GENETIC ACTIVITY OF CERTAIN CHEMICALS IN SALMONELLA-MICROSOMAL SCREENING SYSTEM

SHEIKH A. RASOOL*
ROOHI MUSHTAQ*

SUMMARY: The Ames Salmonella typhimurium microsomal screening system was standardized and six alkaloids and a mosquito repellent were screened for their mutagenic potential. The results indicated that mosquito repellent and all the six (buxenone, ephedradine, protopine, saponin, pleiocarpamine and vindoline) alkaloids exhibited mutagenic activity (based on his $^-$ → his$^+$ reversions, in TA98 tester strain. In addition, vindoline also induced reversion mutations in TA100.

Key Words: Ames test, salmonella, microsomal system, alkaloids.

INTRODUCTION

The Salmonella microsomal test system (1,2) is being used extensively for screening a variety of substances for their genetic activity. The test monitors reversion mutations in a battery of specially constructed mutant strain of *Salmonella typhimurium* LT2. A mammalian (usually of rat) liver homogenate is added to the bacterial suspension as an approximation of mammalian metabolism (3). Over 80% of the organic carcinogens tested so far have been detected as mutagens in this system (4). Since many carcinogens induce mutations in Salmonella microsomal Muta test, it is widely used both as an indicator of mutagenicity and carcinogen city (1).

This paper presents results of the genetic activity of a mosquito repellent and six alkaloids in Ames standard tester strain.

MATERIALS AND METHODS

Chemicals

Mosquito repellent (a green colored coil) was produced from the local market while all the alkaloids were obtained from HEJ Institute of Chemistry, University of Karachi, Karachi, Pakistan. Structural formulae of all the 6 alkaloids are given in Figures 1-6 (5). Nitrosoguanidine (MNNG) (Fluka) was used as the control mutagen. List of all the chemicals (along with their solvents) is given in Table 1.

Tester strains

Salmonella typhimurium tester strains were requested from Dr. Ames of the University of California, Berkeley, USA. All the strains were grown on 'Oxoid' media. The tester strains were checked for different requirements and characteristics according to the methods given by Maron and Ames (3).

Preparation of rat-liver S9 fraction and mix

S9 ($9x10^3$) fraction was obtained from phenobarbitone (Pakistan Pharmaceutical Industry)-Induced rats (a gift from PCSIR Labs., Karachi) according to the method described by Garner *et al.* (6). S9 mix was prepared according to the recipe recommended by Maron and Ames (3) 0.1 ml per plate of the high S9 mix was used in the experiment. The standard direct plate incorporation method was followed (3).

Overlay mutagenesis

For such assays, the following were added in sequence: 2 ml of melted top agar, 0.1 ml of an overnight broth culture, the test compound (500 μg per plate) or the control mutagen (10 μg per plate). The contents were mixed gently and poured onto the minimal medium (7) plates and distributed uniformly. The plates for spontaneous reversions did not contain any chemical. After 60 hours incubation, revertants to histidine prototrophy in the control and test compound plates were scored in the light background lawn of bacterial growth. The background was due to biotin and trace amounts of histidine. The trace amounts of histidine in the top agar enable bacterial strains on the plates to pass through several initial divisions which are essential for many of the mutagens (3). Spontaneous revertants were also scored.

RESULTS

Table 2 shows the induced reversion mutation rate by mosquito repellent. Results of induced his$^-$ → his$^+$ reversion mutations by the alkaloids are presented in Table 3.

* From Department of Microbiology, University of Karachi, Karachi-75270, Pakistan.

Table 1: List of chemicals with their solvents.

S. No.	Chemical	Solvent	Source
1	Mosquito repellent	Distilled water	Local market
2	Buxenone	Methanol	HEJ, Institute of Chemistry, University of Karachi
3	Ephedradeine	Ethanol	
4	Protopine	Methanol	
5	Saponin	Ethanol	
6	Pleiocarpamine	Acetone	
7	Vindoline	Ethanol	
8	MNNG	Citrate phospate buffer (pH6)	Fluka

It is clear that mosquito repellent and the alkaloids are potential mutagens for some of the tester strains.

DISCUSSION

The effect of different alkaloids and a mosquito repellent on the reversion mutability of Ames tester strains has been presented in this paper. It is clear that the spontaneous reversion values fall within the standard value ranges (Table 2). However, the range of spontaneous revertants may vary from experiment to experiment, season or laboratory to laboratory (3). MNNG was used as control mutagen for mutation induction in TA100 and the results agree with the earlier findings (8). Mosquito repellent, a widely used chemical has shown increased mutation frequency only in tester strain TA 100 (only after activation with S9 mix). Thus, the chemical constituents of mosquito repellent have been able to induce base pair substitutions at one of the GC pairs (9). Six different alkaloids were also screened for their mutagenic activity.

Thus, buxenone has induced reversion mutations (without and with microsomal activation seven and eighteen times respectively of spontaneous reversions) in TA98 which detects frame shifts. Rasool *et al.* (2) have observed similar results by trimethoprim. Similarly, ephedradine has also reverted TA98 and TA100 tester strains. Interestingly, this alkaloid has induced mutations only after microsomal activation thereby indicating that ephedradine is a pro-mutagen which is converted into a potential carcinogen after activation. Thus, ephedradine is a mutagen. Similar results were obtained by Rasool *et al.* (10) in the case of snuffs. Protopine and pleiocarpamine have also induced frame shift mutations in TA98 without and with microsomal activation. The reversion rate in TA98 strain (with S9) by both of these alkaloids 10 times more compared with the spontaneous values where as 4 fold increase was observed without S9 mix. It is understood that both of the alkaloids are primarily mutagenic which after activation tend to exert more dynamic mutational potential (11). Saponin, another alkaloid induces frame shift mutation in TA97a and TA98 strains only after activation by S9 mix. A known mutagen benzo (α) pyrene (which also requires similar activation) also behaves alike (2, 12). Vindoline has induced reversions in TA98 both without and

Table 2: Induced reversion (per plate) of tester strains by mosquito repellent.

Particulars	Strains									
	TA97a		TA98		TA100		TA102		TA104	
	$S9^-$	$S9^+$	$S9^-$	$S9^+$	$S9^-$	$S9^+$	$S9^-$	$S9^+$	$S9^-$	$S9^+$
Spotaneous reversions	144	153	31	36	114	187	199	241	306	470
MNNG (10 μg per plate) induced reversion	-	-	-	-	a*	a*	-	-	-	-
Mosquito repellent induced reversions 500 μg/plate	48	100	0	11	245	a*	0	0	152	198

* Innumberable

Table 3: Comparative values of revertans ($his^- \rightarrow his^+$) induced by the alkaloids (500 μg/plate) in the tester strains (without and with microsomal activation) (spont neous reversion values were subtracted).

Particulars	Strains					
	TA97a		TA98		TA100	
	$S9^-$	$S9^+$	$S9^-$	$S9^+$	$S9^-$	$S9^+$
Spotaneous reversions	144	153	31	36	114	187
MNNG (10 μg per plate)	-	-	-	-	a*	a*
Buxenone	92	157	171	528	27	130
Ephedradeine	0	a*	81	778	143	461
Protopine	0	103	105	306	88	124
Saponin	12	1202	39	365	1	50
Pleiocarpamine	0	0	12	314	0	0
Vindoline	0	104	1560	a*	0	1818

* Innumberable

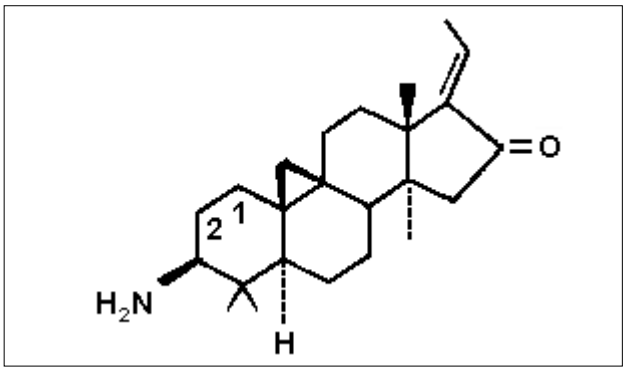

Figure 1: Buxenone.

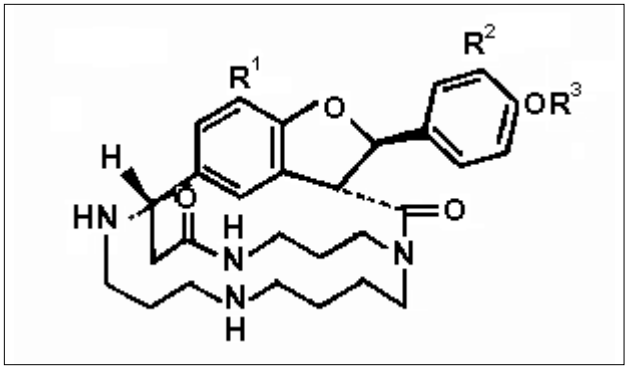

Figure 2: Exhedradine.

with S9 mix activation, whereas microsomal activation is required to induced reversions in TA100 by this alkaloid.

It is interesting to study the chemistry of naturally occurring products because many of these have been used as chemotherapeutics. The mutagenicity of these

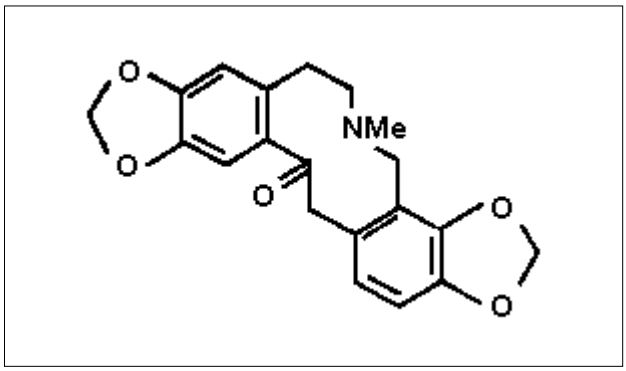

Figure 3: Protopine.

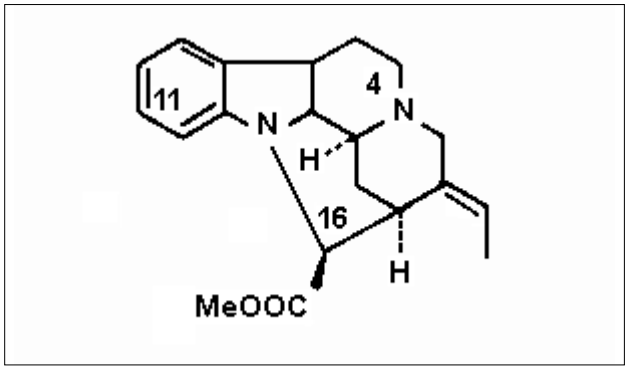

Figure 4: Pleiocarpamine.

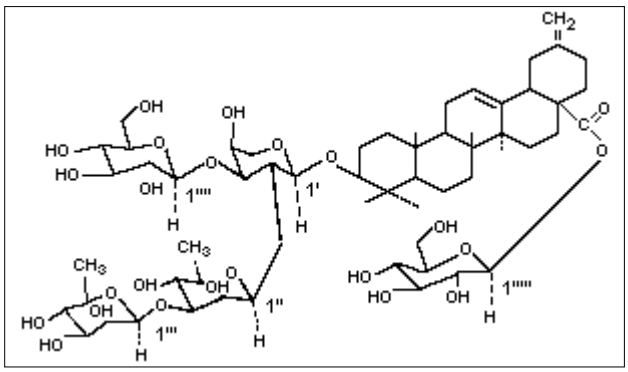

Figure 5: Saponin.

alkaloids has also been determined on the basis of resonance due to the carbonyl group, double bond, nitrogen lone pair or atomic ring and due to compounds having other structures like indole, isoquinoline or aporphine. The presence of sugarmoeity in some of the compounds (e.g. saponin) has also been the cause of reversion mutation induction in the present studies. The structure of ephedradine (Figure 2) reveals the presence of amidic and phenolic groups and cyclic ether. It is already known that amidic group mimics the enol group for the causation of mutations (11). Thus, the presences

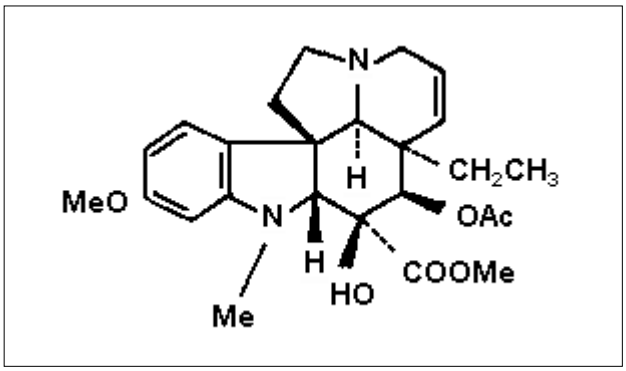

Figure 6: Vindoline.

of resonance in amide group could be responsible for the observed mutagenicity by ephedradine. The structure of vindoline carries an acetate group, a carbon with direct attachment with hydroxyl, carbomethoxyl group and an indole ring. It is well understood that these functional groups (in single or combination) are responsible for mutagenesis (12). Hence vindoline is a valid potential double action mutagen. Protopine has induced $his^- \rightarrow his^+$ reversions in TA98 (without and with S9 mix) only. This alkaloid has an isoquinoline ring, in which benzene ring is attached with 2-oxygen atoms and forms ether linkage. These oxygens have lone pairs which can increase the total resonance with benzene ring. Thus, the resonance plays role in increasing the mutagenicity (14). Buxenone also induced reversions in TA98 presumably due to the presence of enone type resonance in ring D (15). The molecular structure of saponin has an ester position C-28 of terpenoide, which may show resonance with two different double bonds at different positions, thereby fulfilling the criteria for a powerful mutagen (16). Thus, mutation causation by saponin in our assays seems valid .

To conclude, the Ames test is an efficient screening system for genetic activity as 85±5% of the carcinogens have been proved to be mutanges in this system (17).

REFERENCES

1. Ames BN, Durston WE, Yamasaki E, Lee FD : Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proc Natl Acad Sci, 70:2281-2285, USA, 1973.

2. Khan SAMA, Alvi AZ, Umer MN : Genetic activity of trimethoprim in Salmonella/microsomal screening system. Mutation Res, 188:197-200, 1987.

3. Maron DM, Ames BN : Revised methods for Salmonella mutagenicity test. Mutation Res, 113:173-215, 1983.

4. Ames BN, McCann J : Validation of the Salmonella test. A reply to Rinkus and Legator. Cancer Res, 41:4192-4196, 1981.

5. Southern IW, Buckingham J : In: Dictionary of Alkaloids, vol 69, Ed by GA Cordell, JE Saxton and M Shamma, Chapman and Hall, London, 1989.

6. Garner RC, Miller EC, Miller JA : Liver microsomal metabolism of aflatoxin B1 to a reactive derivative toxic to S. Typhimurium TA1530. Cancer Res, 32:2058-2066, 1972.

7. Vogel HJ, Bonner MD : Acetylornithinase of E. coli: Partial purification and some properties. J Biol Chem, 218:97-106, 1956.

8. Shakeel AK, Rassol SA, Roohi M, Saifi ZS, Arfi S : In vitro studies of thiosemicarbazone compounds for antibacterial, antiviral and genetic activity. Kar Univ J Sc, 14:47-52, 1986.

9. Ames BN : The detection of chemical mutagens with enteric bacteria. In: Chemical mutagens, principles and methods for their detection. Ed by Hollaender, Plenum Press, New York, pp 268-282, 1971.

10. Rasool SA, Hashmi AH, Sattar SA : In vitro screening of environmental carcinogens in Salmonella/microsome test system: Assay of indigenously used chemicals. Kar Uni J Sc, 11:175-184, 1983.

11. Marnett LJ, Hurd HK, Hollstein MC, Levin DE, Esterbauer H, Ames BN : Naturally occurring carbonyl compounds are mutagens in S. typhimurium tester strain TA104. Mutation Res, 148:25-34,1985.

12. Levin DE, Yamaski E, Ames BN : A new salmonella tester strain TA97, for detection of frame-shift mutagens: A run of cytosines as a mutational hot-spot. Mutation Res, 94:315-330, 1982.

13. Basu A, Marnett LJ : Molecular requirements for the mutagenicity of malondialdehyde and related acroleins. Cancer Res, 44:2848-2854, 1984.

14. Lutz D, Eder E, Neudecker T, Henschler D : Structure mutagenicity relationship in, ß-unsaturated carboxylic compounds and their corresponding allylic compounds. Mutation Res, 93: 305-316, 1982.

15. Eder E, Henschler D, Neudecker T : Mutagenic properties of allylic and ß-unsaturated compounds: Consideration of alkylating mechanisms. Xenobiootcia, 12:831-848, 1982.

16. Yamaguchi T : Criteria for mutagenicity. Agri Biol Chem, 46:849-860, 1982.

17. Rinkus SJ, Legator MS : Chemical characterization of 465 know or suspected carcinogens and their correlation with mutagenic activity in S. typhimurium system. Cancer Res, 39:3289-3318, 1979.

Correspondence:
Sheikh Ajaz RASOOL
Department of Microbiology,
University of Karachi,
Karachi-75270,
PAKISTAN.