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APPENDIX

The standard curve of the bovine serum albumin standard solutions

A set of bovine serum albumin (BSA) standard solutions were created from the stock solution of 2 mg/mL BSA in Tris-HCl, pH 8.0 buffer solution. Pipette the stock solution and diluted as the table below to give the BSA standard solutions in concentrations of 125, 250, 500, 750, 1000 µg/mL. The UV absorption at 595 nm was monitored and plotted as standard curve.

Table 8. Average absorbance of BSA standard solutions.

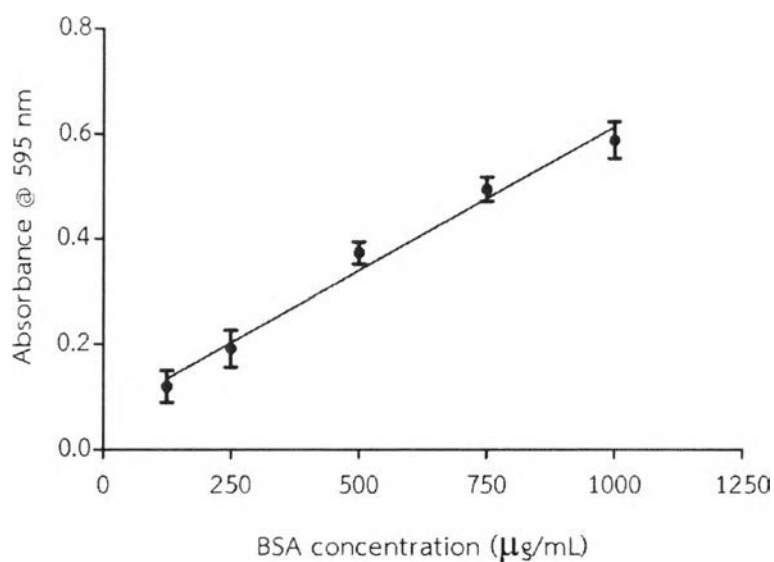| Final concentration of BSA (µg/mL)	Average absorbance (± SD) at 595 nm
125	0.120±0.03
250	0.192±0.03
500	0.373±0.02
750	0.493±0.02
1000	0.587±0.03

BSA standard

Figure 21. The BSA standard curve with calculated linear regression equation; $y = 0.0005x + 0.0661$, $R^2 = 0.9845$. Each bar represents the mean ± SD (n = 3).

The standard curve of Renieramycin M standard solution

Accurately weighed 0.10 mg of renieramycin M (RM) and dissolved in 100 μL of DMSO, then added 900 μL of MeOH to give concentration of 0.1 mg/mL renieramycin M in 10% DMSO/MeOH as stock solution. The stock solution was diluted to give 5 serial concentrations, 1.56, 3.13, 6.25. 12.5, 25 μg/mL, respectively as working solutions. 20 μL of each renieramycin M working solution was injected into HPLC instrument for analysis and gave five renieramycin M amounts, 31.5, 62.5, 125, 250 and 500 ng of renieramycin M in each concentration as a final amount of renieramycin M used in calibration curve.

Table 9. Average area under the curve of HPLC chromatogram for renieramycin M standard curve.

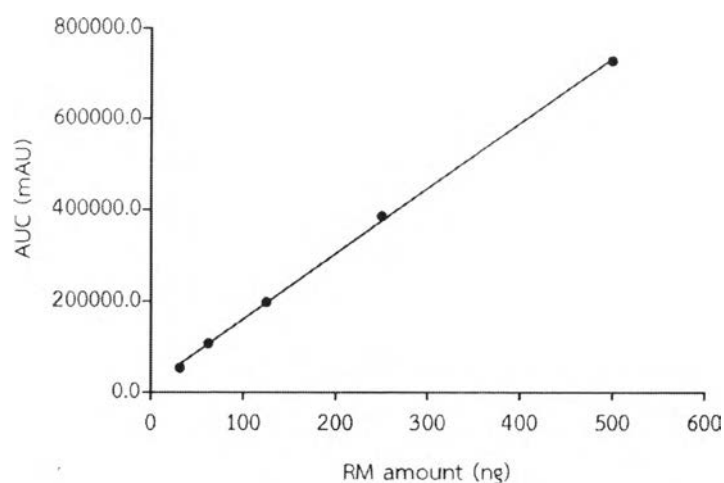| concentration of RM substrates (μg/mL)	Amount of RM (ng) in reaction for HPLC analysis	Average area under the curve (AUC) of HPLC chromatogram ($\pm$ SD) (mAU)
1.56	31.5	54305±6.25
3.13	62.5	107967±11.5
6.25	125	197532±17.1
12.5	250	385479±8.54
25	500	727546±4.58

RM standard curve

Figure 22. The tandard curve of renieramycin M (RM) with calculated linear regression equation; $y = 1430.8x + 17341$, $R^2 = 0.9993$

Table 10. The amount of solid ammonium sulfate to be added to solution to give the desired final saturation at 0 °C*.

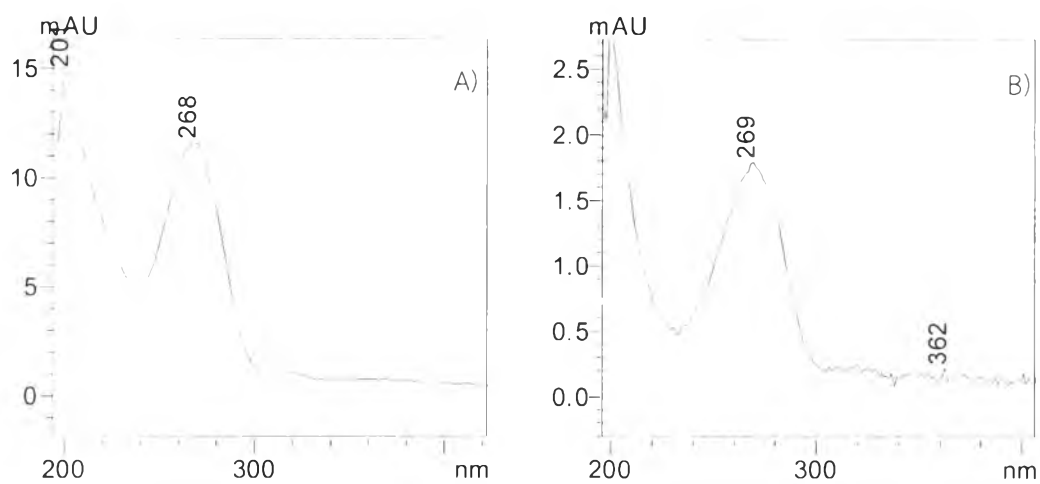|	Final concentration of solid ammonium sulfate, % saturation at 0 °C																
	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
Initial concentration of ammonium sulfate																	
g solid ammonium sulfate to add to 100 mL of solution																	
0	10.7	13.6	16.6	19.3	22.9	26.2	29.5	33.1	36.6	40.4	44.2	48.3	52.3	56.7	61.1	65.9	70.7
5	8.0	10.9	13.9	16.8	20.0	23.2	26.6	30.0	33.6	37.3	41.1	45.0	49.1	53.3	57.8	62.4	67.1
10	5.4	8.2	11.1	14.1	17.1	20.3	23.6	27.0	30.5	34.2	37.9	41.8	45.8	50.0	54.5	58.9	63.6
15	2.6	5.5	8.3	11.3	14.3	17.4	20.7	24.0	27.5	31.0	34.8	38.6	42.6	46.6	51.0	55.5	60.0
20	0	2.7	5.6	8.4	11.5	14.5	17.7	21.0	24.4	28.0	31.6	35.4	39.2	43.3	47.6	51.9	56.5
25		0	2.7	5.7	8.5	11.7	14.8	18.2	21.4	24.8	28.4	32.1	36.0	40.1	44.2	48.5	52.9
30			0	2.8	5.7	8.7	11.9	15.0	18.4	21.7	25.3	28.9	32.8	36.7	40.8	45.1	49.5
35				0	2.8	5.8	8.8	12.0	15.3	18.7	22.1	25.8	29.5	33.4	37.4	41.6	45.9
40					0	2.9	5.9	9.0	12.2	15.5	19.0	22.5	26.2	30.0	34.0	38.1	42.4
45						0	2.9	6.0	9.1	12.5	15.8	19.3	22.9	26.7	30.6	34.7	38.8
50							0	3.0	6.1	9.3	12.7	16.1	19.7	23.3	27.2	31.2	35.5
55								0	3.0	6.2	9.4	12.9	16.3	20.0	23.8	27.7	31.7
60									0	3.1	6.3	9.6	13.1	16.6	20.4	24.2	28.3
65										0	3.1	6.4	9.8	13.4	17.0	20.8	24.7
70											0	3.2	6.6	10.0	13.6	17.3	21.2
75												0	3.2	6.7	10.2	13.9	17.6
80													0	3.3	6.8	10.4	14.1
85														0	3.4	6.9	10.6
90															0	3.4	7.1
95																0	3.5
100																	0

*(Harris and Angal 1989)

Figure 23 UV spectra of A) renieramycin M standard and B) jorunnamycin A standard

Table 11. Total protein contents of each fractionated fraction.

% ammonium sulfate concentration	replicate			average of total protein ±SD (mg)
	#1	#2	#3	
40-45	3.28	3.80	3.94	3.67±0.35
45-50	6.22	4.58	3.61	4.81±1.32
50-55	8.48	6.02	4.24	6.25±2.13
55-60	8.66	6.39	4.45	6.50±2.11
60-65	7.62	7.40	6.70	7.24±0.48
65-70	5.40	5.025	4.84	5.09±0.28
70-75	2.97	2.96	2.89	2.94±0.05
75-80	2.55	0.78	1.94	1.76±0.90
80-85	1.24	0.36	1.22	0.94±0.50

Table 12. The esterase activity for renieramycin M hydrolysis activity of each crude protein/enzyme fraction.

% ammonium sulfate concentration	replicate	Average JA amount* (μmol) x10^{5}	Enzyme unit** (U) x10^{5}	Activity*** (U/mg) x10^{5}	Average activity ±SD (U/mg) x10^{5}
40-45	#1	0.23	0.26	0.64	0.92±0.28
	#2	0.33	0.37	0.92	
	#3	0.43	0.48	1.19	
45-50	#1	0.21	0.23	0.58	0.80±0.27
	#2	0.26	0.29	0.73	
	#3	0.40	0.44	1.10	
50-55	#1	0.77	0.85	2.13	2.12±0.23
	#2	0.68	0.75	1.88	
	#3	0.84	0.94	2.34	
55-60	#1	1.51	1.68	4.21	4.59±0.34
	#2	1.69	1.88	4.69	
	#3	1.75	1.95	4.87	
60-65	#1	2.37	2.63	6.58	6.58±0.07
	#2	2.34	2.60	6.50	
	#3	2.39	2.66	6.65	
65-70	#1	2.60	2.89	7.22	7.50±0.38
	#2	2.65	2.95	7.36	
	#3	2.85	3.17	7.93	
70-75	#1	2.19	2.44	6.10	5.56±0.70
	#2	2.09	2.32	5.81	
	#3	1.72	1.91	4.77	
75-80	#1	0.44	0.50	1.24	1.59±0.40
	#2	0.55	0.61	1.52	
	#3	0.73	0.81	2.03	
80-85	#1	0.02	0.02	0.05	0.09±0.09
	#2	0.01	0.02	0.04	
	#3	0.07	0.08	0.19	

*calculated from renieramycin M (RM) standard curve giving amount of jorunnamycin A (JA) equivalent to RM

**the enzyme unit (U) was calculated as production of the amount of JA (μmol) per minute. In this experiment, the assay was incubated for 90 minutes.

***Activity was calculated by the enzyme units divided by the amount of protein (mg). The protein amount of 0.4 mg was used in each reaction.
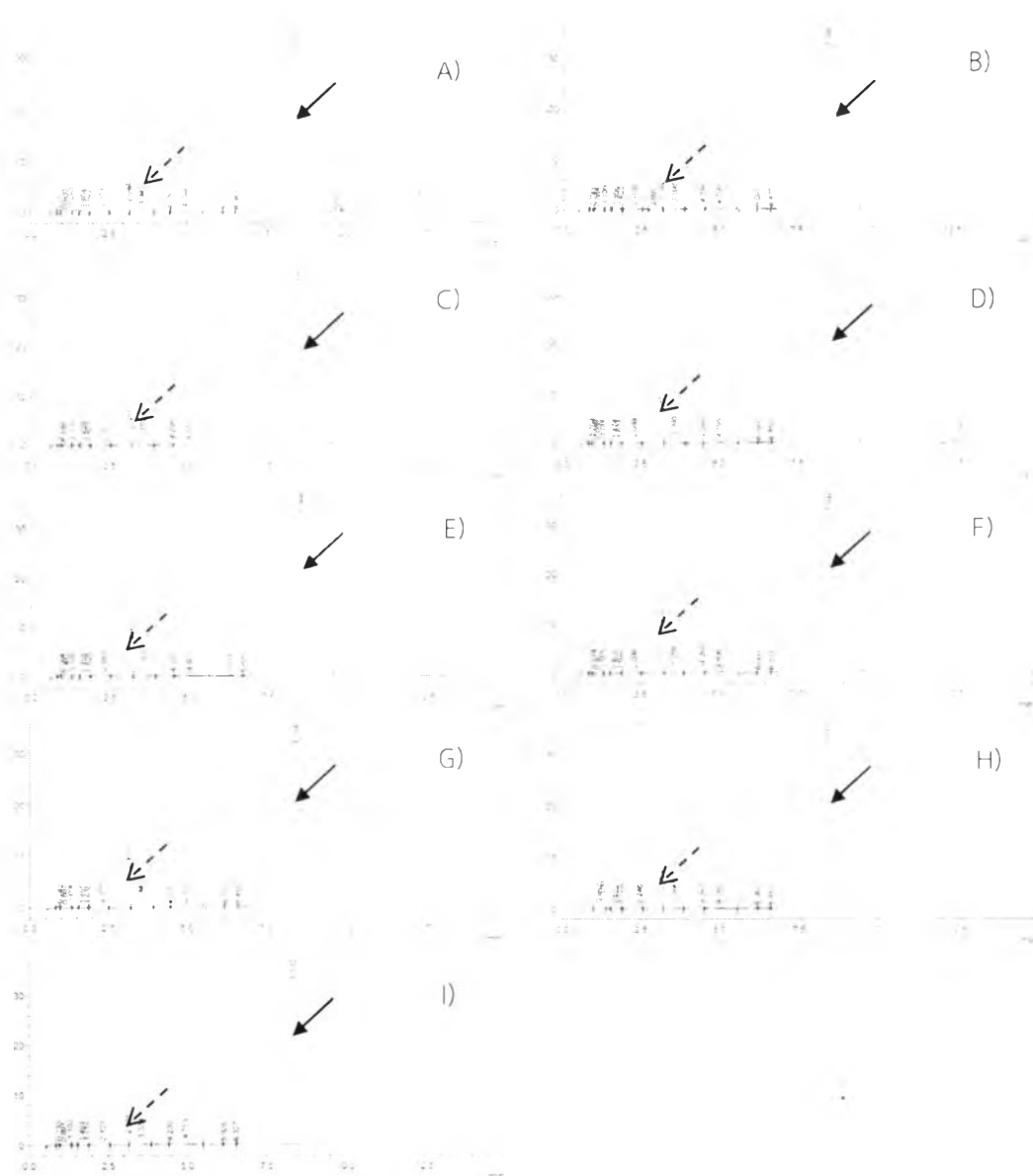
Figure 24. The HPLC chromatograms of jorunnamycin A (dashed arrows) and renieramycin M (black arrows). **A) – I)** represent each renieramycin M hydrolysis reaction by ammonium sulfate fractionated proteins at the concentration of 40-45%, 45-50%, 50-55%, 55-60%, 60-65%, 65-70%, 70-75%, 75-80%, 80-85% ammonium sulfate, respectively.
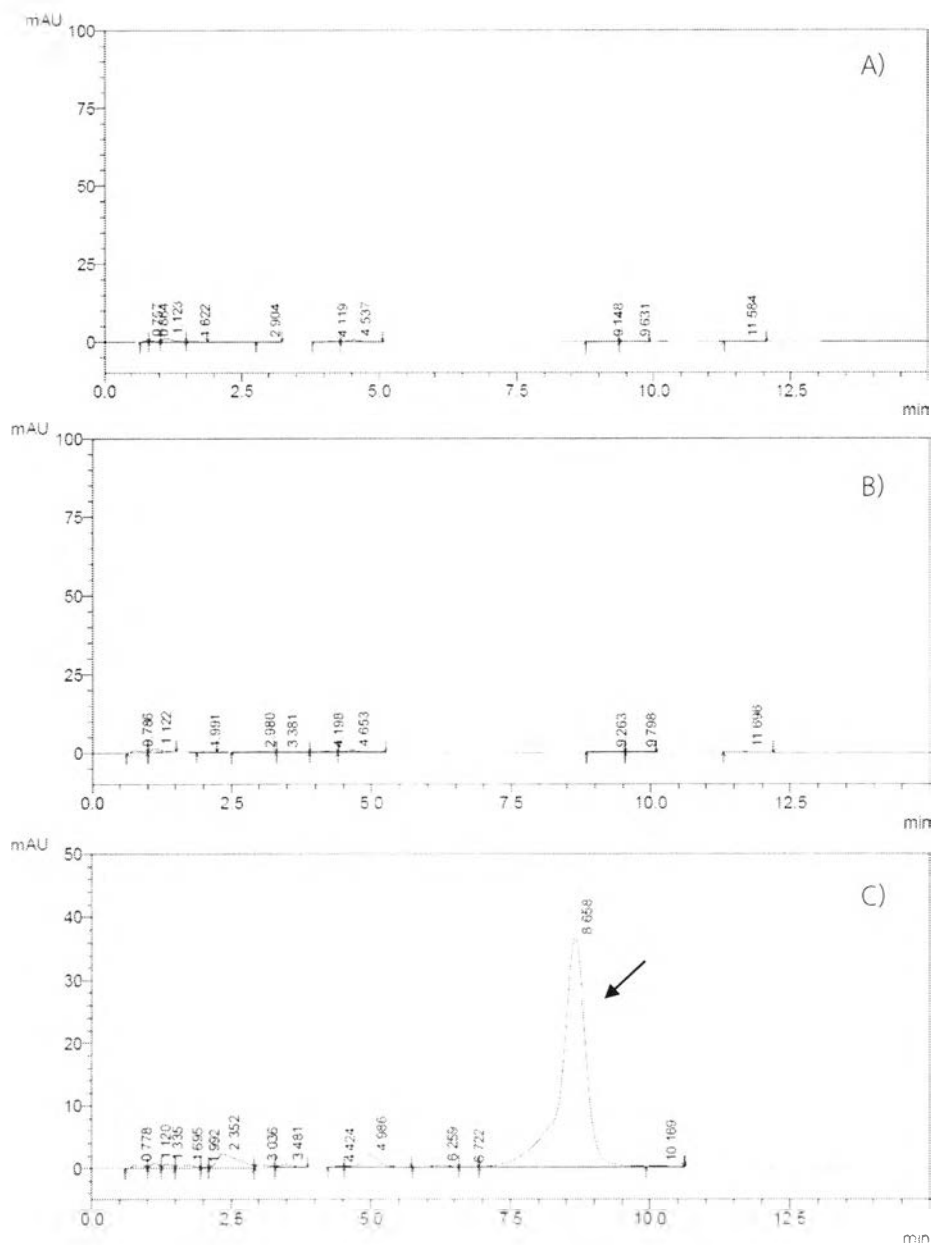
Figure 25. The HPLC chromatograms of **A)** Tris-HCl buffer used in reaction, **B)** boiled crude enzyme from visceral part of *J. funebris* and **C)** reaction of renieramycin M (black arrow) incubated with boiled crude protein.
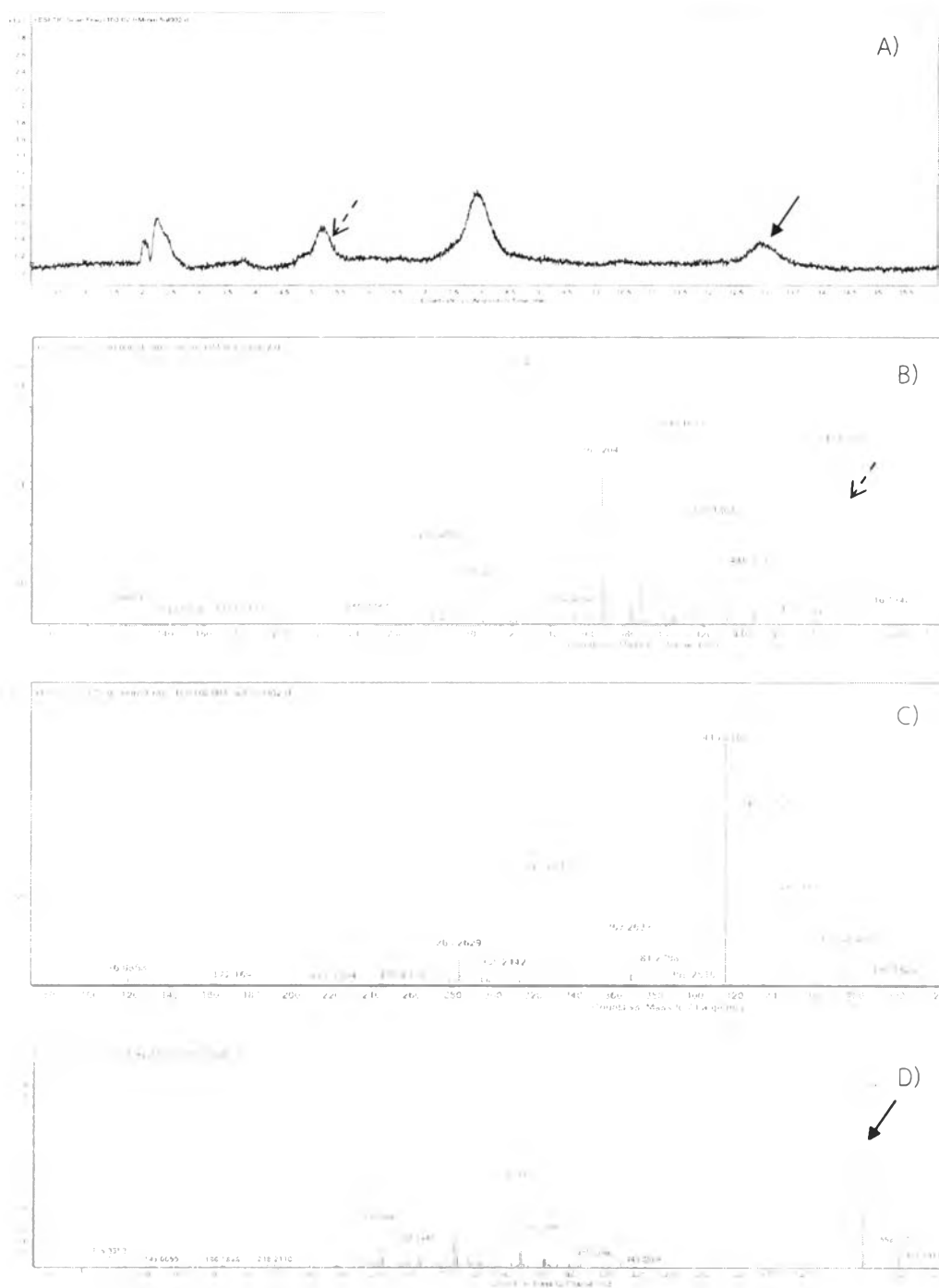
Figure 26. Mass spectra of liquid chromatography-mass spectrometry (LC-MS) analysis of renieramycin M hydrolysis reaction; **A)** LC chromatogram (70% MeOH in water as mobile phase, flow rate 0.5 mL/min), **B)** Mass spectrum of jorunnamycin A (JA) (R_t =5.190 min, dashed arrow), **C)** Mass spectrum of other compounds (R_t = 7.891 min), **D)** Mass spectrum of renieramycin M (RM) (R_t =12.961 min, black arrow).

Table 13. The esterase activity for renieramycin M hydrolysis under different incubation time and concentrations.

final concentration of RM (mM)	Time (min)	replicate	Average JA amount* (nmol)	Average JA amount (nmol)
0.025 mM	30	#1	1.09	0.86±0.22
		#2	0.84	
		#3	0.65	
	60	#1	1.55	1.52±0.15
		#2	1.35	
		#3	1.65	
	90	#1	1.90	2.13±0.22
		#2	2.14	
		#3	2.34	
	120	#1	2.14	2.20±0.16
		#2	2.09	
		#3	2.38	
0.05 mM	30	#1	1.12	1.23±0.12
		#2	1.21	
		#3	1.35	
	60	#1	1.99	2.0±0.10
		#2	1.86	
		#3	2.05	
	90	#1	2.76	2.94±0.20
		#2	3.16	
		#3	2.90	
	120	#1	3.99	3.58±0.40
		#2	3.18	
		#3	3.57	

*calculated from renieramycin M (RM) standard curve giving amount of jorunnamycin A (JA) equivalent to RM

Table 13. (Continued) The esterase activity for renieramycin M hydrolysis under different incubation time and concentrations.

final concentration of RM (mM)	Time (min)	replicate	Average JA amount* (nmol)	Average JA amount (nmol)
0.1 mM	30	#1	1.16	
		#2	1.03	1.14±0.10
		#3	1.23	
	60	#1	2.16	
		#2	2.25	2.18±0.07
		#3	2.12	
	90	#1	3.14	
		#2	3.31	3.24±0.09
		#3	3.26	
	120	#1	4.59	
		#2	4.45	4.73±0.37
		#3	5.148	

*calculated from renieramycin M (RM) standard curve giving amount of jorunnamycin A (JA) equivalent to RM
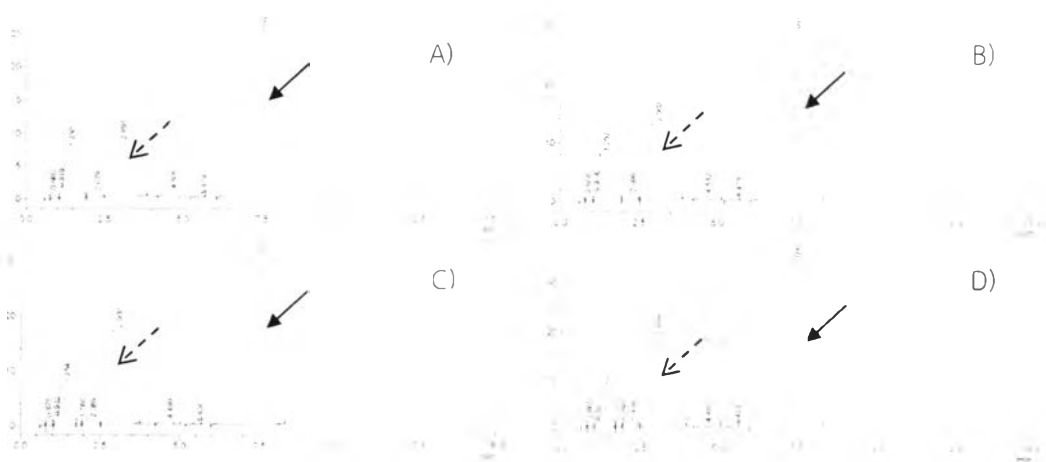

Figure 27. HPLC chromatograms of jorunnamycin A (dashed arrows) and renieramycin M (black arrows) of selected concentration of renieramycin M (0.05 mM) incubated for A) 30 minutes, B) 60 minutes, C) 90 minutes, and D) 120 minutes.

Table 14. The esterase activity for renieramycin M hydrolysis under different temperatures.

Temperature (°C)	replicate	Average JA amount* (µmol) $\times 10^3$	Enzyme unit** (U) $\times 10^5$	Activity*** (U/mg) $\times 10^5$	Average activity ±SD (U/mg) $\times 10^5$
20	#1	1.75	1.94	4.85	5.49±0.60
	#2	2.17	2.41	6.02	
	#3	2.01	2.23	5.58	
25	#1	2.62	2.91	7.29	6.70±1.02
	#2	1.99	2.21	5.53	
	#3	2.63	2.92	7.30	
30	#1	2.63	2.92	7.30	7.24±0.08
	#2	2.62	2.91	7.28	
	#3	2.58	2.86	7.15	
35	#1	2.95	3.28	8.20	7.91±0.34
	#2	2.71	3.01	7.53	
	#3	2.88	3.20	8.01	
40	#1	3.99	4.43	11.1	10.1±1.32
	#2	3.84	4.27	10.7	
	#3	3.10	3.45	8.62	
45	#1	3.92	4.36	10.9	11.4±0.44
	#2	4.22	4.68	11.7	
	#3	4.18	4.64	11.6	
50	#1	3.6	4.01	10.0	10.5±0.70
	#2	3.67	4.08	10.2	
	#3	4.08	4.53	11.3	
55	#1	1.41	1.57	3.93	4.14±0.19
	#2	1.54	1.71	4.27	
	#3	1.52	1.69	4.23	
60	#1	0.30	3.36	8.39	0.96±0.11
	#2	0.37	4.11	1.03	
	#3	0.37	4.11	1.03	

*calculated from renieramycin M (RM) standard curve giving amount of jorunnamycin A (JA) equivalent to RM

**the enzyme unit (U) was calculated as production of the amount of JA (µmol) per minute. In this experiment, the assay was incubated for 90 minutes.

***Activity was calculated by the enzyme units divided by the amount of protein (mg). The protein amount of 0.4 mg was used in each reaction.
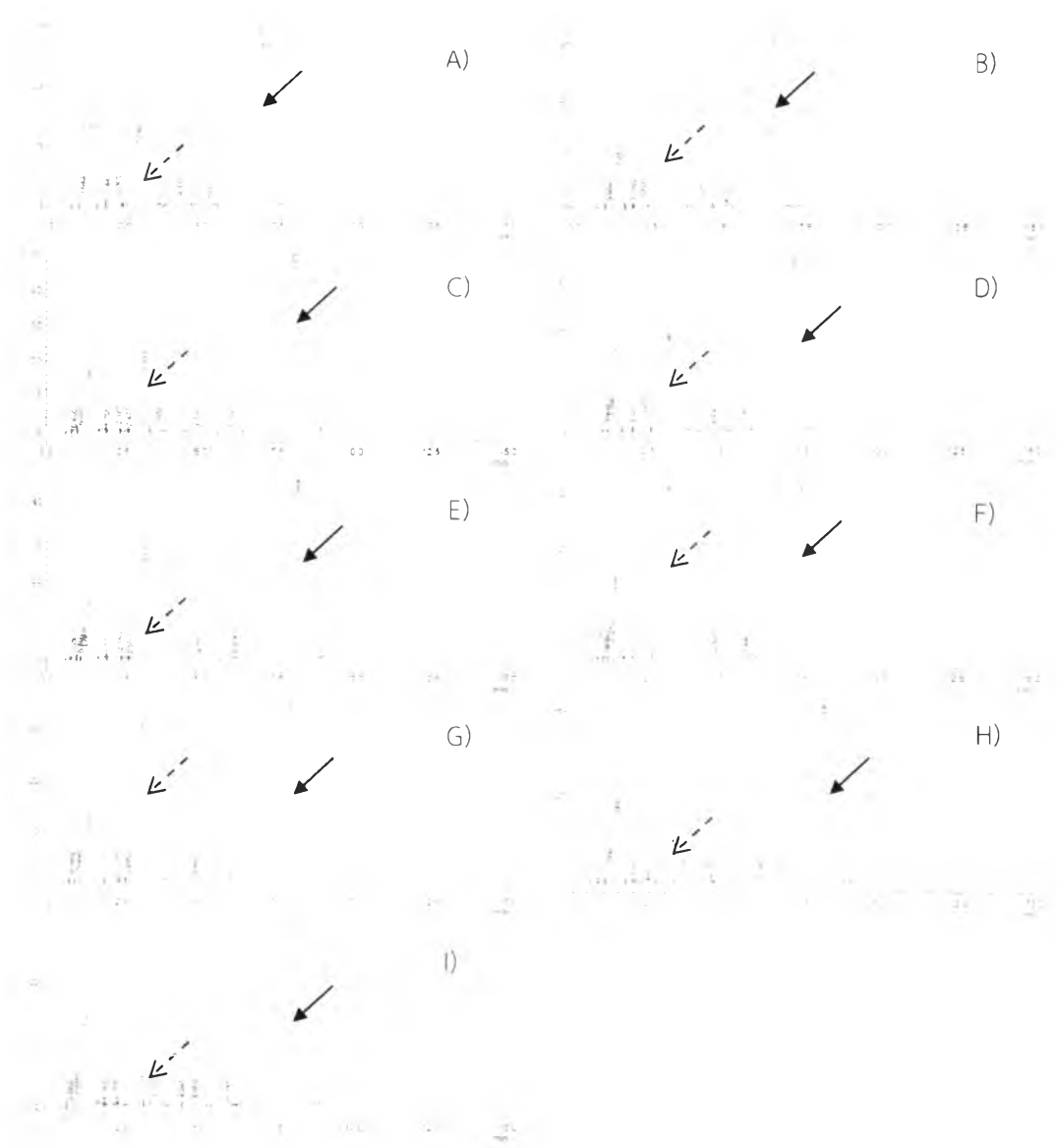
Figure 28. The HPLC chromatograms of jorunnamycin A (dashed arrows) and renieramycin M (black arrows). **A) – I)** represent each renieramycin M hydrolysis reaction under different temperatures at 20, 25, 30, 35, 40, 45, 50, 55 and 60°C, respectively.

Table 15. The esterase activity for renieramycin M hydrolysis under different pH values.

pH	replicate	Average JA amount* (μmol) x10^5	Enzyme unit** (U) x10^5	Activity*** (U/mg) x10^5	Average activity ±SD (U/mg) x10^5
7	#1	2.88	3.20	7.99	7.66±0.34
	#2	2.76	3.07	7.67	
	#3	2.63	2.92	7.31	
7.5	#1	2.73	3.04	7.60	7.67±0.18
	#2	2.84	3.15	7.88	
	#3	2.72	3.02	7.55	
8	#1	2.90	3.22	8.04	8.22±0.29
	#2	2.91	3.23	8.07	
	#3	3.08	3.42	8.55	
8.5	#1	3.09	3.43	8.57	8.49±0.12
	#2	3.08	3.42	8.54	
	#3	3.01	3.34	8.35	
9	#1	3.54	3.93	9.83	9.80±0.19
	#2	3.59	3.99	9.98	
	#3	3.45	3.84	9.59	
9.5	#1	5.11	5.68	14.2	14.0±0.33
	#2	5.08	5.64	14.1	
	#3	4.89	5.44	13.6	
10	#1	5.65	6.28	15.7	15.2±0.65
	#2	5.21	5.79	14.5	
	#3	5.58	6.20	15.5	
10.5	#1	4.81	5.34	13.3	14.0±0.59
	#2	5.05	5.61	14.0	
	#3	5.23	5.81	14.5	
11	#1	4.29	4.77	11.9	12.4±0.77
	#2	4.79	5.33	13.3	
	#3	4.34	4.82	12.0	
11.5	#1	2.83	3.14	7.85	8.96±0.98
	#2	3.48	3.87	9.67	
	#3	3.37	3.75	9.37	

*calculated from renieramycin M (RM) standard curve giving amount of jorunnamycin A (JA) equivalent to RM

**the enzyme unit (U) was calculated as production of the amount of JA (μmol) per minute. In this experiment, the assay was incubated for 90 minutes.

***Activity was calculated by the enzyme units divided by the amount of protein (mg). The protein amount of 0.4 mg was used in each reaction.
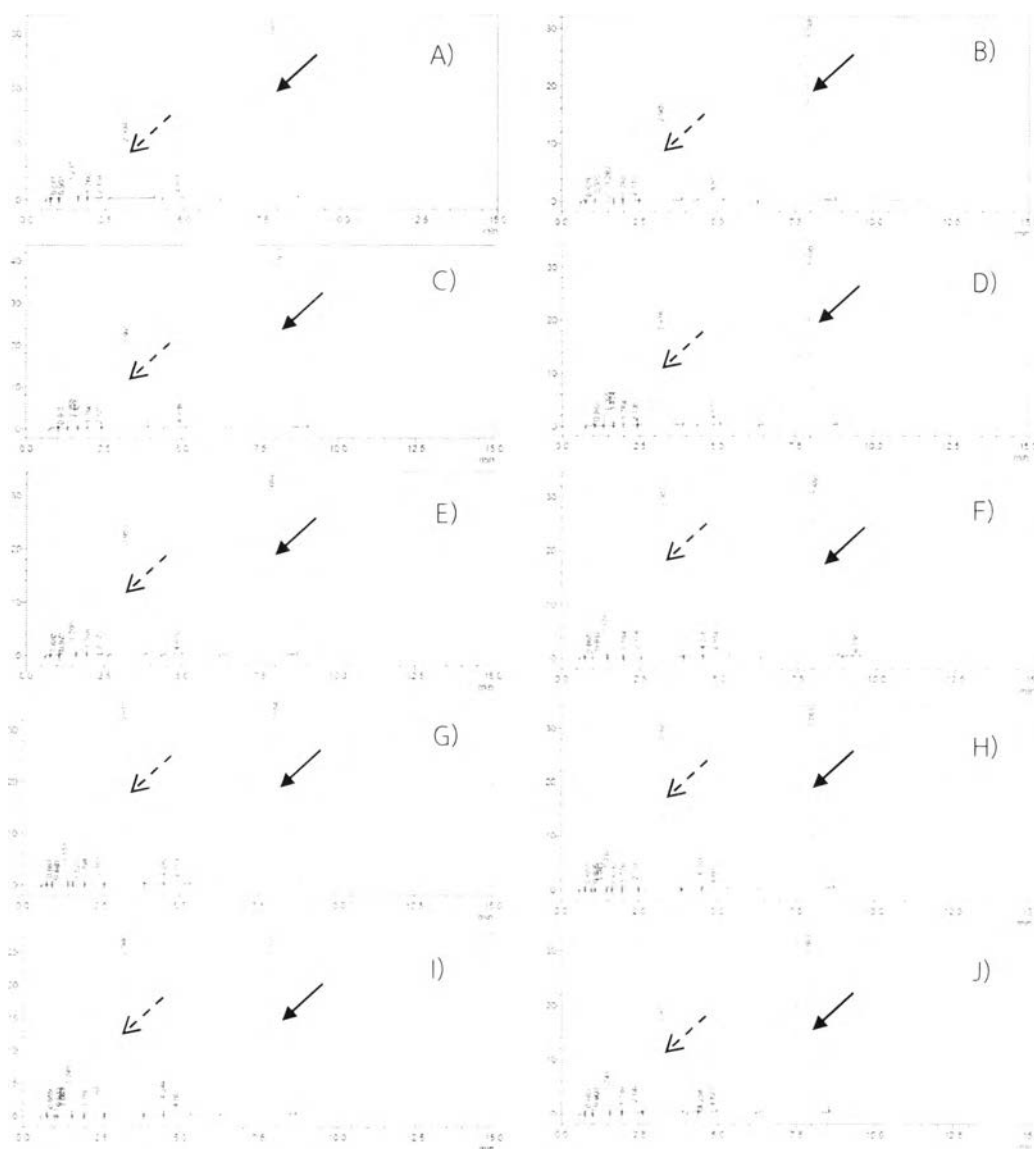
Figure 29. HPLC chromatograms of jorunnamycin A (dashed arrows) and renieramycin M (black arrows). **A) – J)** represent each renieramycin M hydrolysis reaction under different pH values at 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11 and 11.5, respectively.

Table 16. The esterase activity for renieramycin M hydrolysis under different types of buffer.

Buffer	replicate	Average JA amount* (μmol) $\times 10^3$	Enzyme unit** (U) $\times 10^{\cdot}$	Activity*** (U/mg) $\times 10^5$	Average activity ±SD (U/mg) $\times 10^{\varsigma}$
Tris-HCl	#1	2.86	3.18	7.94	8.86±1.50
	#2	2.90	3.22	8.06	
	#3	3.81	4.24	10.60	
Tricine	#1	8.44	9.38	23.4	25.2±1.53
	#2	9.35	10.4	25.9	
	#3	9.43	10.5	26.2	
Phosphate buffer solution	#1	4.20	4.67	11.7	11.6±0.11
	#2	4.21	4.67	11.7	
	#3	4.14	4.60	11.5	

*calculated from renieramycin M (RM) standard curve giving amount of jorunnamycin A (JA) equivalent to RM

**the enzyme unit (U) was calculated as production of the amount of JA (μmol) per minute. In this experiment, the assay was incubated for 90 minutes

***Activity was calculated by the enzyme units divided by the amount of protein (mg). The protein amount of 0.4 mg was used in each reaction.
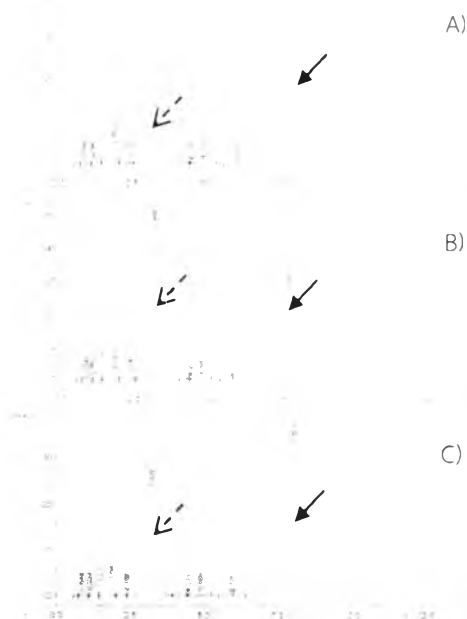

Figure 30. The HPLC chromatograms of jorunnamycin A (dashed arrows) and renieramycin M (black arrows) in reaction using different types of buffer A) Tris HCl, pH 8.0, B) Tricine, pH 8.0, C) Phosphate buffer solution (PBS), pH 8.0.

Table 17. Combination of various parameters used in renieramycin M hydrolysis reaction.

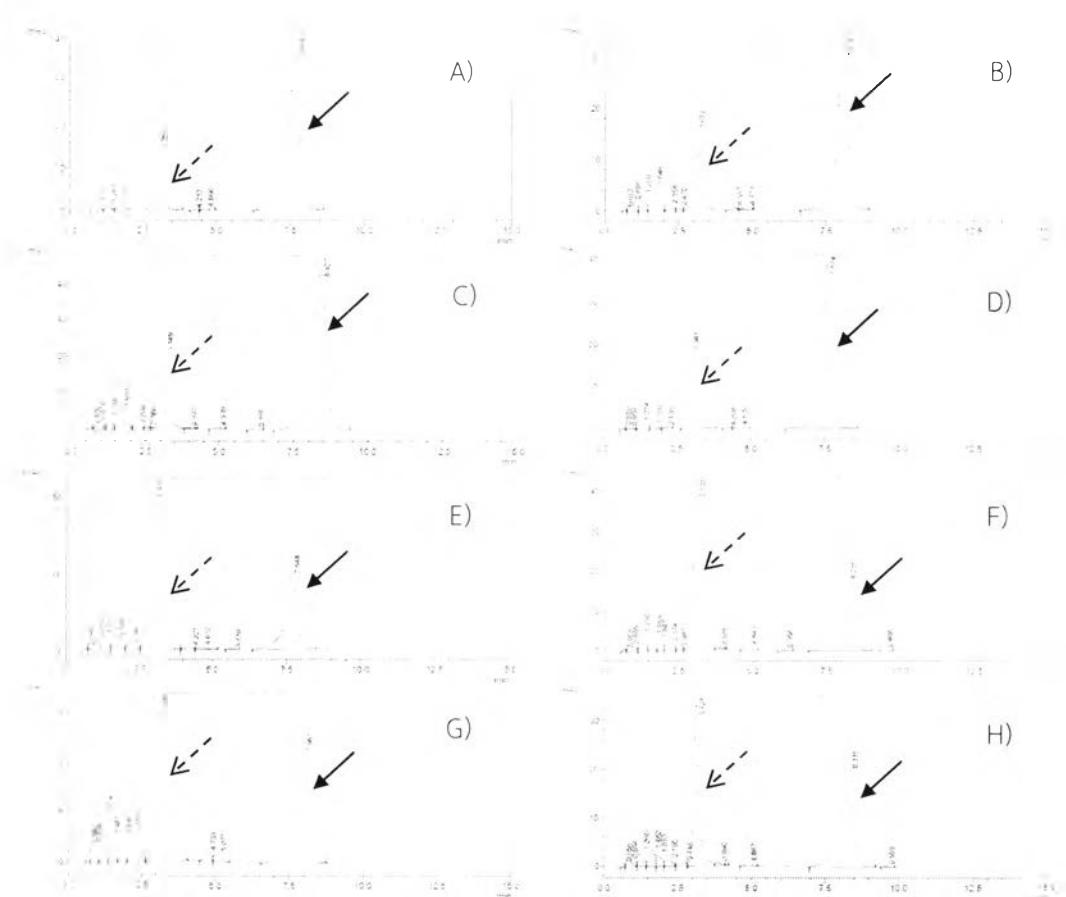| HPLC chromatogram	Assay condition	Parameters		
		Buffer type	pH	Temperature (°C)
A	Typical condition	50 mM Tris-HCl	8.0	25
B	#1	50 mM Tris-HCl	8.0	45
C	#2	50 mM Tris-HCl	10.0	25
D	#3	50 mM Tris-HCl	10.0	45
E	#4	50 mM Tricine	8.0	25
F	#5	50 mM Tricine	8.0	45
G	#6	50 mM Tricine	10.0	25
H	#7	50 mM Tricine	10.0	45

Figure 31. The HPLC chromatograms of jorunnamycin A (dashed arrows) and renieramycin M (black arrows). A) – H) represent each renieramycin M hydrolysis reaction under different conditions (Table 12), respectively.

Table 18. The esterase activity for renieramycin M hydrolysis under various conditions.

condition	replicate	Average JA amount* (μmol) $\times 10^3$	Enzyme unit** (U) $\times 10^5$	Activity*** (U/mg) $\times 10^5$	Average activity ±SD (U/mg) $\times 10^5$
Standard protocol	#1	2.33	2.60	6.50	6.67±0.20
	#2	2.48	2.75	6.89	
	#3	2.38	2.65	6.62	
#1	#1	2.70	3.00	7.49	11.2±3.22
	#2	4.77	5.30	13.3	
	#3	4.63	5.15	12.9	
#2	#1	4.93	5.47	13.7	13.1±2.09
	#2	3.87	4.30	10.8	
	#3	5.33	5.93	14.8	
#3	#1	3.16	3.51	8.78	8.24±0.79
	#2	2.64	2.93	7.33	
	#3	3.10	3.44	8.61	
#4	#1	8.93	9.93	24.8	25.7±0.74
	#2	9.43	10.5	26.2	
	#3	9.35	10.4	26.0	
#5	#1	7.43	8.26	20.6	21±1.03
	#2	7.96	8.84	22.1	
	#3	7.24	8.05	20.1	
#6	#1	3.17	3.53	8.82	8.75±0.39
	#2	3.28	3.64	9.10	
	#3	2.99	3.33	8.32	
#7	#1	5.28	5.86	14.7	14.7±1.10
	#2	5.67	6.30	15.8	
	#3	4.88	5.42	13.6	

*calculated from renieramycin M (RM) standard curve giving amount of jorunnamycin A (JA) equivalent to RM

**the enzyme unit (U) was calculated as production of the amount of JA (μmol) per minute. In this experiment, the assay was incubated for 90 minutes.

***Activity was calculated by the enzyme units divided by the amount of protein (mg). The protein amount of 0.4 mg was used in each reaction.

Table 19. Yield of the produced jorunnamycin A from reaction under various conditions.

condition	replicate	JA amount* (mol)	Percentage of JA compared to theoretical RM** in reaction	Average percentage ±SD
Standard protocol	#1	0.23398	22.4981	23.1±0.69
	#2	0.247909	23.83744	
	#3	0.238162	22.90015	
#1	#1	0.269747	25.93723	38.8±11.2
	#2	0.477143	45.87916	
	#3	0.463435	44.56104	
#2	#1	0.492523	47.35801	45.3±7.24
	#2	0.387419	37.25183	
	#3	0.533394	51.28792	
#3	#1	0.316243	30.40799	28.5±2.74
	#2	0.264041	25.3886	
	#3	0.309838	29.79212	
#4	#1	0.893301	85.89437	88.8±2.55
	#2	0.942822	90.65595	
	#3	0.934541	89.85968	
#5	#1	0.743244	71.46575	72.5±3.56
	#2	0.795747	76.51411	
	#3	0.724393	69.65316	
#6	#1	0.31749	30.52787	30.3±1.36
	#2	0.327654	31.50517	
	#3	0.299698	28.81715	
#7	#1	0.52773	50.74327	50.7±3.81
	#2	0.567404	54.55803	
	#3	0.48807	46.92979	

*calculated from renieramycin M (RM) standard curve giving amount of jorunnamycin A (JA) equivalent to RM

**Theoretical amount of RM substrate used in each reaction was 1.04 mol (600 ng/20 µL) per injection

Table 20. The esterase activity for renieramycin M hydrolysis of five tissue sample batches (Month/Year).

Tissue sample batch	replicate	Average JA amount* (µmol) $\times 10^3$	Enzyme unit** (U) $\times 10^5$	Activity*** (U/mg) $\times 10^5$	Average activity $\pm$SD (U/mg) $\times 10^5$
#1 Oct/2011	#1	1.10	1.22	3.06	2.94±0.26
	#2	0.95	1.05	2.63	
	#3	1.12	1.25	3.12	
#2 May/2012	#1	1.34	1.49	3.71	3.70±0.10
	#2	1.37	1.52	3.80	
	#3	1.29	1.44	3.59	
#3 Nov/2012	#1	1.65	1.83	4.58	4.61±0.03
	#2	1.67	1.85	4.63	
	#3	1.66	1.84	4.61	
#4 Jan/2013	#1	1.80	1.99	4.99	5.06±0.33
	#2	1.72	1.91	4.79	
	#3	1.95	2.17	5.42	
#5 Jun/2013	#1	2.53	2.81	7.01	7.11±0.10
	#2	2.60	2.88	7.21	
	#3	2.56	2.84	7.11	

*calculated from renieramycin M (RM) standard curve giving amount of jorunnamycin A (JA) equivalent to RM

**the enzyme unit (U) was calculated as production of the amount of JA (µmol) per minute. In this experiment, the assay was incubated for 90 minutes

***Activity was calculated by the enzyme units divided by the amount of protein (mg). The protein amount of 0.4 mg was used in each reaction.
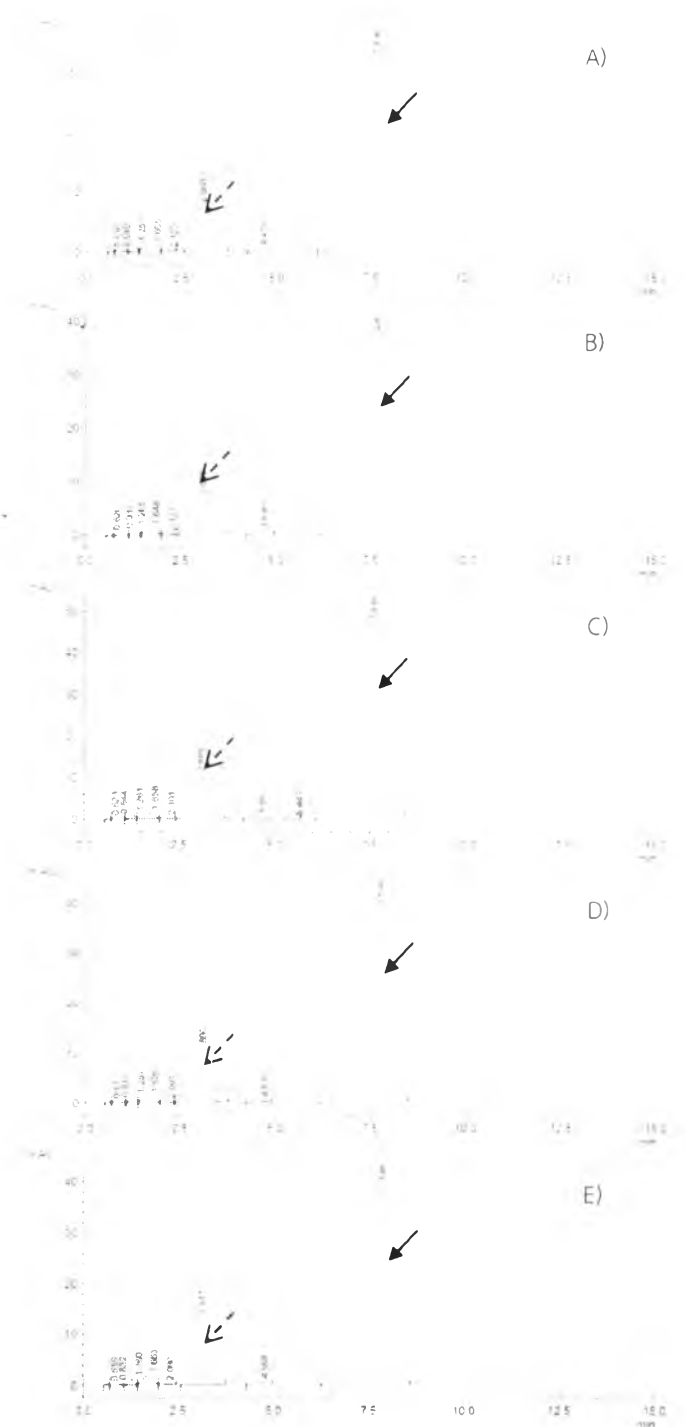
Figure 32. The HPLC chromatograms of jorunnamycin A (dashed arrows) and renieramycin M (black arrows) in reaction incubated with different batches of visceral proteins from *J. funebris*. **A) – E)** represent each renieramycin M hydrolysis reaction of batch #1-#5, respectively.

Table 21. The esterase activity of the crude enzyme stored in different period of time.

Time period (Months)	Temperature (°C)	replicate	Average JA amount* (μmol) x10^{-2}	Enzyme unit** (U) x10^{-5}	Activity*** (U/mg) x10^{-5}	Average activity ±SD (U/mg) x10^{-5}
0	4	#1	3.11	3.46	5.76	5.31±0.40
		#2	2.69	2.99	4.99	
		#3	2.80	3.11	5.19	
	-20	#1	3.14	3.49	5.82	5.47±0.32
		#2	2.92	3.24	5.41	
		#3	2.80	3.12	5.19	
	-80	#1	3.09	3.43	5.72	5.30±0.37
		#2	2.74	3.04	5.07	
		#3	2.76	3.06	5.11	
2	4	#1	1.06	1.18	2.94	3.03±0.10
		#2	1.12	1.25	3.12	
		#3	1.09	1.21	3.01	
	-20	#1	1.75	1.94	4.86	4.65±0.29
		#2	1.55	1.73	4.32	
		#3	1.72	1.91	4.77	
	-80	#1	1.80	2.00	5.01	4.97±0.05
		#2	1.77	1.97	4.92	
		#3	1.79	1.99	4.99	
4	4	#1	0.83	9.18	2.30	2.25±0.10
		#2	0.77	8.58	2.14	
		#3	0.83	9.21	2.30	
	-20	#1	1.40	1.55	3.89	4.04±0.23
		#2	1.41	1.57	3.93	
		#3	1.55	1.72	4.31	
	-80	#1	1.80	2.00	5.01	4.92±0.08
		#2	1.77	1.96	4.91	
		#3	1.75	1.94	4.85	

*calculated from renieramycin M (RM) standard curve giving amount of jorunnamycin A (JA) equivalent to RM

**the enzyme unit (U) was calculated as production of the amount of JA (μmol) per minute. In this experiment, the assay was incubated for 90 minutes.

***Activity was calculated by the enzyme units divided by the amount of protein (mg). The protein amount of 0.4 mg was used in each reaction.
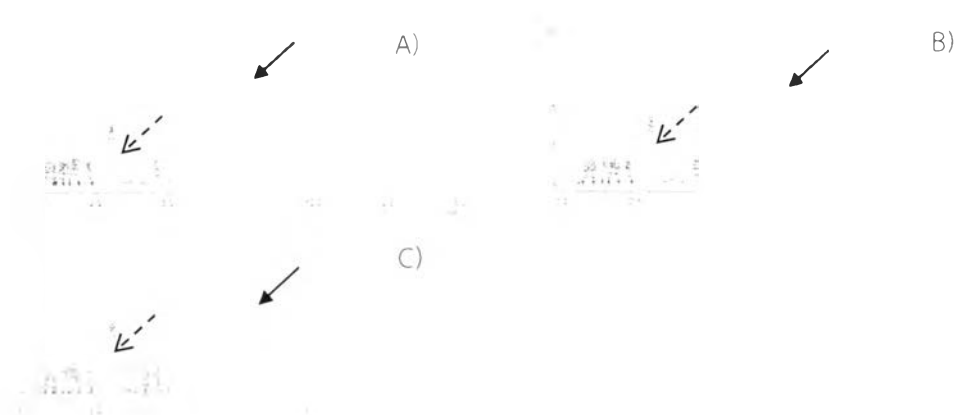
Figure 33. The HPLC chromatograms of jorunnamycin A (dashed arrows) and renieramycin M (black arrows). **A)-C)** represent reaction at 4, -20, -80 °C at starting time (0 month), respectively.
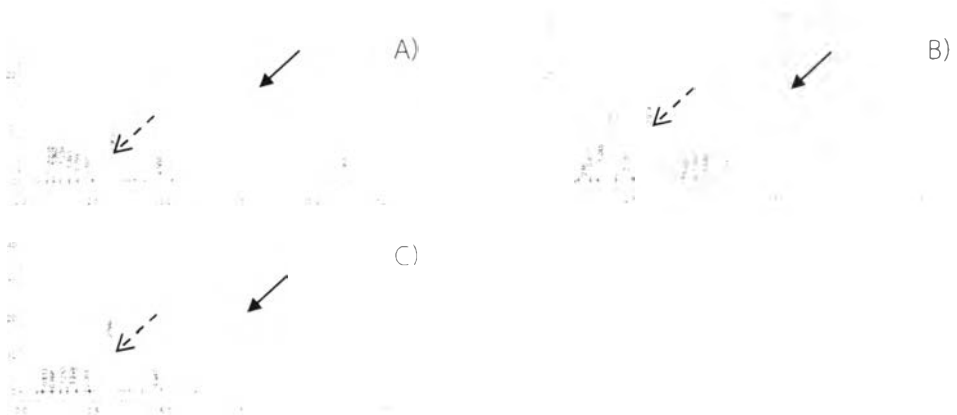

Figure 34. The HPLC chromatograms of jorunnamycin A (dashed arrows) and renieramycin M (black arrows). **A)-C)** represent reaction at 4, -20, -80 °C after 2 months of storage, respectively.
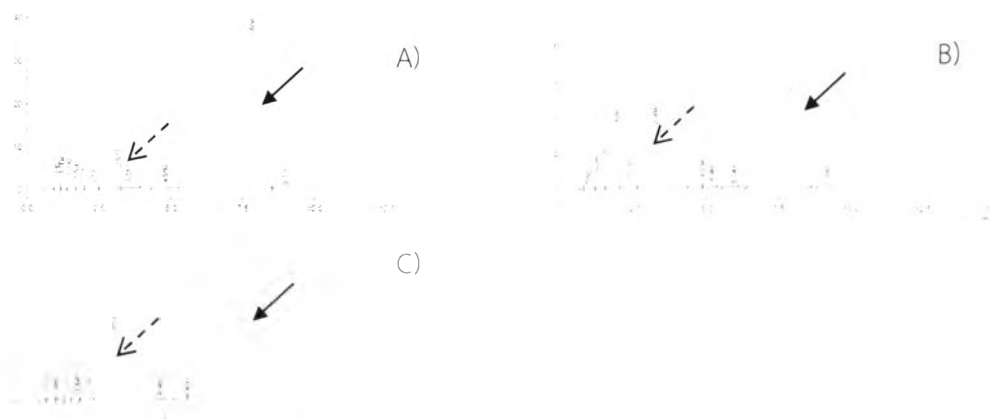
Figure 35. The HPLC chromatograms of jorunnamycin A (dashed arrows) and renieramycin M (black arrows). **A)-C)** represent reaction at 4, -20, -80 °C after 4 months of storage, respectively.
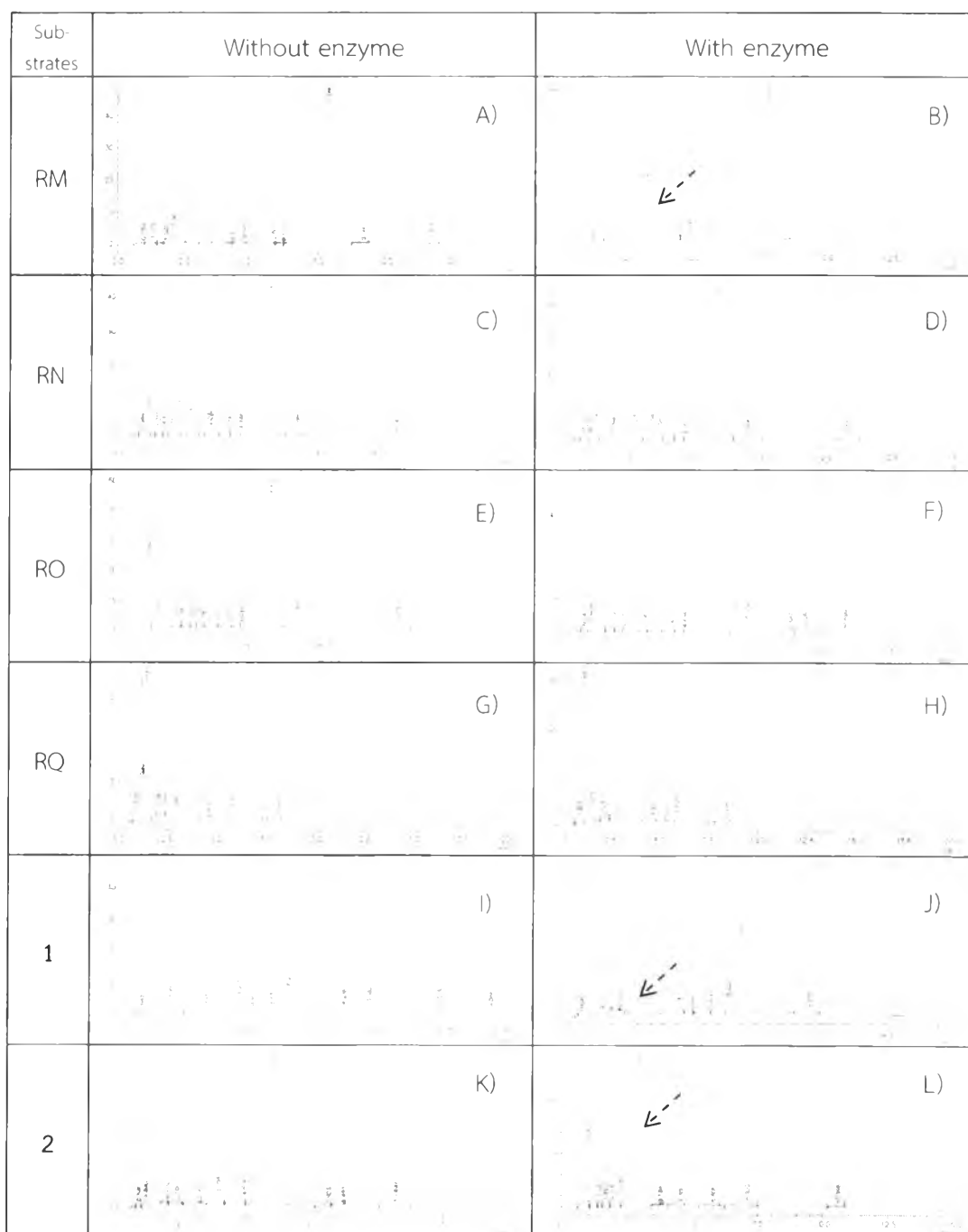
Figure 36. The HPLC chromatograms of hydrolysis reaction by incubated various substrates without/with crude enzyme. Dashed arrows show the peak of jorunnamycin A. **A) – L)** represent each hydrolysis reaction with different substrates.

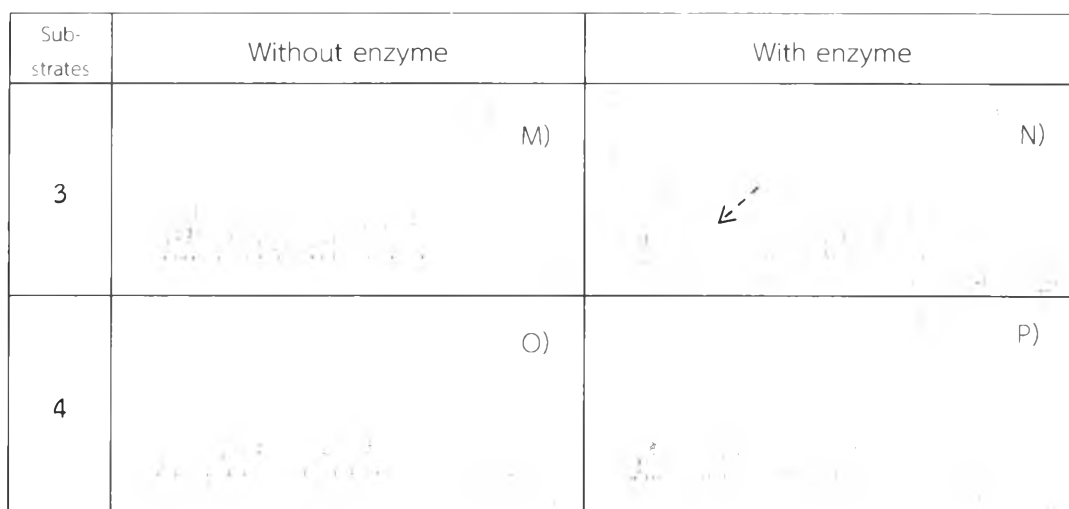| Sub-strates	Without enzyme	With enzyme
3	M)	N)
4	O)	P)

Figure 36. (continue) The HPLC chromatograms of hydrolysis reaction by incubated various substrates without/with crude enzyme. Dashed arrow shows the peak of jorunnamycin A. **M) – P)** represent each renieramycin M hydrolysis reaction with different substrates.

Table 22. The esterase activity for renieramycin M hydrolysis using renieramycin M (RM), 2, and 3 as substrates.

compound	replicate	Average JA amount* (μmol) $\times10^{-}$	Enzyme unit** (U) $\times10^{-5}$	Activity*** (U/mg) $\times10^{5}$	Average activity $\pm$SD (U/mg) $\times10^{5}$
RM	#1	2.57	2.86	7.15	6.70$\pm$0.40
	#2	2.31	2.57	6.42	
	#3	2.35	2.61	6.52	
1	#1	0.14	0.16	0.39	0.31$\pm$0.03
	#2	0.12	0.13	0.32	
	#3	0.13	0.15	0.37	
2	#1	13.4	14.8	37.1	37.4$\pm$0.33
	#2	13.4	14.9	37.3	
	#3	13.6	15.1	37.7	
3	#1	1.74	1.93	4.83	5.03$\pm$0.21
	#2	1.81	2.01	5.02	
	#3	1.89	2.10	5.24	

*calculated from renieramycin M (RM) standard curve giving amount of jorunnamycin A (JA) equivalent to RM
**the enzyme unit (U) was calculated as production of the amount of JA (μmol) per minute. In this experiment, the assay was incubated for 90 minutes.
***Activity was calculated by the enzyme units divided by the amount of protein (mg). The protein amount of 0.4 mg was used in each reaction.

VITA

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Poster presentation

Waropastrakul, D., Suwanborirux, K., De-Eknamkul, W., and Chuanasa, T. Detection of esterase activity converting cytotoxic renieramycin M to jorunnamycin A in the crude enzyme of the nudibranch Jorunna funebris. Proceeding of the 30th Annual Research Conference in Pharmaceutical Sciences, December 6, 2013. Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. p. 41-44.