Formation of a Novel Macrocyclic Alkaloid from the Unnatural Farnesyl Diphosphate Analogue Anilinogeranyl Diphosphate by 5-Epi-Aristolochene Synthase

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Table S1. Data collection and refinement statistics.

TEAS-geraniline complex (Space group: $P4_12_12$, Cell parameters: $a = b = 126.75$ Å, $c = 124.3$ Å, $\alpha = \beta = \gamma = 90.0°$)

Data collection[a]

Source	Berkeley ALS Beamline BL8.2.1
Wavelength (Å)	1.00
Resolution (Å)	44.37 - 2.47 (2.56 - 2.47)
Multiplicity	8.2 (4.0)
Completeness (%)	95.66 (79.13)
<I> / σ<I>	11.19 (1.13)
Wilson B-factors	47.89

Refinement

No. reflections	291261 (11363)
No. unique reflections	35313 (2870)
No. of non-hydrogen atoms	4,481
R_{work} (%)[b]	17.57 (0.3320)
R_{free} (%)[c]	22.51 (0.3782)

Model	# of non–hydrogen atoms	Average B-factor
Macromolecules	4,362	71.9
Ligands	59	114.2
Solvent	60	54.3

Rmsd bond (Å)[d]	0.008
Rmsd angle (°)[d]	1.03
Ramachandran plot analysis[d] %favored (% outliers)	96 (0.19)
Average B-factor	71.90

[a]$R_{sym} = \Sigma|I-\langle I\rangle|/\Sigma I$ where I is the integrated intensity of a given reflection, [b]$R_{work} = \Sigma|F(obs)-F(calc)|/\Sigma F(obs)$, [c]$R_{free} = \Sigma|F(obs)-F(calc)|/\Sigma F(obs)$, calculated using 5% of the data, [d]From *PHENIX Comprehensive Validation*

Table S2. Summary of kinetic constants determined for catalytic turnover of FPP to 5-epi-aristolochene in independent biological replicates. Bacterial expressed TEAS protein was isolated by affinity chromatography and incubated with a range of 1-^{3}H-FPP concentrations (0.1 to 80 μM) as well as 0.1 to 40 μM AGPP for 30 min prior to determining the amount of hexane extractable, radiolabled reaction products formed by scintillation counting.

	replicate 1		replicate 2		replicate 3		replicate 4		replicate 5		Average
	Value	± S.E.	Value	± S.E.	Value	± S.E.	Value	± S.E.	Value	± S.E.	
V_{max} (pmol/hr)	42.81	3.13	33.01	1.23	375.12	15.24	16.84	1.22	408.9	11.17	175.34
K_M (μM)	9.85	2.68	4.28	0.70	7.43	1.16	5.58	1.69	4.47	0.58	6.32
K_i (μM)	0.03	0.01	1.24	0.34	0.16	0.04	0.03	0.01	.386	0.04	0.37
k_{cat} $^{s-1}$	6.00E-03		0.54E-03		20.8E-03		0.28E-03		31.5E-03		.012
Enzyme	40 nM		333 nM		100 nM		333 nM		72 nM		

Table S3. Summary of kinetic constants determined for catalytic turnover of AGPP to geraniline by the TEAS enzyme in independent biological replicates. Bacterial expressed TEAS protein was isolated by affinity chromatography and incubated with 0.4 to 65 μM AGPP for 0.5 to 1 hr at 37^{o}C prior to determining the amount of geraniline formed by GC-MS.

	replicate 1		replicate 2		replicate 3		replicate 4		replicate 5		Average
	Value	± S.E.	Value	± S.E.	Value	± S.E.	Value	± S.E.	Value	± S.E.	
V_{max} (pmol/hr)	351	53.5	194	27.2	105	7.5	75	27.3	370	109	219
K_M (μM)	12.9	5.24	11	4.35	3.4	0.75	11.2	12.9	2.4	3.8	8.18
k_{cat} $^{s-1}$	0.195E-03		0.11E-03		0.058E-03		0.042E-03		0. 21E-03		0. 12E-03
Enzyme	1 μM										

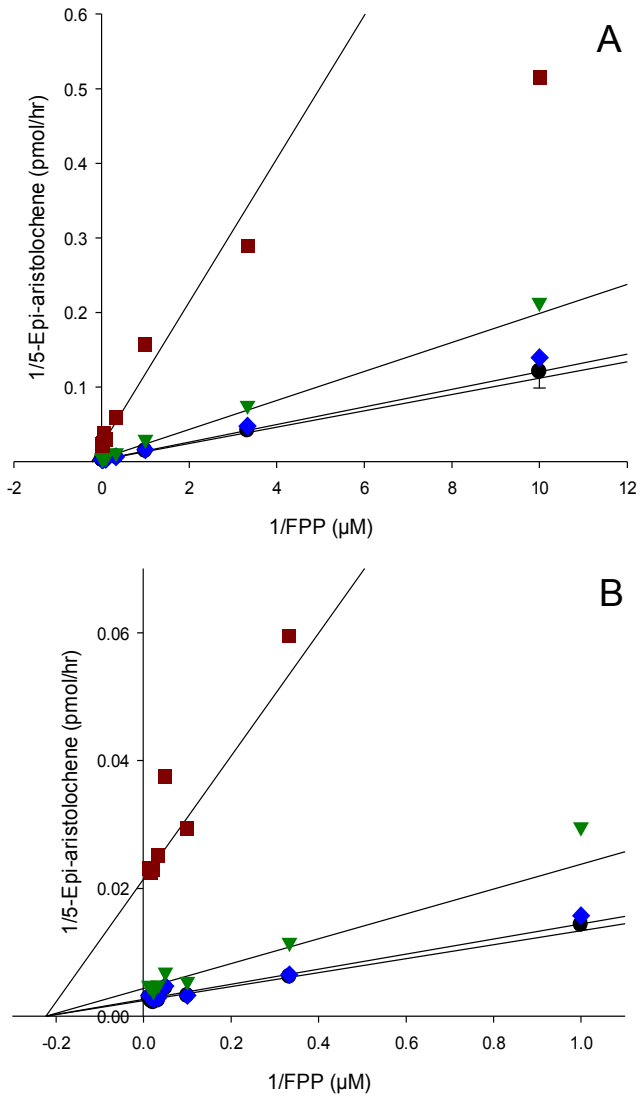

Figure S1. Lineweaver-Burk plots of AGPP inhibition of FPP to hydrocarbon (~98% 5-epi-aristolochene, see Fig. S3 upper panel), non-competititve, non-linear regression line fitting. 5-Epi-aristolochene synthase (72 nM) was incubated at the indicated concentrations of ^{3}H-FPP in the absence (●) or presence of 0.03 (◆), 0.3 (▼), 3.0 (■) μM AGPP in 250 mM Tris, pH 7.5 and 50 mM $MgCl_2$ at room temperature for 10 min. The reactions (50 μl) were terminated by addition of 50 μl stop solution and then extracted with 300 μl of hexane to partition the reaction products into the organic phase, which was treated with silica to remove any FPP and FOH formed from chemical decay or aborted catalysis. The amount of radioactivity incorporated into hydrocarbon reaction products was determined by scintillation counting an aliquot of the silica scrubbed hexane extract. Data were analyzed using the Enzyme Kinetics 1.3 software suite. Panel B is an enlargement of axis intercepts of panel A.

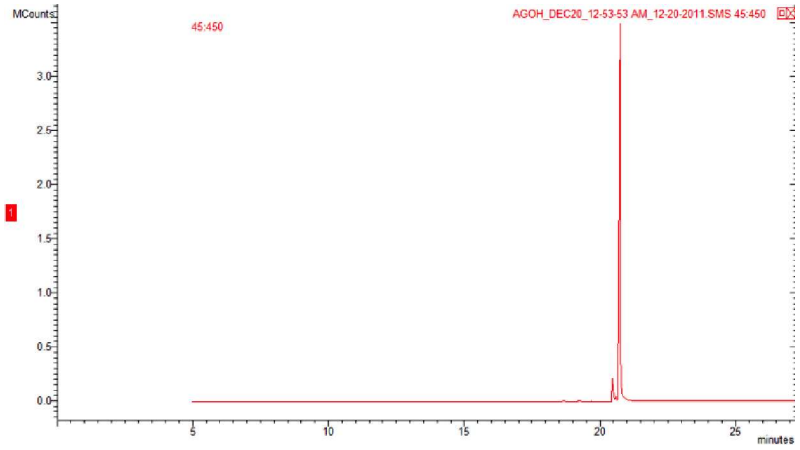

Figure S2. GC-MS profile of AGOH.

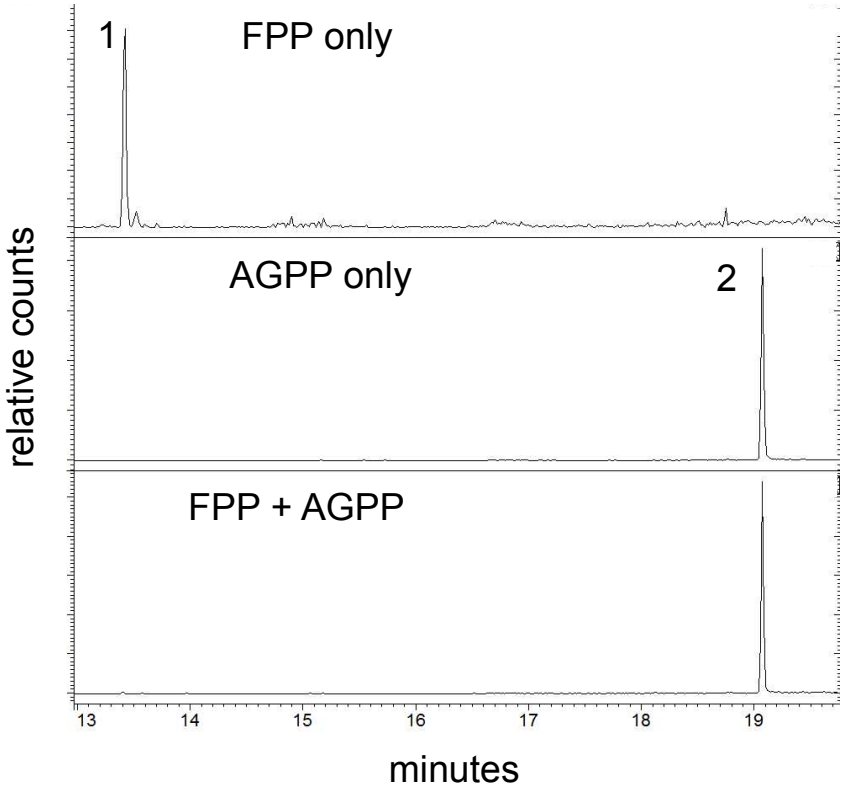

Figure S3. GC-MS reaction product profiles of the TEAS enzyme incubated with 20 μM FPP (upper panel), 20 μM AGPP (middle panel), or both (lower panel). Purified 5-epi-aristolochene synthase (1 μM) was incubated with the indicated substrates for 0.5 hr at 37^{o}C before the reaction products were extracted with hexane and evaluated by GC-MS. The MS for the peak appearing at 13.42 min (1) was identical to 5-epi-aristolochene, while the MS of the peak at 19.08 (2) was identical to that of geraniline.

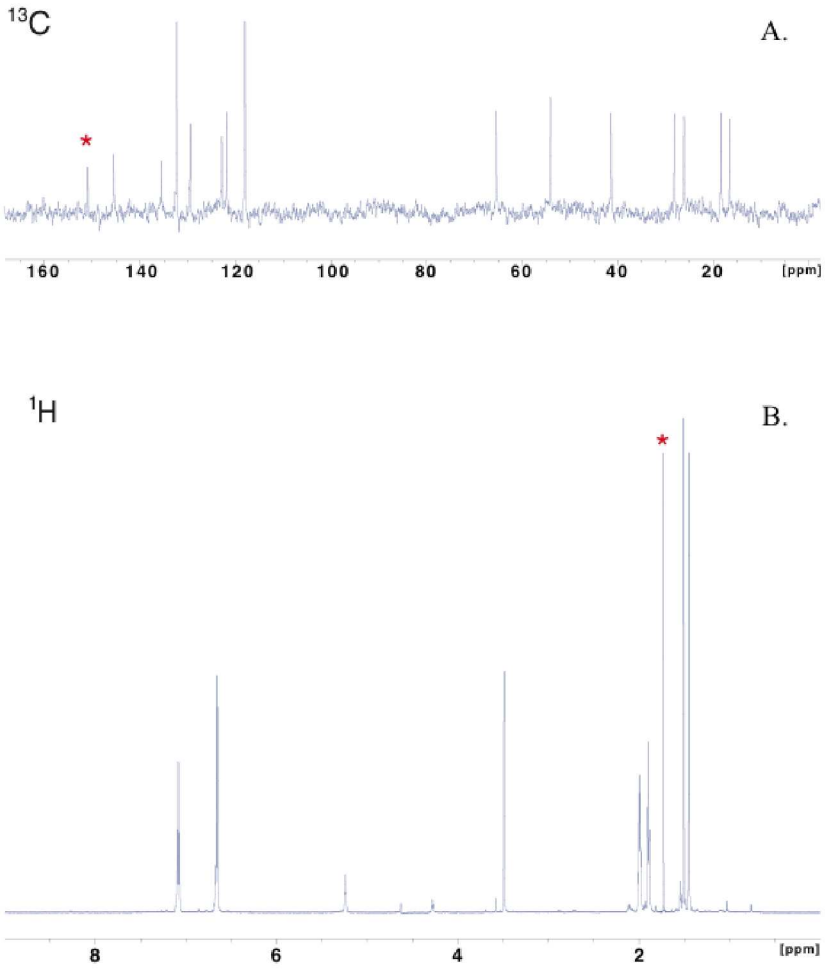

Figure S4. ^{13}C (A) and ^{1}H (B) NMR spectra of AGPP in D_2O. The red asterisks denote a contaminate in this preparation.

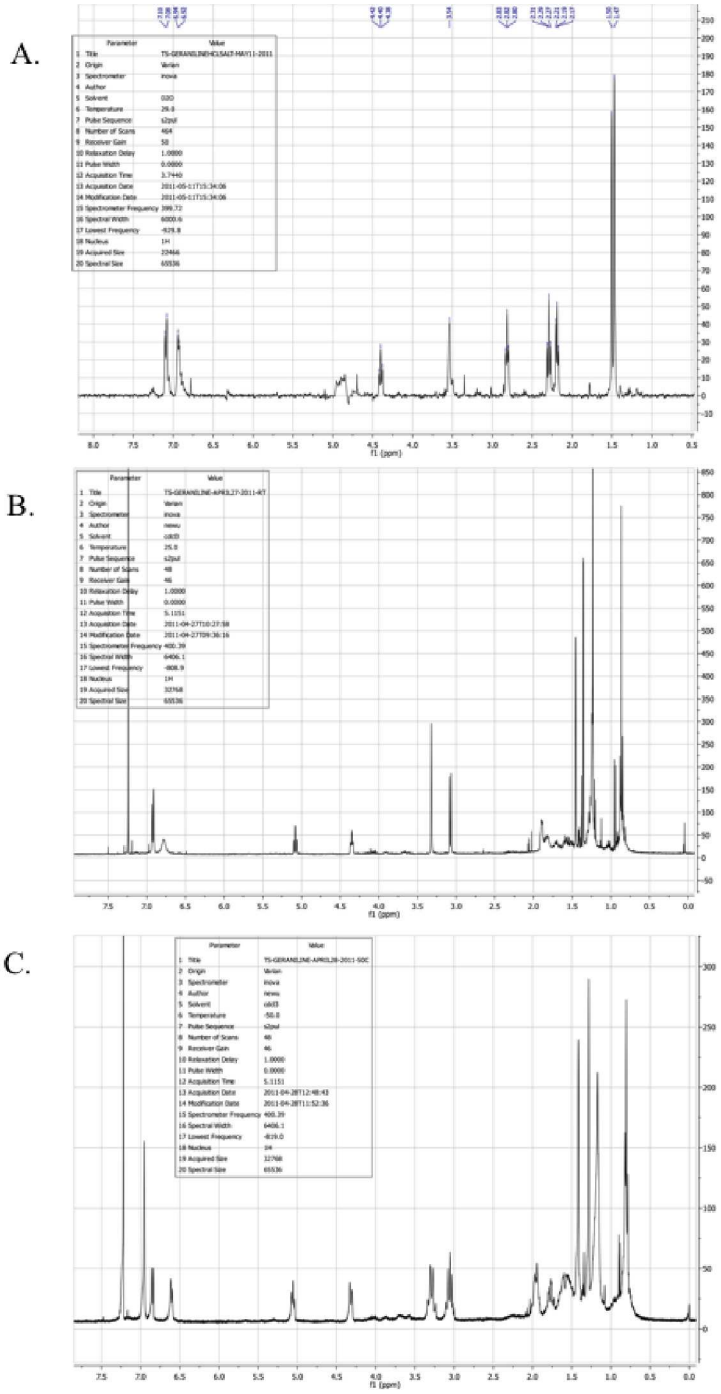

Figure S5. NMR spectra of geraniline. ^{1}H NMR spectrum of geraniline•HCl in D_2O (A). One of the geraniline methine resonances is missing because it was suppressed by the pulse sequence and post-acquisition processing along with the residual HDO peak; ^{1}H NMR spectrum of geraniline at 25˚ in $CDCl_3$ (B); and ^{1}H NMR spectrum of geraniline at -50˚ in $CDCl_3$ (C).