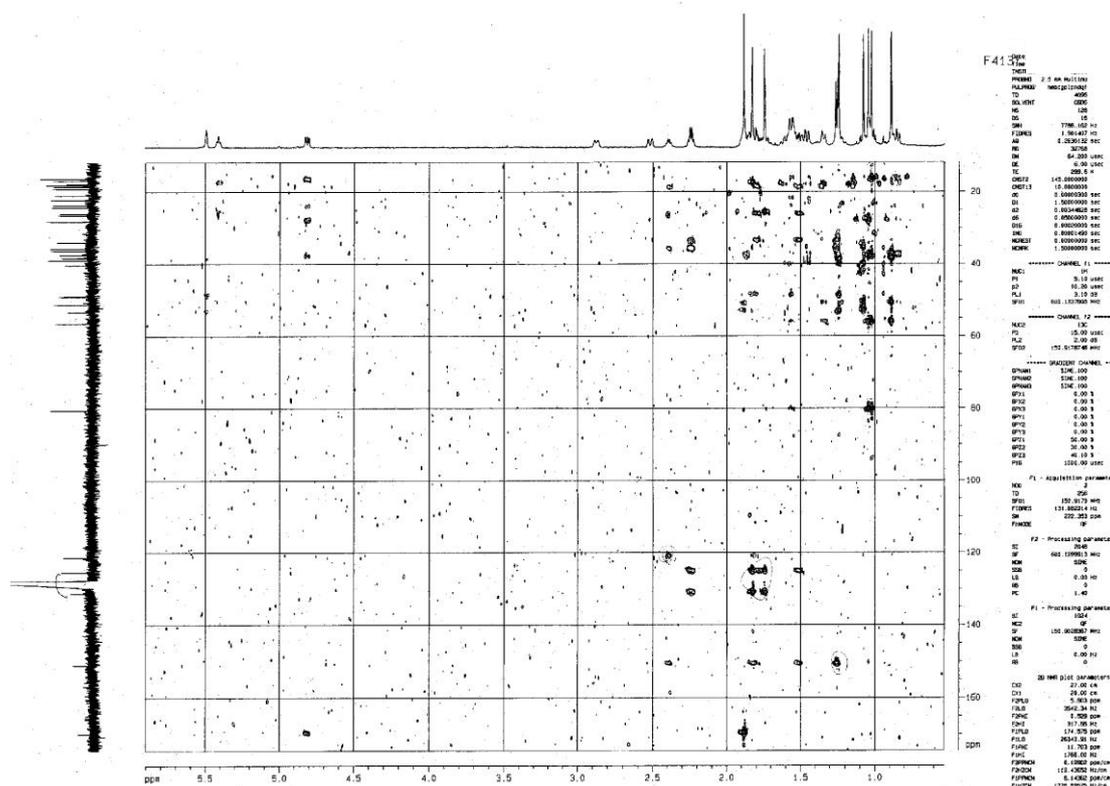
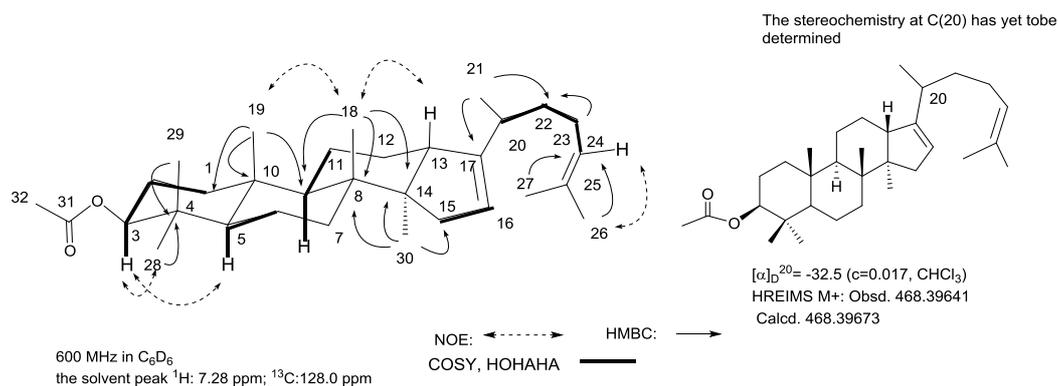
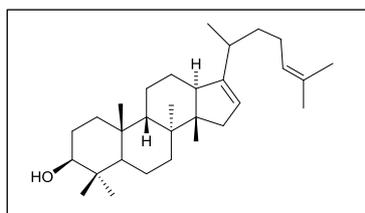


Fig. S2-9. NMR data analyses for proposing structure of **10-Ac**, optical rotation and HRMS data.



NO.	1H	^{13}C	NO.	1H	^{13}C	NO.	1H	^{13}C	NO.	1H	^{13}C
1	0.88(m);1.57(m)	38.75	9	1.456 (bd, J=12.5 Hz)	51.05	17	—	150.9	25	—	131.1
2	1.75(m);1.86(m)	24.13	10	—	37.39	18	1.077(3H,s)	18.34	26	1.827(3H, s)	25.84
3	4.81 (dd, J=11.7, 4.6Hz)	80.49	11	1.24(m);1.58(m)	22.08	19	0.889(3H, s)	16.33	27	1.744(3H,s)	17.71
4	—	38.13	12	1.57(m);1.89(m)	23.60	20	2.394(q, J=6.6 Hz)	33.81	28	1.024(3H, s)	28.09
5	0.844 (bd, J=10.1 Hz)	56.33	13	2.875(bd, J=10.9Hz)	48.84	21	1.256(3H, d, J=6.8 Hz)	19.01	29	1.044(3H, s)	16.76
6	1.46(m);1.58(m)	18.50	14	—	53.30	22	1.51(m);1.80(m)	36.12	30	1.241(3H, s)	17.96
7	1.35(m);1.62(m)	35.62	15	1.81(m);2.52(bd, J=14.0Hz)	38.86	23	2.24(dt, J=7.3, 7.3 Hz)	26.36	31	—	169.9
8	—	40.18	16	5.49(bs)	121.2	24	5.41(t, 7.0 Hz)	125.4	32	1.883(3H, s)	20.83

The stereoisomer of **10**, described below, was isolated by Wu's group from the mutants of *S. cerevisiae* lanosterol synthase, which was reported in the following references.



1. Wu, Tung-Kung; Chang, Yi-Chun; Liu, Yuan-Ting; Chang, Cheng-Hsiang; Wen, Hao-Yu; Li, Wen-Hsuan; Shie, Wen-Shiang, *Organic & Biomolecular Chemistry* (2011), 9(4), 1092-1097.
2. Liu, Yuan-Ting; Hu, Tain-Chang; Chang, Cheng-Hsiang; Shie, Wen-Shiang; Wu, Tung-Kung, *Organic Letters* (2012), 14(20), 5222-5225.

Fig. S3. EIMS and NMR spectra of Product **12**-acetate.

Fig. S3-1. EIMS spectrum of product **12**-Ac

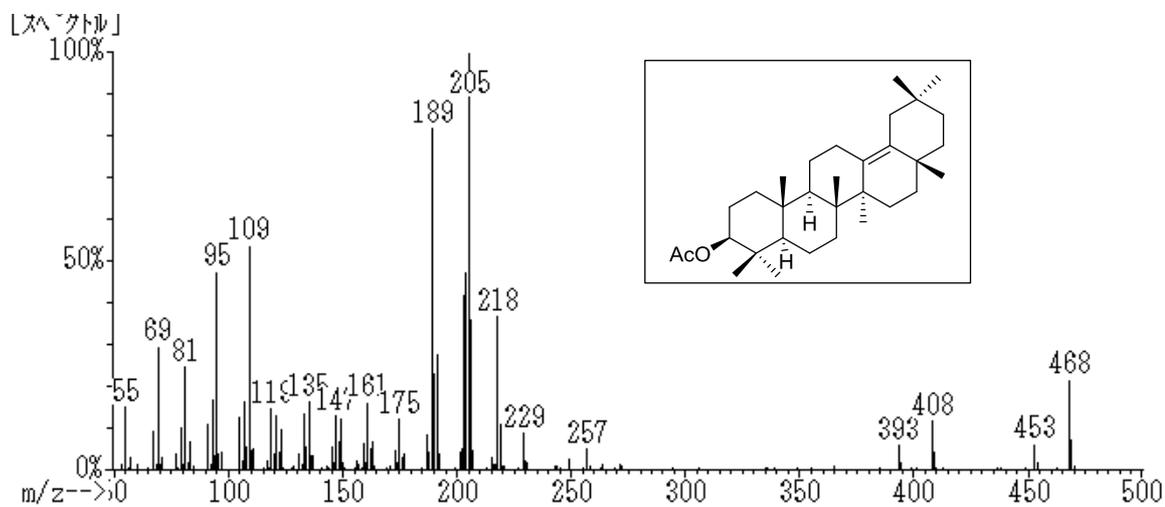


Fig. S3-2. $^1\text{H-NMR}$ spectrum of product **12**-Ac in C_6D_6 (400 MHz).

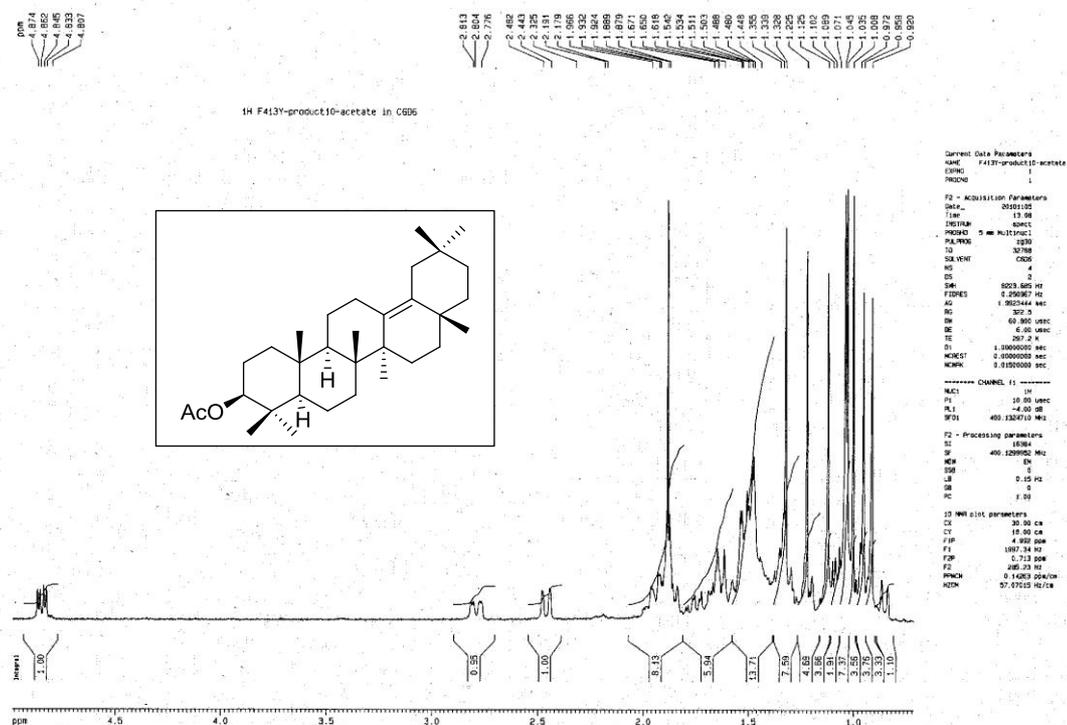


Fig. S3-3. ^{13}C -NMR spectrum of product 12-Ac in C_6D_6 (100 MHz).



Fig. S3-4. ^1H - ^1H COSY spectrum of product 12-Ac in C_6D_6 .

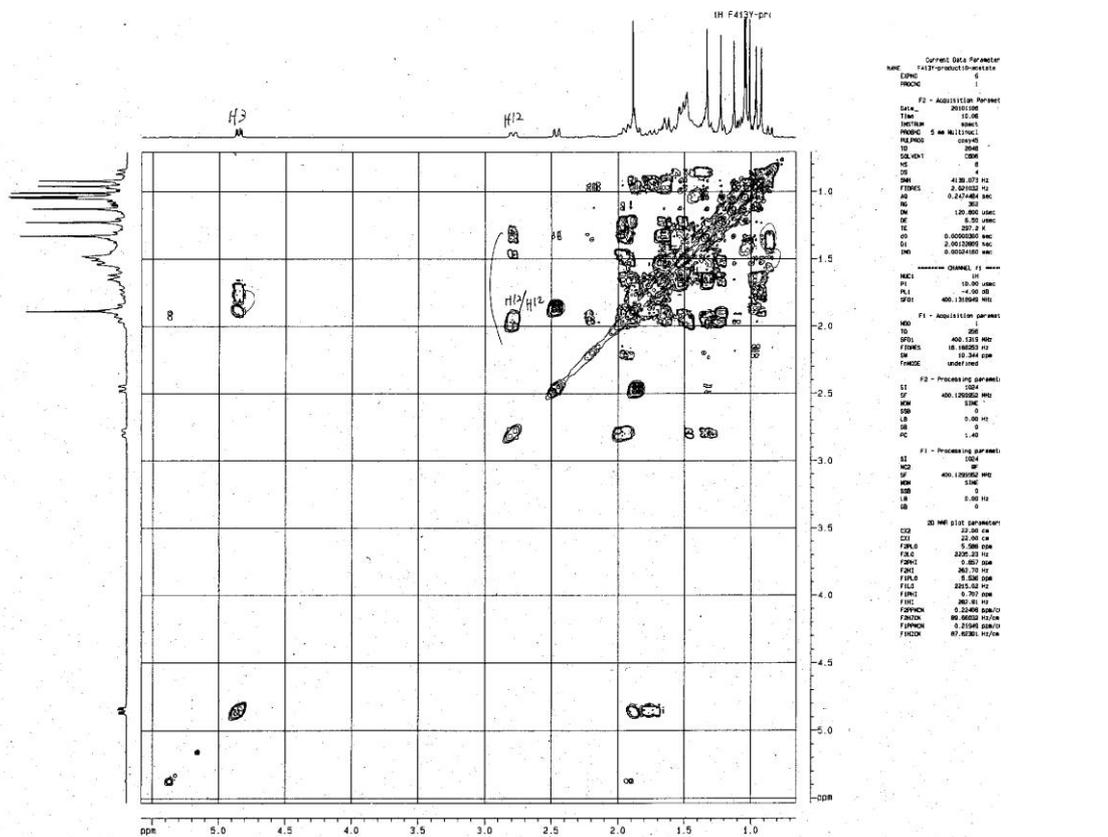


Fig. S3-7. NOESY spectrum of product **12-Ac** in C_6D_6 (600 MHz)-Expanded Region)

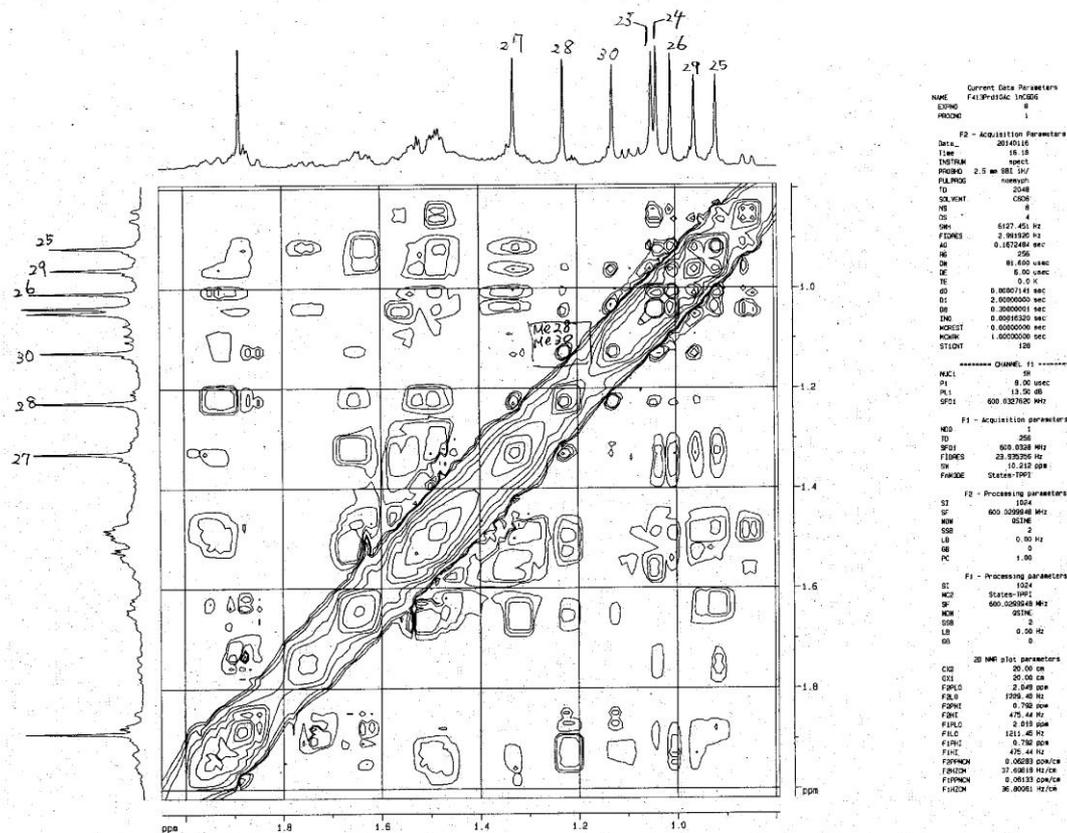


Fig. S3-8. HSQC spectrum of product **12-Ac** in C_6D_6

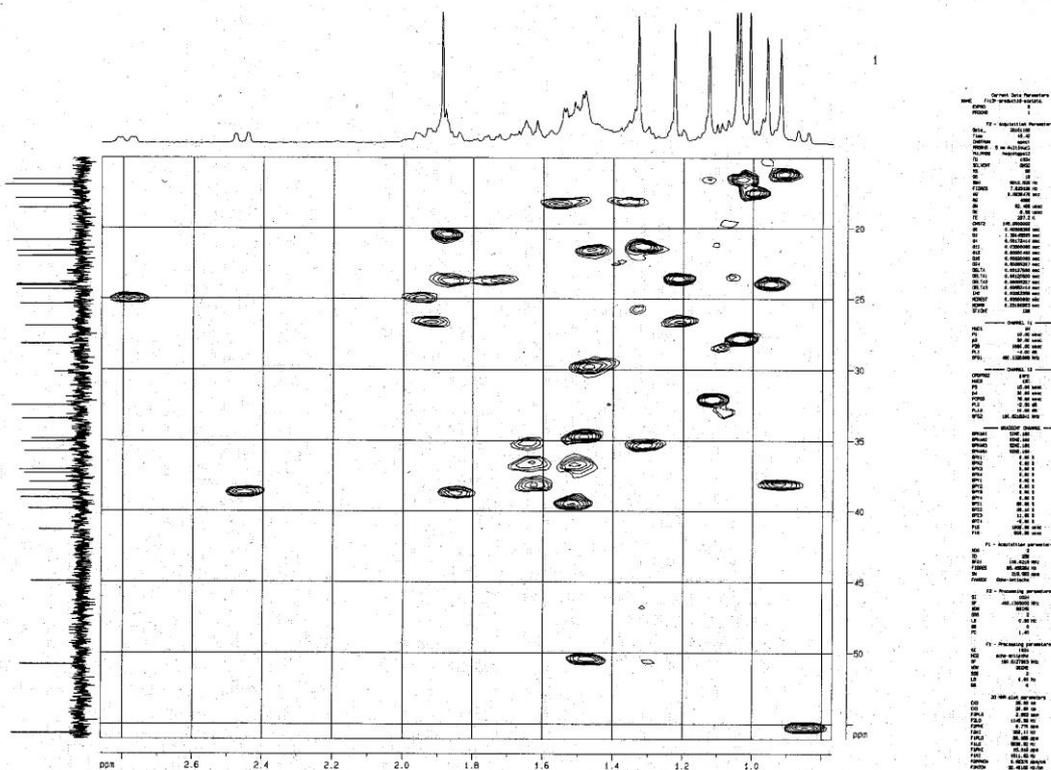


Fig. S3-9. HMBC spectrum of product 12-Ac in C₆D₆

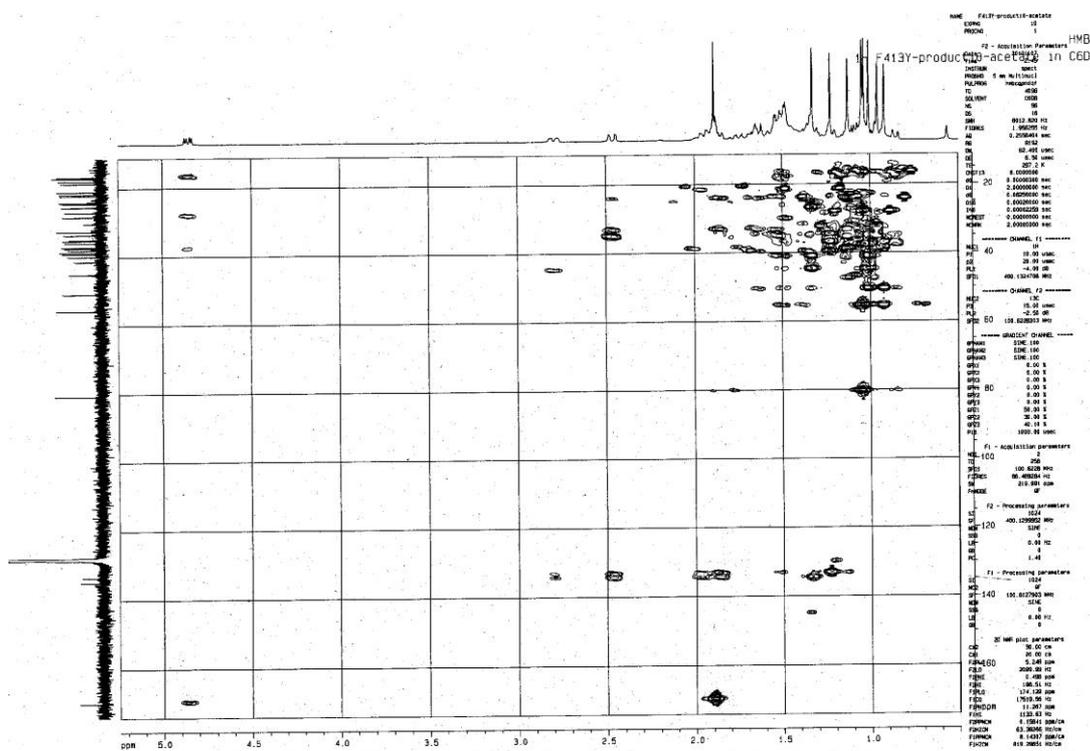
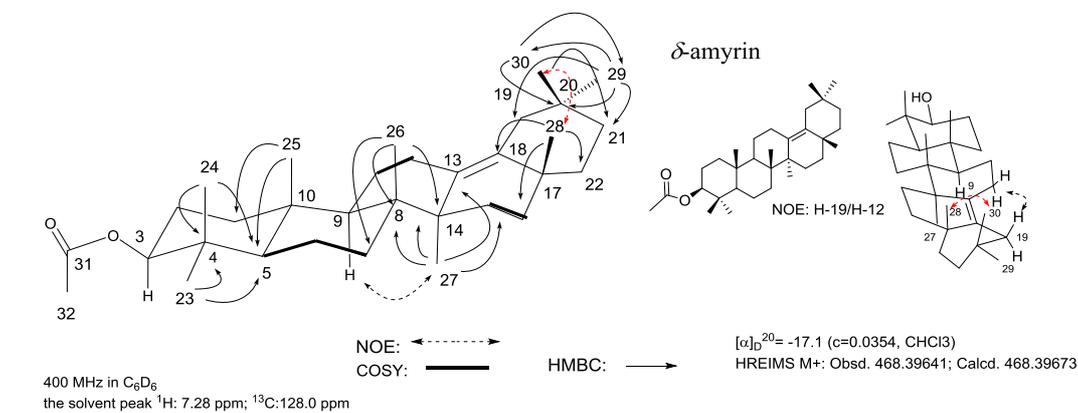


Fig. S3-10. NMR data analyses for proposing structure of 12-Ac, optical rotation and HRMS data.



NO.	¹ H	¹³ C	NO.	¹ H	¹³ C	NO.	¹ H	¹³ C	NO.	¹ H	¹³ C
1	0.92(m);1.63(m)	38.54(t)	9	1.47 (m)	50.80(d)	17	—	34.87(s)	25	0.920(3H,s)	16.58(q)
2	1.73(m);1.88(m)	24.07(t)	10	—	37.32(s)	18	—	133.4(s)	26	1.008(3H,s)	17.90(q)
3	4.85(dd, 11.6, 4.8 Hz)	80.53 (d)	11	1.31(m);1.47(m)	22.00(t)	19	1.84 (m);2.46 (bd, J=15.6 Hz)	39.04 (t)	27	1.328(3H,s)	21.63 (q)
4	—	37.95(s)	12	1.95(m);2.78(m)	25.36(t)	20	—	33.45(s)	28	1.225(3H, s)	23.83 (q)
5	0.855 (bd, J=11.6 Hz)	55.64(d)	13	—	134.9(s)	21	1.31(m);1.63(m)	35.74 (t)	29	0.959 (3H, s)	32.52(q)
6	1.35(m);1.54(m)	18.59(t)	14	—	44.96 (s)	22	1.51 (2H, m)	39.80 (t)	30	1.125(3H, s)	24.34 (q)
7	1.48(2H,m)	35.08(t)	15	1.21(m);1.90 (m)	26.92 (t)	23	1.045(3H, s)	28.18(q)	31	—	169.9(s)
8	—	41.29 (s)	16	1.51(m); 1.63(m)	37.06(t)	24	1.035(3H,s)	16.95(q)	32	1.889 (3H, s)	20.86 (q)

Fig. S4. EIMS and NMR spectra of product **15** acetate.

Fig. S4-1. EIMS spectrum of **15**-Ac.

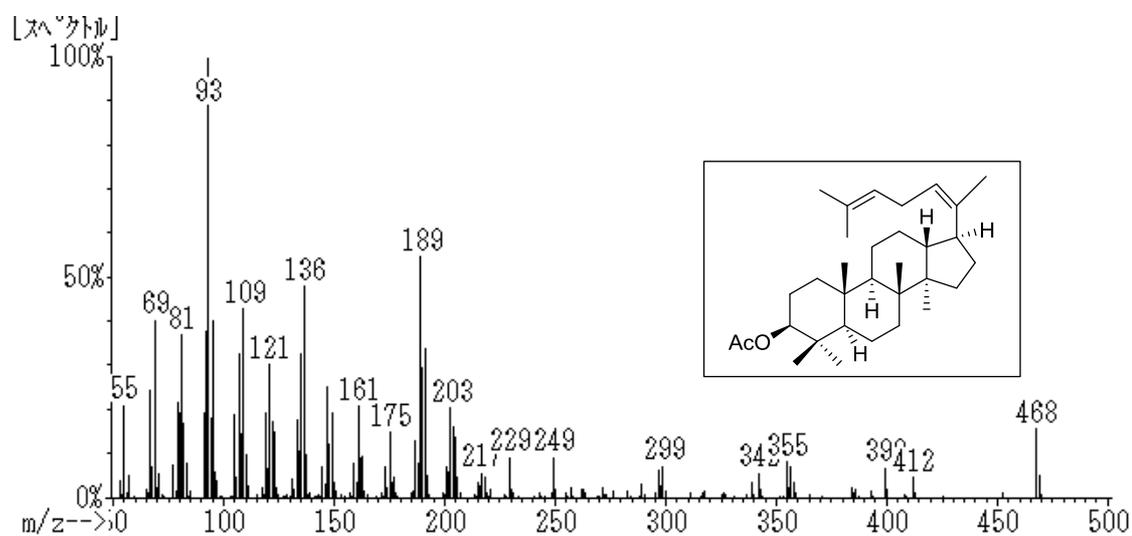


Fig. S4-2. ¹H-NMR spectrum of **15**-Ac in CDCl₃ (400 MHz).

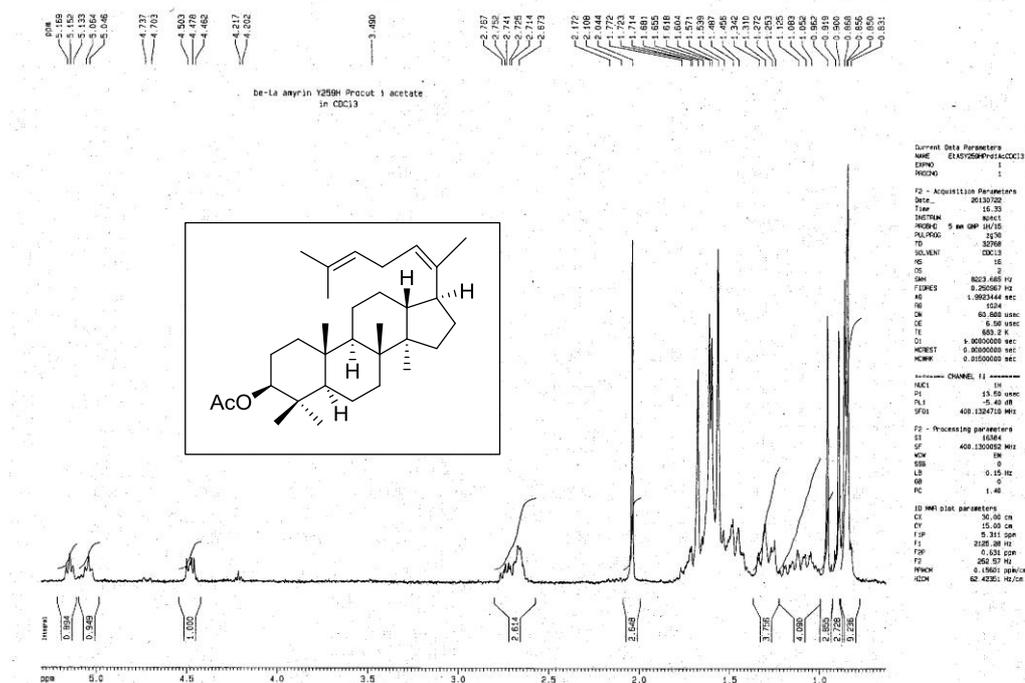


Fig. S4-3. ^{13}C NMR spectrum of **15-Ac** in CDCl_3 (100 MHz).

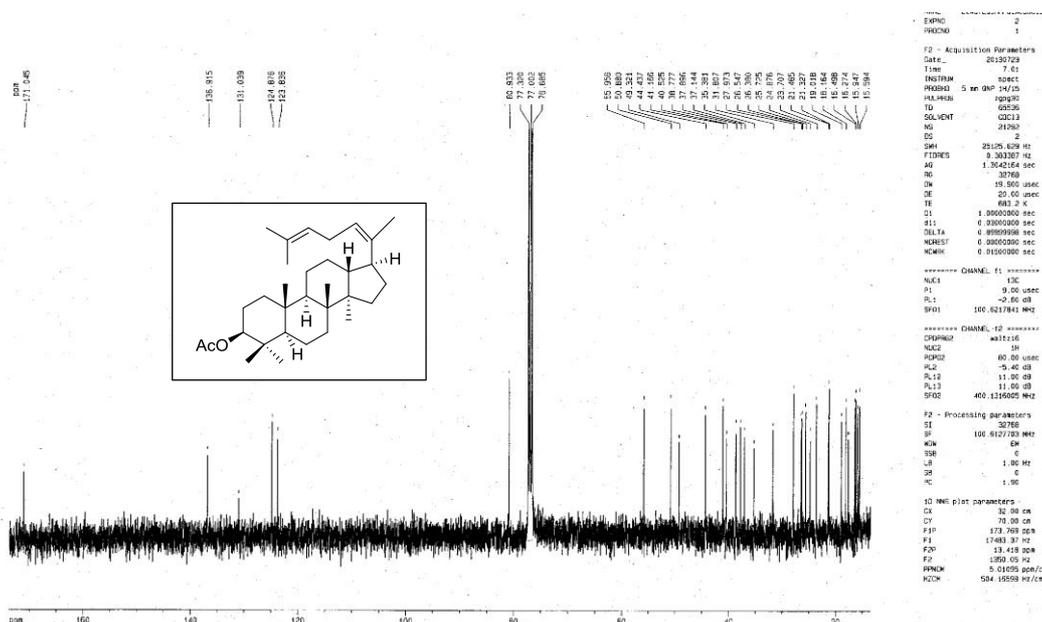


Fig. S4-4. ^1H - ^1H COSY spectrum of **15-Ac** in CDCl_3 .

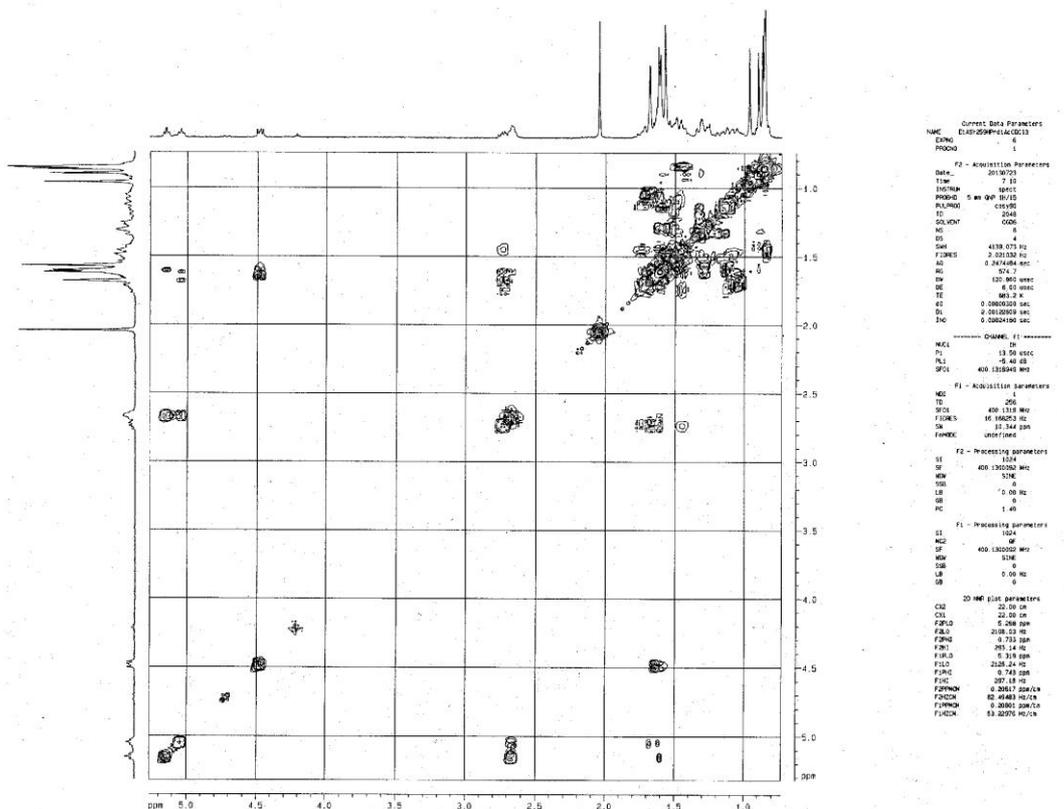


Fig. S4-5. HOHAHA spectrum of 15-Ac in CDCl₃.

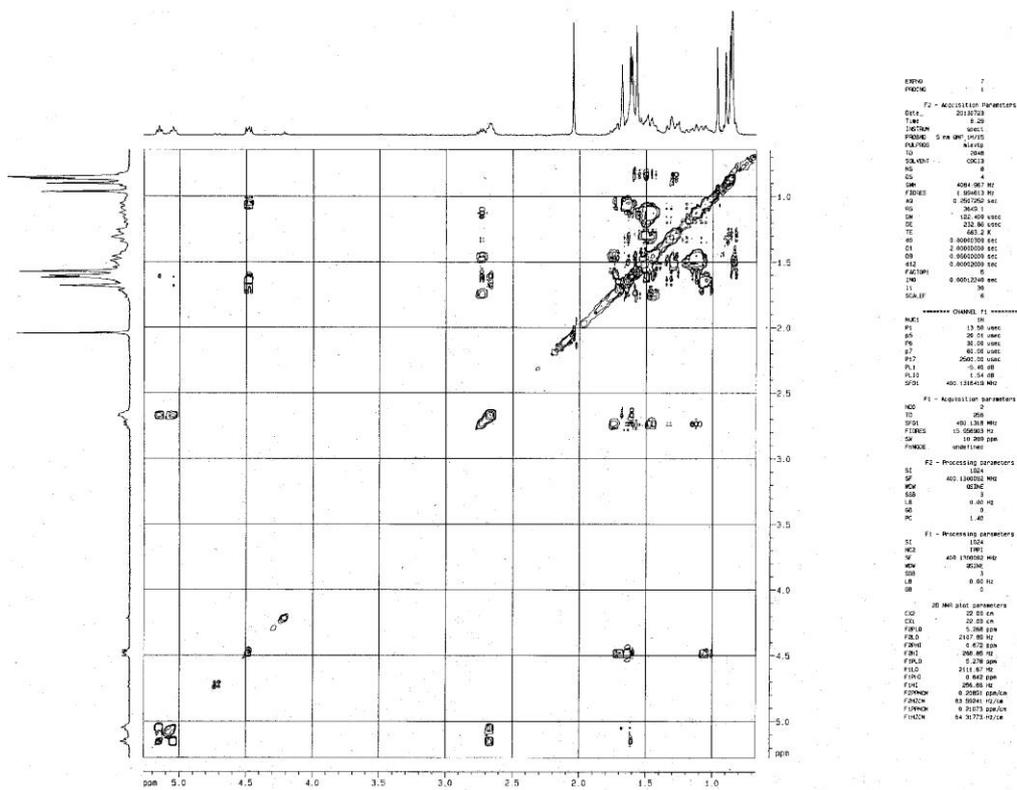


Fig. S4-6. NOESY spectrum of 15-Ac in CDCl₃.

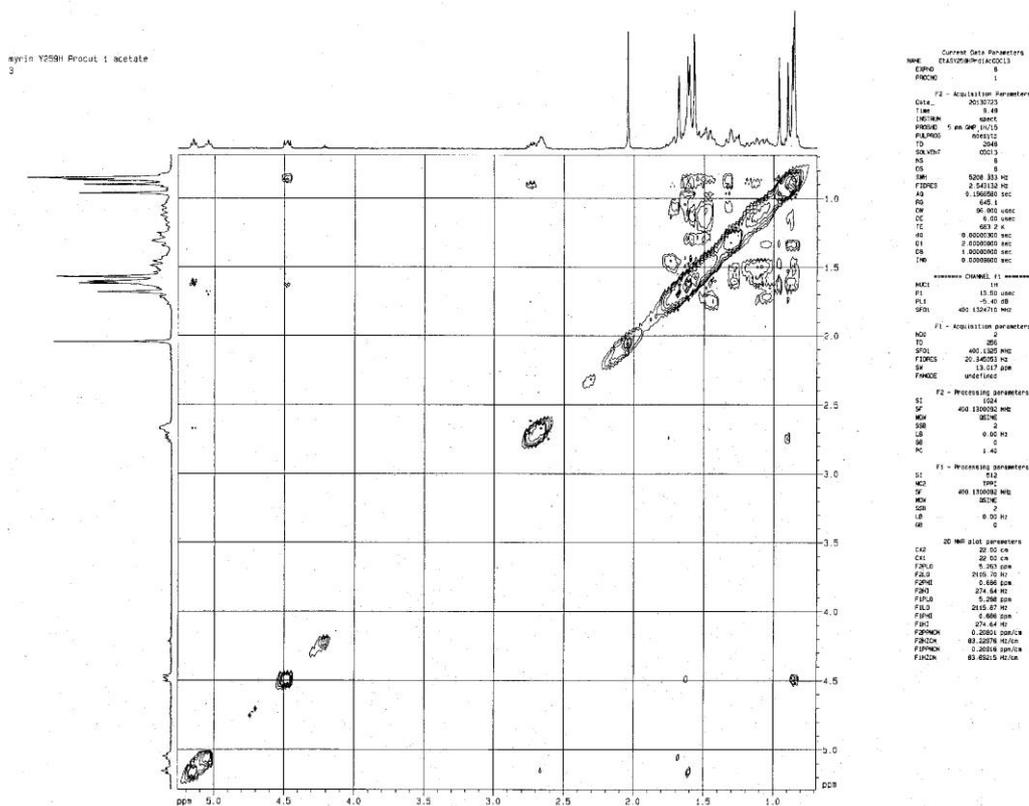
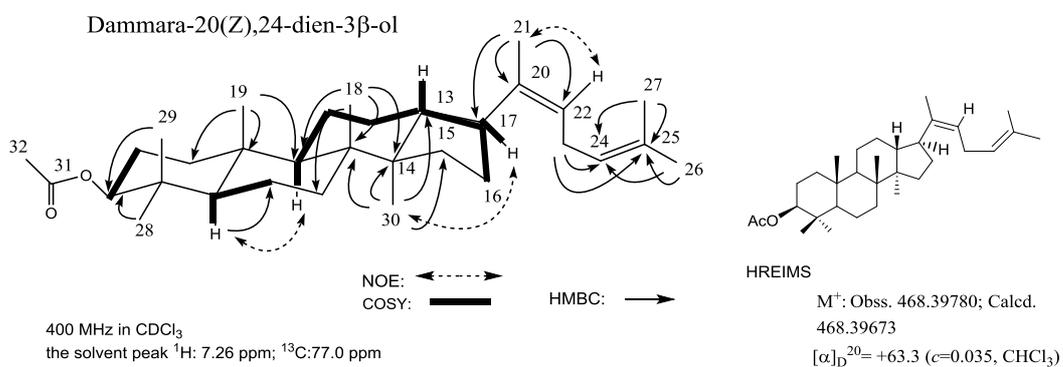


Fig. S4-9. NMR data analyses for proposing structure of **15-Ac**, optical rotation and HRMS data.



NO.	^1H	^{13}C	NO.	^1H	^{13}C	NO.	^1H	^{13}C	NO.	^1H	^{13}C
1	1.05 (m); 1.71(m)	38.77	9	1.34 (m)	50.88	17	2.73 (1H, m)	41.17	25	—	131.0
2	1.64 (2H, m)	23.71	10	—	37.14	18	0.962 (3H, s)	15.59	26	1.681 (3H, s)	25.72
3	4.48 (dd, $J=10.3, 6.4$ Hz)	80.93	11	1.17 (m); 1.52(m)	21.47	19	0.868 (3H, s)	16.27	27	1.618 (3H, s)	17.71
4	—	37.90	12	1.06 (m); 1.47(m)	24.88	20	—	136.9	28	0.856 (3H, s)	27.97
5	0.84 (1H, m)	55.96	13	1.63(1H, m)	44.44	21	1.604 (3H, s)	19.02	29	0.850 (3H, s)	16.50
6	1.50 (2H, m)	18.16	14	—	49.32	22	5.15 (t, $J=7.2$ Hz)	124.8	30	0.900 (3H, s)	15.95
7	1.28(m); 1.58(m)	35.38	15	1.12(m); 1.58(m)	31.81	23	2.66 (2H, m)	26.38	31	—	171.0
8	—	40.53	16	1.45 (m); 1.73 (m)	26.55	24	5.06 (t, $J=7.2$ Hz)	123.8	32	2.044(3H, s)	21.33

Fig. S5-3. ¹³C-NMR spectrum of product 17-Ac (100 MHz, C₆D₆).

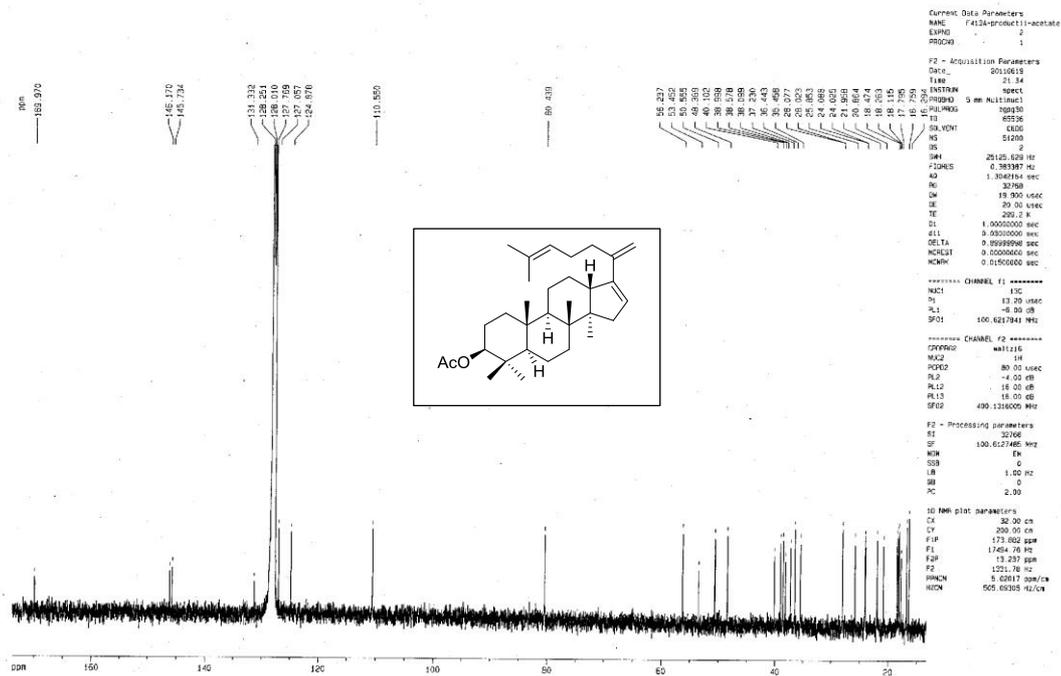


Fig. S5-4. ¹H-¹H COSY spectrum of product 17-Ac.

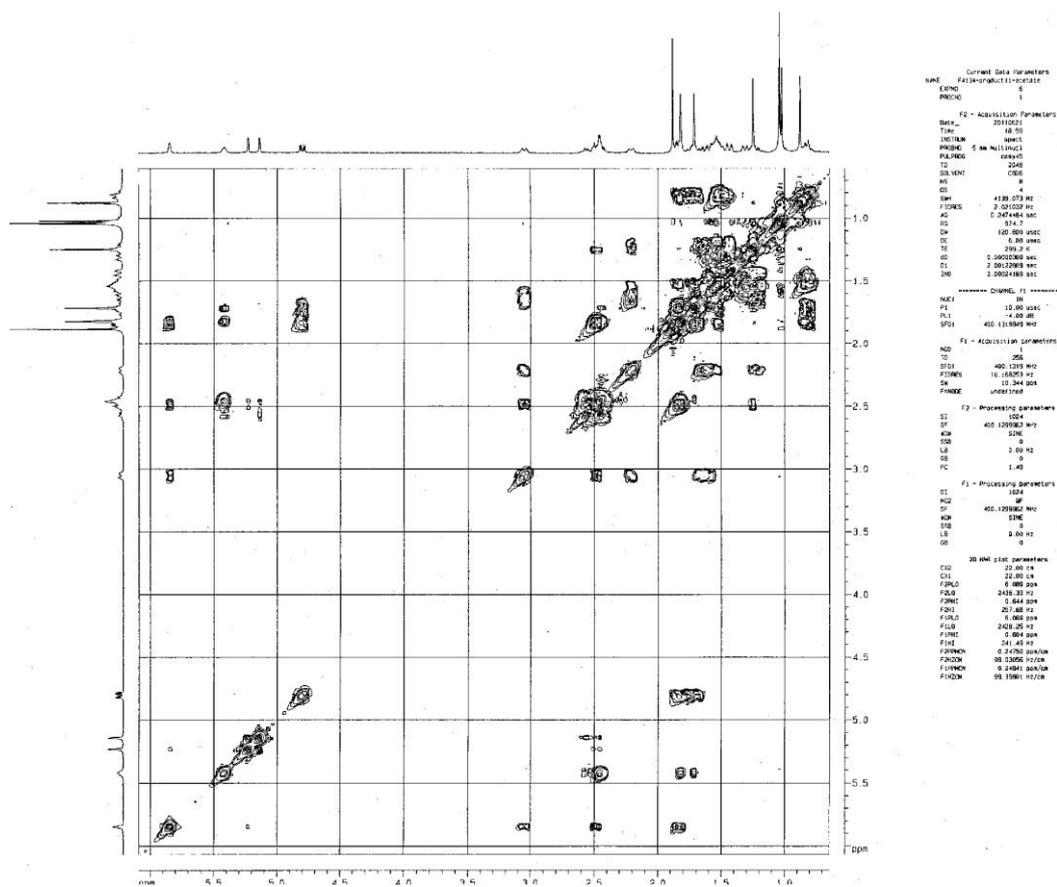
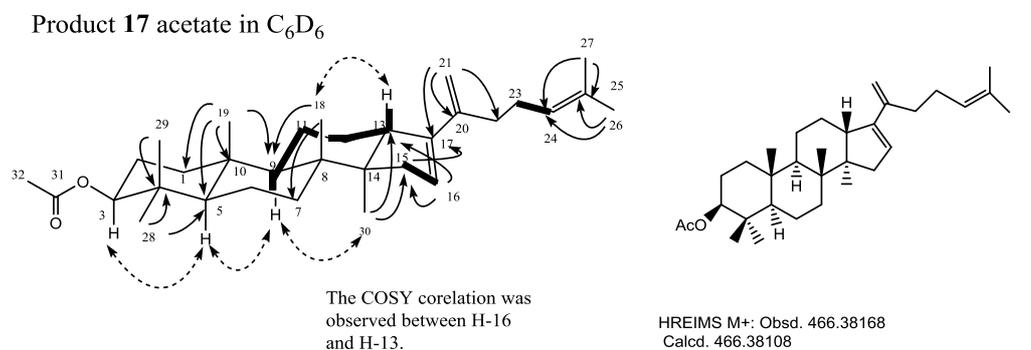


Fig. S5-9. NMR data analyses for proposing structure of **17-Ac** and HREIMS data. Reliable optical rotation was not obtained due to the sample loss during isolation and the instrumental analyses.



400 MHz in C_6D_6
the solvent peak 1H : 7.28 ppm; ^{13}C :128.0 ppm

NOE: \dashrightarrow
COSY: \longrightarrow HMBC: \longrightarrow

NO.	1H	^{13}C	NO.	1H	^{13}C	NO.	1H	^{13}C	NO.	1H	^{13}C
1	0.82 (m);1.52 (m)	38.58	9	1.43 (bd, 12.0 Hz)	50.56	17	—	146.2	25	—	131.3
2	1.71 (m); 1.84(m)	24.09 ^a	10	—	37.23	18	1.038 (3H, s)	18.26 ^b	26	1.822 (3H, s)	25.85
3	4.80 (dd, J=11.6, 4.8 Hz)	80.44	11	1.22 (m); 1.56(m)	21.96	19	0.876 (3H, s)	16.29	27	1.713 (3H,s)	17.80
4	—	38.09	12	1.63 (m);2.21 (bd, J=12.5 Hz)	24.03 ^a	20	—	145.7	28	1.019 (3H, s)	28.08 ^b
5	0.83 (m)	56.24	13	3.05 (1H,dd, J=13.0, 3.2 Hz)	48.37	21	5.23 (s); 5.14 (s)	110.5	29	1.038 (3H, s)	16.76 ^b
6	1.46 (m); 1.56(m)	18.47	14	—	53.45	22	2.47 (m); 2.57(m)	35.44	30	1.247 (3H,s)	16.12
7	1.31(bd, J=12.5Hz); 1.58(m)	35.46	15	1.84 (m); 2.49 (m)	39.00	23	2.45 (2H, m)	28.02	31	—	170.0
8	—	40.10	16	5.84 (bs)	127.1	24	5.42 (Very broad s)	124.9	32	1.882 (3H, s)	20.86

a and *b*: The assignments are exchangeable between the same letters.

Fig. S6. GCMS spectrum of the acetate of the hexane-extract obtained from the Y259H mutant. **19** acetate. The EIMS of **19** acetate was almost identical to that of dammara-(*E*)-20(22),(*E*)24(25)dien-3 β -ol acetate that was isolated from F728H mutant (Ito, R.; Hashimoto I.; Masukawa Y.; Hoshino, T. *Chem.-Eur. J.* **2013**, *19*, 17150-17158, see Supporting Information, Fig. S36, EIMS) and is quite similar as that of EIMS of **15**-acetate.

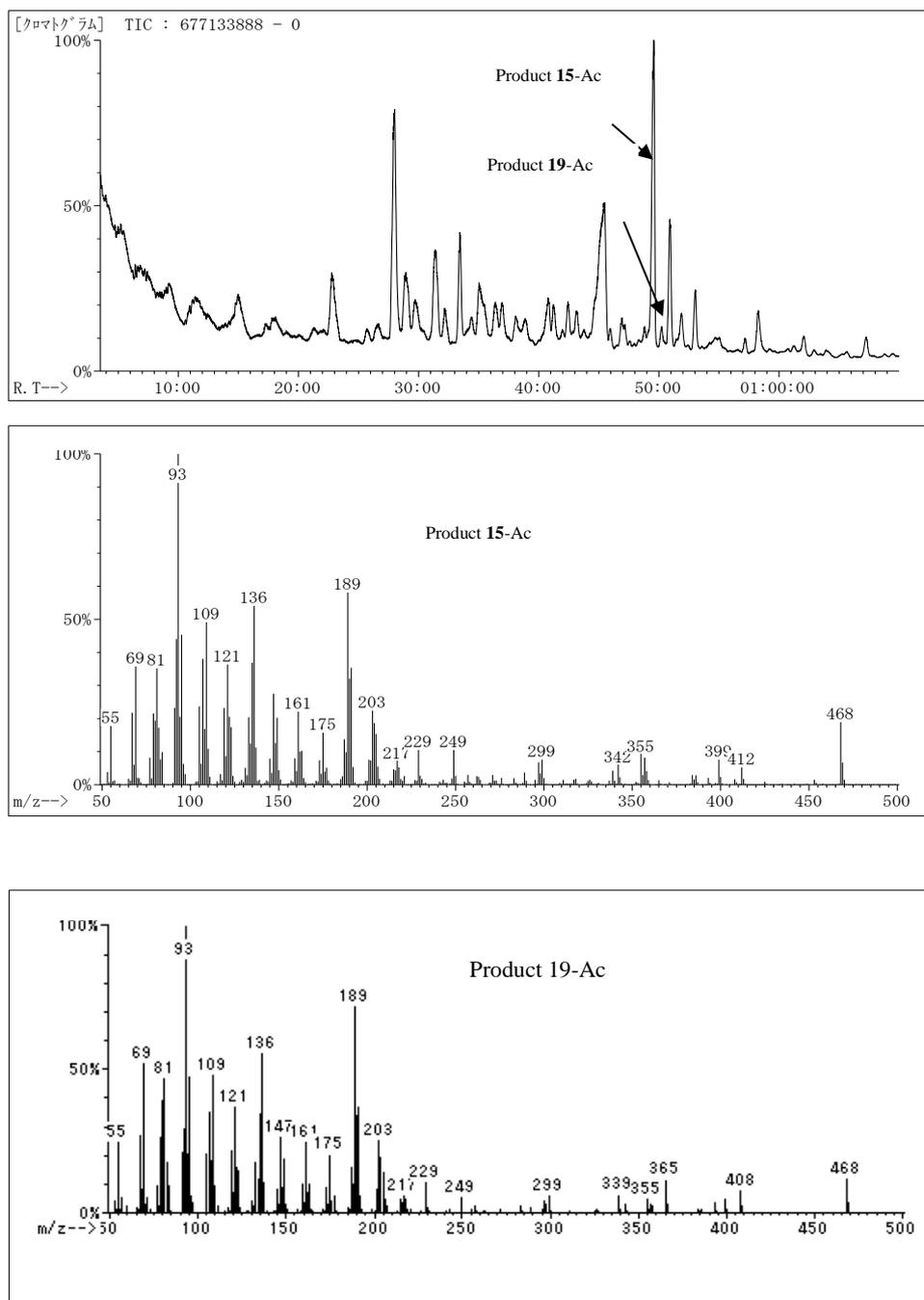
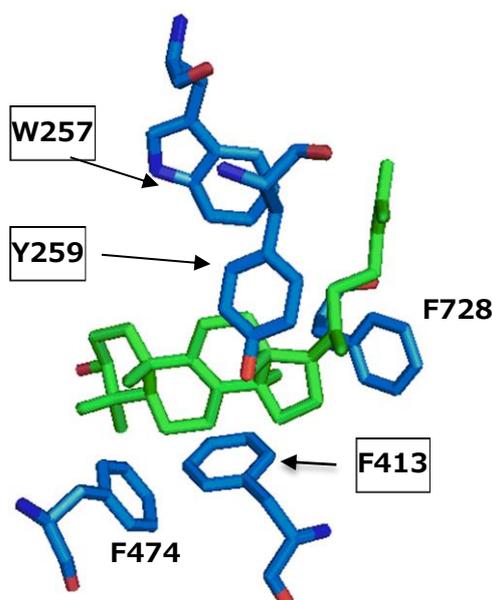
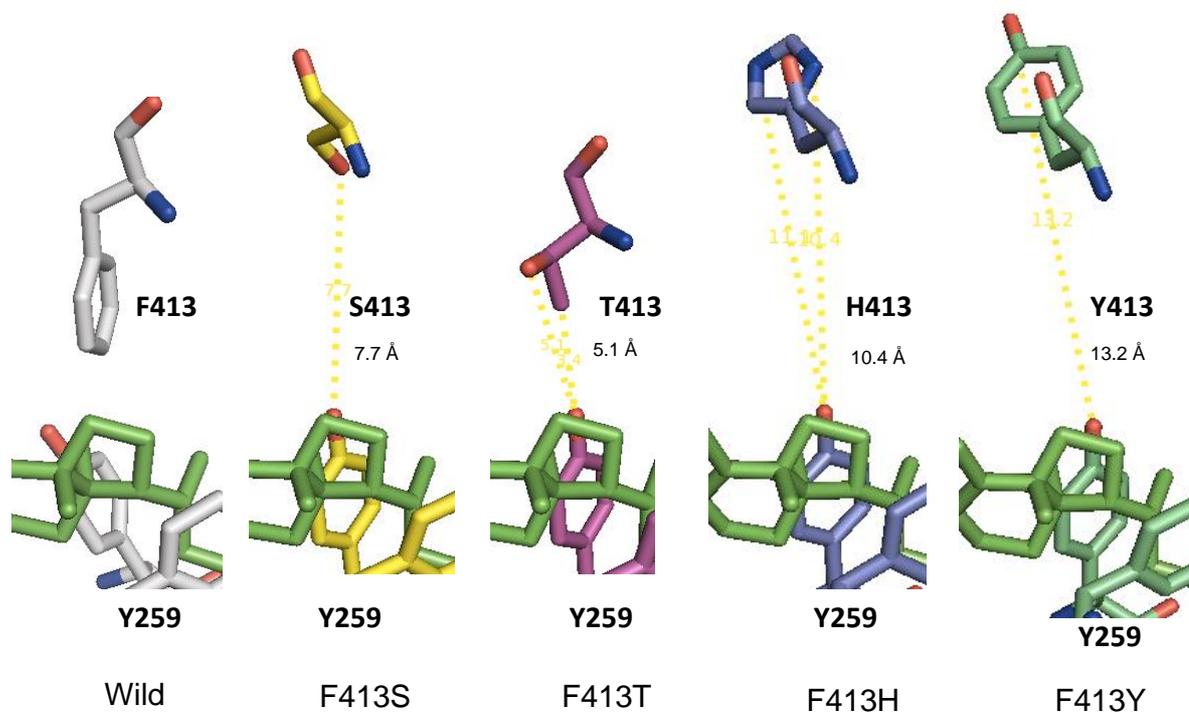


Fig. S7.1. Homology modeling of β -amyirin synthase prepared with Pymol (<http://www.pymol.org>). This model was constructed by ESyPred3D (<http://www.unamur.be/sciences/biologie/urbm/bioinfo/esypred/>), based on the X-ray crystal structure of human lanosterol cyclase (pdb ID: 1w6k, *Nature* **432**, 118-122, 2004). Lambert C, Leonard N, De Bolle X & Depiereux E (2002) ESyPred3D: Prediction of proteins 3D structures *Bioinformatics*. **18**, 1250-1256.



Lanosterol molecule is shown with green color. We have reported the detailed functional analysis of F728 in the previous paper (R. Ito, I. Hashimoto, Y. Masukawa and T. Hoshino, *Chem. Eur. J.* **2013**, *19*, 17150-17158). The site-directed mutagenesis experiments have demonstrated that the function of cation/ π interaction was assigned to the F728 residue. On the other hand, the steric bulk at 474 position is critical to the accurate folding of oxidosqualene to complete the polycyclization reaction (Ito, R.; Masukawa, Y.; Nakada, C.; Amari, K.; Nakano, C.; Hoshino, T. *Org. Biomol. Chem.* **2014**, *12*, 3836-3846). By the mutagenesis experiments, we found that D⁴⁸⁵C⁴⁸⁶TA motif triggers the polycyclization reaction and the C564 is involved in hydrogen bond formation with the carboxyl residue of D485, resulting in enhancement of the acidity (R. Ito, Y. Masukawa and T. Hoshino, *FEBS J.*, **2013**;280:1267-1280). F474 is situated in the vicinity to B-ring formation site. F413 residue is located in approximate to the C/D ring. Y259 and W257 that correspond to Y261 and to W259, respectively, of *P. ginseng* PNY β -amyirin synthase, are also marked in this model. It was reported that the Y261H mutant of PNY gave the tetracyclic products (see the Text and the ref. T. Kushiro, M. Shibuya, K. Masuda, Y. Ebizuka, *J. Am. Chem. Soc.*, **2000**, *122*, 6816-6824). The hydrogen bonding may occur between OH of the polar amino acids (Ser, Thr or Tyr mutants) and the phenolic OH of Y259, because of the proximal distance between them (see Fig. S7.2), which would have brought about the inappropriate placement of Y259, resulting in the decreased activities of the polar amino acid-substituted mutants (see Figure 3 in Text).

Fig. S7.2. Distances between OH of Y259 and OH of polar amino acids (Ser and Thr), OH of Tyr and NH or N of His, which were estimated from the modeling constructed by the methods of ESyPred3D. Lanosterol molecule is shown with green color.



Hydrogen bonding is presumed between OH of Y259 and the polar group of Ser, Thr, His and Tyr, and water molecule(s) is also likely to intervene between the OH of Y259 and the polar groups of the substituted amino acids.

Fig. S8. GC traces of the lipophilic acetylated materials produced by various mutants targeted for F413. A 100 mL culture of each mutants were subjected to centrifugation. The cell pellets were subjected to saponification with 15% KOH/MeOH under reflux condition, followed by the extraction of the lipophilic materials with hexane extract (3 x 10 mL). The triterpene fraction including products was obtained by partial purification with a SiO₂ column to remove oxidosqualene, dioxidosqualene and nontriterpene impurities (hexane/EtOAc=100:1), followed by acetylation with Ac₂O/Py. The acetate mixture was dissolved in 1.0 mL of hexane. A 0.5 μL of the hexane was injected to the GC apparatus. The GC conditions were as follows: J & W, DB-1 capillary column (Length 30 m, I.D. 0.32 mm, Film Thickness 0.25 mm); column injection temp., 300°C; column temperature, 245-270°C (0.35 °C/min).

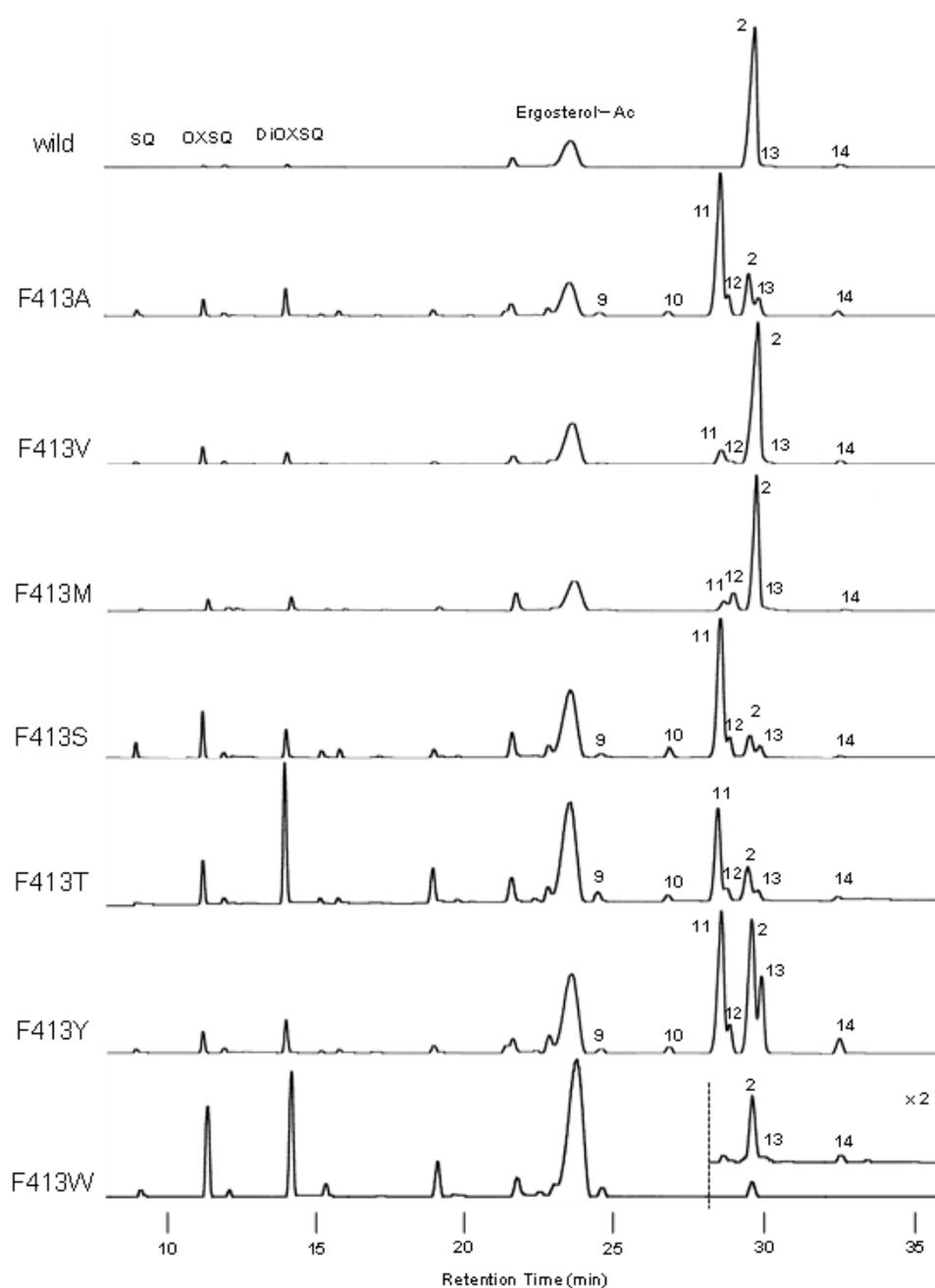


Fig. S9. Estimation of the EtAS enzymatic activities for the wild-type and the F413X mutants.

<Expression level of β -amylin synthase (EtAS)>

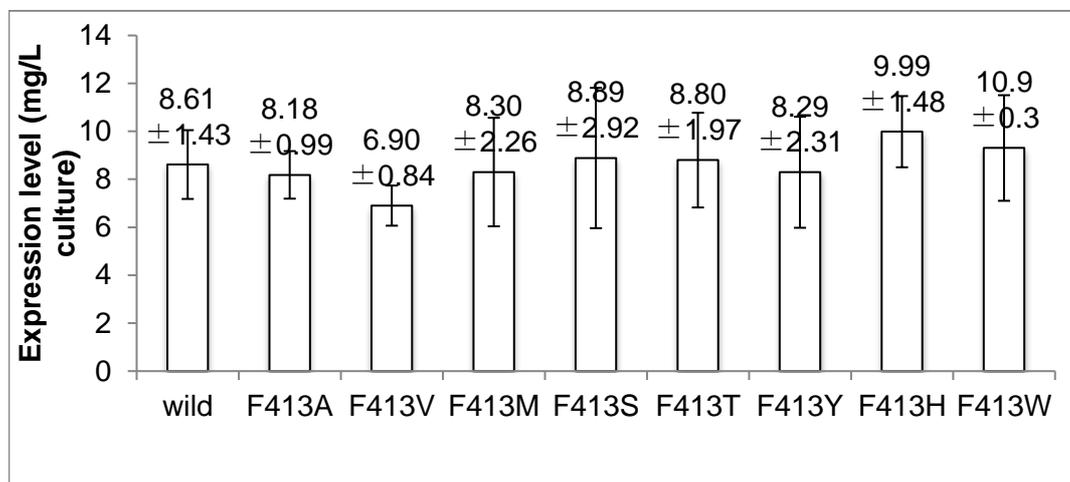


Figure S9.1. Expression level (mg/L) of the EtAS enzymes of the wild-type and the site-directed mutant. The yeast cells grown in 1L-medium were collected and the protein amounts (mg) were quantified by Western blot analysis.

<GC analyses for the quantities of oleanane-type triterpenes>

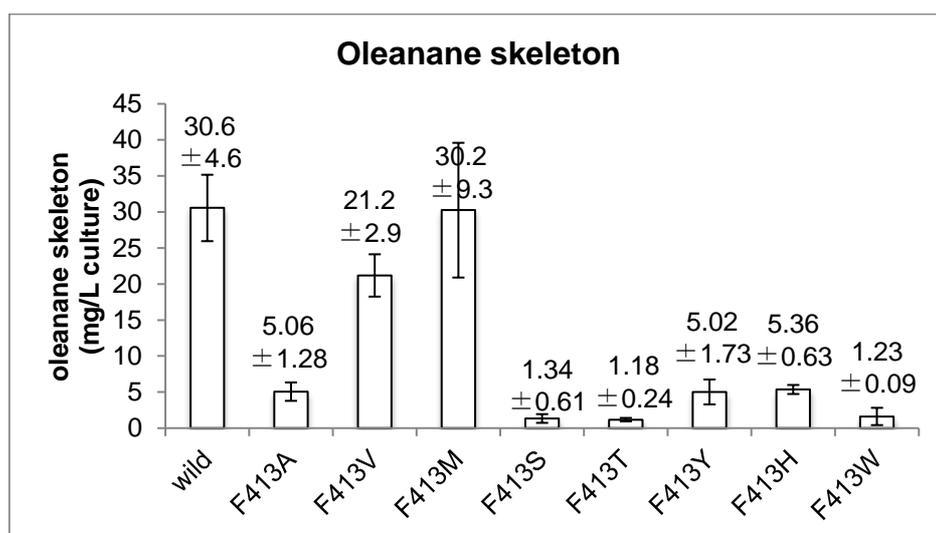


Fig. S9.2. The quantities of products **2** and **12** (oleanane skeleton) produced by 1L-culture of each of the mutant strains that were determined by GC analyses using GGOH (geranylgeraniol) as an internal standard.

<Enzyme activities for the production of oleanane-type triterpenes>

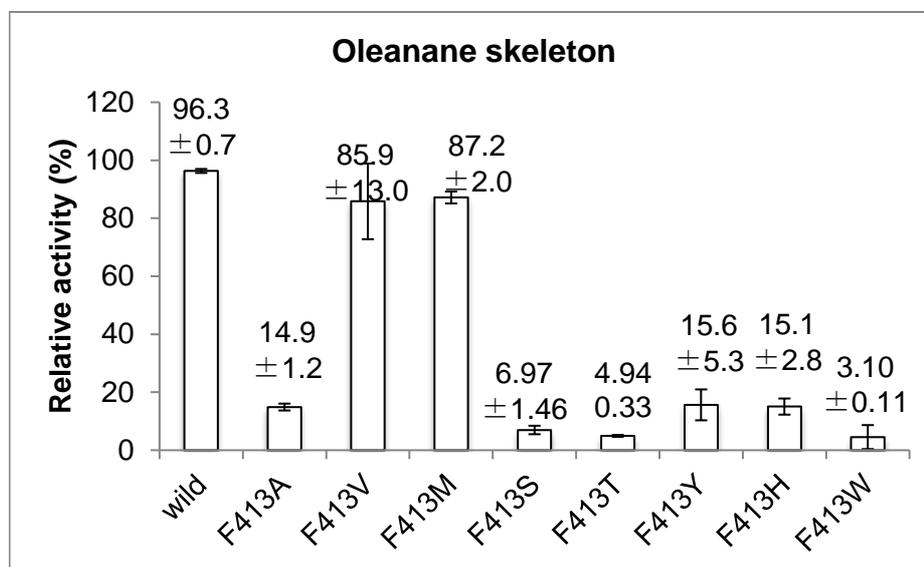


Fig. S9.3. Enzyme activities of the mutants relative to that of the wild-type for the production of oleanane skeleton. The wild-type activity (100%) indicates the sum of the relative activities shown in Figs. S9.3, Fig. S9.5 and S9.7. These enzyme activities were estimated by dividing the amounts of oleanane-type products (**2** and **12**) by the expressed quantities of EtAS enzymes. This means that the values of Fig. S9.2 were divided by those of Fig. S9.1. The wild-type did not show 100% activity, i.e., 96.3 ± 0.7 %, because the wild-type produced the tetracycles **13** and **14** in a small amount (see Figs. S9.5 and S9.7).

< GC analyses for the quantities of Dammarane-types >

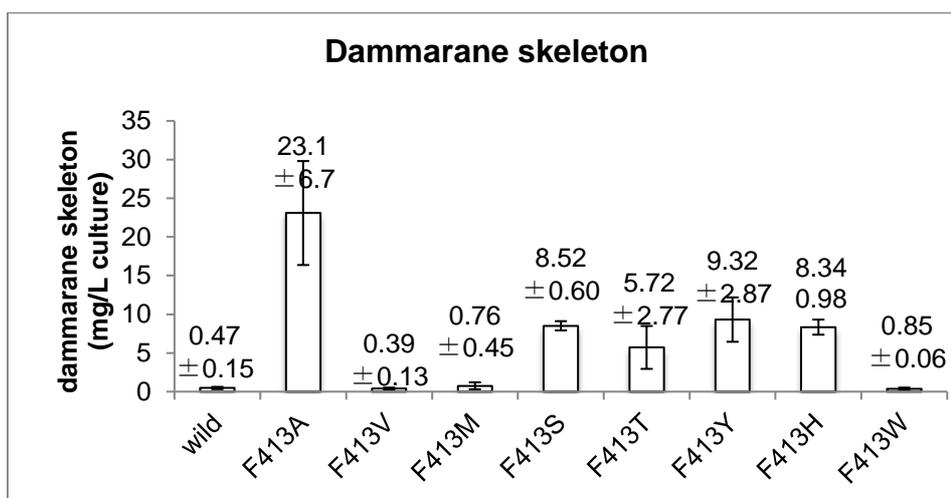


Fig. S9.4. The quantities of products **10**, **11**, **13** (dammarane skeleton) produced by 1L-culture of each of the mutant strains that were determined by GC analyses using GGOH (geranylgeraniol) as an internal standard. Product **10** is tentatively categorized as Dammarane skeleton, but not 17-epi-dammaraen skeleton. To make sense, the stereochemistry of C-20 must be determined.

< Enzyme activities for the production of Dammarane-types >

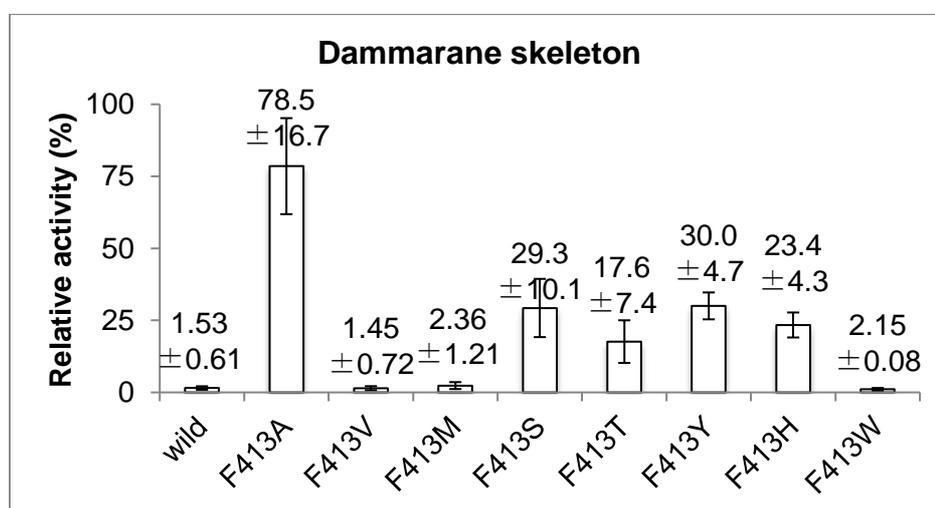


Figure S9.5. Enzyme activities of the mutants relative to that of the wild-type. These enzyme activities were estimated by dividing the amounts of dammarane-type products (**10**, **11**, **13**) by the expressed quantities of EtAS enzymes, that is, the values of Fig. S9.4 were divided by those of Fig. S9.1. The activity of the wild-type for the production of **13** and **14** was very low, i.e., $1.53 \pm 0.61\%$, thus, the relative activity does not correspond to 100%. The total values of Fig.S9.3 and Fig. S9.5 for the wild-type corresponds to 100%.

< GC analyses for the quantities of 17-*epi*-Dammarane-type >

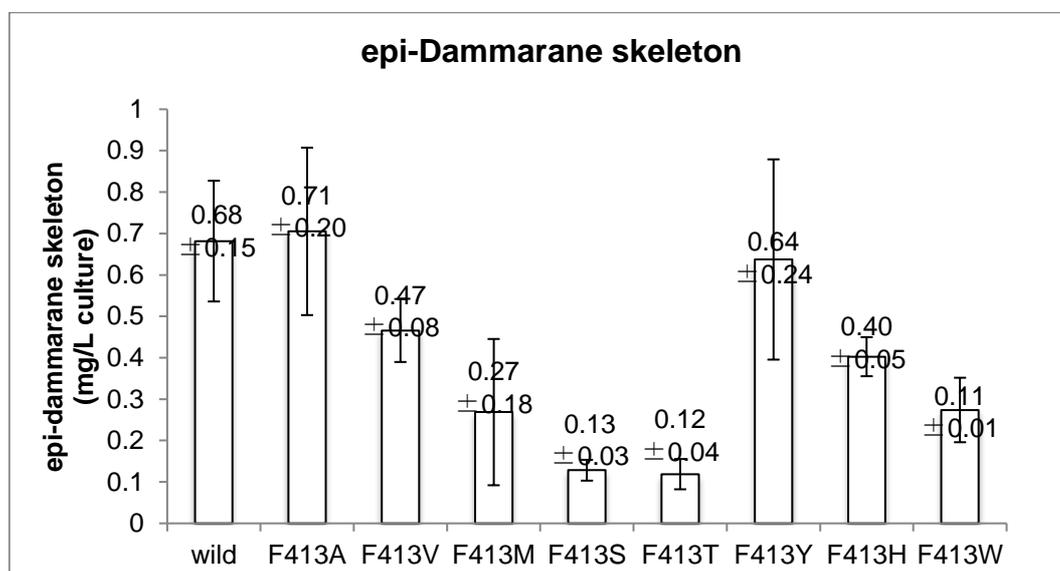


Fig. S9.6. The quantities of products **14** (17-*epi*-dammarane skeleton) produced by 1L-culture of each of the mutant strains, which were determined by GC analyses using GGOH (geranylgeraniol) as an internal standard.

< Enzyme activities for the production of 17-*epi*-Dammarane-types >

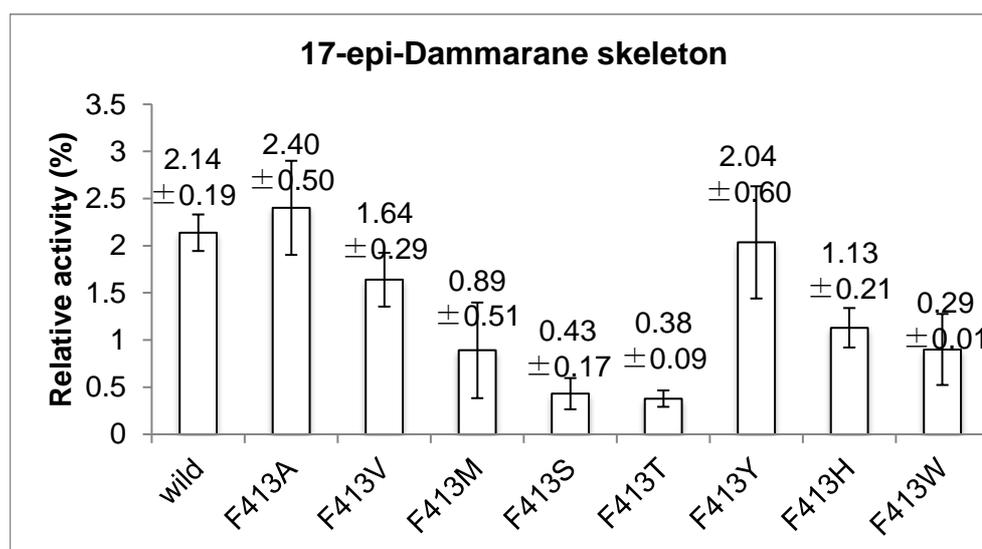


Figure S9.7. Enzyme activities of the mutants relative to that of the wild-type. These enzyme activities were estimated by dividing the amounts of 17-*epi*-dammarane-type product **14** by the expressed quantities of EtAS enzymes (%).

< GC analyses for the quantities of bicyclic product >

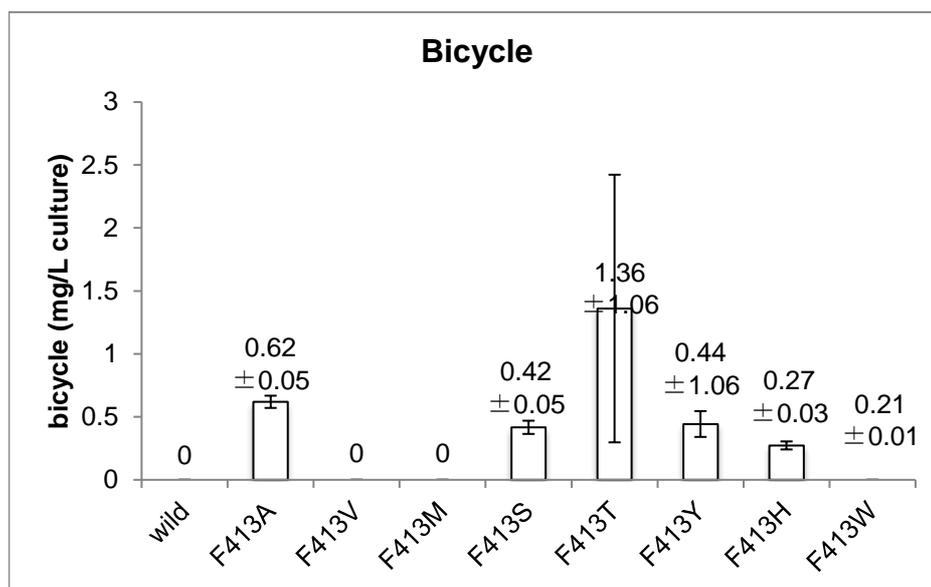


Fig. S9.8. The quantities of products **9** produced by 1L-culture of each of the mutant strains, which were determined by GC analyses using GGOH (geranylgeraniol) as an internal standard.

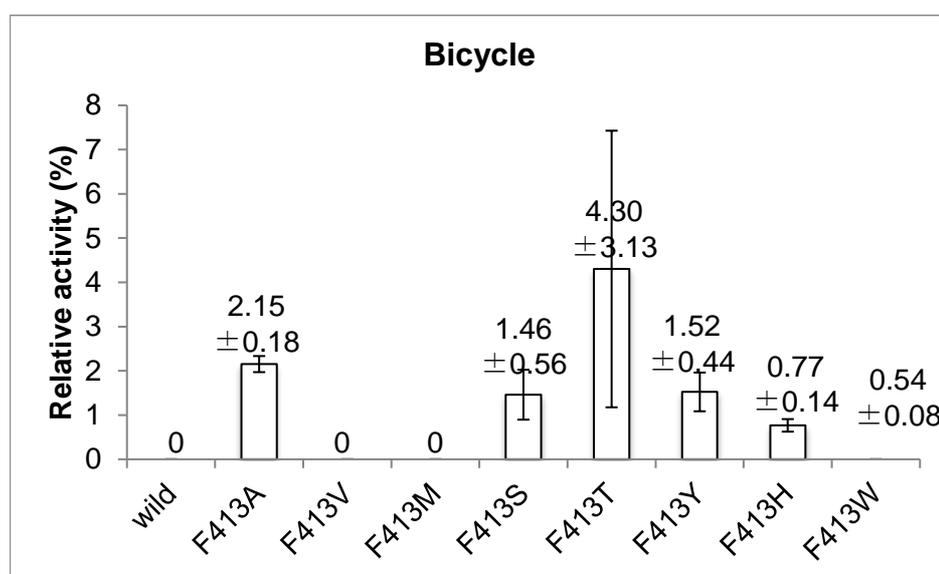


Figure S9.9. Enzyme activities of the mutants relative to that of the wild-type. These enzyme activities were estimated by dividing the amounts of bicyclic product **9** by the expressed quantities of EtAS enzymes (%).

Fig. S10. Estimation of the EtAS enzyme activities for the Y259X mutants.

<Expression level of β -amyrin synthase (EtAS)>

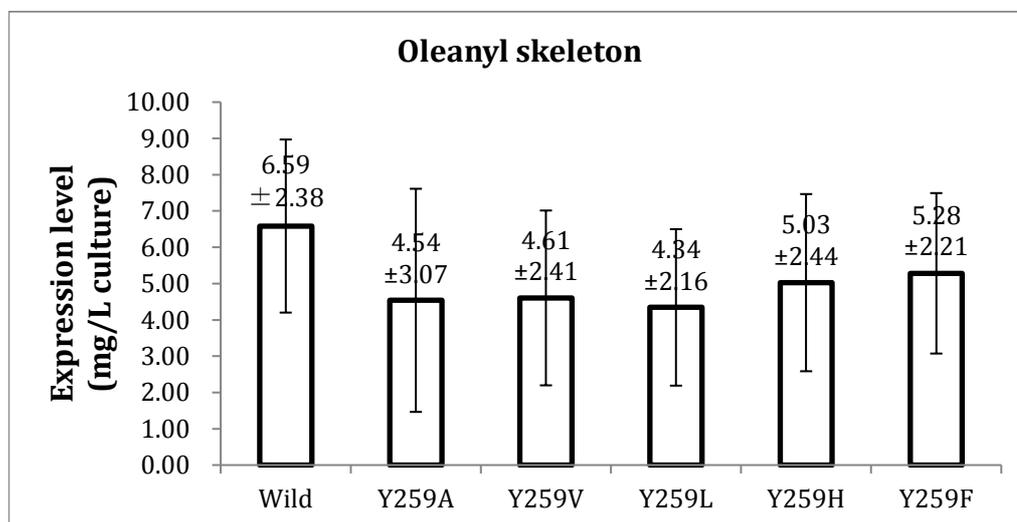


Fig. S10.1. Expression level (mg/L) of the EtAS enzymes of the wild-type and the site-directed mutant. The yeast cells grown in 1L-medium were collected and the protein amounts (mg) were quantified by Western blot analysis.

<GC analyses for the quantities of oleanane-type triterpenes>

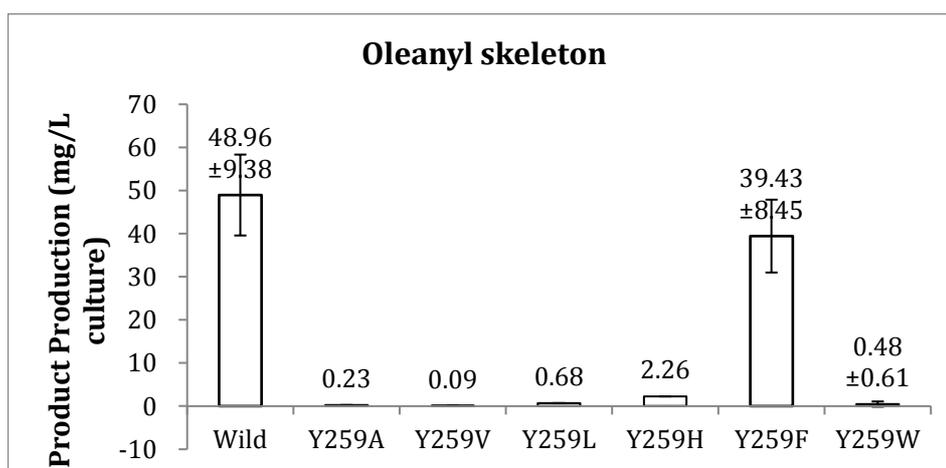


Fig. S10.2. The quantities of products 2, 16 and 18 (oleanane skeleton) produced by 1L-culture of each of the mutant strains that were determined by GC analyses using GGOH (geranylgeraniol) as an internal standard.

<Enzyme activities for the production of oleanane-type triterpenes>

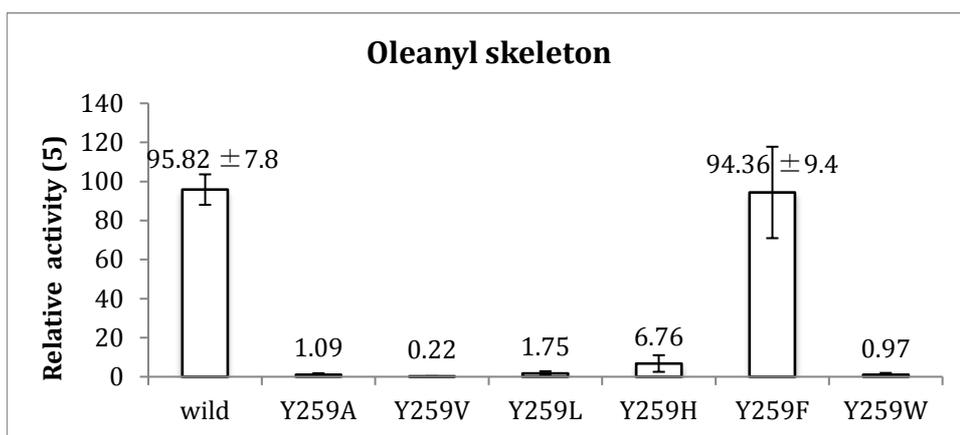


Fig. S10.3. Enzyme activities of the mutants relative to that of the wild-type for the production of oleanane skeleton. See the legend to Fig. S9.3 for the calculation method.

<GC analyses for the quantities of lupanyl-type triterpene, lupeol>

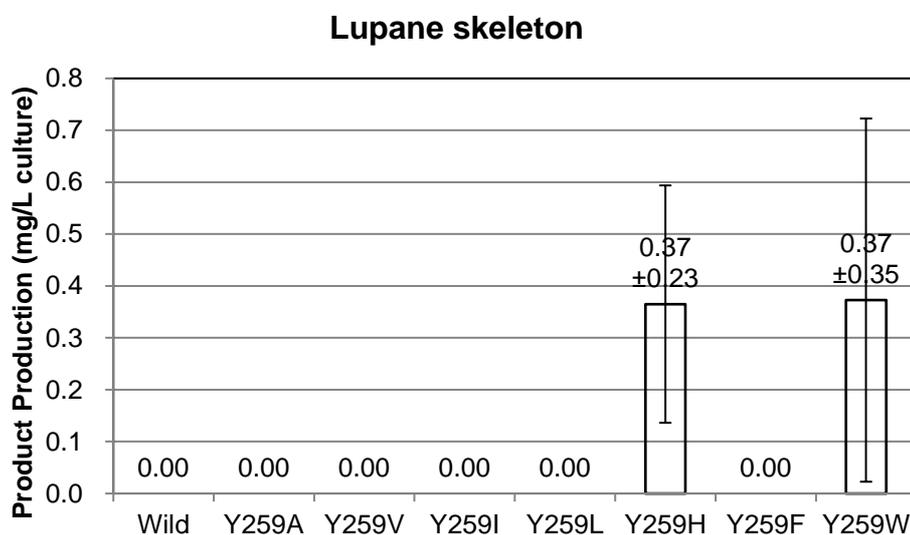


Fig. S10.4. The quantities of product **20** (lupeol, 6/6/6/6/5-fused pentacycle) produced by 1L-culture of each of the mutant strains that were determined by GC analyses using GGOH (geranylgeraniol) as an internal standard.

<Enzyme activities for the production of lupeol>

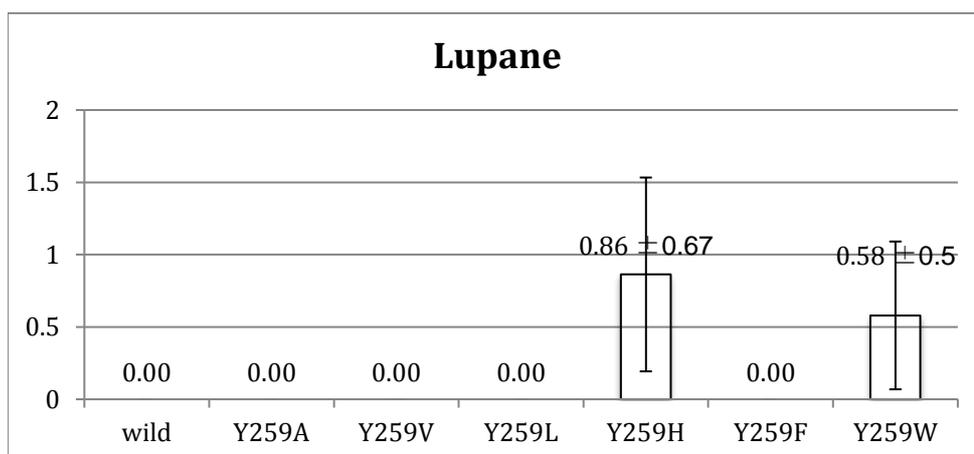


Fig. S10.5. Enzyme activities of the mutants relative to that of the wild-type. These enzyme activities were estimated by dividing the amounts of lupanyl-type product **20** by the expressed quantities of EtAS enzymes.

<GC analyses for the quantities of tetracyclic triterpenes>

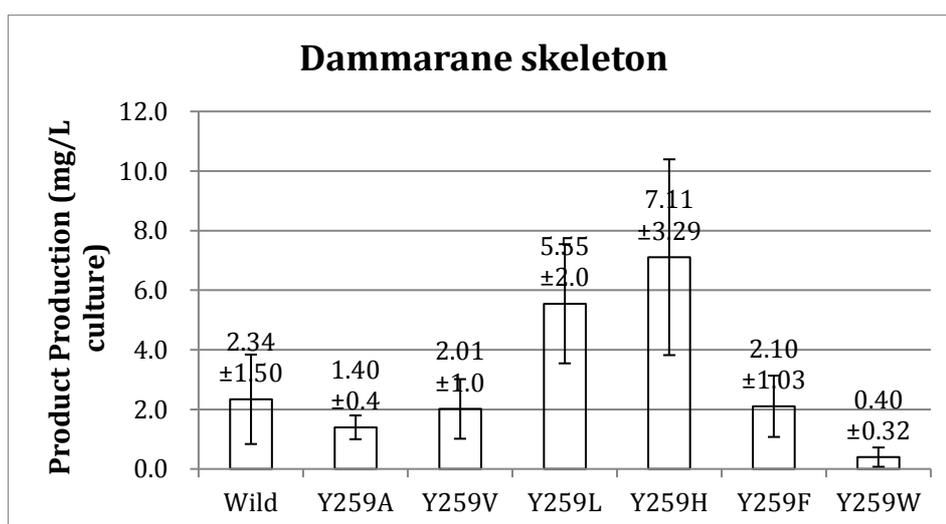


Fig. S10.6. The total quantities of products **11**, **13**, **14**, **15**, **17** and **19** produced by 1L-culture of each of the mutant strains that were determined by GC analyses using GGOH (geranylgeraniol) as an internal standard.

<Enzyme activities for the production of dammarenyl skeleton>

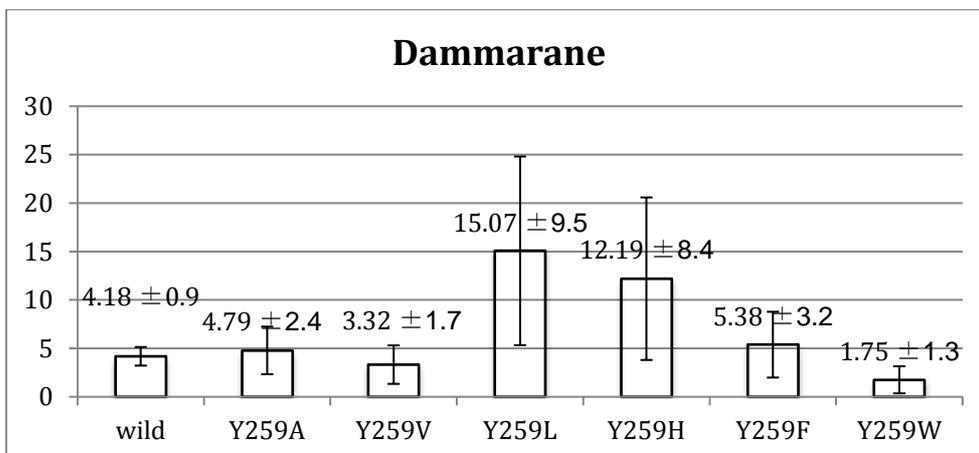


Fig. S10.7. Enzyme activities of the mutants relative to that of the wild-type. These enzyme activities were estimated by dividing the amounts of tetracyclic products **11**, **13**, **14**, **15**, **17** and **19** by the expressed quantities of EtAS enzymes.

Fig. S11. Estimation of the EtAS enzyme activities for the W257X mutants.

<Expression level of β -amyrin synthase (EtAS)>

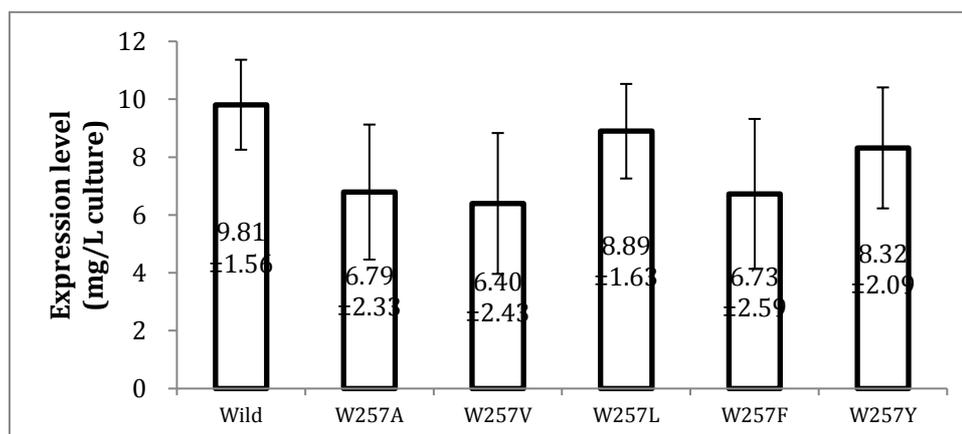


Fig. S11.1. Expression level of β -amyrin synthase (EtAS) for the wild-type and the mutants. The protein amounts (mg) were quantified by Western blot analysis.

<GC analyses for the quantities of oleanane-type triterpenes>

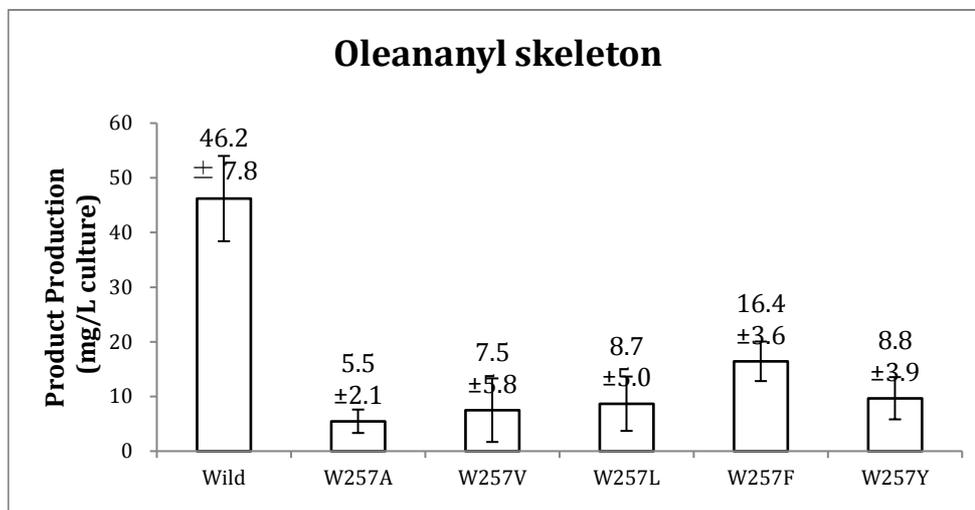


Fig. S11.2. The quantities of oleanyl products (**2** and **16**) for wild-type and the variants, which were determined by GC analyses.

<Enzyme activities for the production of oleanane-type triterpenes>

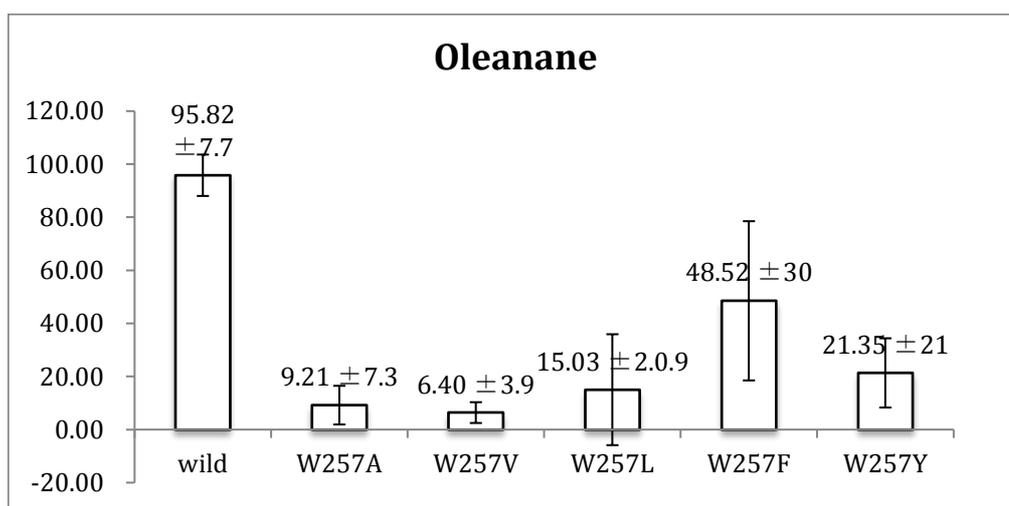


Fig. S11.3. Enzyme activities of the mutants relative to that of the wild-type. These enzyme activities were estimated by dividing the amounts of oleany products **2** and **16** by the expressed quantities of EtAS enzymes.

<GC analyses for the quantities of lupanyl-type triterpene, lupeol>

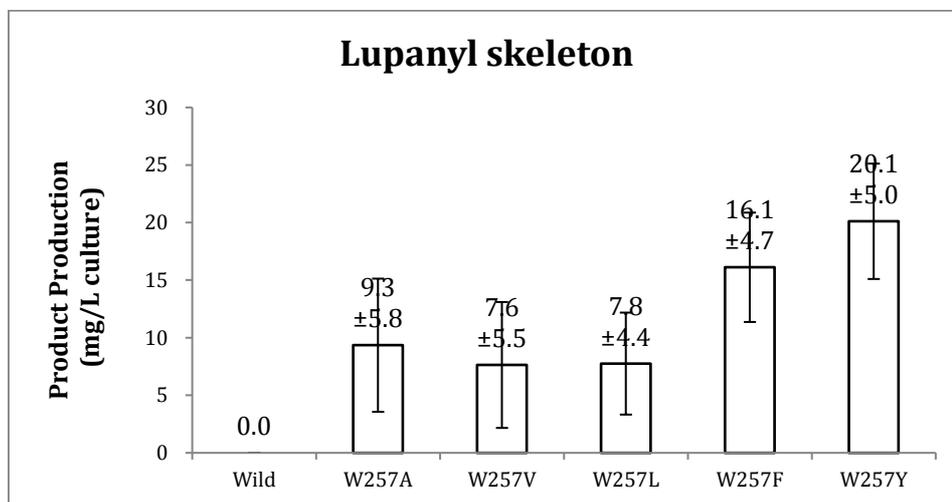


Fig. S11.4. The quantities of lupane skeleton (**20**, lupeol) for wild-type and the variants, which were determined by GC analyses.

<Enzyme activities for the production of lupeol>

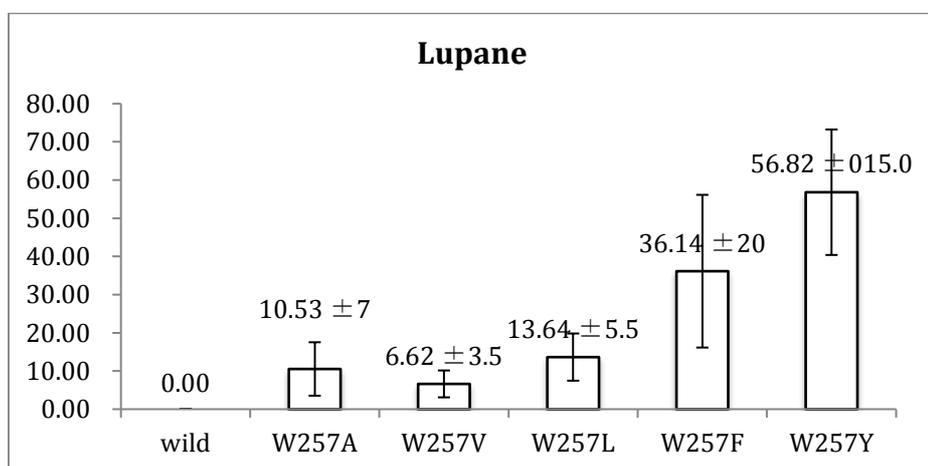


Fig. 11.5. Enzyme activities of the mutants relative to that of the wild-type. These enzyme activities were estimated by dividing the amounts of lupeol **20** by the expressed quantities of EtAS enzymes.

<GC analyses for the quantities of tetracyclic triterpenes>

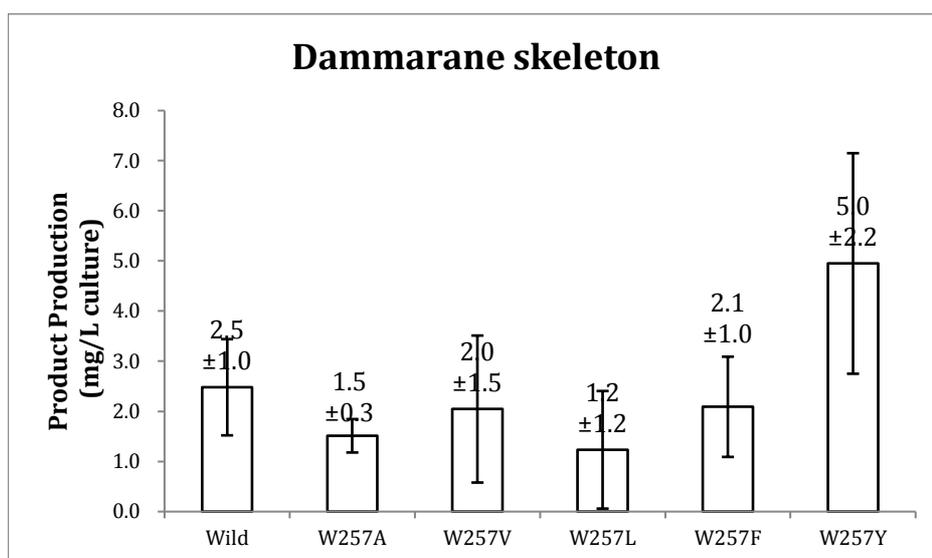


Fig. S11.6. The quantities of dammarenyl skeleton (**13** and **14**) for wild-type and the variants, which were determined by GC analyses.

<Enzyme activities for the production of dammarenyl skeleton>

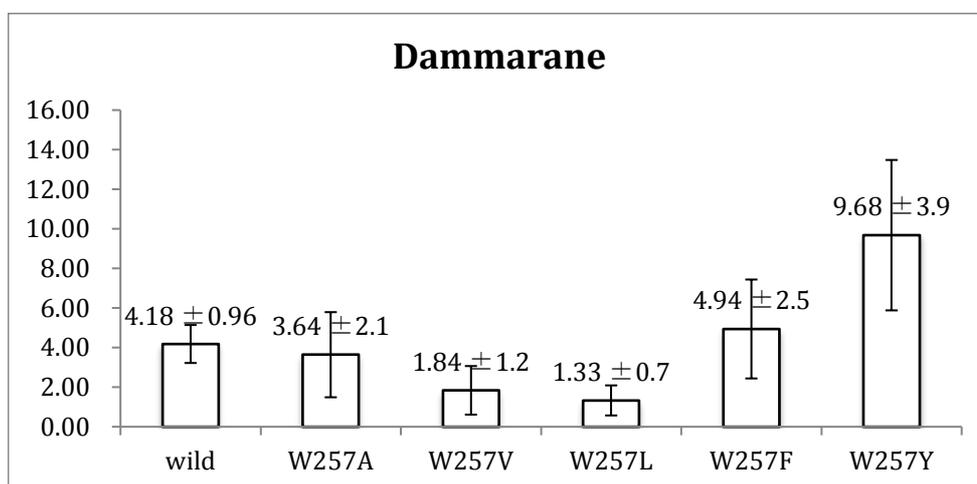


Fig. S11.7. Enzyme activities of the mutants relative to that of the wild-type. These enzyme activities were estimated by dividing the amounts of dammarenyl skeleton (**13** and **14**) by the expressed quantities of EtAS enzymes.