MUTAGENICITY OF ALKYL AZIDES

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Alkyl azides showed mutagenicity for *S. typhimurium* TA100 strain with S9 mix. However, no significant activity was observed for TA98 either with or without S9 mix or for TA100 without S9 mix. On the other hand, 3-azido-1,2-propanediol showed the enantioselective activity regardless of S9 mix. Two aryl azides tested were not mutagenic, and trimethylsilyl azide exhibited potent activity for TA100. Their structure-activity relationships are discussed.

KEY WORDS mutagenicity; alkyl azide; steroselectivity; *S. typhimurium*

Originally, organic azides belong to artificially synthesized compounds and have not yet been found in natural products. However, various organic azides are widely used as synthetic intermediates leading to amines, amides or heterocyclic compounds, *etc.*[1] One of the well known compounds is 3'-azido-3'-deoxythymidine (AZT) which has been used as an anti-AIDS drug.[2] Therefore, to clarify the toxicity of such azido compounds is important for the health protection not only of chemists but also of the public at large because no one can rule out the possibility of their release into the environment.

Mutagenicity of azido compounds has been well studied on inorganic azides such as sodium azide and its metabolite, β-azidoalanine (AZAL).[3a] Recently, some azido saccharides, azido alcohols, and azido nucleosides were also reported to show mutagenic activity in the absence of S9 mix.[4] We found that 18 alkyl azides synthesized were all mutagenic for *S. typhimurium* TA100 in the presence of S9 mix, and discuss them in terms of structure-activity relationships.

MATERIALS AND METHODS

Sodium azide, silyl azides, and aryl azides were purchased from Wako Pure Chemical Co., Ltd., Tokyo Chemical Co., Ltd., and Aldrich Chemical Co., Inc. Benzyl azide (**1**), diphenylmethyl azide (**2**), phenethyl azide (**3**), 1-azido-3-phenylbutane (**5**), 1-azido-3,3-diphenylpropane (**6**), and *t*-butyl azidoacetate (**12**) were synthesized from corresponding halides and sodium azide. Both enantiomers (*R* and *S*) of 1-azido-2-phenylpropane (**4**), 2-azido-1-phenylethanol (**7**), 3-azido-2-benzylpropanol (**8**), methyl 2-azidomethylpropionate (**9**), 3-azido-1,2-propanediol (**10**), and 3-azido-3-phenyl-1,2-propanediol (**11**) were synthesized from chiral building blocks which are commercially available or were prepared previously by enzymatic method.[5] The details will be reported elsewhere.

Salmonella typhmurium strains TA98 and TA100 were provided by Dr. B. N. Ames, University of California.[6] All compounds tested were dissolved in DMSO. Mutagenic activity was quantitatively determined by the preincubation method.[7] S9 was prepared from the liver of male Sprague-Dawley rats pretreated with PCB, and mixed with NADPH, NADH, and glucose-6-phosphoric acid according to the procedure developed by Matsushita, *et al.*[8] Benzo[*a*]pyrene and 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide were used as positive controls in the mutation assay with and without S9 mix, respectively. The dose response curves of some typical compounds are displayed in Fig 1.

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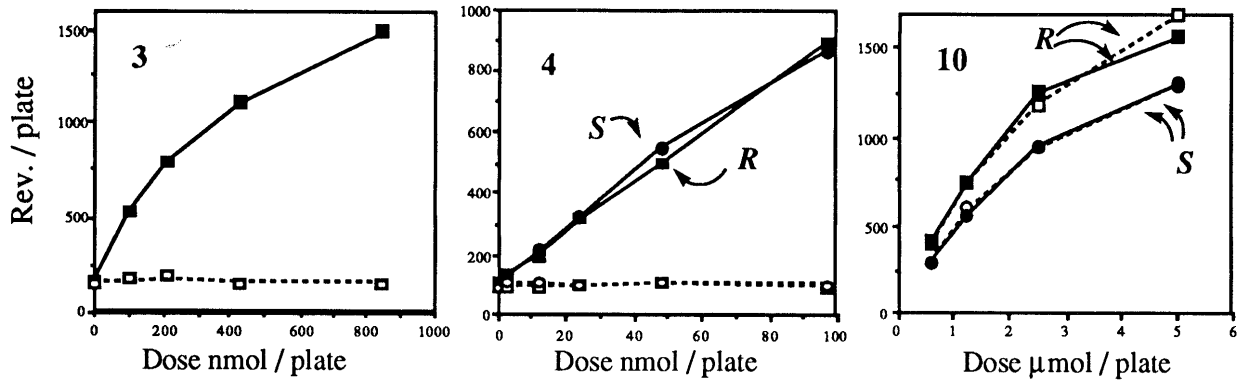
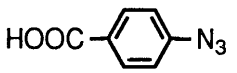
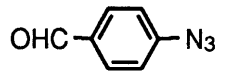


Fig 1. Dose Response Curves of Phenethyl azide (**3**), (*R* & *S*)-2-Phenylpropyl azide (**4**), and (*R* & *S*)-3-Azido-1,2-propanediol (**10**) i n TA100 Strain (+S9 : — , -S9 :---)

Table 1. Mutagenic Activity of Azido Compounds for TA100 Strain

Structure (comp. no.)	Rev./μmol/plate [a)]	Structure (comp. no.)	Rev./μmol/plate [a)]
$PhCH_2N_3$ (**1**)	2,871	$Ph\text{-}\overset{*}{C}H(OH)\text{-}CH_2N_3$ (**7**)	290 (*R*) 315 (*S*)
$Ph\text{-}CH(Ph)N_3$ (**2**)	6,560	$Ph\text{-}CH_2\text{-}\overset{*}{C}H(CH_2OH)\text{-}CH_2N_3$ (**8**)	395 (*R*) 375 (*S*)
$Ph\text{-}CH_2CH_2N_3$ (**3**)	1.850	$H_3COOC\text{-}\overset{*}{C}H(CH_3)\text{-}CH_2N_3$ (**9**)	172 (*R*) 157 (*S*)
$Ph\text{-}\overset{*}{C}H(CH_3)CH_2N_3$ (**4**)	8,141 (*R*) 7,888 (*S*)	$HOH_2C\text{-}\overset{*}{C}H(OH)\text{-}CH_2\text{-}N_3$ (**10**)	283(-S9), 276 (+S9) (*R*) 215(-S9), 222 (+S9) (*S*)
$Ph\text{-}CH(CH_3)CH_2CH_2N_3$ (**5**)	8.632 (*RS*)	$HOH_2C\text{-}\overset{*}{C}H(OH)\text{-}\overset{*}{C}H(Ph)\text{-}N_3$ (**11**)	215 (*R,R*) 124 (*S,S*)
$Ph\text{-}CH(Ph)\text{-}CH_2CH_2N_3$ (**6**)	5,305	$(H_3C)_3COOC\text{-}CH_2\text{-}N_3$ (**12**)	216
	ND (±S9)	NaN_3	8,257 (+S9) 17,350 (-S9)
	ND (±S9)	$(CH_3)_3SiN_3$	7,517 (+S9) 34,808 (-S9)
		$(CH_3)_3SiCH_2N_3$	237 (+S9) ND (-S9)

a) Results obtained in the presence of S9 mix unless otherwise noted. ND : No significant mutagenicity was observed.

Table 1 depicts the specific mutagenic activity (revertants per μmol) of each compound obtained from the slope of the linear portion of the reversion-dose curve.

RESULTS AND DISCUSSION

Alkyl azides tested showed mutagenic activity for TA100 with S9 mix, but not for TA100 without S9 mix nor for TA98 either with or without S9 mix, except 3-azido-1,2-propanediol (**10**) and trimethylsilyl azide, the mutagenicity of which was observed for TA100 without S9 mix, too. These results reveal that S9 mix activates certainly alkyl azides for mutation. In relation to stereoselective

activity, only 3-azide-1,2-propanediol (**10**) and its 3-phenyl derivatives (**11**) showed obvious difference in the potency for each enantiomer. Trimethylsilyl azide and trimethylsilylmethyl azide, being useful tools for organic syntheses, are also mutagenic. Especially, it is noteworthy that trimethylsilyl azide exhibited two times higher potency for TA100 without S9 mix than sodium azide. In addition, no significant mutagenicity of two simple aryl azides tested was observed for TA100 and TA98 either with or without S9 mix.

Owais *et al.* reported that sodium azide is metabolized in a bacterial system to a mutagenic intermediate, AZAL, which was suggested to be further activated to the ultimate mutagenic species.[3b] In the present data, the possibility of the direct conversion of alkyl azides to AZAL with S9 mix seems unlikely because the same mutagenic potency with and without S9 mix was observed for 3-azido-1,2-propandiol (Fig 1). If the AZAL production occurs, it seems likely to be in a bacterial system. When the metabolites of diphenylpropyl azide and benzyl azide with S9 mix were checked, we could isolate diphenylpropionitrile and diphenylpropyl alcohol from the former and only benzyl alcohol from the latter.[9] They did not show mutagenicity under the same conditions using TA100 without S9 mix. From a chemical viewpoint, the nitrile may be produced *via* a nitrene intermediate expected to react with DNA. Another mechanistic possibility is the release of azide associated with alcohol formation. It is likely that no mutagenic aryl azides tested have more difficulty releasing the azide than alkyl azide. Thus, clarification of the mechanism is difficult on the basis of the present data. However, it is evident that hydrophobic azido compounds (**1**-**6**) show higher activity by over one order of magnitude than hydrophilic compounds (**7**-**12**), and a very hydrophilic compound such as 3-azido-1,2-propanediol exhibits mutagenicity even in the absence of S9 mix as described in the literatures.[4] It seems to be due to the characteristics of enzymes included in the S9 mix that apparent enantioselectivity was not observed for several chiral azides (**4**,**7**,**8**, and **9**). Further investigation is being undertaken for the mechanistic approach.

REFERENCES AND NOTES

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5) The optical rotation, $[\alpha]_D$ (*c*, solv.); (*R*)-**4**, 31.2° (1.0, $CHCl_3$), (*S*)-**4**, -29.5° (1.0 ,$CHCl_3$), (*R*)-**7**,-85.9° (1.0, MeOH), (*S*)-**7**, 86.7° (1.0, MeOH), (*R*)-**8**, 37.0° (1.1, $CHCl_3$), (*S*)-**8**, -37.6° (1.0, $CHCl_3$), (*R*)-**9**, -11.3° (2.5, $CHCl_3$), (*S*)-**9**, 13.3° (2.5, $CHCl_3$), (*R*)-**10**, -1.7° (2.0, $CHCl_3$), (*S*)- **10**, 2.0° (1.7, $CHCl_3$), (*R*,*R*)-**11**,-154.2° (1.0, MeOH), (*S*,*S*)-**11**, 158.2° (1.0, MeOH).
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9) After incubation for 24 h at 37° C with S9 mix, the mixture was extracted with ether and CH_2Cl_2. Structures of the metabolites were determinated by GC-MS and ^{1}H-NMR spectroscopy analyses.

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