Structure–activity relationships of the *N*-methylcarbamate series in *Salmonella typhimurium*

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Summary

Aromatic hydrocarbons of low molecular weight, hydroxy and *N*-methylcarbamate derivatives were tested for mutagenicity by the reversion of histidine-dependent *Salmonella typhimurium* TA98 and TA1535 in the presence of a rat-liver 9000 × *g* supernatant fraction. The presence of 2 or 3 aromatic rings resulted in a weak increase in revertants. Hydroxylation and carbamylation of aromatic rings increased the mutagenic activity of these aromatic compounds. In order to evaluate the structure–activity relationship, the specific molecular connectivity indices were calculated. A significant inverse relationship exists between mutagenicity and zero- and second-order specific molecular connectivity indices. Only compounds with second-order specific molecular connectivity indices lower than 0.300 increased mutagenic activity.

The methyl carbamate pesticides have been available for use since 1957. During the past 10 years, the mutagenic (and carcinogenic) properties of these compounds have been tested (Woo, 1983). These compounds have been reported to be non-mutagenic but when carbaryl is used, a slight increase above the control values is observed (Rani et al., 1980; Chauvit, 1984). These increases, however, were not statistically verified. 1-Naphthol is the major hydrolysis product from carbaryl and this compound was reported to give a positive reversion of *Salmonella typhimurium* TA1538 (Purchase et al., 1978). Moreover, the phenyl carbamates like propham and chlorpropham showed no increased reversion in Salmonella strains (Chauvit, 1984). This discrepancy between the results of carbamates and their metabolites for *Salmonella typhimurium* led us to test *N*-methylcarbamate and a series of parent compounds for mutagenicity.

In a recent paper, Murakami and Fukami (1986) used a concept of the specific molecular connectivity index to calculate a relationship with the mutagenic and carcinogenic effects of these

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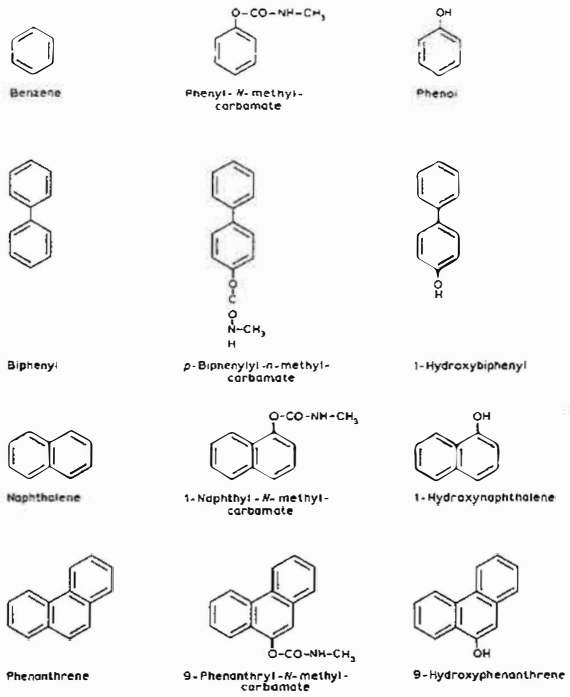

Fig. 1. Structures of chemicals tested.

pesticides as reported in the literature. We report here the relationship between these indices and the mammalian enzyme-activated mutagenic activity of 12 compounds whose structures are shown in Fig. 1.

Materials and methods

Chemicals

Benzene, phenol, biphenyl, 1-hydroxybiphenyl, naphthalene, 1-hydroxynaphthalene, phenanthrene and 9-hydroxyphenanthrene were obtained from Aldrich Chemicals. Phenyl *N*-methylcarbamate, *p*-biphenylyl *N*-methylcarbamate, 1-naphthyl *N*-methylcarbamate and 9-phenanthryl *N*-methylcarbamate, were synthesized as follows.

The carbamates were prepared by reacting a mixture of the appropriate phenol (0.15 mole) and anhydrous ether with methylisocyanate (0.15 mole) using a trace of triethylamine as a catalyst (Kolbezen, 1954). High grade phenols were obtained from commercial sources; 1-naphthol was purified by sublimation prior to use. The mixture was stirred for 10 h at room temperature. For 1-naphthyl and 9-phenanthryl *N*-methylcarbamate, the reaction was carried out under an inert atmosphere and in darkness to avoid any oxidation.

Recrystallizations from toluene–hexane gave colorless crystals: phenyl *N*-methylcarbamate m.p. 86°C [85–86°C (Kolbezen, 1954)], *p*-biphenylyl *N*-methylcarbamate m.p. 136°C [133.5°C (Metcalf, 1967)] and 1-naphthyl *N*-methylcarbamate (carbaryl) m.p. 144°C [142–145°C (Haynes, 1957)]. The 9-phenanthryl *N*-methylcarbamate was recrystallized from chloroform–hexane [1:2], m.p. 164°C.

The test compounds were stored in the dark at 4°C and dissolved in dimethylsulfoxide on the day of the mutagenicity experiment.

S9 preparation

Male Sprague–Dawley rats (180 g) received a single i.p. injection of Aroclor 1254 (500 mg/kg body weight: Aroclor was diluted in corn oil 1:5 v/v). 5 days later, the rats were killed. The livers were homogenized in 3 vol. of sterile, cold KCl, 150 mM. The homogenate was centrifuged at 9000 × *g* for 10 min. This S9 fraction was mixed with the NADPH-regenerating system, containing 4 mM $MgCl_2 \cdot 6H_2O$, 33 mM KCl, 5 mM glucose 6-phosphate, 4 mM NADP, 0.2 M Na_2HPO_4, 0.2 M NaH_2PO_4, pH 7.4.

Reversion of histidine auxotrophic Salmonella typhimurium tester strains to histidine prototrophy

These mutagenicity experiments were performed as described by Maron and Ames (1983). *Salmonella typhimurium* strains TA98 and TA1535 were grown overnight in nutrient broth (8 g Bacto Nutrient Difco and 5 g NaCl per liter). 100 µl of the test compound dissolved in DMSO, 500 µl S9 activating system, 100 µl of the bacterial overnight culture and 2500 µl top agar (0.5 mM L-histidine HCl/0.5 mM biotin, 6 g agar Difco and 5 g NaCl per liter; 45°C) were mixed in a test tube and poured onto minimal agar (1.5% agar, Vogel-Bonner E medium with 2% glucose) in a petri dish. After incubation in the dark for 2 days at 37°C, colonies (his^+ revertants) were counted.

TABLE 1

REVERSION OF his⁻ *Salmonella typhimurium* BY PHENYL AND DIPHENYL SERIES[a]

Test compound	μg/plate	TA98 (μl S9/plate)			TA1535 (μl S9/plate)		
		20	50	100	20	50	100
Dimethyl sulfoxide, blank		19 ± 4	20 ± 3	20 ± 5	18 ± 5	18 ± 4	17 ± 3
Benzene	5	26	25	27	21	24	19
	10	28	27	30	12	15	15
	50	33	25	16	21	19	20
	100	30	29	19	20	24	11
	1000	8	10	10	0	5	6
Phenol	5	21	21	19	19	12	20
	10	28	25	27	26	25	24
	50	29	26	25	26	22	25
	100	28	25	23	28	25	29
	1000	16	24	22	29	27	23
Phenyl *N*-methyl-carbamate	5	20	25	20	21	18	12
	10	25	24	25	24	19	17
	50	30	23	29	25	18	15
	100	23	25	26	26	20	10
	1000	16	24	22	28	26	23
Biphenyl	5	26	22	30	21	17	19
	10	33	27	35	25	23	23
	50	30	30	22	19	28	13
	100	27	24	24	11	19	13
	1000	18	12	20	3	11	8
1-Hydroxybiphenyl	5	29	24	30	28	13	16
	10	63	87	40	55	10	27
	50	30	33	33	27	21	15
	100	25	38	39	30	27	17
	1000[b]	0	0	4	3	11	7
p-Biphenylyl *N*-methyl-carbamate	5	34	28	36	36	31	35
	10	33	33	37	39	37	47
	50	36	40	25	40	40	48
	100	31	32	41	43	39	37
	1000	27	9	24	10	12	17

[a] The compounds were tested for mammalian activated mutagenicity as described by Maron et al. (1983). Values represent mean values of 3 plates. For solvent blanks, 6 plates were used (the standard deviation is shown in the table). All experiments were repeated at least once and gave similar results.

[b] Strong precipitation of test compound in the top agar.

Calculation of molecular connectivity index

The zero-order molecular connectivity index, ${}^0\chi$ was calculated in the following fashion (Sabljić, 1983). Each non-hydrogen atom in a molecule is described by its delta value, δ which is equal to the number of adjacent non-hydrogen atoms. The index is then calculated for each compound according to the expression:

$$ {}^0\chi = \sum_{j=1}^{n} (\delta_j)^{-0.5} $$

TABLE 2

REVERSION OF his⁻ *Salmonella typhimurium* BY NAPHTHALENE AND PHENANTHRENE[a] SERIES

Test compound	μg/plate	TA98 (μl S9/plate)			TA1535 (μl S9/plate)		
		20	50	100	20	50	100
Naphthalene	5	17	22	27	32	45	18
	10	19	37	36	35	47	22
	50	23	35	28	30	36	26
	100	21	32	22	49	33	27
	1000	0	0	0	25	10	14
1-Hydroxynaphthalene	5	26	26	29	37	38	38
	10	39	34	36	51	39	43
	50	29	40	36	43	51	47
	100	28	26	34	42	62	41
	1000	23	23	18	31	42	36
1-Naphthyl-*N*-methyl-carbamate	5	23	24	26	15	19	19
	10	30	23	31	25	20	21
	50	33	38	35	29	18	19
	100	39	29	32	32	22	13
	1000	17	21	15	26	20	18
Phenanthrene	5	19	24	19	70	48	39
	10	22	34	25	34	38	40
	50	22	25	23	26	27	29
	100	24	26	24	28	25	28
	1000[b]	0	0	0	0	0	0
9-Hydroxyphenanthrene	5	29	24	20	42	42	54
	10	39	28	26	49	37	46
	50	42	35	28	55	48	45
	100	27	27	38	59	40	36
9-Phenanthryl *N*-methylcarbamate	5	52	37	34	39	40	28
	10	30	35	39	34	25	28
	50	36	30	33	47	30	38
	100	25	25	27	40	25	23

[a,b] see footnotes to Table 1.

where '*n*' is the number of non-hydrogen atoms in a molecule. The zero-order specific molecular connectivity index is expressed as ${}^0\chi/n$. The second-order molecular connectivity index ${}^2\chi$, refers to a molecular fragment consisting of 3 adjacent and consecutive non-hydrogen atoms (i, j and k) (Kier et al., 1977). The value of (i j k) is computed as the reciprocal square root of the product of the delta values, $(\delta_i\ \delta_j\ \delta_k)^{-0.5}$. These c_{ijk} values are then summed by considering all molecular fragments in the molecule.

The summarizing expression becomes:

$$ {}^2\chi = \Sigma\, c_{ijk} = \sum_s (\delta_i\ \delta_j\ \delta_k)_s^{-0.5} $$

where *s* is the number of molecular fragments consisting of 3 adjacent and consecutive non-hydrogen atoms in a molecule. The second-order specific molecular connectivity index is expressed as: ${}^2\chi/s$.

Calculation of structure–activity relationship

The structure–activity relationship is the correla-

TABLE 3

ZERO- AND SECOND-ORDER SPECIFIC MOLECULAR CONNECTIVITY INDICES OF TEST COMPOUNDS

Compounds		$^0\chi/n$	$^2\chi$	$^2\chi/s$
Benzene	4.242	0.707	2.120	0.353
Phenol	5.112	0.730	2.743	0.343
Phenyl *N*-methylcarbamate	8.104	0.736	4.253	0.327
Biphenyl	8.225	0.685	4.560	0.304
1-Hydroxybiphenyl	9.095	0.699	4.649	0.273
p-Biphenylyl *N*-methyl-carbamate	12.087	0.711	6.692	0.304
Naphthalene	6.811	0.681	3.853	0.296
1-Hydroxynaphthalene	7.682	0.698	3.972	0.264
1-Naphthyl *N*-methyl-carbamate	10.674	0.712	6.200	0.295
Phenanthrene	9.380	0.670	5.758	0.274
9-Hydroxyphenanthrene	10.250	0.683	6.351	0.276
9-Phenanthryl *N*-methyl-carbamate	13.242	0.697	7.829	0.279

tion coefficient between the calculated connectivity index and *Salmonella typhimurium* revertants from plates incubated with 10 μg compound and 20 μl, 50 μl or 100 μl S9 according to the method described by Schwartz (1963).

Results and discussion

The results of testing the mutagenic potency of 12 compounds of a carbamate series in TA98 and TA1535 in the presence of S9, are summarized in Tables 1 and 2. A positive response is one that gives twice the average spontaneous level. For TA98 only, 1-hydroxybiphenyl is positive, while 1-naphthol, carbaryl, 9-hydroxyphenanthrene and 9-phenanthryl *N*-methylcarbamate approach a significant value. For TA1535, positive values are obtained for naphthalene, *p*-biphenylyl *N*-methyl-

TABLE 4

CALCULATION OF RELATIONSHIP BETWEEN SPECIFIC MOLECULAR CONNECTIVITY INDEX AND MUTAGENICITY

Indices	Compounds[c]	Strain	Slope	Regression parameters		
					F	r
$^0\chi/n$	Whole (12)	TA98	−77	0.726	0.527	0.123
		TA1535	−276	2.899[a]	8.409[b]	0.445
	AH (4)	TA98	10	0.079	0.0062	0.025
		TA1535	−654	4.562[b]	20.81[b]	0.821
	OH (4)	TA98	−124	1.515	2.297	0.432
		TA1535	−387	2.542[a]	6.466[b]	0.626
	Carb. (4)	TA98	−261	3.328[b]	11.075[b]	0.725
		TA1535	−279	1.554	2.417	0.441
$^2\chi/s$	Whole (12)	TA98	−161	2.370	5.62	0.376
		TA1535	−253	4.381[b]	19.19[b]	0.601
	AH (4)	TA98	6	0.112	0.012	0.035
		TA1535	−146	1.662	2.764	0.465
	OH (4)	TA98	−223	1.388	1.926	0.402
		TA1535	−207	1.827	3.341	0.500
	Carb. (4)	TA98	−179	2.482[a]	6.160[b]	0.617
		TA1535	−128	0.820	0.673	0.251

The significance of the correlation coefficient is given by t and F values as reported by Schwartz (1963). [a]significant at 0.01 level; [b]significant at 0.001 level; [c]AH: aromatic hydrocarbons, OH: hydroxy compounds, carb: *N*-methylcarbamate compounds; ( ): number of compounds used for calculation.

Calculation was carried out using zero- and second-order specific molecular connectivity values reported in Table 3 and numbers of revertants from plates incubated with 10 μg of test compound in the presence of 20, 50 and 100 μl of 9000 × *g* rat-liver supernatant fraction.

carbamate, phenanthrene, 9-hydroxyphenanthrene and 9-phenanthryl *N*-methylcarbamate.

As reported by Oesch et al. (1981), phenanthrene was converted to a mutagen for base-substitution strains (TA100, TA1535) by the S9 fraction of liver homogenate from rats treated with Aroclor 1254, whereas TA98 was not mutable.

As shown in Table 2, 9-hydroxyphenanthrene is mutagenic with TA1535. Bücker et al. (1979) reported that this compound was not a direct mutagen for TA98 but the reversion of TA1535 was dose-related.

For 1-naphthol, De Flora et al. (1984) showed no mutagenic effect on TA98 and TA1535, but Purchase et al. (1978) reported a positive result with TA1538. The low level of reversion, however, indicates that there are no mutagens in the compound tested, and makes it difficult to interpret the structure-activity relationship.

The zero- and second-order molecular connectivity indices and their corresponding specific indices for the 12 compounds tested are listed in Table 3. Table 4 shows the results of a relationship calculation between connectivity indices and level of reversion.

When the second-order specific molecular connectivity index is used for calculation, a high inverse relationship is found, significant at the 0.001 level. When the compounds are arranged according to function class, and a calculation is carried out using the zero-order molecular connectivity index, the regression is significant for aromatic hydrocarbons versus TA1535 and is significant for methylcarbamates versus TA98. These results suggest that the aromatic hydrocarbons are efficient in reversion of a base-substitution strain, whereas methylcarbamates are more efficient in frameshift strains. Murakami and Fukami (1986) compared the zero- and second-order specific molecular connectivity indices of 12 carbamates to their mutagenic, carcinogenic and teratogenic potencies reported in the literature. They indicate that carbaryl and benomyl with the lowest zero- and second-order specific molecular connectivity indices show teratogenic activity.

From our results, it seems that compounds with second-order specific molecular connectivity indices lower than 0.300 produce an increase in mutagenic activity for physicochemical reasons. A low molecular connectivity index indicates that the molecule has a massed and complicated structure (Murakami and Fukami, 1985). The kinetics of metabolism, the capability for activation, and the ability of the activated molecule to bind to DNA must be related to molecular conformation and can explain differences in mutagenic potencies. However, in order to increase the safety of prediction for mutagenic potencies of chemical compounds, more elaborate methods for molecular description should be developed.

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