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STUDIES ON CHEMICAL CARCINOGENS AND MUTAGENS. XXVII [1] ALKYLATING PROPERTY OF MUTAGENIC N-TRIMETHYLSILYLMETHYL-N-NITROSOUREA, A SILICON-ANALOGUE OF N-NEOPENTYL-N-NITROSOUREA, IN AQUEOUS MEDIA

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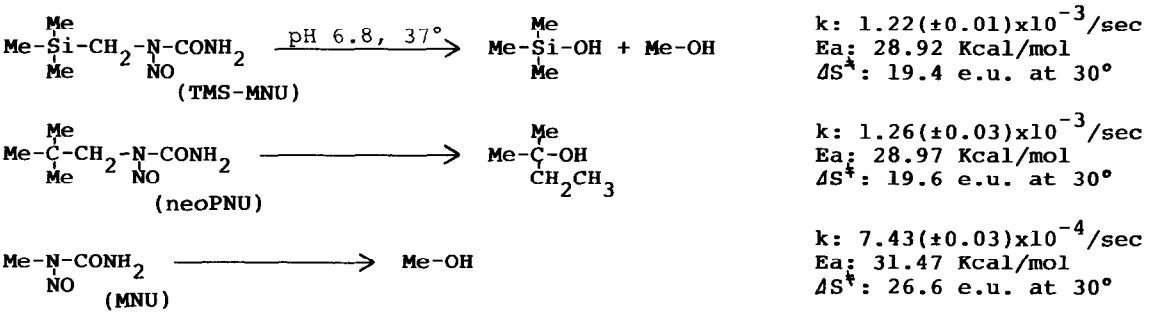
The kinetics for hydrolysis and the chemoselectivity toward nucleophiles of mutagenic N-trimethylsilylmethyl-N-nitrosourea, a silicon analogue of N-neopentyl-N-nitrosourea, were studied.

It is known that the genotoxicity of N-alkyl-N-nitrosoureas is attributed to their alkylating ability toward the informational biopolymer of DNA.[2] Thus, N-alkyl-N-nitrosoureas are hydrolyzed to alkanediazohydroxides which alkylate the target nucleophilic sites of DNA.[3] The size of the genotoxic effect thereby produced is thought to depend substantially on the structure of the alkyl moiety introduced onto DNA. The present study was undertaken to obtain information on the hydrolytic activation and the alkylating characteristics of N-trimethylsilylmethyl-N-nitrosourea (TMS-MNU),[4,5] a silicon-containing mutagenic N-alkyl-N-nitrosourea. N-Methyl-N-nitrosourea (MNU) and N-neopentyl-N-nitrosourea[6] were also examined for comparison.

Rate of Hydrolysis



The rates for hydrolyses of TMS-MNU, neoPNU, and MNU were determined in 1/15 M phosphate buffer (pH 6.8) containing 5% dimethylsulfoxide at 25°, 30°, and 37°C, by following the decrease in UV absorbances at 243, 264, and 244 nm, respectively. The pseudo-first order rate constants (k), the activation energies (Ea) and the entropies of activation ($\Delta S^{\ddagger}$) are shown in the above Chart. It is of interest to note that all the kinetic parameters, k, Ea, and

$\Delta S^{\ddagger}$ of TMS-MNU were almost the same as those of neoPNU, respectively, and that MNU was hydrolyzed at a slower rate with significantly larger Ea and $\Delta S^{\ddagger}$ than those of TMS-MNU and neoPNU, respectively.

Chemoselectivity toward 4-(p-Nitrobenzyl)pyridine, S_{NBP}, in Phosphate Buffer[7)]

The chemoselectvity of these nitrosoureas was evaluated in terms of the ratio of the molar fraction of the nitrosourea which is consumed for alkylation of the ring nitrogen of 4-(p-nitrobenzyl)pyridine (NBP) versus the molar fraction of the residual nitrosourea which is hydrolyzed in an phosphate buffer (pH 6.0) containing 60% acetone. S_{NBP} is a good measure of chemoselectivity and is defined as follows:

$$S_{NBP} = \log \left\{ \frac{[H_2O]}{[NBP]} \times \frac{N}{(100-N)} \right\} \quad \text{-------} \quad \text{eq. (1)}$$

where N is the % molar fraction of the nitrosourea having reacted with the nitrogen of the NBP molecule after completion of the alkylation reaction and the residual % fraction, (100-N), is that consumed in hydrolysis, mainly alkylation of H_2O. $[H_2O]$ and [NBP] are the concentrations of H_2O and NBP in the reaction medium, respectively. As previously reported,[7)] S_{NBP} correlates well with the substrate constant, s, in the Swain-Scott equation.[8)] A linear correlation was found between s and S_{NBP}, the regression equation for the correlation being given below.

$$s = 0.123(\pm 0.0078)S_{NBP} + 0.318(\pm 0.019) \quad \text{-------} \quad \text{eq.(2)}$$

The correlation coefficient was 0.997 (10 samples) and the 95% confidence limits are given in parentheses in the equation.[7)]

The S_{NBP} values of these nitrosoureas, in addition to the Swain-Scott's substrate constants (s) estimated from S_{NBP}, are given in Table I, with those of other nitrosoureas so far reported.[7)] It is obvious that S_{NBP} of TMS-MNU is almost the same as that of MNU and significantly differs from those of other alkylnitrosoureas including neoPNU.

Table I **Chemoselectivity (S_{NBP}) of N-Alkyl-N-nitrosoureas toward 4-(p-Nitrobenzyl)pyridine (NBP) at 37°C**

	S_{NBP}	Subst. const. (s)
TMS-MNU	0.96(±0.02)	0.44
MNU	0.98(±0.02)	0.44
neoPNU	(—)*	(—)*
N-ethyl-N-nitrosourea	-0.35	0.27
N-propyl-N-nitrosourea	-0.27	0.28
N-butyl-N-nitrosourea	-0.45	0.26
N-pentyl-N-nitrosourea	-0.57	0.25
N-benzyl-N-nitrosourea	0.02	0.32

* This nitrosourea did not give any coloring material in the NBP test over the background level.

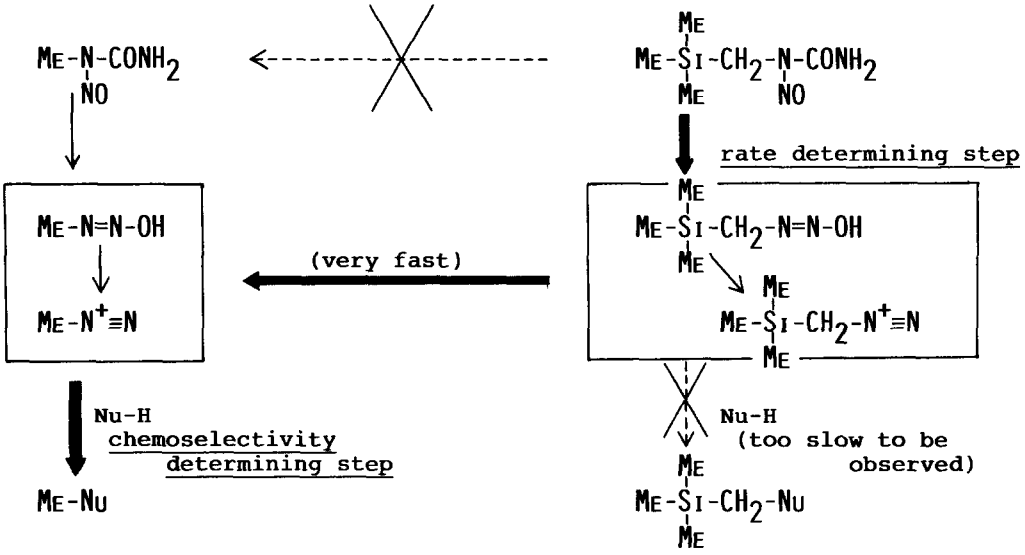
Reactions with Nucleophiles in Aqueous Medium

The nitrosoureas were hydrolyzed in acetone-water (1 : 4 v/v) at 37°C for 2 days and the hydrolytic products were analyzed by gas-chromatography using a Porapak Q column. TMS-MNU was quantitatively converted to methanol and trimethylsilanol. Trimethylsilylmethanol was not detected thereby. As expected, neoPNU gave 2-methylbutan-2-ol quantitatively, which is the rearranged product of neopentyl cation. The treatment of TMS-MNU with aniline in acetone-phosphate buffer (pH 6.0) at 37°C for 1 day gave N-methylaniline in 4.39% yield. N-Trimethylsilylmethylaniline was thereby not detected by gas chromatography, although the authentic preparation[9] of N-trimethylsilylmethylaniline proved to be stable enough under the reaction condition employed. The treatment of MNU under the same reaction condition as the above gave N-methylaniline in almost the same yield of 4.68%, while any alkylated anilines was not detected by the treatment of neoPNU under the same reaction condition.

Discussion

It is worth noting that TMS-MNU and its carbon-analogue, neoPNU, undergo hydrolyses with the almost same kinetical parameters; reaction rate, activation energy, and entropy of activation. It is, therefore, suggested that the same reaction mechanism is involved in the rate-determining steps of the alkylating processes of both TMS-MNU and neoPNU and that the silicon atom does not have any appreciable substituent effects on the hydrolytic activation process. In contrast, TMS-MNU is not a Si-containing alkylating but a methylating agent and its chemoselectivity toward nucleophiles is the same as that of MNU, differing remarkably from those of all the other N-alkyl-N-nitrosoureas so far examined, as shown in Table I. These results indicate that the reactive species of TMS-MNU in the alkylating step, in such aqueous media as examined here, must be the same as that of MNU. As a conclusion, TMS-MNU undergoes hydrolysis at least in aqueous media at around neutrality to give trimethylsilylmethanediazohydroxide at a similar rate to that of neoPNU, and that the diazohydroxide thus produced (or its diazonium ion) is readily hydrolyzed to trimethylsilanol and methanediazohydroxide (or its diazonium ion), prior to the trimethylsilylmethylation of nucleophiles present in the reaction medium. The fact that the hydrolysis rate of MNU is lower than that of TMS-MNU eliminates the possibility that TMS-MNU might be hydrolyzed to MNU prior to the hydrolysis of the nitrosourea structure.

Here, it is to be noted that mutagenicity and cyotoxicity of TMS-MNU on both bacterial and mammalian tester strains are demonstrated in our preliminary study[10] to be very similar to those of MNU, but remarkably different from those of all the other N-alkyl-N-nitrosoureas examined including neoPNU. These biological data support that TMS-MNU is a biological methylating agent equivalent to MNU as described in this paper.



(Nu-H: nucleophiles including H_2O)

References

1) Part XXVI: A. Hakura, S. Ninomiya, K. Kohda and Y. Kawazoe, Chem. Pharm. Bull., **32**, (No.9) in press (1984).

2) J. A. Miller, Cancer Res., **30**, 559 (1970); A. R. Peterson, J. R. Landolph, H. Peterson, S. P. Spears and C. Heidelberger, Cancer Res., **41**, 3095 (1981) and literatures cited therein.

3) J. K. Snyder and L. M. Stock, J. Org. Chem., **45**, 1990 (1980).

4) T. Aoyama and T. Shioiri, Chem. Pharm. Bull.,**29**, 3249 (1981).

5) D. Seyferth, H. Menzel, A. W. Dow and T. C. Flood, J. Organometal. Chem., **44**, 279 (1972).

6) W. Kirmse and H. Schutte, Liebigs. Ann. Chem., **718**, 86 (1968).

7) Y. Kawazoe, N. Tamura and T. Yoshimura, Chem. Pharm. Bull., **30**, 2077 (1982).

8) C. G. Swain and C. B. Scott, J. Am. Chem. Soc., **75**, 141 (1953).

9) J. M. Duff and A. G. Brook, Canad. J. Chem. **55**, 2589 (1977); J. E. Noll, J. L. Speier and B. F. Daubert, J. Am. Chem. Soc., **73**, 3867 (1951).

10) To be published.

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