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Mutagenicity of Quinoline Derivatives and Analogs—Quinoxaline 1,4-Dioxide is a Potent Mutagen

Quinoline derivatives and analogs were surveyed for their mutagenicity using the *Salmonella*/microsome assay. 4-Methylquinoline, 4-methylquinoline 1-oxide, quinoxaline 1-oxide, quinoxaline 1,4-dioxide, 2,3-dimethylquinoxaline 1,4-dioxide, benzo[*f*]quinoline, 1-methylbenzo[*f*]quinoline, and 4-methylbenzo[*h*]quinoline were mutagenic on *S. typhimurium* TA 100 in the presence of the rat liver 9000 × *g* supernatant ("S-9 mix"). Quinoxaline 1-oxide, quinoxaline 1,4-dioxide, and 2,3-dimethylquinoxaline 1,4-dioxide were also mutagenic on TA 100 in the absence of S–9 mix. 2-Methylquinoxaline was mutagenic on strain TA 98 in the presence of S-9 mix. Among these mutagenic compounds, quinoxaline 1,4-dioxide was the strongest mutagen. The following compounds showed no significant mutagenicity on TA 98 and TA 100 both in the presence and absence of S-9 mix: 2-methylquinoline, 2,4-dimethylquinoline, quinoline-2-carboxamide, 4-methylquinoline-2-carboxamide, N-methylquinoline-2-carboxamide, N,4-dimethylquinoline-2-carboxamide, 2-(2-pyrazinyl)quinoline, 2-(5-pyrimidinyl)quinoline, benzo[*h*]quinoline, 1,10-phenanthroline, quinoline 1-oxide, 2-methylquinoline 1-oxide, 2,4-dimethylquinoline 1-oxide, isoquinoline, 3-methylisoquinoline, isoquinoline-1-carboxamide, 4-chloroquinazoline, quinoxaline, 2,3-dimethylquinoxaline, 3-methylquinoxaline-2-carboxamide, 2,3-bis(2-pyridyl)-quinoxaline and acridine.

Keywords——mutagenicity; quinoline derivatives; quinoline analogs; quinoxaline 1,4-dioxide; *Salmonella typhimurium* TA 98, 100; metabolic activation; S-9

Introduction

Current interest in the mutagenic and carcinogenic activity of quinoline derivatives[1] prompted us to undertake a survey of the mutagenic activity of quinoline derivatives and analogs. A large number of this class of compounds have been synthesized in the chemistry laboratories of this country. As an initial step of this program, we investigated the mutagenicity of 33 compounds by use of the *Salmonella*/microsome system.[2–4]

Materials and Methods

The following compounds used were commercial samples; quinoline, 2-methylquinoline, 4-methylquinoline, 2,4-dimethylquinoline, benzo[*f*]quinoline, benzo[*h*]quinoline, 1-methylbenzo[*f*]quinoline, 4-methylbenzo[*h*]quinoline, quinoxaline, 2,3-dimethylquinoxaline, 1,10-phenanthroline, isoquinoline, 3-methylisoquinoline and acridine. The following compounds were prepared by methods described in literature quoted for each compound; quinoline 1-oxide,[5] 2-methylquinoline 1-oxide,[6] 4-methylquinoline 1-oxide,[7] 2,4-dimethylquinoline 1-oxide,[8] 2-methylquinoxaline,[9] quinoxaline 1-oxide,[10] quinoxaline 1,4-dioxide,[10] 2,3-dimethylquinoxaline 1,4-dioxide,[11] quinoline-2-carboxamide,[12] 4-methylquinoline-2-carboxamide,[12] N-methylquinoline-2-carboxamide,[13] N,4-dimethylquinoline-2-carboxamide,[13] 2-(2-pyrazinyl)quinoline,[14] 2-(5-

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pyrazinyl)quinoline,[14] isoquinoline-1-carboxamide,[12] 4-chloroquinazoline,[15] 3-methylquinoxaline-2-carboxamide[16] and 2,3-bis(2-pyridyl)quinoxaline.[13] 4-Nitroquinoline 1-oxide was a gift of Prof. Y. Kawazoe of Nagoya City University.

Mutagenicity of these compounds was assayed on *Salmonella typhimurium* strains TA 98 and TA 100 in the presence and absence of "S-9 mix" as described by Yahagi *et al.*[3] In this assay, the mixture containing bacteria, reagent and S-9 mix (or only buffer) was incubated at 37° for 20 min (the "preincubation"); soft-agar was added to the mixture; and the whole mixture was poured on agar plate. The plate was maintained at 37° for two days and the His^--to-His^+ revertant colonies were counted. S-9 fraction was prepared from the livers of rats to which polychlorinated biphenyl had been intraperitoneally administered. The mutagenicity test was carried out using various doses of compounds; usually up to the doses at which either killing of the bacteria was observed or the compound no longer completely dissolved in the test medium. The results were regarded as positive only when a dose-response relationship was observed.

Results

Of 33 compounds examined, 11 showed positive results in the mutagenicity test, and the results are given in Tables I and II. The other 22 compounds gave no significant mutagenicity in the test systems employed. Among the active compounds were quinoline, 4-nitroquinoline 1-oxide, and benzo[*f*]quinoline, which are known to show mutagenicity in the *Salmonella*/microsome assay. Our results are consistent with those reported in literature: quinoline is positive in the system TA 100+S-9,[1] 4-nitroquinoline 1-oxide in the systems TA 98±S-9 and TA 100±S-9,[17] and benzo[*f*]quinoline in the system TA 100+S-9.[18]

Among the 8 compounds whose mutagenicity was newly discovered, quinoxaline 1,4-dioxide is the strongest mutagen. It showed potent mutagenicity in all the four systems employed; TA 98±S-9 and TA 100±S-9. Since this compound is known to be photosensitive,[10] we examined the effect of irradiation of near-ultraviolet light on the mutagenicity

Table I. Mutagenicity of Quinoline Derivatives and Analogs[a]

Compound	Dose (μg/plate)	Number of His^+ revertants per plate			
		TA 98		TA 100	
		−S-9	+S-9	−S-9	+S-9
Quinoline	500	25	94	153	**947**[b]
4-Methylquinoline	500	14	138	146	**1486**
4-Methylquinoline 1-oxide	7500	—	73	—	**390**
4-Nitroquinoline 1-oxide	0.1	**139**	46	**2552**	101
	10	Killing	**166**	Killing	**3045**
2-Methylquinoxaline	500	19	**169**	189	157
Quinoxaline 1-oxide	5000	—	—	**784**	**497**
2,3-Dimethylquinoxaline 1,4-dioxide	190	**87**	**127**	**405**	**747**
Benzo[*f*]quinoline	50	25	131	104	**660**
1-Methylbenzo[*f*]quinoline	10	25	91	150	**499**
4-Methylbenzo[*h*]quinoline	50	20	100	154	**348**
Control		15—61	26—52	110—202	99—245

a) For reason of space, only typical results are presented for each compound. Those who need more information may ask the author for details.
b) Positive results are represented by gothic numbers. These numbers are averages of results in two to five independent experiments.

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Table II. Mutagenicity of Quinoxaline 1,4-Dioxide

Dose (μg/plate)	Number of revertants per plate[a]			
	TA 98		TA 100	
	−S-9	+S-9	−S-9	+S-9
0	14	45	155±20	110
1	24	50	173±10	125
5	41	51	348±51	493
10	52	200±95	515±65	1009±47
50	380±99	524±30	3122±453	3602±575
100	831±52	848±153	6080±2583	5955±1438
500	Killing	Killing	Killing	Killing

a) Results of three independent experiments are presented in average±standard deviation.

Table III. Effect of Irradiation of Near-ultraviolet Light on the Mutagenicity of Quinoxaline 1,4-Dioxide

Reagent	Irradiation during the period of preincubation[a]	Number of revertants per plate
Quinoxaline 1,4-dioxide	−	6786
	+	1398
None	+	234
	−	150

a) Irradiation was done with Black light (wavelength 300—400 nm) at a distance of 15 cm from the test tube containing the reagent and bacteria (TA 100, −S-9). The concentration of quinoxaline 1,4-dioxide in the mixture was 1 mM. The preincubation was for 40 min.

of this compound. As Table III shows, the irradiation during the incubation of this compound with the bacteria caused significant decrease in the number of revertant colonies formed. Irradiation of a solution of this compound before its contact with the bacteria also diminished the mutagenicity (data not shown). The irradiation itself was without effect on the bacteria. Therefore, it is clear that quinoxaline 1,4-dioxide, but not the photo-decomposition product, is mutagenic.

Discussion

Quinoxaline 1,4-dioxide is used as a feed additive to promote growth in chickens and pigs. Its handling by man has been cautioned owing to its carcinogenicity.[19] The potent mutagenicity observed for this compound (Table II) suggests that it can also be a genetic hazard.

Comparison of the structures of active and inactive compounds indicates that there is no apparent structure-activity relationship. For example, introduction of a methyl group or an N-oxide function into the structure results in abolishment of the mutagenicity in some cases but it also results in generation of mutagenicity in other cases.

It is remarkable that more than one-fourth (8 out of 30) of the compounds which we chose rather arbitrarily from the great number of available quinoline derivatives have been found to be mutagenic. This indicates that continuation of the survey in this class of compounds is desirable.

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