Mutagenicity of Condensed Pyridazines with Different Substituents

Tamotsu MORITA,*,[a] Hirotaka NAITOU,[a] and Etsuo OISHI[b]

Laboratory of Environmental Microbiology[a] and Laboratory of Organic Chemistry,[b] Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, 52–1 Yada, Shizuoka 422, Japan.
Received August 1, 1994; accepted October 27, 1994

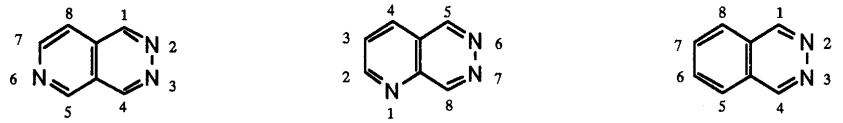
A total of 24 compounds were prepared by introducing an *N*-oxide, a hydrazino group, a methoxy group or a chloro group into 3 kinds of condensed pyridazines: pyrido[3,4-*d*]pyridazines, pyrido[2,3-*d*]pyridazines and phthalazines. The mutagenicity of these 24 compounds was assessed by the Ames method using two tester strains (*Salmonella typhimurium* TA98 and TA100). No mutagenic activity was detected with any of the 3 condensed pyridazines without substituents or any of the 5 condensed pyridazines with a methoxy group. The compounds with *N*-oxide in the pyridazine ring showed no or only very weak mutagenicity. However, when an oxide was introduced into the nitrogen of the pyridine ring, the mutagenicity against strain TA98 was higher than that of any other test compound. All compounds with a hydrazino group were mutagenic against strains TA98 and TA100, irrespective of the presence or absence of S9 mix-induced metabolic activation. 1-Hydrazinophthalazine (hydralazine) which has been clinically used as an antihypertensive agent was weakly mutagenic. The introduction of a chloro group increased the bactericidal effects of the condensed pyridazines, thus hampering the assessment of mutagenicity. A majority of the compounds which were found to be mutagenic in this study required no metabolic activation with S9 mix.

Key words condensed pyridazine; mutagenic activity; *N*-oxide; hydrazino substituent; methoxy substituent; chloro substituent

To assess the mutagenicity of the increasing number of new compounds being synthesized, it is essential to determine the relationship between the type or position of substituents and the mutagenicity of the compounds. The relationship between the mutagenicity and the structure of several heterocyclic compounds (*e.g.*, quinoline,[1] imidazole[2] and imidazo[4,5-*f*]quinoxaline[3]) was reported recently. Condensed pyridazine derivatives are used as antihypertensive agents, cardiotonics, antibacterials and Luminol reagent. We synthesized 24 mono-substituted compounds by introducing an *N*-oxide, a hydrazino group, a methoxy group or a chloro group into condensed pyridazines: pyrido[3,4-*d*]pyridazine, pyrido[2,3-*d*]pyridazine and phthalazine (benzo[*d*]pyridazine) of which mutagenicity had not been assessed except 1-hydrazinophthalazine. Assessment was done using the Ames method to examine the relationship between mutagenicity and substituents.

MATERIALS AND METHODS

Preparation of Compounds Chemical structures of the test compounds are shown in Fig. 1. Non-substituted pyrido[3,4-*d*]pyridazine (A1) was prepared according to the literature.[4] Other pyrido[3,4-*d*]pyridazines (group A) were prepared as follows. A2, A3 and A4: To a stirred solution of A1 (1000 mg) in $CHCl_3$ (6 ml), portions of *m*-chloroperbenzoic acid (1600 mg) in $CHCl_3$ (20 ml) were added over 30 min. After standing at room temperature for 24 h, the mixture was chromatographed on SiO_2 using $CHCl_3$ as eluate and gave successively: *m*-chlorobenzoic acid (1423 mg, 98%), unchanged A1 (264 mg, 26%), A2 (216 mg, 19%), A3 (340 mg, 30%) and A4 (35 mg, 3%). A2; colorless needles from MeOH, mp 202—204 °C (dec.). *Anal.* Calcd for $C_7H_5N_3O$: C, 57.14; H, 3.43; N, 28.56. Found: C, 57.39; H, 3.40; N, 28.31. MS *m/z*: 147 (M^+), 131 (M^+-16). ^{1}H-NMR ($(CD_3)_2SO$): 9.57 (1H, s, C4-H), 9.45 (1H, s, C5-H), 8.89 (1H, s, C1-H), 8.80 (1H, d, $J=5.9$,



pyrido[3,4-*d*]pyridazine (A1)	pyrido[2,3-*d*]pyridazine (B1)	phthalazine (C1)
2-oxide (A2)	6-oxide (B2)	2-oxide (C2)
3-oxide (A3)	7-oxide (B3)	
6-oxide (A4)		
1-$NHNH_2$ (A5)	5-$NHNH_2$ (B5)	1-$NHNH_2$ (C5)
4-$NHNH_2$ (A6)	8-$NHNH_2$ (B6)	
1-OCH_3 (A7)	5-OCH_3 (B7)	1-OCH_3 (C7)
4-OCH_3 (A8)	8-OCH_3 (B8)	
1-Cl (A9)	5-Cl (B9)	1-Cl (C9)
4-Cl (A10)	8-Cl (B10)	

Fig. 1. Chemical Structures of Pyrido[3,4-*d*]pyridazines (A), Pyrido[2,3-*d*]pyridazines (B) and Phthalazines (C)

* To whom correspondence should be addressed.

C7-H), 7.75 (1H, d, $J=5.9$, C8-H). ^{13}C-NMR ($(CD_3)_2SO$): 153.3 (C4), 151.3 (C5), 149.7 (C7), 135.6 (C8a), 125.5 (C1), 115.2 (C8), 114.7 (C4a). A3; pale yellow needles from MeOH, mp 222—223 °C (dec.). *Anal.* Calcd for $C_7H_5N_3O$: C, 57.14; H, 3.43; N, 28.56. Found: C, 56.92; H, 3.38; N, 28.42. MS *m/z*: 147 (M^+), 131 (M^+-16). ^{1}H-NMR ($(CD_3)_2SO$): 9.52 (1H, s, C1-H), 9.33 (1H, s, C5-H), 9.00 (1H, s, C4-H), 8.77 (1H, d, $J=5.6$, C7-H), 8.00 (1H, d, $J=5.6$, C8-H). ^{13}C-NMR ($(CD_3)_2SO$): 153.6 (C1), 148.3 (C5), 146.3 (C7), 126.9 (C8a), 126.0 (C4), 122.3 (C4a), 118.8 (C8). A4; pale yellow needles from MeOH, mp 250 °C (dec.). *Anal.* Calcd for $C_7H_5N_3O$: C, 57.14; H, 3.43; N, 28.56. Found: C, 56.91; H, 3.34; N, 28.34. MS *m/z*: 147 (M^+), 131 (M^+-16). ^{1}H-NMR ($(CD_3)_2SO$): 9.68 (1H, s, C1-H or C4-H), 9.62 (1H, s, C1-H or C4-H), 9.11 (1H, s, C5-H), 8.60 (1H, d, $J=6.7$, C7-H), 8.17 (1H, d, $J=6.7$, C8-H). ^{13}C-NMR ($(CD_3)_2SO$): 149.1 (C1 or C4), 147.4 (C1 or C4), 143.8 (C7), 134.7 (C5), 123.5 (C8a), 123.4 (C8), 120.4 (C4a). A2 and A3 structure was determined by their reaction with $POCl_3$ in $CHCl_3$ to give A9 and A10, respectively; A5 and A6: Finely ground A9 (500 mg) was added during 20 min to a stirred solution of hydrazine hydrate (0.4 ml) in MeOH (5 ml). The mixture was stirred for an additional 22 h at room temperature. The precipitate was collected and recrystallized from MeOH to give A5, orange-red needles, mp 290—291 °C (dec.) in 65% yield (313 mg). *Anal.* Calcd for $C_7H_7N_5$: C, 52.16; H, 4.38; N, 43.46. Found: C, 51.95; H, 4.23; N, 43.23. IR (KBr): 3340—2800 cm^{-1} (NH). A10 similarly gave A6 (71%) which crystallized from MeOH as orange-red needles, mp 301—302 °C (dec.). *Anal.* Calcd for $C_7H_7N_5$: C, 52.16; H, 4.38; N, 43.46. Found: C, 52.13; H, 4.32; N, 43.26. IR (KBr): 3350—2800 cm^{-1} (NH); A7 and A8: A9 (8.0 g) was added to the solution of MeONa [prepared from Na (2.2 g) and MeOH (180 ml)] and the mixture was refluxed for 1 h. After removal of MeOH under reduced pressure, $CHCl_3$ was added to the residue and filtered. The extract was passed through a column of SiO_2 to remove impurities. Recrystallization from benzene–petr. ether gave colorless needles (A7), mp 182—183 °C (dec.) in 84% yield (6.5 g). *Anal.* Calcd for $C_8H_7N_3O$: C, 59.62; H, 4.38; N, 26.07. Found: C, 59.78; H, 4.38; N, 25.87. ^{1}H-NMR ($CDCl_3$): 9.38 (1H, d, $J=1.0$), 9.33 (1H, d, $J=1.0$), 9.03 (1H, d, $J=5.7$), 7.97 (1H, dt, $J=5.7$, 1.0), 4.32 (3H, s, OCH_3). ^{13}C-NMR ($CDCl_3$): 159.7, 150.4, 150.1, 146.0, 123.3, 122.8, 115.0, 55.2. A8 was obtained in 87% yield from the reaction of A10 with MeONa in essentially the same manner as described above. Recrystallization from benzene–petr. ether gave colorless needles, mp 187—188 °C (dec.). *Anal.* Calcd for $C_8H_7N_3O$: C, 59.62; H, 4.38; N, 26.07. Found: C, 59.39; H, 4.39; N, 25.82. ^{1}H-NMR ($CDCl_3$): 9.63 (1H, dd, $J=1.0$, 0.5), 9.28 (1H, d, $J=0.5$), 9.04 (1H, d, $J=5.4$), 7.72 (1H, dd, $J=5.4$, 1.0), 4.34 (3H, s, OCH_3). ^{13}C-NMR ($CDCl_3$): 160.0, 150.6, 148.2, 147.0, 131.5, 117.4, 114.1, 55.26; A9 and A10: Finely ground 1-chloro-4-hydrazinopyrido[3,4-*d*]pyridazine[5] (14.0 g) was added during 45 min to a stirred suspension of yellow mercuric oxide (70.0 g) in water (1150 ml). The mixture was stirred a further 4 h and filtered. The filtrate was extracted with $CHCl_3$ (3×300 ml). The combined extracts were washed with H_2O, dried over Na_2SO_4, and passed through a column of SiO_2 eluted with $CHCl_3$. Recrystallization from benzene–petr. benzin gave A9 (colorless needles) in 68% yield (8.1 g). This compound had no definite melting point and on heating slowly decomposed above 103 °C to another black solid which melted at 120 °C (dec.). *Anal.* Calcd for $C_7H_4ClN_3$: C, 50.77; H, 2.44; N, 25.38. Found: C, 50.55; H, 2.63; N, 25.55. ^{1}H-NMR ($CDCl_3$): 9.71 (1H, d, $J=0.7$), 9.61 (1H, d, $J=1.0$), 9.21 (1H, d, $J=5.9$), 8.10 (1H, ddd, $J=5.9$, 1.0, 0.7). ^{13}C-NMR ($CDCl_3$): 154.6, 151.5, 151.2, 149.5, 128.9, 121.9, 116.2. 4-Chloro-1-hydrazinopyrido[3,4-*d*]-pyridazine[5] (8.0 g) was similarly treated with mercuric oxide (40.0 g) to give A10 8colorless scales) in 68% yield (4.6 g). This compound also decomposed slowly above 145 °C to another black solid which melted at 156 °C (dec.). *Anal.* Calcd for $C_7H_4ClN_3$: C, 50.77; H, 2.44; N, 25.38. Found: C, 50.48; H, 2.47; N, 25.25. ^{1}H-NMR ($CDCl_3$): 9.77 (1H, dd, $J=1.0$, 0.7), 9.57 (1H, d, $J=0.7$), 9.17 (1H, d, $J=5.6$), 7.85 (1H, dd, $J=5.6$, 1.0). ^{13}C-NMR ($CDCl_3$): 154.5, 151.4, 150.6, 150.0, 130.9, 120.1, 117.6. All melting points are uncorrected. Infrared (IR) absorption spectra were recorded on a Jasco A-102 diffraction grating IR spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were measured at 270 MHz on a JEOL JNM-GSX270. Chemical shifts are quoted in parts per million (ppm) with tetramethylsilane as an internal standard, and coupling constants (J) are given in hertz (Hz). The following abbreviations are used: s = singlet, d = doublet, t = triplet. Mass spectra (MS) were recorded on a JEOL JMS D-100 mass spectrometer. Samples were vaporized in a direct inlet system.

Pyrido[2,3-*d*]pyridazines (group B) and phthalazines (group C) were prepared according to the literature: B1[4]; B2 and B3[6]; B5, B6, B9 and B10[7]; B7 and B8[8]; C1, C2, C5, C7 and C9.[9]

The purity of these compounds was determined based on the NMR spectra. They showed one spot on the thin layer chromatograph.

Mutagenicity Assay Mutagenicity was assayed by the preincubation method described by Yahagi *et al.*[10] and Maron and Ames.[11] Two tester strains, *Salmonella typhimurium* TA98 and TA100, obtained from the National Institute of Public Health, Tokyo, were used. The number of background (spontaneous) revertant colonies of TA98 in the presence and absence of S9 mix were 26 ± 6 and 19 ± 6 per plate, and those of TA100 were 119 ± 17 and 111 ± 16 per plate throughout the experiment ($n=26$). Rat liver S9 extract was prepared from 500 mg/kg of polychlorinated biphenyl (Kanechrol 500) treated Sprague-Dawley male rats as described by Maron and Ames.[11] All test compounds (Fig. 1) were dissolved in dimethyl sulfoxide (DMSO). The highest concentration of each test compound was set at 5000 μg/plate. At each concentration level, the compound was tested at least twice (three times if the data fluctuated), using 2 agar plates. Deviations in the number of induced revertant colonies between the two plates were less than 24% (average 8%) in TA98 and 11% (average 4%) in TA100.

The relative mutagenic activity was calculated using the following equation: (No. of induced revertant colonies) − (No. of spontaneous revertant colonies)/concentra-

TABLE I. Relative Mutagenic Activity[a] of Condensed Pyridazines

Compound	Substituent	TA98		TA100	
		−S9	+S9	−S9	+S9
Pyrido[3,4-*d*]pyridazines					
A1	None	—	—	—	—
A2	2-Oxide	—	—	±[b]	±
A3	3-Oxide	34[c]	30	—	—
A4	6-Oxide	438	246	—	238
A5	1-Hydrazino	120	53	615	901
A6	4-Hydrazino	140	88	1028	1208
A7	1-Methoxy	—	—	—	—
A8	4-Methoxy	—	—	—	—
A9	1-Chloro	—	—	—	—
A10	4-Chloro	—	—	—	—
Pyrido[2,3-*d*]pyridazines					
B1	None	—	—	—	—
B2	6-Oxide	K[d]	—	K	—
B3	7-Oxide	—	—	—	—
B5	5-Hydrazino	272	144	1128	1176
B6	8-Hydrazino	43	25	193	251
B7	5-Methoxy	—	—	—	—
B8	8-Methoxy	—	—	—	—
B9	5-Chloro	K	—	K	—
B10	8-Chloro	K	160	K	540
Phthalazines					
C1	None	—	—	—	—
C2	2-Oxide	—	—	—	—
C5	1-Hydrazino	34	48	354	514
C7	1-Methoxy	—	—	—	—
C9	1-Chloro	—	—	—	—

a) Relative mutagenic activity = (No. of induced revertants − No. of background)/mg. *b*) Number of induced revertant colonies increased dose-responsively but was not double the number of background colonies. *c*) Number of induced revertant colonies increased dose-responsively and was at least twice the number of background colonies. Each value indicates the relative mutagenic activity. *d*) Compound showed killing effect at low concentration.

tion of compound (mg). This calculation was done for the concentration range in which the number of induced revertants showed a concentration-dependent increase. The relative mutaganic activity is shown in Table I.

RESULTS AND DISCUSSION

Mutagenicity of Non-substituted Condensed Pyridazines Of the three compounds without substituents, A1 and B1 showed no bactericidal or mutagenic activity against any of the two tester strains even at their highest concentration (500 μg/plate) (Fig. 2). C1 showed weak bactericidal effects on TA98 in the absence of S9 mix, although this compound showed no mutagenicity.

Mutagenicity of Condensed Pyridazines with *N*-Oxide A2 showed no mutagenicity against TA98 even at a concentration of 5000 μg/plate; however, the compound caused a dose-dependent increase in the number of induced revertants of TA100 although the number was less than twice the number of background revertants. A3 was mutagenic only against TA98. A4, obtained by introducing an oxide to the nitrogen of the pyridine ring, was tested at only one concentration (2500 μg/plate), because the amount of this compound synthesized was very small. The relative mutagenic activity of this compound against TA98 was higher than that of any other compound tested (Table I). A4 was also weakly mutagenic against TA100 in the presence of S9 mix. The results for this compound suggest that the introduction of an oxide to the nitrogen of pyridine ring makes the compound more likely to bind with or intercalate itself into the DNA. B2 and B3 showed no mutagenicity against TA98 or TA100 even at a concentration of 2500 μg/plate. In the absence of S9 mix, B2 completely suppressed the survival of both strains at a concentration of 625 μg/plate (Fig. 2). In the presence of S9 mix, however, B2 showed no bactericidal activity even at a concentration of 2500 μg/plate, probably because S9 mix detoxicated this compound by enzymatically modifying the oxide at N6. C2 showed no bactericidal or mutagenic activity against either of the two strains even at a concentration of 5000 μg/plate.

Mutagenicity of Condensed Pyridazines with Hydrazino Substituent Five condensed pyridazines with a hydrazino substituent (A5, A6, B5, B6, C5) were tested in this study. All these compounds were clearly mutagenic against both strains, irrespective of the presence or absence of S9 mix (Fig. 2). The relative mutagenic activity was high with A5, A6 and B5 (Table I). The relative mutagenic activity of B6 against both strains was 1/5 to 1/6 the activity of B5. These results suggest that the nitrogen atom of the pyridine ring affects the mutagenicity.

C5 (1-hydrazinophthalazine) is called hydralazine and its hydrochloride is often used clinically as an antihypertensive agent. Although the relative mutagenic activity of this compound against TA98 and TA100 was not high (Table I), there was a clear dose–response relationship (Fig. 2). It has been reported that hydralazine is mutagenic in the Ames test,[12] genotoxic in *in vivo* and/or *in vitro* tests,[12c,f,g,13] clastogenic[14] and carcinogenic.[15] Considering that hydralazine is often used as an antihypertensive agent and that this compound is orally administered for long periods of time at a daily dose of 30 to 200 mg, many more *in vivo* cumulative tests of this compound should be carried out.

Mutagenicity of Condensed Pyridazines with Methoxy Substituent Five compounds with a methoxy group (A7, A8, B7, B8, C7) were tested. None of them was mutagenic against TA98 or TA100 in the presence or absence of S9 mix (Table I). However, the methoxy group seems to affect the mutagenicity and/or carcinogenicity of cyclic compounds in a complicated manner considering reports that: the mutagenicity of quinoline against TA100 was affected by the position at which a methoxy substituent was introduced[1]; that nitrobenzene, which is not mutagenic against TA98 or TA100, became mutagenic against these strains after introduction of a methoxy substituent at particular positions[16]; and that the carcinogenicity of 4-aminoazobenzene was increased or suppressed depending on the position at which a methoxy substituent was introduced.[17] Our experiment also revealed that the mutagenicity of compound A3 against TA98 was completely suppressed by the introduction of a methoxy group to the C1 of A3 (data not shown).

Mutagenicity of Condensed Pyridazines with Chloro Substituent Of the five compounds with a chloro substituent (A9, A10, B9, B10, C9), only B10 was mutagenic against both strains in the presence of S9 mix (Fig. 2).

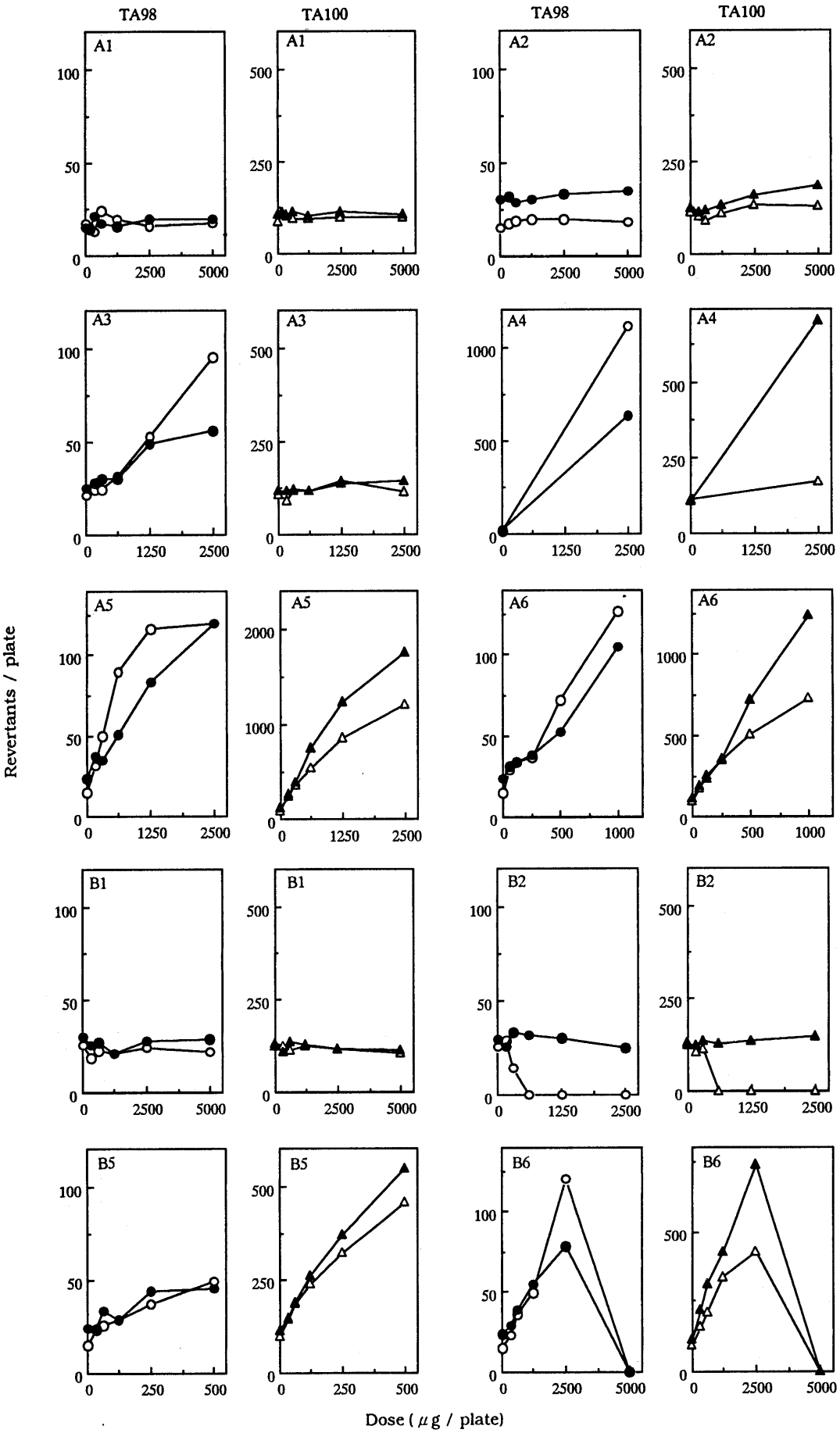


Fig. 2

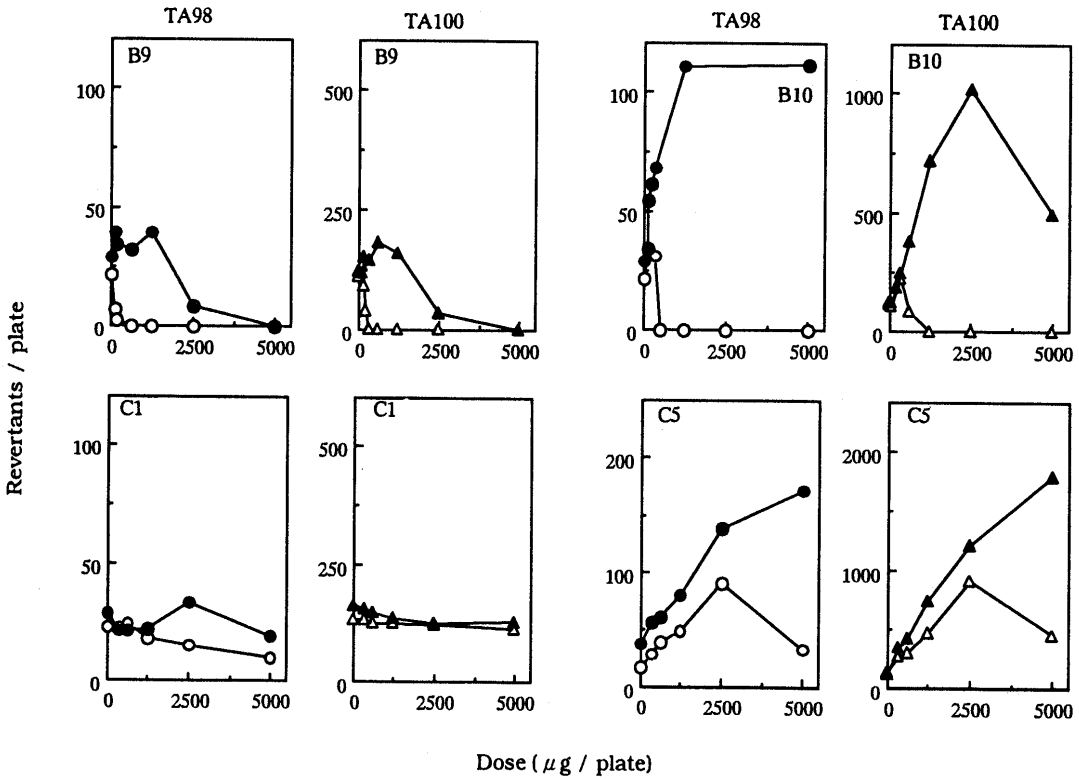


Fig. 2. Dose–Response Curves for Condensed Pyridazines in *Salmonella typhimurium* TA98 and TA100 with or without S9 Activation

Dose–response curves of compounds which showed positive results and/or killing effects are shown. Each point represents the average of two plates. ○, TA98 without S9; ●, TA98 with S9; △, TA100 without S9; ▲, TA100 with S9.

The introduction of a chloro group into the condensed pyridazines increased the bactericidal activity of these compounds. B9 and B10 completely suppressed the survival of both strains at a concentration of 250 and 1000 μg/plate, respectively. When S9 mix was added to the assay medium, B10 lost its bactericidal activity and the mutagenicity of B10 was observed (Fig. 2). On the other hand, the bactericidal effect of B9 was not suppressed in the presence of S9 mix, and this hindered the detection of mutagenicity of B9 (Fig. 2). The chloro group of the pyridazine ring of B10 seems to be highly reactive because it is close to the nitrogen atom of the pyridine ring. We speculated that the exposure of B10 to S9 mix resulted in enzymatic modification of the chloro group, leading to detoxication of B10. The mechanism of the detoxication, however, remains unknown.

The mutagenicity of a larger number of mono-substituted condensed pyridazines should be examined, and condensed pyridazines with multiple substituents synthesized for the analysis of interactions among these substituents in mutagenesis.

REFERENCES AND NOTES

1) A. K. Debnath, R. L. L. de Compadre, C. Hansch, *Mutation Res.*, **280**, 55 (1992).
2) R. Forster, S. D. Blowers, S. Cinelli, H. Marquardt, J. Westendorf, *Mutation Res.*, **298**, 71 (1992).
3) R. Vikse, A. Knapstad, L. Klungsoyr, S. Grivas, *Mutation Res.*, **298**, 207 (1993).
4) D. B. Paul, H. J. Rodda, *Aust. J. Chem.*, **21**, 1291 (1968).
5) I. Matsuura, K. Okui, *Chem. Pharm. Bull.*, **17**, 2266 (1969).
6) D. B. Paul, H. J. Rodda, *Aust. J. Chem.*, **22**, 1745 (1969).
7) D. B. Paul, H. J. Rodda, *Aust. J. Chem.*, **22**, 1759 (1969).
8) I. Matsuura, *Heterocycles*, **3**, 381 (1975).
9) E. Hayashi, T. Higashino, C. Iijima, Y. Kono, T. Doihara, *Yakugaku Zasshi*, **82**, 584 (1962).
10) T. Yahagi, M. Degawa, Y. Seino, T. Matsushima, M. Nagao, T. Sugimura, Y. Hashimoto, *Cancer Lett.*, **1**, 91 (1975).
11) D. M. Maron, B. N. Ames, *Mutation Res.*, **113**, 173 (1983).
12) *a*) C. R. Shaw, M. A. Butler, J.-P. Thenot, K. D. Haegle, T. S. Matney, *Mutation Res.*, **68**, 79 (1979); *b*) J. Tosk, I. Schmeltz, D. Hoffmann, *ibid.*, **66**, 247 (1979); *c*) G. M. Williams, G. Mazue, C. A. McQueen, T. Shimada, *Science*, **210**, 329 (1980); *d*) S. Parodi, S. De Flora, M. Cavanna, A. Pino, L. Robbiano, C. Bennicelli, G. Brambilla, *Cancer Res.*, **41**, 1469 (1981); *e*) D. E. Levin, E. Yamasaki, B. N. Ames, *Mutation Res.*, **94**, 315 (1982); *f*) S. De Flora, P. Zanacchi, C. Bennicelli, M. Camoirano, L. Cavanna, L. Sciaba, E. Cajelli, P. Faggin, G. Brambilla, *Environ. Mutagen.*, **4**, 605 (1982); *g*) J. Kitamura, H. Mori, S. Sugie, K. Kawai, *Res. Commun. Pathol. Pharmacol.*, **49**, 414 (1985); *h*) H. Glatt, F. Oesch, *Teratogen. Carcinogen. Mutagen.*, **5**, 773 (1985).
13) C. A. McQueen, C. J. Maslansky, I. B. Glowinsky, S. B. Crescenzi, W. W. Weber, G. M. Williams, *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 1269 (1982); C. A. McQueen, G. M. Williams, *Cell Biol. Toxicol.*, **3**, 209 (1987); H. Mori, S. Sugie, N. Yoshimi, H. Iwata, A. Nishikawa, K. Matsukubo, H. Shimizu, I. Hirono, *Jpn. J. Cancer Res. (Gann)*, **79**, 204 (1988); P. Garberg, E. L. Aakerblom, G. Bolcsfoldi, *Mutation Res.*, **203**, 155 (1988); A. Jendryczko, M. Drozdz, *Exp. Pathol.*, **36**, 53 (1989); C. A. McQueen, B. M. Way, S. M. Queener, *Toxicol. Appl. Pharmacol.*, **118**, 135 (1993).
14) F. Kodama, K. Fukushima, M. Umeda, *J. Toxicol. Sci.*, **5**, 141 (1980); M. Jr. Ishidate, K. Yoshikawa, *Arch. Toxicol., Suppl. 4*, 41 (1980).
15) B. Toth, *J. Natl. Cancer Inst.*, **61**, 1363 (1978); M. Drozdz, M. Luciak, A. Jendryczko, K. Magner, *Exp. Pathol.*, **32**, 119 (1987).
16) A. Kawai, S. Goto, Y. Matsumoto, H. Matsushita, *Jpn. J. Ind. Health*, **29**, 34 (1987).
17) H. K. Watanabe, Y. Hashimoto, I. Abe, H. Sato, *Gann*, (*Jpn. J. Cancer Res.*) **73**, 136 (1982).