

MUTAGENIC STUDY OF 8-APM

K. NARUI

Ajinomoto Co., Inc.

Mutagenic Study of β -APM

I. Summary

β -APM was examined for mutagenic activities in microbial systems such as reverse mutation assays using *Salmonella typhimurium* strains (TA98, TA100, TA1535, TA1537 and TA1538) and *Escherichia coli* strains (WP₂uvrA) and "Rec-assay" using *Bacillus* strains (H17Rec⁺ and M45Rec⁻).

β -APM was not mutagenic in the bacterial system used.

II. Experimental Procedure

1) Reverse Mutation Assay

Mutagenicities were tested by a modification (Nagao et al., 1977) of the Ames method (Ames et al., 1975). For each experiment, an inoculum from the stock cultures was grown overnight at 37°C in nutrient broth. A mixture of the test substance in 0.1 ml of DMSO or aqueous (β -APM or control substance), 0.5 ml of S-9 Mix or 0.1 M Sodium phosphate buffer (PH 7.4) and 0.1 ml of a culture of bacterial tester strain was incubated at 37° and shaken at the same time for 20 minutes. After 2 ml of Soft Agar at 45°C were added, the mixture was poured over minimal glucose agar plates. With the incubation for 2 days at 37°C, revertant colonies on the plates were counted. Two plates were used for each dose.

2) Rec-Assay

Rec-Assay was tested by the method of Kada et al. (Kada et al., 1976, 1980). Strains H17Rec⁺ and M45Rec⁻ were grown overnight in B₂ broth (meat extract 10g, NaCl 5g, distilled water 100 ml, PH to 7.0). 1 ml of 50% glycerol (w/v) and 3 ml of fully grown bacterial broth culture were combined and stored at -85°C. On the very day of these experiments, each culture was thawed and streaked on the "dry" surface of the broth agar plate. The paper disk (diameter: 16 mm) which contains the test material solution (40 μ l) was placed over each starting point of the streaks. The plates were kept

at 4-5°C for 24 hours, and then incubated at 37°C for about 20 hours. The length of the inhibition zone was then measured.

III. Materials

- 1) Test Materials: β -APM Lot No. 830708
(β -L-Aspartyl-L-phenylalanine Methyl Ester)
- 2) Control Materials
 - (1) Positive Control
 1. AF-2; Furyl furamide
2-(2-furyl)-3-(5-nitro-2-furyl) acryl amide
 2. ENNG; N-Ethyl-N-nitrosoguanidine
 3. 2-NF; 2-Nitrofluorene
 4. 9-AA·HCl; 9-Amino acridine hydrochloride
 5. 2-AA; 2-Amino anthracene
 6. Mitomycin
 - (2) Negative Control
 - Kanamycin sulfate
 - (Solvent)
 - DMSO; Dimethyl sulfoxide
 - Distilled water

TABLE 1. Results of Reverse Mutation Assays, Nonactivated.

Compound $\mu\text{g}/\text{plate}$	Revertants/Plate					
	Base Pair Change Type Strains			Frame Shift Type Strains		
	TA100	TA1535	WP ₂ uvrA	TA98	TA1537	TA1538
B-APM 20000	152	4	32	34	9	22
10000	142	4	36	33	9	27
5000	142	3	41	33	8	23
1000	150	5	35	41	9	25
500	146	4	36	37	8	24
100	149	6	39	34	8	19
50	149	4	44	40	9	22
10	152	4	34	39	8	22
Negative Control:						
DMSO (Solvent)	¹⁵² 159	4	37	35	9	20
Distilled Water	¹⁵⁸ 169	4	29	37	7	24
Positive Control:						
AF-2 0.1			1632	308		
0.05	1056		441	244		
0.02	691					
ENNG 4		884				
2		67				
9-AA·HCl 100					363	
80					294	
2+NF 5						984
2						467

Note: Each figure of revertants is always given as a mean of 2 plates.

TABLE 2. Results of Reverse Mutation Assays, Activated.

Compound $\mu\text{g}/\text{plate}$		Revertants/Plate					
		Base Pair Change Type Strains			Frame Shift Type Strains		
		TA100	TA1535	WP ₂ uvrA	TA98	TA1537	TA1538
β -APM	20000	162	7	37	68	11	49
	10000	152	7	33	72	11	48
	5000	160	11	37	68	14	54
	1000	155	7	37	76	12	53
	500	150	9	32	78	13	56
	100	162	7	42	76	12	56
	50	147	6	40	84	10	52
	10	145	7	34	75	10	61
Negative Control:							
DMSO (Solvent)		161	8	34		11	53
Distilled Water		169	10	39		9	50
Positive Control:							
2-AA	10		446	777			
	5		300	561			
	2					317	
	1	612			560	91	656
	0.5	308			248		196
	0.2						

Note: Each figure of revertants is always given as a mean of 2 plates.

TABLE 3. Results of Rec-Assay

Compound	$\mu\text{g/ml}$	Inhibition Zones (mm)		Rec-Effect	Conclusion
		H17 (Rec ⁺)	M45 (Rec ⁻)		
β -APM	200	0	0	0	-
	100	0	0	0	-
	50	0	0	0	-
Negative Control:					
Kanamycin sulfate					
	200	8.3	9.3	1.0	-
	100	5.3	6.5	1.2	-
Distilled Water					
		0	0	0	-
DMSO (Solvent)					
		0	0	0	-
Positive Control:					
Mitomycin C					
	20	8.3	22.0	13.7	+
	10	3.7	21.1	17.4	+

Note: The symbol (-) indicates negative of mutagenicity. The symbol (+) indicates positive of Mutagenicity.

TABLE 4. Summary of Mutagenicity Assays

Strains	Reverse Mutation Assays												Rec-Assay
	Base Change Type						Frame Shift Type						H17 (Rec ⁺) M45 (Rec ⁻)
	TA100		TA1535		WP ₂ uvrA		TA98		TA1537		TA1538		
Metabolic Activation System	-S ₉	+S ₉	-S ₉	+S ₉	-S ₉	+S ₉	-S ₉	+S ₉	-S ₉	+S ₉	-S ₉	+S ₉	-S ₉
β-APM	-	-	-	-	-	-	-	-	-	-	-	-	-

Note: The symbol (-) indicates negative of mutagenicity.