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SYNTHESIS AND PROPERTIES OF N-TRIMETHYLGERMYLMETHYL-N-NITROSOUREA, A GERMANIUM ANALOGUE OF N-NEOPENTYL-N-NITROSOUREA[1)]

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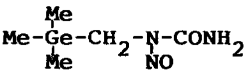
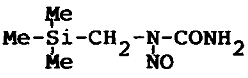
The rate for hydrolysis, chemoselectivity toward nucleophiles, and partition property of N-neopentyl-, N-trimethylsilylmethyl-, and N-trimethylgermylmethyl-N-nitrosoureas were compared. The latter two compounds are silicon and germanium analogues of the neopentyl derivative. The substitution effect of Ge in place of Si or C are discussed on the chemical and physicochemical properties.

As previously reported,[3)] N-trimethylsilylmethyl-N-nitrosourea (TMS-MNU) and N-neopentyl-N-nitrosourea (neoPNU) undergo hydrolyses with almost the same kinetical parameters, suggesting that the same reaction mechanism is involved in the rate-determining step of alkylation without any appreciable substitution effect between Si and C atoms. However, alkylating characteristics involved in the product-determining step of TMS-MNU is almost the same as that of N-methyl-N-nitrosourea (MNU).[3)] Biologically, TMS-MNU was proved to be a methylating agent equivalent to MNU in cytocidal and mutagenic processes.[4)] The present paper describes synthesis and chemical/physico-chemical properties of N-trimethylgermylmethyl-N-nitrosourea (TMG-MNU), a germanium (Ge) analogue of TMS-MNU and neoPNU. The purpose was to determine the substitution effect of Ge in place of Si or C on the chemical and physico-chemical behavior of these genotoxic nitrosoureas.[4)]



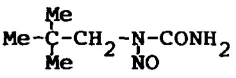
$\mathrm{Me-\overset{Me}{\underset{Me}{Ge}}-CH_2-\underset{NO}{N}-CONH_2}$

TMG-MNU



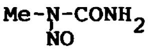
$\mathrm{Me-\overset{Me}{\underset{Me}{Si}}-CH_2-\underset{NO}{N}-CONH_2}$

TMS-MNU



$\mathrm{Me-\overset{Me}{\underset{Me}{C}}-CH_2-\underset{NO}{N}-CONH_2}$

neoPNU



$\mathrm{Me-\underset{NO}{N}-CONH_2}$

MNU

Synthesis of TMG-MNU

A mixture of KOCN (4.83 g), tetraethylammonium iodide (0.414 g), and trimethylgermylmethyl chloride (6.65 g) was dissolved in 38 ml of dimethylformamide and heated at 100-105°C for 45 min. After cooled to 2°C, ammonia was bubbled into the reaction mixture for 1 hr. The temperature was maintained below 14°C. The inorganic salt was removed by filtration and washed with benzene. The filtrate and the washings were combined, evaporated in vacuo, and the resulting residue, once dissolved in 10 ml of water, was extracted with benzene-ethyl acetate (1:1 v/v). The extract was washed with H_2O and then saturated aq. NaCl and dried over $MgSO_4$. After evaporation, the residue was recrystallized from benzene to give 3.61 g of N-trimethylgermylmethylurea as white needles (50.2% yield). mp: 114-115°C. Anal. Calcd. for $C_5H_{14}N_2OGe$: C, 31.48; H , 7.40; N, 14.68. Found: C, 31.35; H, 7.57; N, 14.87. NMR (in $CDCl_3$) δ ppm: 0.22 (s, $3xCH_3$); 2.80 (s, CH_2); 4.63 (broad s, NH_2); 5.2 (broad s, NH). IR (KBr) cm^{-1}: 3200, 3350 (NH); 1660 (C=O); 830, 600 (Ge-C). The urea thus prepared was finely pulverized and mixed with 1.24 g of $NaNO_2$ in 15 ml of H_2O. To this slurry was added 2 g of 50% H_2SO_4 with stirring under cooling. After 10 min, the precipitates were washed with H_2O, followed by vacuum drying. The yellow residue was recrystallized from hexane to give 2.17 g of TMG-MNU as yellow needles (70% yield). mp: 82-85°C. Anal. Calcd. for $C_5H_{13}N_3O_2Ge$: C,27.32; H, 5.96; N 19.12. Found: C, 27.34; H, 5.88; N, 19.45. NMR (in $CDCl_3$) δ ppm: 0.18 (s, $3xCH_3$); 3.73 (s, CH_2); 7.0 (broad s, NH_2). IR (KBr) cm^{-1}: 3200, 3300 (NH); 1720 (C=O); 830, 600 (Ge-C).

Kinetics of Hydrolysis

The rate for hydrolysis of TMG-MNU was measured in 1/15 M phosphate buffer (pH 6.8) containing 5% dimethyl sulfoxide at 30.0, 35.7, and 39.7°C, by following the decrease in UV absorbance at 273 nm. The observed (peudo-first order) rate constant (k_{obs}), the second-order rate constant (k_2), the activation energy (Ea), and the entropy of activation ($\Delta S^{\ddagger}$) calculated from k_2 are shown in Table I, together with those of TMS-MNU, neoPNU, and MNU.[3] The activation parameters of the Ge-analogue are appreciably different from those of the C- and Si-analogues, although there is no difference in the rate at 37°C for them. MNU is hydrolyzed more slowly at 37°C than the others and its activation energy is the largest.

Table I Kinetical Data at 37°C in Phosphate Buffer (pH 6.8)

	$t_{1/2}$	k_{obs}/sec	k_2/M·sec	Ea(Kcal/mol)	$\Delta S^{\ddagger}$ (e.u.)
TMG-MNU	587 sec	$1.18x10^{-3}$	$2.96x10^{4}$	26.0	43.7
TMS-MNU	568	$1.22x10^{-3}$	$3.07x10^{4}$	28.9	53.2
neoPNU	551	$1.26x10^{-3}$	$3.17x10^{4}$	29.0	53.4
MNU	933	$7.43x10^{-4}$	$1.87x10^{4}$	31.5	60.5

Rates are the means of more than three measurements, respectively. k_2 *was calculated provided that Kw = 14.4, since pH of neutral phosphate buffer (pH=7.00) containing 5% DMSO was 7.20.* $\Delta S^{\ddagger}$ *was calculated with* k_2 *at* 37°C.

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

Chemoselectivity, S_{NBP}, was obtained by the procedure previously described[3,5] and was converted to the substrate constant (s) in Swain-Scott equation[6] according to the following correlation equation.[5)]

$$s = 0.123\ S_{NBP} + 0.318$$

The chemoselectivity constant thus obtained is shown in Table II, together with those of some other nitrosoureas of interest for comparison.[3)] It is worth noting that the chemoselectivity of the Ge-analogue is the same as those of MNU and TMS-MNU, suggesting that a common reaction intermediate is produced in the product-determining steps among these derivatives.

Table II Chemoselectivity and Partition Property

	$\underline{S}_{NBP}$	$\underline{s}$	log k′
TMG-MNU	0.99	0.44	0.733
TMS-MNU	0.96	0.44	0.717
neoPNU	—*	—*	0.667
MNU	0.98	0.44	0.116
N-ethyl-N-nitrosourea	-0.35	0.27	0.224
N-propyl-N-nitrosourea	-0.27	0.28	0.362
N-butyl-N-nitrosourea	-0.45	0.26	0.539
N-pentyl-N-nitrosourea	-0.57	0.25	0.751
N-benzyl-N-nitrosourea	0.02	0.32	0.520

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

Products of Hydrolysis

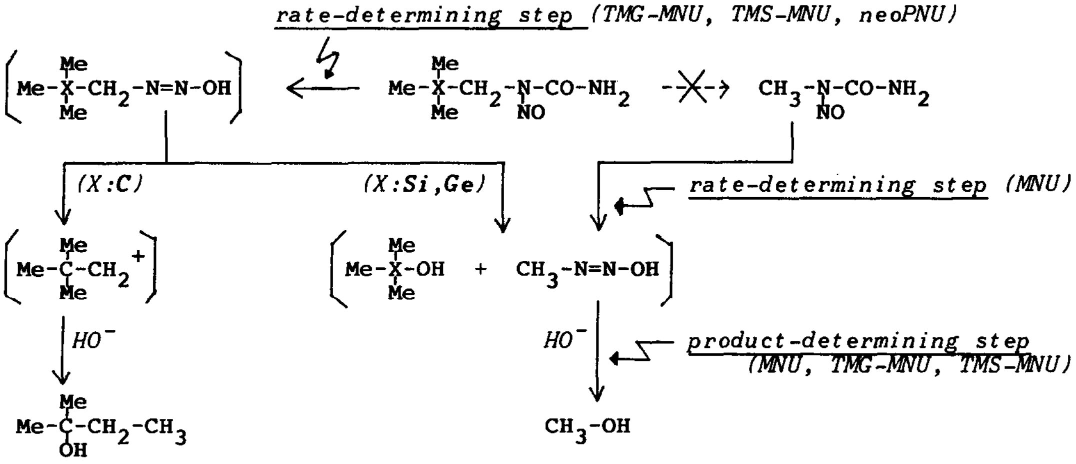
After TMG-MNU was hydrolyzed in acetone-water (1:4 v/v) at 37°C, the hydrolyzate was analyzed by gas-chromatography using a Porapak Q column, indicating that a stoichiometric amount of methanol was produced.

Partition Property

Since these nitrosoureas are not very stable in aqueous media, the method of partitioning between n-octanol and water is not adequate. Therefore, the relative partition property among these nitrosoureas was estimated from the retention time in HPLC.[7)] The value of log k′, as a measure for the partition property, is defined as log $(R_x-R_o)/R_o$, where R_x and R_o are the retention times of the nitrosourea in question and the solvent used for the HPLC measuremnt, respectively.[7)] HPLC was carried out with a JASCO TWINCL type apparatus equipped with a 4.6 mm x 250 mm JASCO SIL C_{18} column at 25°C by eluting with 60% methanol in water. The log K′ values thus obtained are shown in Table II. The lipophilicity increases with an increase in the number of carbons of the alkyl group, as expected from the readily estimated van der Waals volume.[8)] The Ge-analogue is more lipophilic than the Si-analogue, which is followed by the C-analogue, although the difference is not very large.

Discussion

Evaluating previously obtained data with the Si-analogue,[3)] the reaction characteristics may be summarized as shown in the chart. In conclusion, the Ge-analogue undergoes hydrolysis through a very similar transition state to those of the C- and Si-analogues in the rate-determining step, but its reaction intermediate (alkanediazohydroxide) readily undergoes a further hydrolytic cleavage of the Ge-CH_2 bond to release trimethylgermanium hydroxide[9] and methanediazohydroxide, the latter being the reaction intermediate of MNU. Therefore, TMG-MNU is a methylating agent equivalent to MNU in the product-determining step in aqueous media. It is worth noting that the activation parameters of hydrolysis of TMG-MNU appreciably differ from those of the C- and Si-analogues, in other words, the substitution effect between Ge and Si may be larger than that between Si and C in the rate-determining step. It is evident from the partition property, as expected, that the hydrophobicity monotonously increases in order of the C-, Si-, and Ge-analogues.



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References and Notes

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